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The Association of SAM68 Gene Expression and Several Biochemical Markers Measurements in Patients with Carcinoma of prostate

A Thesis

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Muneer Kadhum khudhair Hamadi

M.SC. Clinical Biochemistry (2013)

Supervisor

Prof. Dr. Maha Fadhil Smaism

Dept. of Biochemistry, College of Medicine

University of Babylon

Supervisor

Prof.Dr. Mohammed Ridha Joodi A.Jalo

Dept. of Surgery, College of Medicine

University of Babylon.

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سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَى كُلِّ شَيْءٍ شَهِيدٌ.

صدق الله العلي العظيم

فصلت (53).

Dedication

This thesis is dedicated to one of the houses of the old Najaf, to each drop of the blood shed on the land of this country, to my parents, my Family, my brothers and my sisters.

My endless love for all from my support and encouragement.

Muneer

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Muneer k. khudhair

Supervisor Certification

We certify that this thesis were prepared under my supervision at the College of Medicine, University of Babylon as a partial fulfillment of the requirement for the degree of PhD of Biochemistry .

Supervisor

Professor

Dr. Maha Fahdil Smaism

Professor

Dr. Mohammed Ridha Joodi A.Jalo

In the view of the available recommendation, I forward the present thesis for debate by the examining committee

Signature:

Prof .Dr. AbdulSamie H. Al-Ta'ee

Head of Department of Clinical Biochemistry

College of Medicine –University of Babylon.

Summary

Prostate cancer is a heterogeneous age-related disorder that is a leading cause of cancer related deaths among men. Prostate cancer is the most common cancer in the world specifically in Iraq, compared to other cancers, that affects men of old ages of 50 years and more, but it rarely affects men that lesser than this age category. Carcinogenesis of prostate is a multistep accumulation of genetic lesions that may result in uncontrolled cellular proliferation, a decrease in cell death or apoptosis, invasion, metastatic spread and blockade of prostatic cell differentiation. Benign prostate hyperplasia is not cancer and does not develop into cancer. But it can be a serious medical problem for some men.

Benign prostatic hyperplasia (BPH) is a histologic diagnosis that refers to the proliferation of smooth muscle and epithelial cells within the prostatic transition zone. The exact etiology is unknown; however, the similarity between BPH and the embryonic morphogenesis of the prostate has led to the hypothesis that BPH may result from a "reawakening" in adulthood of embryonic induction processes. Sam68 is up-regulated in many human cancers, including prostate cancer (PCa) where it promotes cell proliferation and survival.

The expression of the Sam68 gene in the patient's blood with Prostate tumors was estimated in the 120 specimens of cDNA prepared from RNA of Prostate cancer and Benign Prostate Hyperplasia.

The concentration of RNA was estimated using two techniques, the first is Nanophotometer, where the concentration was (55 ±13)*1000ng/ml, and another technique is Quantus Fluorometer, and the concentration was (58 ±4.21)*1000ng/ml. The products were amplified with the use primer level of 10 pmole was considered to be the best. The current study proved that the sam68 gene

was highly expressed in prostate cancer, where were highly significant P<0.001 when compared to the gene expression in benign prostate hyperplasia by using real time- PCR in the presence of the housekeeping gene.

The studied chemical markers were estimated by the ELISA technique. The current study showed that the enzyme AKT1 was high activity in prostate cancer and benign prostate patients but not significance, as it recorded a P >0.001.

According to statistical results of the β-catenin concentration were significantly increased in patients with prostate cancer to benign prostate hyperplasia, where P value <0.001

Also, the current study showed that Interleukin 8 was highly significant in prostate cancer, it recorded a value of P <0.001, compared with the concentration in benign prostate hyperplasia patients

Statistical significance was assigned in this study as P <0.001, indicating that the concentration of Vascular Endothelial Growth Factor in prostate cancer patients was high when compared with benign prostate hyperplasia patients.

Serum Vascular Endothelial Growth Factor Receptor-2 was statistically significantly higher in PCa compared to benign prostate hyperplasia patients (p<0.001)

Our finding that Sam68 is required for optimal proliferation and survival of prostate cancer cells may demonstrate that Sam68 has separable effects on AR-regulated transcriptional activity and alternative splicing, both of which may affect PCa

Serine/threonine kinase-1 activity and substrate phosphorylation in cells, suggesting that active kinase is exclusively associated with cellular membranes, ensures that Akt-1 activity play a role in creating an anti-apoptotic threshold

The activation of canonical Wnt signalling and the AR are related in prostate cancer progression, it was associated with PSA progression in prostate tumors patients.

Chemokines and cancer are very much intertwined concepts, the surprisingly high production of IL-8 in most tumors, is due to various roles for IL-8 in cancer stem cells, angiogenesis, and inflammatory infiltrates

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List of Abbreviations

Abbreviate	Term
5α-Ri	5α -Reductase inhibitors
ABX-IL-8	Anti-Body-IL-8
Akt	serine/threonine kinase
Ang	Angiopoitein factor
AR	Androgen receptors
AUA	American Urological Association
BPH	Benign Prostate Hyperplasia
BPO	Benign Prostatic Obstruction
CXCL8	Interleukin-8
DHT	5-α-dihydrotestosterone
DRE	Digital Rectal Examination
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	endoplasmic reticulum
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
HIF	Hypoxiainducible factor
IL8	Interleukin-8
IPSS	International Prostate Symptom Score
IV	intravenous infusion
LH	luteinizing hormone
LHRH	Hypothalamic luteinising hormone-releasing hormone
LUTS	lower urinary tract symptoms
PCa	Prostate Cancer
PH	pleckstrin homology
PIGF	placental growth factor
PKB	protein kinase B
PSA	Prostate specific antigen
QoL	Quality of Life
RBP	RNA binding proteins
Real time PCR	Real time-Polymerase Chain Reaction.
SAM68	SRC associated in mitosis of 68 kDa
STAR	signal transduction and activation of RNA metabolism
TK	tyrosine kinase
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

Introduction

The prostate goes through two main growth periods as a man ages, the first is early in puberty, when the prostate doubles in size, the second begins around age 25years and continues during most of a man's life. Benign Prostate Hyperplasia is when it gets large enough to cause problems, as the prostate enlarges, it squeezes the urethra. The bladder wall becomes thicker. Eventually, the bladder may weaken and lose the ability to empty completely urine then remains in the bladder. These problems cause many of the lower urinary tract symptoms (LUTS) of BPH. Benign Prostate Hyperplasia (BPH) is a significant problem can be defined as an abnormal anatomy (pathology)and/or function(physiology) that may cause harm to the human body.

In clinical Benign Prostatic Hyperplasia(BPH), is prostate adenoma, resulting in a varying degree of Benign Prostatic Obstruction (BPO) that may cause harm to the human bladder or kidneys(1). The need for intervention depends on the whether the disease is: first, life threatening; second, affecting the functions of other organs; and last, affecting the patient's Quality of Life (QoL), in order of priority. Though clinical BPH is seldom life threatening disease , severe obstruction leading to hydronephrosis and infection in an immunocompromised patient that may cause death(2). Benign Prostatic Hyperplasia (BPH) is a pathological change that occurs in the prostate, leading to enlargement. It's a condition that greatly affects the quality of life of middle-aged and elderly men.

Histopathologically, hyperplastic changes frequently occur in the prostate tissue of elderly men, the incidence of which has been reported to reach approximately 80%in men in their 70s.Approximately 25% of men with histologic

BPH are assumed to experience Lower Urinary Tract Symptoms (LUTS) and receive some kind of treatment(1).

Prostate Cancer is the first most common cancer in men before of breast cancer (in women) and colon cancer, Prostate cells can begin to mutate and can metastasis into surrounding tissue, such as bone(3), which is a metabolically active tissue being continuously remodeled throughout life(4). However, there are several risk factors associated with prostate cancer, such as family history, race, diet, and the age, which age being the main factor (3). During the last twenty years, several biochemical markers of both bone formation and resorption have been introduced.

Prostate specific antigen (PSA) is widely accepted as the most important marker for detecting prostate tumers and for monitoring treatment(5). There is no specific normal or abnormal level of PSA in the blood, and levels may vary over time in the same man(6). In the past, most doctors considered PSA levels of 4.0 ng/mL and lower as normal. Therefore, if a man had a PSA level above 4.0 ng/mL, doctors would often recommend a prostate biopsy to determine whether prostate cancer was present. However, more recent studies have shown that some men with PSA levels below 4.0 ng/mL have prostate cancer and that many men with higher levels do not have prostate cancer(7). In addition, various factors can cause a man’s PSA level to fluctuate. For example, a man’s PSA level often rises if he has prostatitis or a urinary tract infection. Prostate biopsies and prostate surgery also increase PSA level. The concentration of bound or complexed PSA is higher in prostate carcinoma, whereas that of free PSA is higher in BPH. The ratio of free to total PSA is lower in men with prostatic carcinoma. The PSA index is expressed as the percentage of the total plasma PSA that is free; an index above about 17 per cent is suggestive of BPH and one of less than 17 per cent of prostate carcinoma(8).

1.1 Definition

• BPH is a term that describes when the smooth muscles and cells of the prostate enlarge. This term is used for any prostate enlargement.

• BPE, benign prostatic enlargement, a term that means the prostate gland itself has enlarged. This is a condition of BPH. Not all men with BPH will develop BPE. About 50 percent will.

• BPO, benign prostatic obstruction, a term that means the enlarged gland and surrounding tissue causes a blockage. About 50 percent of men with BPE have a problem with obstruction(9).

1.1.1 Anatomy

Prostate is a gland found only in males. It is located in front of the rectum and below the urinary bladder (Figure 1-1). The size of the prostate varies with the age. In younger men, it is about the size of a walnut, but it can be much larger in older men(10). The function of the prostate is to produce fluid that protects and nourishes sperm cells in semen, making the semen more liquid. Prostatic fluid is rich in enzymes, proteins and minerals. Just behind the prostate are glands called seminal vesicles that make most of the fluid for semen. The urethra, which is the tube that carries urine and semen out of the body, goes though the center of the prostate(11).

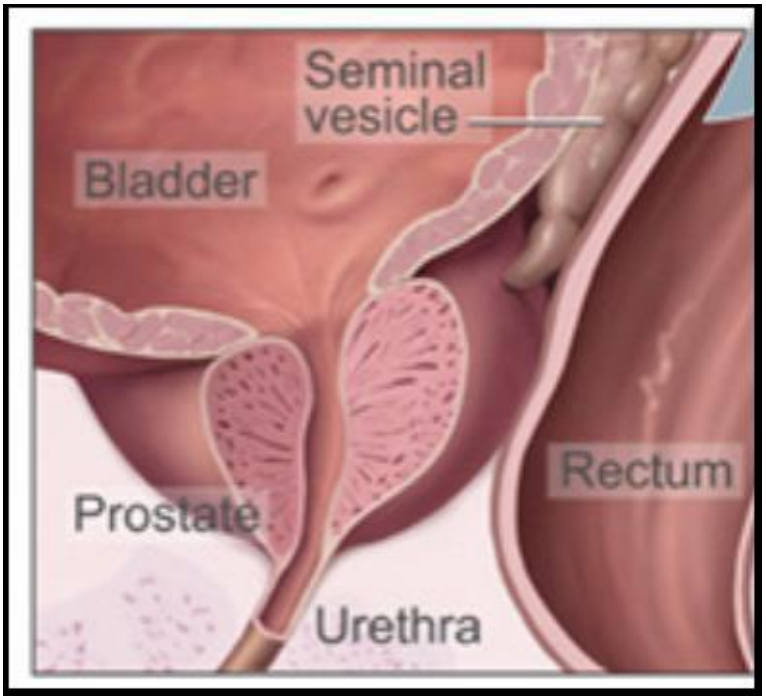


Figure 1-1: Male lower abdominal anatomy (10).

1.1.2 Physiology

Prostate cells are physiologically dependent on androgens to stimulate growth, function and proliferation. Testosterone, although not tumorigenic, is essential for the growth and perpetuation of tumor cells(12). The testes are the source of most androgens, with adrenal biosynthesis providing only 5-10% of androgens (i.e. androstenedione, dihydroepiandrosterone and dihydroepiandrosterone sulphate) (13). Testosterone secretion is regulated by the hypothalamic-pituitarygonadal axis. Hypothalamic luteinising hormone-releasing hormone (LHRH) stimulates the anterior pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Luteinising hormone stimulates the Leydig cells of the testes to secrete testosterone. Within the prostate cell, testosterone is converted to 5-α-dihydrotestosterone)DHT) by the enzyme 5-α-reductase; DHT is an androgenic stimulant about 10 times more powerful than testosterone. Meanwhile, circulating testosterone is peripherally aromatized and

converted to oestrogens ,which together with circulating androgens; exert a negative feedback control on hypothalamic LH secretion (13). If prostate cells are deprived of androgenic stimulation, they undergo apoptosis (programmed cell death). Any treatment that results ultimately in suppression of androgen activity is referred to as androgen deprivation therapy (13).

1.2 Tumor

A tumor, also known as a neoplasm, is an abnormal mass of tissue which may be solid or fluid-filled. A tumor does not mean cancer. Tumors can be benign (not cancerous), pre-malignant (pre-cancerous), or malignant (cancerous). There are many different types of tumors and a variety of names for them; their names usually reflect their shape and the kind of tissue that appear in it. But simply, a tumor is a kind of lump or swelling, it does not necessarily pose a health threat (14).

1.2.1 Prostate Cancer

Carcinogenesis of prostate is a multistep accumulation of genetic lesions that may result in uncontrolled cellular proliferation, a decrease in cell death or apoptosis, invasion, metastatic spread and blockade of prostatic cell differentiation (15).

In the prostate, the expression of oncogene is a driven malignant conversion and expression of tumor suppressor genes that inhibit this process(16). Several types of cells are found in the prostate, but almost all prostate cancers develop from the gland cells. Gland cells make the prostate fluid that is added to the semen. The medical term for a cancer that starts in gland cells is adenocarcinoma(17).

Other types of cancer can also start in the prostate gland, including sarcomas, small cell carcinomas, and transitional cell carcinomas. Some prostate cancers can grow and spread quickly, but most of them grow slowly. In fact, autopsy studies show that many older men (and even some younger men) who died

of other diseases also had prostate cancer that never affected them during their lives. In many cases neither they nor their doctors even knew they had it(17).

Prostate cancer is the most prevalent cancer in men, with a median age at diagnosis of 68 years. Two-thirds of prostate cancer-related deaths occur in men aged > 75 years (18). Older men tend to have larger tumors of a higher grade than younger patients(19).

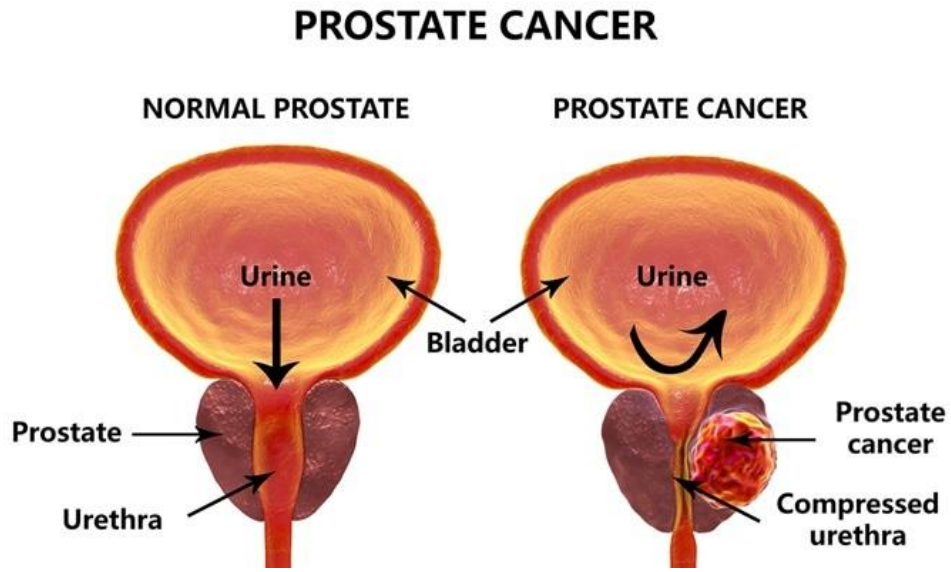


Figure 1-2: Prostate cancer illustration(20)

Prostate cancer (PCa) is a heterogeneous age-related disorder that is a leading cause of cancer related deaths among men (21); it is the second most common cancer among men worldwide (22), and the most common cancer among American and European males (23). Approximately 1.1 million cases of PCa were reported in 2012, of which 70% were in developing countries(24). The prevalence of PCa, which varies among ethnic groups, may be related to genetic and environmental factors. Nevertheless, globally each year about 270,000 men die from PCa (24). PCa, the third most common cancer in men in Iran, is less common than in Western countries(24)(25). Iran has a low incidence of PCa (9.11 per 100,000)(26) while PCa patients who were admitted to a Middle Euphrates Cancer Center in Al-Najaf Al-Ashraf Province during 2018 were 8.27 per 100,000

According to the statistics documented in the aforementioned center. Such differences of PCa incidence in different countries may be associated with such factors as lifestyle and socioeconomic conditions(26).

1.2.2 Benign prostate hyperplasia

Benign prostate hyperplasia (B.P.H) is not cancer and does not develop into cancer. But it can be a serious medical problem for some men. If it requires treatment, medicines can often be used to shrink the size of the prostate or to relax the muscles within it, which usually helps with urine flow. If medicines aren't helpful (some type of surgery) such as a transurethral resection of the prostate may be needed(10).

It seems likely that the nature of B.P.H is a failure of apoptosis (natural programmed death of cells) and that some of the drugs used to treat it may induce that process (27).

Benign prostatic hyperplasia (BPH) is a histologic diagnosis that refers to the proliferation of smooth muscle and epithelial cells within the prostatic transition zone(28) The exact etiology is unknown; however, the similarity between BPH and the embryonic morphogenesis of the prostate has led to the hypothesis that BPH may result from a “reawakening” in adulthood of embryonic induction processes. The enlarged gland has been proposed to contribute to the overall lower urinary tract symptoms (LUTS) complex via at least two routes:

(1) Direct bladder outlet obstruction (BOO) from enlarged tissue (static component)

(2) From increased smooth muscle tone and resistance within the enlarged gland (dynamic component).

LUTS are best quantified by validated questionnaires, such as the International Prostate Symptom Score (IPSS) or the American Urological Association (AUA)

symptom score(1). LUTS in elderly men are dominantly caused by urodynamic changes of the lower urinary tract, such as benign prostatic obstruction and detrusor overactivity or underactivity (28).

In parallel to BPH, also BPE, LUTS and abnormal urodynamic patterns increase with age. The prevalence ofmoderate/severe LUTS (IPSS > 7) is around 20% in the 5th, 30% in the 6th and 40% in the 8th decade of life. In a prevalence study based on 2,096 men living in Austria, it was calculated that currently approximately 350,000 men older than 40 years living in Austria (total population 8.7 million) have moderate to severe LUTS (29). Due to demographic changes in countries, The morbidity rate will be higherو substantially to approximately 500,000 in the next two decades, emphasizing also its socioeconomic relevance (29). Despite its high prevalence and socioeconomic impact, the pathophysiology of BPH is only incompletely understood. It is, for example, still largely unknown why some men develop a 40-g prostate and others a 200-g prostate.

1. 3. Androgen Pathway & Androgen receptors

The prostate, like other sex-accessory tissues, is stimulated in its growth, maintenance and secretory function by the continued presence of certain hormones and growth factors. Foremost among these is testosterone. Serum testosterone is under the control of the hypothalamic (LHRH)- pituitary (LH/FSH)-testicular (testosterone) hormone axis. Testosterone originating from the testis (95%) and adrenal gland (5%) is the major serum androgen stimulating prostate growth. Although testosterone is the primary plasma androgen, it appears to function as a pro-hormone in that the most active form of the androgen in the prostate is dihydrotestosterone (DHT)(30).

Hormonal regulation of BPH is dependent on the presence of androgen and oestrogen receptors. In addition, activity of the enzyme 5α-reductase plays an important role in the BPH pathogenesis. Androgen receptors (AR) are widely

expressed in benign epithelium and adjacent stroma. Importantly, alterations in AR expression comparable to those observed in cancer tissue have not been observed in benign disorders. Thus, there are no reports in the literature on AR gene amplifications, mutations or enhanced interaction with coactivators in BPH. Inhibitory approaches in BPH primarily target 5α-reductase (31).

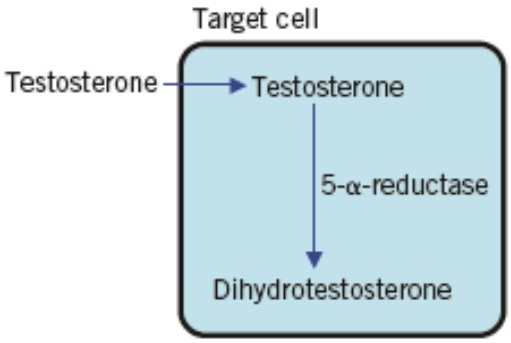


Figure 1-3: Scheme the conversion of testosterone to the active form(8)

1.4. Symptoms and signs

Prostate Tumors may not cause signs or symptoms in its early stages. There are several symptoms as listed below:

• Incomplete emptying: the feeling your bladder is full, even after passing urine
• Frequency: the need to pass urine often, about every one to two hours
• Intermittency: the need to stop and start several times when passing urine
• Urgency: feeling the urgent need to pass urine as if you can't wait
• Weak stream: a weak urine flow
• Straining: trouble starting to pass urine or the need to push or strain to pass urine
• Nocturia: the need to wake up at night more than two times to pass urine(32) (33)

1.5. Risk factors

The risk factors include Age, metabolic syndrome, diabetes, obesity, hypertension, diet and sex hormone levels. Typically, these factors do not occur in combination, but in certain men they can overlap.

1.5.1 Age

Benign prostate hyperplasia is increases with age, which has been confirmed by numerous studies. It was reported that prostate volume increased at a median rate of 0.6 ml per year (range: −9.9 to 62.1 ml), which represented a median annual change of 2.5% (34). Although symptom severity cannot be correlated directly with prostate volume, having a large prostate volume is a risk factor for the development of LUTS. That is, larger prostates are associated with increased risks of urinary retention, increased future need for surgery and clinical progression of BPH (35).

Also Age is one of the risk factors of prostate cancer which increase with the age. More than 90% of men diagnosed with prostate cancer are older than 50 years (36). The incidence of LUTS also increases with age. For example, a prospective study examined the incidence and progression of LUTS in men enrolled in the Health Professionals Follow-up Study, incidence and progression rates for LUTS significantly increased with age, and progression rates were higher than incidence rates (37).

1.5.2. Metabolic syndrome

Metabolic syndrome includes hypertension, dyslipidaemia, glucose intolerance, obesity and insulin resistance with compensatory hyper-insulinaemia(38). New findings on the development of BPH and BOO support the notion that metabolic syndrome can influence the natural course of these condi-

tions (29). Other study showed that the differences in prostate volume were significantly higher in obese patients, older patients and in those with low serum concentrations of HDL cholesterol (39).

Men with LUTS also have significantly higher levels of glycosylated haemoglobin (indicating poorer control of blood glucose) than men without LUTS (40).

Obesity Increased levels of adipose tissue have been shown to be associated with greater prostate volume (41)(42). Also, alcohols abuse and cigarette smoking may also be risk factors for prostate cancer(43).

1.5.3.Diet

Like many other risk factors, a clear correlation between diet and the development of BPH and LUTS has not been shown. However, some evidence suggests that various macronutrients and micronutrients might influence the risk of developing BPH and LUTS. Initially, milk and dairy products were thought to increase the risk of BPH and LUTS(28); however, a later study has shown no association (44). In addition, fruit might have a protective role in preventing BPH(28). Furthermore, one review suggested that the low occurrence of BPH in Asian men, as well as in vegetarian men, can be attributed to low-fat and high-fibre diets (45).

1.5.4.Genetic

To date the vast majority of genetic studies in prostatic diseases have focused on prostate cancer rather than BPH. However, a study from China reported that variants in 2q31 and 5p15 are associated with aggressive (defined by multiple criteria) BPH(46). Another study reported that rs103294, a single-nucleotide polymorphism of *LILRA3* (encoding leukocyte immunoglobulin-like receptor A3), which has been shown to be associated with prostate cancer risk in a Chinese population, was associated with risk of developing BPH (47).

Family history, men with a brother or father with prostate cancer has a two-fold risk of developing the disease. Those with both an affected brother and father have an eight-fold increased risk (36). Race, African-American men have a higher risk for prostate cancer due to a genetic factor (48).

1.6. Diagnosis of Prostate Tumor

1.6.1 Prostate Specific Antigen (PSA)

Prostate specific antigen (PSA) is the optimal tumor marker for prostate tumor, would be effective for early detection, staging and monitoring patients after definitive treatment, the PSA as a tumor marker would have a high sensitivity, specificity and positive predictive value for distinguishing men with BPH from men with prostate cancer (49). The measurement of PSA level has revolutionized the diagnosis of prostate cancer (50)

1.6.2. International Prostate Symptom Score (IPSS):

International Prostate Symptom Score (IPSS) are subjective questionnaires that can be used to help evaluate lower urinary tract symptoms and their effect on patients suffering from BPH (51). These questionnaires have patients rate symptoms of incomplete bladder emptying, frequency intermittency, urgency, weak stream, straining, and nocturia on scales from 0 (not at all) to 5 (almost always). The scores are then tallied, and classified as mild (0-7), moderate (8-19), or severe (20-35) (52). These rankings help to guide treatment decisions and responses. The IPSS contain an additional quality of life measure, asking patients to classify their feelings if they had to live with their urinary symptoms for the rest of their lives on scale of 0(delighted) to 6(terrible). The maximum IPSS is 35,and patients are classified as having severe symptoms if they have an IPSS of 20 to 35, After medical treatment is initiated, the IPS questionnaire can be used to Monitor

Response to therapy (53)

1.6.3. Digital Rectal Examination (DRE)

Perform a DRE to assess the size, shape, and consistency of the prostate gland. An enlarged prostate often presents on examination as soft, smooth, boggy, mobile, and with an obscured sulcus. Note any nodules or indurations, which may suggest prostate cancer(51) . Prostate cancer nodule usually is hard and firm and any asymmetry of each lobe of the Prostate should be further evaluated (53). If tissue looks suspicious, a biopsy is taken. Pathologists evaluate a biopsy using a subjective rubric called the Gleason scoring system which gives an overall summary of progression and aggressiveness of the cancer. Cancer's grade is based on a comparison of the prostate tissue as seen under a microscope to a discrete model structure. The scale runs from 1 to 5, where 1 represents cells that are very nearly normal, and 5 represents cells that do not resemble native-normal cells. Two grades of the most prevalent tissue structures are summed to create a Gleason score between 2 and 10. The more advanced the cancer is, the higher its Gleason score (54). Most prostate cancers are located in the peripheral zone (Figure 1.3) of the prostate and may be detected by DRE when the volume is about 0.2 ml or larger

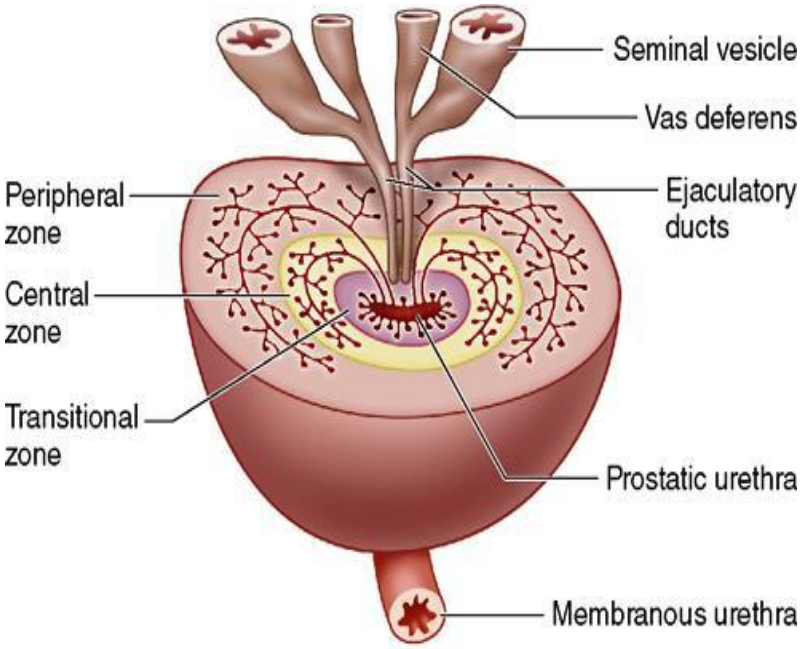


Figure 1.3 Prostatic Zones (55)

1.6.4. Prostatic Ultrasound

Trans abdominal or trans rectal prostatic ultrasound also be considered to accurately evaluate the size, shape, anatomy, and potential pathology of the prostate in a minimally invasive, cost-effective, and reproducible way (56).

1.7. Treatment of BPH

A more profound knowledge of the pathogenesis. The natural history, and the risk of the Progression enabled more differentiated therapy of elderly men with BPH. specific approach used to treat BPH depends upon a number of factors like age, prostate size, weight, prostate-specific antigen level ,and severity of the symptoms(57).

Treatment options are as follows:

1.7.1. Watchful waiting

Watchful waiting is the monitoring of a patient without medical or surgical intervention; it generally entails education, reassurance, periodic review and lifestyle advice(58).

1.7.2. Pharmacological treatment

Medical therapy for clinical benign prostatic hyperplasia (BPH) has advanced significantly in the last 2 decades. Many new α1 antagonists and 5α - Reductase inhibitors (5α-Ri) are now commercially available. The practicing urologist must decide on the most appropriate medication for his patients, taking into consideration various factors like efficacy, dosing regime, adverse effects, cost, patient's socioeconomic background, expectations, drug availability and his own clinical experience (59) .

1.7.3. Surgical treatment

Surgical treatment of BPH is necessary, and referral to a urologist is warranted if medical treatments fail, or if Benign Prostatic Obstruction causes renal insufficiency, urinary retention, recurrent urinary tract infections, bladder calculi or hydronephrosis, may be contributing to a patient's symptoms(60) .

1.8. Treatment of prostate cancer

1.8.1. Hormone therapy

Hormonal therapy is the cornerstone of treatment for men with metastatic prostate cancer. At first, prostate cancer cells need the male hormone testosterone in order to grow. In almost all men with metastatic prostate cancer, treatments to reduce testosterone levels are helpful but they are not cures. Testosterone levels can be lowered by surgical removal of both testes (bilateral orchiectomies) or treatment with medicines termed gonadotropin-releasing hormone (GnRH agonists) (61) .

1.8.2. Chemotherapy

Chemotherapy may provide extra help in men with metastatic prostate cancer that has grown despite hormone therapy. Docetaxel (Taxotere®) and mitoxantrone (Novantrone®) are the most commonly used chemotherapy drugs for prostate cancer. Because of potential side effects, chemotherapy may not be suitable for all of these men(62).

Along with hormone and chemotherapy treatments for prostate cancer, there are other therapies designed to treat or prevent the problems that are due to spread of prostate cancer to bone(61).

1.8.3. External Beam Radiation Therapy

External beam radiation relieves pain in the majority of men and is most useful for treatment of one or two sites of pain. It used to treat early stage prostate cancer, also can be aimed at sites of painful bone metastasis. Radiopharmaceuticals are drugs given by intravenous infusion (IV), such as strontium-89 (Metastron®) or samarium-153 (Quadramet®) (63).

1.9. The Studied Markers

1.9.1. Genetic Study of SAM68 mRNA

SAM68 (SRC associated in mitosis of 68 kDa) was originally identified as a protein physically associated with and phosphorylated by the tyrosine kinase c-SRC during mitosis (64)(65), opening the interesting possibility of a signaling circuitry driven by c-SRC and affecting RNA processing and trafficking in a cell-cycle-dependent manner.

SAM68 belongs to the STAR (signal transduction and activation of RNA metabolism) family of RNA binding proteins (RBPs) that link signaling pathways to RNA processing (66). They contain a GRP33/SAM68/GLD-1 (GSG) domain for

RNA binding and homodimerization, flanked by regulatory regions harboring motifs for protein-protein interactions, often mediated by conserved amino acid residues targeted by posttranslational modifications(67). SAM68 contains six proline-rich sequences and a tyrosine-rich region at the C-terminus, which form docking sites for signaling proteins containing SRC homology 3 (SH3) and 2 (SH2) domains (Figure 1)(64)(65)(68). Notably, tyrosine phosphorylation by SRC-related kinases impairs SAM68 homodimerization (69). Additional posttranslational modifications were also reported to affect the functions of this RBP. SAM68 binds to and is methylated by the arginine methyltransferase PRMT1 (70), thus affecting SAM68 interaction with SH3 domains (71) and its nuclear localization (70). SAM68 acetylation, described in tumorigenic breast cancer cell lines (72), by the acetyltransferase CBP increases Post-translational modifications greatly influence the biochemical properties of SAM68 and finely tune its subcellular localization, interaction with signaling proteins, and RNA binding affinity(70)

1.9.2. Role SAM 68 In human Cancer

Elevated expression and high tyrosine phosphorylation of SAM68 of prostate cancer (PCa) patients correlated with enhanced activation of SRC(73). Moreover, SAM68 is frequently up-regulated in PCa patients and promotes PCa cell proliferation and survival to chemotherapeutic agents (74), suggesting a role for this pathway in prostate cancer biology.

Proteomic analyses revealed that SAM68 is able to form two (large and small) protein complexes, interacting with several RBPs and regulators of cytoskeletal organization and signal transduction pathways (75). In accordance with this, SAM68-deficient fibroblasts displayed defects in cell migration and an increase in SRC kinase activity (76). These observations suggest that SAM68 is

required for a negative feedback inhibition of SRC and that deregulated SRC activity could be responsible for the defects in actin cytoskeleton and cell migration observed in SAM68- deficient fibroblasts (75).

Direct recruitment of SAM68 to a promoter region resulted in strong transcriptional repression and mutation of the SAM68 RNA binding domain had no influence on this effect, thus suggesting that SAM68 transcriptional activity occurs in a RNA-independent fashion (77). In PCa cells, SAM68 was proposed to function as a transcriptional coregulator and to promote the transcriptional activity of the androgen receptor (78).

Figure 1.4 Role of SAM68 on Androgen Receptor

The interaction of the proto-oncogenic transcription factor FBI-1 with SAM68 in PCa cells was shown to inhibit SAM68 induction on the Anti-apoptosis protein (*BCL-X*), thus affecting apoptosis (79). Thus, the transcription co-factor SND1/SAM68 complex might be an important determinant of PCa progression and the concomitant up-regulation of these proteins might provide an advantage for cancer cells to invade other tissues, consequently favoring the spreading of metastatic cells (80). In summary, growing evidence documents the involvement of SAM68 in the transcriptional regulation of gene expression of cancer related genes, both by direct binding to the chromatin and by recruitment of specific transcription factors, which in turn affect its splicing activity. As mentioned above, several posttranslational modifications regulate the function and/or localization of SAM68. In particular, serine-threonine and tyrosine phosphorylation of SAM68,

which often occurs in cancer cells, are important for SAM68 homodimerization and RNA affinity (Figure 1.4)(81).

Sam68 may also be acting as a co-activator of ER-dependent transcription in mammary development and tumorigenesis (82). Moreover, Sam68 is required to guarantee proper expression of the gonadotropin receptor transcripts in pre-ovulatory follicles from adult ovary with a possible role upregulating both the FSH and LH receptor transcripts(83).

1.10. Biochemical parameters

1.10.1. Prostate-Specific Antigen

Prostate-Specific Antigen (PSA) is the main secretory protein of the prostate gland was isolated in seminal plasma in 1966; for a long time, PSA has been used in forensic evidence of rape (84)It has been estimated that the PSA concentration in seminal plasma was very high(1.5 mg/ml) and lower in urine (250 ng/ml)(85) .

It is a single chain glycoprotein with a molecular weight of about 34 KDa (86). PSA consists of 237 amino acids and 4 carbohydrates side chains(87).

PSA is a kallikrein-like serine protease produced almost exclusively by the epithelial cells of the prostate (88). PSA is produced by the columnar epithelium of prostatic tissue. Following production, it passes through the basal cell layer, endothelial cells, and capillary membranes to enter the systemic circulation, Semenogelin and fibronectin proteins are responsible for the gel-like consistency of the seminal fluid. Locally, PSA prevents seminal coagulation by breaking

down these proteins into smaller peptides and thereby, helping facilitate impregnation(89).

For practical purposes, it is organ-specific but not cancer-specific. Thus, serum levels may be elevated in the presence of benign prostatic hypertrophy, prostatitis and other non-malignant conditions. The level of PSA as an independent variable is a better predictor of prostate cancer than suspicious findings on digital rectal exam (DRE) or trans rectal ultrasonography (TRUS) (90).There are many different commercial test kits for measuring PSA, but no commonly agreed international standard exists(91)

Even though the PSA test can detect small tumors, finding a small tumor does not necessarily reduce a man probability of dying from prostate cancer, PSA testing may identify very slow-growing tumors that are unlikely to threaten a man life. Also, PSA testing may not help a man with a fast-growing or aggressive cancer that has already spread to other parts of his body before being detected(84).

1.10.2. AKT serine/threonine kinase 1

A serine/threonine kinase is a central node of many signaling pathways and it is frequently deregulated in many types of human cancers. Akt, also known as protein kinase B (PKB) is a 57-kDa serine/ threonine kinase, a critical mediator of growth factor-induced cell survival. Activation of Akt induced by the survival factors can suppress apoptosis in a transcription-independent manner by phosphorylation and inactivation of components of the apoptotic machinery(92)

Akt is a serine/threonine kinase and it participates in the key role of the PI3K signaling pathway. The Akt can be activated by a wide range of growth signals and the biochemical mechanisms leading to Akt activation are well defined. Once activated, Akt modulates the function of many downstream proteins involved in cellular survival, proliferation, migration, metabolism, and angiogenesis(93).

Akt family proteins contain a central kinase domain with specificity for serine or threonine residues of substrate proteins and amino terminus pleckstrin homology (PH) domain, which mediates lipid-protein and protein-protein interactions and the carboxyl terminus hydrophobic and proline-rich domain. Except for the carboxy-terminal tail, the primary structure of Akt is conserved across evolution (94).

The active functioning of Akt follows four steps – induction by survival factors, translocation to the plasma membrane, phosphorylation, and activating downstream effectors. For the phosphorylation process of Akt, translocation of Akt from the cytoplasm to the inner surface of the cell membrane is critically important(95). Although the status of all the activators, modulators and downstream effectors of Akt play a crucial role in tumor development, aberrant Akt activation itself is highly oncogenic and is observed in various human cancers(96).

Akt can also phosphorylate and activate numerous oncogenic proteins involved in cell cycle progression and tumorigenesis, such as Suppression of apoptosis is not the only function of Akt in promoting oncogenesis since Akt can also induce cell cycle progression in different ways(97).

1.10.3. β-Catenin:

β-Catenin is a 781-amino-acid protein. Ctnnb1 encodes the multi-functional protein β-catenin, two major functions of β-catenin are distinguished: on the one hand, β-catenin constitutes a part of the intracellular anchoring of cell–cell connections when associated with cadherin proteins. On the other hand, β-catenin can also function as a co-activator of transcription factors from the T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) family in the so-called canonical Wnt/β- catenin signaling pathway(98).

A cytosolic multiprotein complex tightly controls the levels of free cytosolic, non-membrane-bound β-catenin by catalyzing the phosphorylation of β-catenin at various amino acid residues near its N-terminus. Phosphorylation of these sites primes β-catenin for subsequent ubiquitinylation and proteasomal degradation. The activity of β-catenin as a transcriptional co-activator is physiologically controlled by so-called Wnt molecules, which bind extracellularly to Frizzled receptors. Activation of Frizzled receptors destabilizes the multi-protein complex responsible for β-catenin phosphorylation. This in turn leads to cytoplasmic accumulation and nuclear translocation of β-catenin. are clearly linked to cell proliferation and survival, thereby providing a selective advantage to tumor cells that possess activated β-catenin signaling (99).

Aberrant Wnt/β-catenin signaling has been linked to a number of human cancers (100)(101) including prostate cancer (102)(103)(104) (105).

A studies have shown that small subpopulations of cancer cells, termed “cancer stem cells (CSCs)” or “tumor-initiating cells” based on their ability to self-renew as well as differentiate to a daughter cell type, play a critical role in both initiation and maintenance of tumors. It has been suggested that these cells are resistant to conventional chemotherapy and radiation, making it important to develop therapeutic approaches to selectively target them(106), perhaps by interfering with cell specific signaling pathways that regulate self-renewal. In PCa, it is possible that CSCs survive after androgen ablation therapy, causing castration-resistant disease(107). Growing evidence shows that Wnt/β-catenin signaling is highly active in CSCs, and may have a role in prostate
stem cell self-renewal (108).

1.10.4. Interleukin-8

Interleukin-8 (IL-8), also known as CXCL8, is a proinflammatory chemokine of CXC type that is processed to give rise to a functionally competent

protein of 77 amino acids in the case of IL-8 produced by parenchymal cells and 72 amino acids in the case of the one produced by monocytes and macrophages(109).

IL-8 determines in endothelial cells proangiogenic effects that include the proliferation, survival, and migration of vascular endothelial cells(110). It is also thought that IL-8 has beneficial autocrine and paracrine effects for the tumor cells themselves (111).

IL-8 plays a very important role in the recruitment of certain tumor development, including the way in which tumors are capable of developing metastasis. The influence of IL-8 is like an actor who has different roles in the same tumor movie(112).

IL-8 is a chemokine produced by cancer cells and whose serum concentration correlates with the tumor burden of patients and with a poor prognosis of the disease .Further evidence was reported in a clinical study of prostate cancer patients who had rising levels of circulating MDSCs associated with increasing serum IL-6 and IL-8 levels, increasing with stage and leading to defective T-cell function(113).

IL-8 expression has been detected in numerous cancer types, including solid tumors (brain, breast, cervical, colon, gastric, lung, melanoma, mesothelioma, ovarian, prostate, renal, and thyroid). Because of a short serum half-life, serum concentrations closely follow in time the changes occurring in IL8- producing cells, this study provided clear evidence that serum IL-8 levels correlate with tumor burden and prognosis in patients with different tumor types. Because IL-8 serum concentrations rapidly reflect changes in bioproduction by cancer cells, the levels of IL-8 may become a helpful dynamic biomarker to monitor the clinical activity of novel cancer therapies, such as iBRAF or immunomodulatory mAbs (114).

IL-8 concentration change may be a useful tool to monitor response to therapy in metastatic melanoma patients treated with antibodies against immune checkpoint inhibitors, allowing early identification of patients who will or will not respond(115).

IL8 is elevated in serum of men with prostate cancer and is associated with poorer outcomes (116). Further, IL8 protein expression measured in the stroma surrounding prostate tumors is reportedly elevated compared with stroma surrounding normal-appearing prostate epithelium(117). The cellular localization of IL8 production and expression in tumor versus benign regions is not fully described. Although IL1b, IL6, IL8, and IL10 have been assessed in serum or via meta-analysis of RNA, the spatial localization of these cytokines in prostate cancer tissues is largely unreported. Whether these cytokines are produced primarily by prostatic or immune cells is to be determined. Further, whether expression patterns within the prostate differ by cancer grade or between races is unknown(118).

1.10.5. Vascular Endothelial Growth Factor

Vasculogenesis is the *de novo* formation of a vascular network whereas angiogenesis is sprouting of new blood vessels from pre-existing ones. Both processes are highly dependent on regulation by vascular endothelial growth factors (VEGFs) and their interaction with membrane receptors expressed on different cell types (119) (120) .

Abnormal angiogenesis is associated with a variety of diseases such as tumor neovascularisation, diabetic retinopathy, rheumatoid arthritis(121). Tumor neovascularisation is triggered by cancer cells to stimulate supply of nutrients and enable metastasis. In the absence of a functional blood supply, tumors are either dormant or necrotic (122).

Angiogenesis is thus a major contributory factor in the growth and spread of a variety of cancers which cause substantial mortality. The VEGF family has complexity with multiple isoforms encoded by each VEGF-related gene, and differences in biological activity between closely related variants(123). Seven VEGF-related genes encoding numerous splice isoforms have been identified. In most metazoan species, genes encoding VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF) are present(124) .

The major trigger inducing angiogenesis is hypoxia, but other factors may also be responsible: hypoglycemia, hypertension, low pH, mechanical stress, chronic inflammation, etc (125). On the other hand, in the presence of VEGF and HIF-1, Ang-2 acts as antagonist of Ang-1, destabilizes the interaction between endothelial cells and the supporting cells, promoting vessel instability and formation of disorganized and immature new blood vessels (126); it also induces the proliferation and migration of endothelial cells, thus favoring tissue neovascularization and pathological angiogenesis (126)(127).

VEGF is overexpressed in a variety of hematological malignancies(128) and the vast majority of solid tumors, including PCa (129), where it is associated with poorer outcomes (130). In PCa, in addition to its expression in blood and lymphatic endothelial cells, VEGF is also expressed at low levels in prostatic glandular epithelial cells and in nonvascular cells, such as macrophages, fibroblast cells, and mast cells(131). Increased expression of TLRs is able to induce VEGF expression, which in turn triggers the proliferation and migratory ability of PCa cells (132).

1.10.6. Vascular Endothelial Growth Factor-2

Human VEGFR2 contain 1337 amino acids and the mature protein is a ~200–230 kDa glycoprotein expressed in both vascular and lymphatic endothelial cells. VEGFR2 binds with high a nity to VEGF-A, VEGF-C, VEGF-D and VEGF-

E. Although VEGFR2 homodimers are implicated in functional regulation, VEGF-A binding can also promote VEGFR1 and VEGFR2 heterodimer formation (133).

However, VEGFR1 levels are relatively low, with tyrosine kinase activity at least ten-fold lower compared to VEGFR2 in endothelial cells; therefore, it can be argued that VEGFR2 have important role, VEGFRs possess a cytoplasmic tyrosine kinase (TK) domain which regulates signal transduction pathways linked to cell proliferation, migration, metabolism, vasodilation, blood vessel formation and remodelling(133).

VEGFR1 and VEGFR2 play important roles in physiological and pathological angiogenesis, whereas VEGFR3 is mainly involved in lymphangiogenesis (134). VEGF ligands regulate embryogenesis, blood vessel development, sprouting and homeostasis (135).

During tumor neoangiogenesis, there are numerous paracrine interactions between endothelial cells and tumor cells(136). Binding VEGF to VEGFR-2 stimulates the secretion of von Willebrand factor (vWF) by endothelial cells (137); the activation of endothelial cells is an essential event for tumor progression (137).

VEGFR-2 is involved in vasculogenesis, normal and pathological angiogenesis, acting through different mechanisms, such as: migration of the hemangioblastsn towards the Yolk sac(138) and differentiation into endothelial cells (139), formation of vascular tubes (tubulogenesis) (140), proliferation of endothelial cells (mitogen effect) , increase of vascular permeability, migration of endothelial cells, transmission of signals which promote the endothelial cells survival, preventing their apoptosis and formation of endothelial fenestrae (139).

In pathological processes, VEGFR-2 is often involved in tumoral angiogenesis. It is considered that VEGFR-2 has the strongest proangiogenic activity, thus blocking VEGFR-2 may have useful clinical implications.(136).

Advantage is that, the weak expression of VEGFR-2 is observed in the healthy tissue or cells while, the overexpression is reported in various types of

cancer namely bladder carcinoma (50%), brain glioma (71.4%), breast cancer (64.5%), cervical cancer (73.3%), colon cancer (71.4%), esophageal cancer (100%), kidney clear cell cancer (35%), NSCLC (54.2%), oral cancer, ovarian cancer (100%), pancreatic cancer (80%), prostate cancer (100%), skin melanoma and likewise(141)

1.11. Aim of present study :

Study the gene expression of Sam68 mRNA and its correlation with studied biochemical parameters and its role in promoting oncogenesis for progression of benign prostatic hyperplasia(BPHP) to prostate cancer(PC).

1.12. Objective of study

1-Assessment of gene expression of the sam68 gene in prostate cancer and benign prostate hyperplasia patients.

2-Study the difference in Benign Prostate Hyperplasia (BPH) indicators consist of (AKT-1, B-catenin, IL-8, VEGF , VEGFR2) among patients used in this study.

3-Study the difference in Prostate cancer (PCa) indicators consist of (AKT-1, B-catenin, IL-8, VEGF , VEGFR2) among patients used in this study.

4-Evaluating the correlation between Age and gene expression of the sam68 gene in BPH and PCa patients.

5- Evaluating the correlation between Biomarker above and gene expression of the sam68 gene in BPH and PCa patients.

6- The selection of these biochemical parameters above because of their positive role in the growth of prostate tumors and metastasise.

2. Materials and Methods

2.1 Materials

2.1.1Chemicals

The standard kits and the chemicals used in this study were shown in the following table.

Table 2-1: Chemicals and kits used in this study

NO	Chemical substance	Origin
1	Ethanol	Biosolve Company (USA)
3	RNA extraction kit (blood)	Geneiad Biotechnology (Tiwan)
4	cDNA Kit	Theromfisher (USA)
5	RT-qPCR Master Mix Kit	Promega USA
6	Primers	Microgene (Korea)
7	Serin/therionine Kinase 1 ELISA kit	Bioassay Technology Laboratory (China).
8	Beta-Catenin ELISA kit	Bioassay Technology Laboratory (China).
9	Interlukine-8 ELISA kit	Bioassay Technology Laboratory (China).
10	Vascular endothelial growth factor ELISA kit	Bioassay Technology Laboratory (China).
11	Vascular endothelial growth factor receptor-2 ELISA kit	Bioassay Technology Laboratory (China).

2.1.2 Instruments and Equipment

The Table below shows the instruments used in this study

Table 2-2: Instruments and Equipment's Used

NO	Instrument and Materials	Origin
1	Cooling centrifuged	PrismR /USA
2	Deep Freeze	GFL / Germany
3	Distiller	GFL / Germany
4	Disposable syringe (5ml)	China
5	EDTA tube (5 ml)	AFCO, Jordan
6	Eppendorf tube (1.5 ml)	China
7	micro centrifuged	Wisespin/ Germany
8	Gel tube	AFCO, Jordan
9	Microcentrifuge	APLE/ Japan
10	Micropipettes	Slamed / Germany
11	Microwave	LG
12	Nano photometer	IMPLEN NP 80
13	pipette tips (0.01,0.1,1) ml	China
14	Plain tube	AFCO, Jordan
15	Sensitive balance	Sartorius/ Germany
16	Spectrophotometer PD-303	APEL/ Japan
17	Real time-PCR	analytik jena/ Germany
18	Incubator	Memmert/Germany
19	Vortex	Kunkel/ Germany
20	Water bath	GFL/Germany
21	ElISA	Paramedical PKL/Italy

2.2 Subject and Study Design

A case-control study recruited 60 BPH patients (48-75 years), who were presented to a urological center which including Al-Sadder Medical City in Al-Najaf Al-Ashraf Province, 60 PCa patients (aged 47-75 years), who were admitted to a Middle Euphrates Cancer Center in Al-Najaf Al-Ashraf Province, and 12 healthy subjects (aged 40-45 years) who had no history of Prostate diseases as controls. This study was established at the laboratory of the Biochemistry Department in Collage of Medicine / University of Babylon. The collection of samples was performed during the period from 1-July to 22-December/2020. The information has been reported using a questionnaire on every individual by face-to-face interviews, to obtain information on their smoking status and on age, weight, height, exercise, family history, past history of diseases and medications.

2.2.1 Inclusion Criteria

All patients presented clinical characteristics of BPH and PCa, confirmed by PSA, Rectal examination, and Ultrasound. PCa patients should be diagnosed by Prostate Biopsy. A permission was taken from all subjects to contribute to this study after were told about the aim and advantages of this study.

2.2.2 Exclusion Criteria

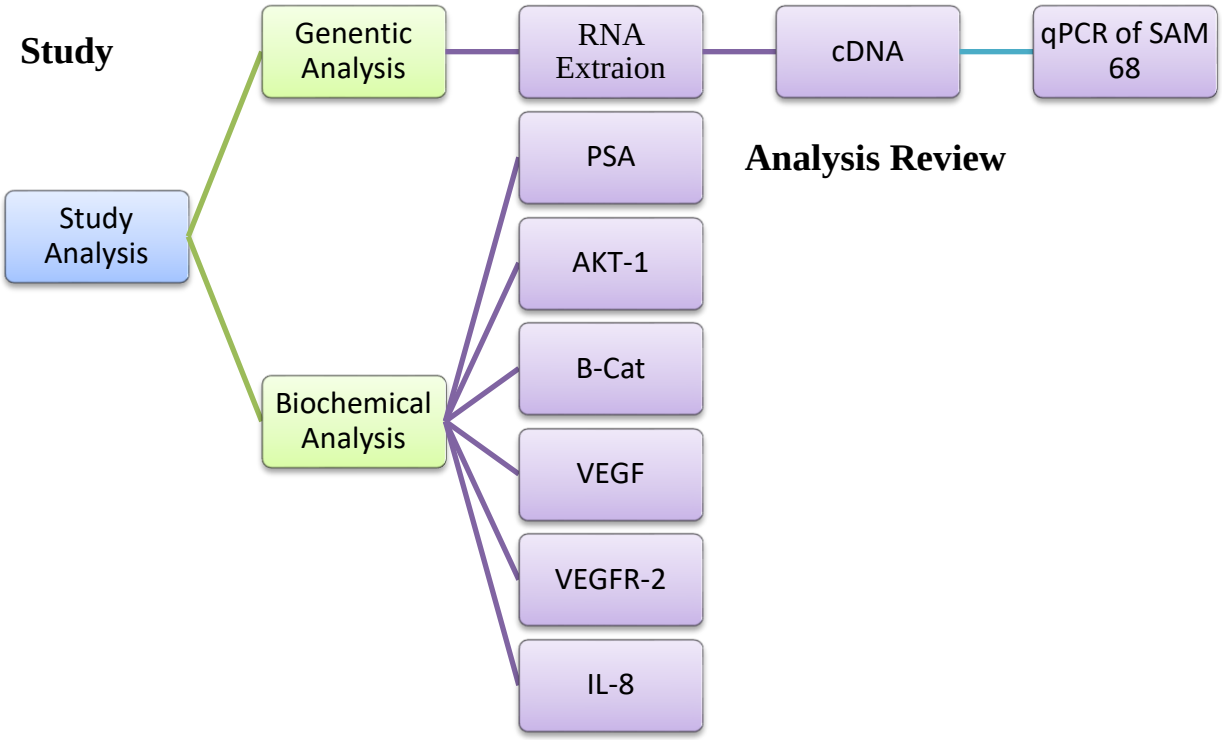
1- Previous malignant and immune diseases

2- Patients who underwent previous transurethral resection of the prostate (TURP) were excluded.

3- None of the patients received hormonal therapy, radiotherapy or chemotherapy before taking a blood sample.

2.3 Methods

2.3.1 Study



Figure(2-1): Scheme of Study Analysis Review

2.3.2 Collection of Blood Samples

Blood samples were collected using a disposable syringe from individuals. About 5 ml of blood were obtained from each individual by vein puncture, 2 ml was collected in EDTA tubes, the remaining 3 ml pushed into a disposable gel tube. Blood in the EDTA tubes used for RNA analysis while the blood in the gel tube was allowed to clot at room temperature for 20 minutes and then centrifuged at 1500 x g for 5 minutes and used for determination of biochemical parameter levels within from collection.

2.3.3.RNA Purification Protocol Procedure

RNA according to GENEzol™ TriRNA Pure Kit from Geneaid company

Principle

The GENEzol™ TriRNA Pure Kit is a phenol and guanidine isothiocyanate plus spin column system for convenient purification of high-quality total RNA from a variety of samples. Initially, samples are homogenized in GENEzol™ Reagent without chloroform phase separation or isopropanol RNA precipitation. Following sample homogenization, simply bind, wash and elute the high-quality, total RNA in RNase-free Water and use in a variety of sensitive downstream applications.

Table 2-3: Component of RNA Extraction Kit

Item	Contents
GENEzol™ Reagent	160 ml
Pre-Wash Buffer 1 (Add Ethanol)	70 ml
DNase I[2] (2U/µl)	550 µl * 2 vial
DNase I Reaction Buffer	5 ml * 2 vial
Wash Buffer 3 (Add Ethanol)	300 ml
RNase-free Water	15 ml
RB Columns	200 Pcs
2 ml Collection Tubes	400

Procedures

A. Sample Homogenization and Lysis Sample preparation

Samples was performed at room temperature.

1. Two hundred microliters of liquid sample was transferred up to a 1.5 ml of microcentrifuge tube (RNase-free).

2. Three volumes of GENEzol™ Reagent was added per 1 volume of sample (3:1) then mixed well by vortex.

3. The sample mixture was incubated for 5 minutes at room temperature.

B. RNA Binding

1. The sample was centrifuged at 12-16,000 x g for 1 minute to remove cell debris then the clear supernatant was transfered to a new 1.5 ml microcentrifuge tube (RNase-free).

NOTE: When extracting RNA from cultured cell samples, cell debris will not commonly collect on the bottom of the microcentrifuge tube. In this case, It can be continued without transferring the supernatant

2. one volume of absolute ethanol was added directly to 1 volume of sample mixture (1:1).

3. Then mixed well by vortex then RB Column was placed in a 2 ml Collection Tube.

4. seven hundred microliter of the sample mixture was transferred to the RB Column. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through.

5. The RNA Binding Step was repeated by transferring the remaining sample mixture to the RB Column.

6. The mixture was centrifuged at 14-16,000 x g for 1 minute then discarded the flow-through. The RB Column was placed in a new 2 ml Collection Tube.

C. **RNA Wash**

1. Four hundred microlitersof Pre-Wash Buffer (make sure ethanol was added) was added to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.

2. The flow-through was discarded then the RB Column was placed back in the 2 ml Collection Tube.

3. Six hundred microliters of Wash Buffer (make sure ethanol was added) was added to the RB Column.

4. Centrifuge at 14-16,000 x g for 30 seconds then the flow-through was discarded . The RB Column was placed back in the 2 ml Collection Tube.

5. Six hundred microliters of Wash Buffer (make sure ethanol was added)was added to the RB Column.

6. Centrifuge at 14-16,000 x g for 30 seconds then the flow-through was discarded.

7. The RB Column was placed back in the 2 ml Collection Tube.

NOTE: For blood samples only, the RB Column was washed again with 600 µl of Wash Buffer.

8. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

D. RNA Elution

1. The dry RB Column was placed in a clean 1.5 ml microcentrifuge tube (RNase-free).

2. About 25-50 µl of RNase-free Water was added into the CENTER of the column matrix.

3. Let stand for at least 3 minutes to ensure the RNase-free Water is completely absorbed by the matrix.

4. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

2.3.4. Determination the Concentration and Purity of RNA Sample.

Various methods were used to determine the RNA yield. The two methods were useful but have varying requirements such as equipment required, ease to use and calculation needed.

2.3.4.1 Estimation of RNA Purity and Concentration by Using Nano Photometer (Absorbance Method)

The most common method used to estimate RNA yield and purity measurement of absorbance. RNA has a strong absorption at 260nm. The calculation used for the measurement of the purity of RNA was performed by estimation of the ratio of the absorbance at A260/A280 & A260/A230 > 1.8 will

indicate that RNA has good-quality and RNA is ready for RT/qPCR. The lower ratio indicates more contaminants were presented.

However, DNA also has a absorption at 260nm and aromatic amino acids which present in protein also has a great absorbance at 280nm, so RNA is not the only molecule capable of absorbing UV light at 260nm, both contaminants, if found in the RNA solution, will lead to the total measurement at 260nm. Besides, the presence of guanidine will contribute to higher 260nm absorbance. This means the RNA quantity may be overestimated if the calculation of RNA yield depends on the absorbance at 260nm (142).

2.3.4.2 Quantus™ Fluorometer

The Quantus™ fluorometer is a two-channel compact fluorometer designed to provide a highly sensitive fluorescent finder in the measurement of nucleic acids. The Quantus™ Fluorometer is optimized with preprogrammed settings for Promega QuantiFluor® Dye Systems (QuantiFluor® dsDNA, RNA and ssDNA Systems) to quantitate nucleic acids and offers the flexibility to create customized methods and quantitation settings for other fluorescent dyes. The calibration process eliminates the need to generate a standard curve for each set of unknown samples.

The fluorescence of the blank sample and the standard that represents the highest point on a standard curve was measured. The Quantus™ Fluorometer is equipped with two fluorescence channels for nucleic acid and protein quantitation: Blue fluorescence channel: Excitation 495nm shortpass (wavelengths up to 495nm), emission 510–580nm. Red fluorescence channel: Excitation 640nm shortpass (wavelengths up to 640nm), emission 660–720nm. When used with the QuantiFluor® Dyes, the Quantus™ Fluorometer provides sensitivities that are up

to 40,000 times higher than that of absorbance-based quantitation, depending on the assay used

2.3.5. cDNA preparation

1- Prepare the 2X RT master mix (20-µL reaction) according to High Capacity cDNA Reverse Transcription Kit (thermofisher)

1. The kit components allowed to thaw on ice.

2. The volume of components needed to prepare the required number of reactions was calculated.

Table (2-4) cDNA preparation Compenenets

Compenenets	Volume
10X RT Buffer	2 µL
25X dNTP Mix (100 mM)	0.8 µL
10X RT Random Primers	2 µL
MultiScribe™ Reverse Transcriptase	1 µL
RNase Inhibitor	1µL
Nuclease-free H2O	3.2 µL
Total per reaction	10 µL

3. The 2X RT master mix Placed on ice and mix gently.

2- Prepare the reverse transcription reactions

1. pipette 10 µL of 2X RT master mixed into each well of a 96-well reaction plate or individual tube.

2. Ten microliter of RNA sample was pipetted into each well, pipette up and down two times to mix, Then seal the plates or tubes.

4. To spin down the contents and to eliminate any air bubbles, briefly centrifuge the plate or tubes

5. The plate or tubes was Placed on ice until you are ready to load the thermal cycler.

3- Perform reverse transcription

1. Program the thermal cycler using the conditions below.

Settings	Step1	Step2	Step3	Step4
Temp.	25°C	37°C	85°C	4°C
Time	10 minutes	120 minutes	5 minutes	Hold

2. The reaction volume was Setting to 20 µL.

3. The reaction plates or tubes was Loaded into the thermal cycler.

4. The thermal cycler run then started.

2.3.6. Primers Preparation and Storage

Primer sequences used for qPCR (5′ to 3′)			
qPCR	Gene	Forward primer	Reverse primer
	Sam68	CTCCTGCTAGGCCAGTGAA	TTGTGGGTAAAGCAACAGGA
	GAPDH	GACTCATGACCACAGTCCATGC	AGAGGCAGGGATGATGTTCTG

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; qPCR, quantitative polymerase chain reaction

Primers were coming in lyophilized or dried form and the units of the primers were given in Pico moles. So, it was needed to reconstitute the primer by adding a suitable amount of nuclease-free water which was supplied by the company to obtain 100 µm/l stock (143)

The Following Steps were Followed for The Preparation of Primers:

The water bath was preheated to a 50℃. Under the aseptic condition, the oligoes were spin down for 30 seconds at 7,000 x g before opening the cap.

1-The optimum amount of sterile, nuclease-free water was added to get 100 µm stock.

2-The stock was vortexed for 30 seconds at 2,000 x g. Then, it was placed in 50℃ water bath for 10 minutes

3-The stock was vortexed again for 30 seconds at 2,000 x g and it was placed in a water bath for 10 minutes.

4-Under aseptic condition a volume of 10 µl of the stock was added to a 90 µl of sterile, nuclease-free water in a 0.2 ml Eppendorf tube to obtain working stock. The stock then was stored at -20℃.

5-The working stock was vortexed for 30 seconds at 2,000 x g and sealed. Then, stored at -20℃. It was recommended that the primers should be

divided into single-use aliquots to limits the freeze-thawing of primers and extend their life.

2.3.7.Real time PCR Amplification

2.3.7.1. principle

The polymerase chain reaction depends on the ability of cDNA polymerase to produce a new strand of cDNA which is complementary to the offered template strand. DNA polymerase can add a nucleotide just onto a preexisting 3'-OH group, so it needs a primer to add the first nucleotide to it. This property makes it able to delineate a specific region of template sequence that wants to amplify. When the PCR reaction finishes, the specific sequence will be increased in billions of copies which are called amplicons (144).

2.3.7.2. Quantification of the RNA product

The SYBR green method was used to quantify PCR product. The reactions were prepared (**Table 2.5**) using 2x qPCR SYBR Green Master Mix (Primer Design Precision) according to the manufacturer's instructions. All samples were loaded into a 96 well plate and centrifuged at 200xg for 2min. Then the qPCR plate was placed into the 7900HT AbiPrism sequence detection system and run according to the following cycles (**Table 2.6**).

In addition, the melting point of the primers was added to machine setting to detect primer specificity.

Table (2-5) 2x qPCR mastermix components.

Materials	Volume (µl)
2x master mix with SYBR green (with ROX)	5

Forward primer	0.5
Reverse primer	0.5
Nuclease-free H_2O	3
cDNA	1
A total volume	10

Table (2-6) Parameters of qPCR reaction.

qPCR reaction	x1 cycle	x40 cycles	
Reaction step	Enzyme activation	Denaturation	Data collection
Cycle length	10min	15sec	1min
Temperature	95^{o}C	95^{o}C	60^{o}C

2.3.8 Determination of Human Serine-Threonine-protein Kinase.

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human AKT1 antibody. AKT1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human AKT1 Antibody is added and binds to AKT1 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated AKT1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human AKT1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

-All reagents should be brought to room temperature before use.

-Standard**:** the 120µl of the standard (25.6ng/ml) was reconstituted with 120µl of standard diluent to generate a 12.8ng/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation before making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (12.8ng/ml) 1:2 with standard diluent to produce 6.4ng/ml, 3.2ng/ml, 1.6ng/ml and 0.8ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month.

-Wash Buffer: 20ml of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Table(2-7): Standard Solutions Concentration of AKT-1

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
25.6ng/ml	12.8ng/ml	6.4ng/ml	3.2ng/ml	1.6ng/ml	0.8ng/ml

Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. Brought all reagents to room temperature before used. The assay is performed at room temperature.

2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips should be stored at 2-8°C.

3. fifty microliter of standard was added to standard well.

4. forty microliter of sample was added to sample wells and then 10µl anti-AKT1 antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.

6. fifty microliter of substrate solution A was added to each well and then 50µl substrate solution B was add to each well. plate was covered incubate with a new sealer for 10 minutes at 37°C in the dark.

7. fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) of each well is determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

A standard curve is constructed by plotting the average Abs for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software.

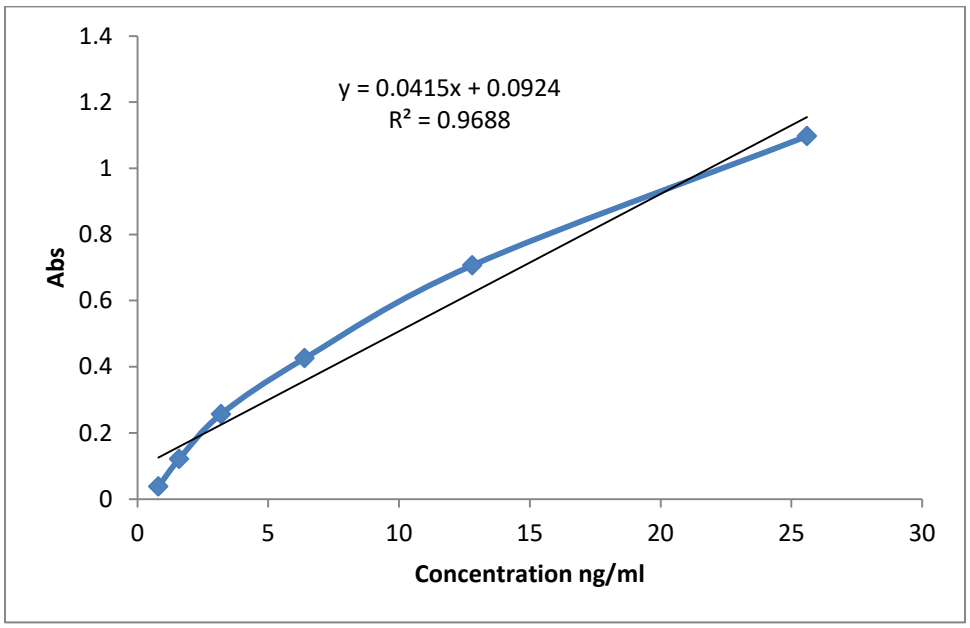


Figure (2-2): Standard Curve of Serine -Threonine-protein Kinase

2.3.9. Determination of Human Beta Catenin.

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human B catenin antibody. B catenin present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human B catenin Antibody is added and binds to B catenin in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated B catenin antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human B catenin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

-All reagents should be brought to room temperature before use.

-Standard**:** the 120µl of the standard (80 ng/ml) was reconstituted with 120µl of standard diluent to generate a 40 ng/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation before making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (40ng/ml) 1:2 with standard diluent to produce 20ng/ml, 10ng/ml, 5 ng/ml and 2.5ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month.

-Wash Buffer: 20ml of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Table(2-8): Standard Solutions Concentration

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
80 ng/ml	40 ng/ml	20 ng/ml	10 ng/ml	5 ng/ml	2.5ng/ml

Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. Brought all reagents to room temperature before used. The assay is performed at room temperature.

2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips should be stored at 2-8°C.

3. fifty microliter of standard was added to standard well.

4. forty microliter of sample was added to sample wells and then 10µl anti-B-Cat. antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.

6. fifty microliter of substrate solution A was added to each well and then 50µl substrate solution B was add to each well. plate was covered incubate with a new sealer for 10 minutes at 37°C in the dark.

7. fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) of each well is determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

A standard curve is constructed by plotting the average Abs for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software.

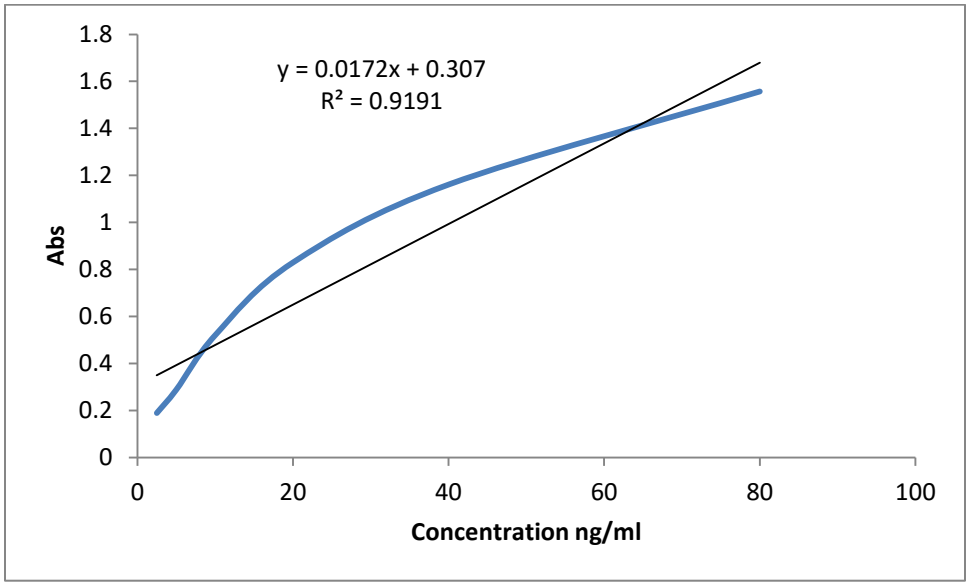


Figure (2-3): Standard Curve of Humam Beta-Catenin

2.3.10. Human Interleukin 8

Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IL-8 antibody. IL-8 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human IL-8 Antibody is added and binds to IL-8 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL-8 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then

added and color develops in proportion to the amount of human IL-8. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

-All reagents should be brought to room temperature before use.

-Standard**:** the 120µl of the standard (1280 ng/l) was reconstituted with 120µl of standard diluent to generate a 640 ng/l standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation before making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (640ng/l) 1:2 with standard diluent to produce 320 ng/l, 160 ng/l, 80 ng/l and 40 ng/l solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month.

-Wash Buffer: 20ml of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Table(2-9): Standard Solutions Concentration

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
Concentration	640 ng/l	320ng/l	160ng/l	80ng/l	40ng/l

Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. Brought all reagents to room temperature before used. The assay is performed at room temperature.

2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips should be stored at 2-8°C.

3. fifty microliter of standard was added to standard well.

4. forty microliter of sample was added to sample wells and then 10µl anti-IL-8 antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.

6. fifty microliter of substrate solution A was added to each well and then 50µl substrate solution B was add to each well. plate was covered incubate with a new sealer for 10 minutes at 37°C in the dark.

7. fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) of each well is determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

A standard curve is constructed by plotting the average Abs for each standard on the vertical (Y) axis against the concentration on the

horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software.

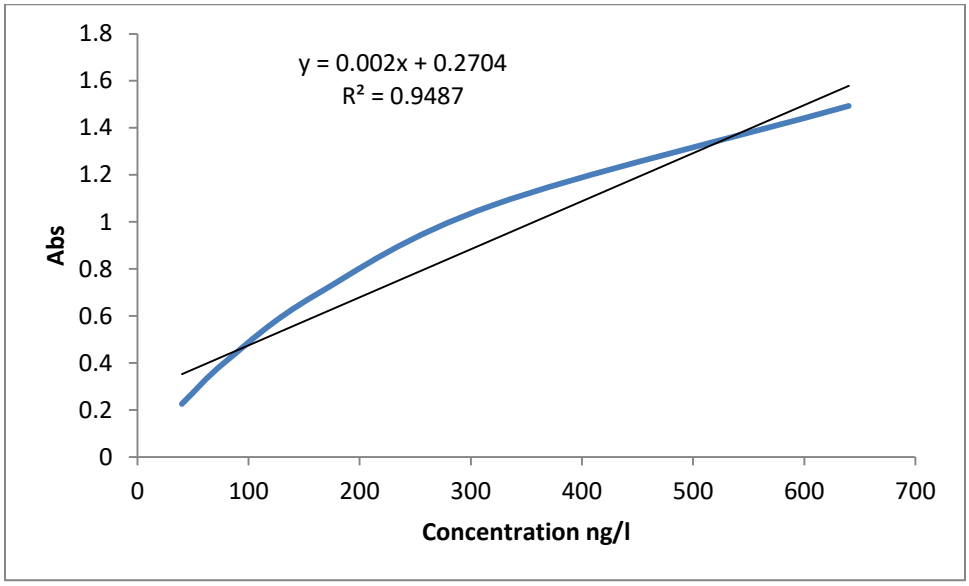


Figure (2-4): Standard Curve of Humam IL-8

2.3.11. Determination of Human Vascular Endothelial Cell Growth Factor

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human VEGF antibody. VEGF present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human VEGF Antibody is added and binds to VEGF in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated VEGF antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human VEGF. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

-All reagents should be brought to room temperature before use.

-Standard**:** the 120µl of the standard (6400ng/l) was reconstituted with 120µl of standard diluent to generate a 3200 ng/l standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation before making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (3200ng/l) 1:2 with standard diluent to produce 1600 ng/l, 800 ng/l, 400 ng/l and 200 ng/l solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month.

-Wash Buffer: 20ml of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Table(2-10): Standard Solutions Concentration

Standard	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
Concentration ng/l	3200ng/l	1600ng/l	800ng/l	400 ng/l	200ng/l

Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. Brought all reagents to room temperature before used. The assay is performed at room temperature.

2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips should be stored at 2-8°C.

3. fifty microliter of standard was added to standard well.

4. forty microliter of sample was added to sample wells and then 10µl anti-VEGF antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.

6. fifty microliter of substrate solution A was added to each well and then 50µl substrate solution B was add to each well. plate was covered incubate with a new sealer for 10 minutes at 37°C in the dark.

7. fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) of each well is determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

A standard curve is constructed by plotting the average Abs for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software.

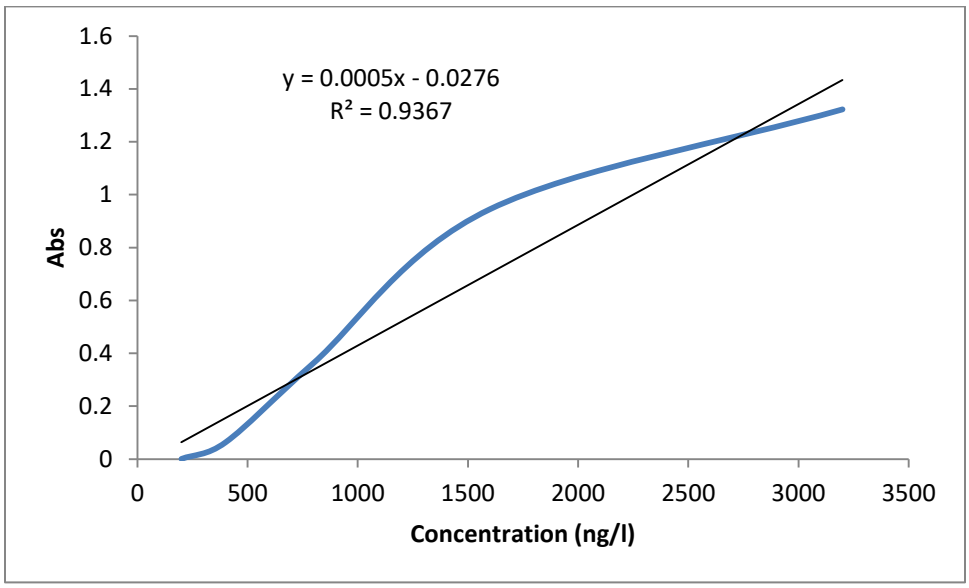


Figure (2-5): Standard Curve of Humam VEGF

2.3.12. Determination of Human Vascuoar Endothelial Cell Growth Factor Receptor 2

Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human VEGFR-2/Flk-1 antibody. VEGFR-2/Flk-1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human VEGFR-2/Flk-1 Antibody is added and binds to VEGFR-2/Flk-1 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated VEGFR-2/Flk-1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human VEGFR-2/Flk-1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

-All reagents should be brought to room temperature before use.

-Standard**:** the 120µl of the standard (48ng/ml) was reconstituted with 120µl of standard diluent to generate a 24 ng/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation before making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (24ng/ml) 1:2 with standard diluent to produce 12 ng/ml, 6ng/ml, 3ng/ml and 1.5ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month.

-Wash Buffer: 20ml of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Table(2-11): Standard Solutions Concentration

Standard	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
Concentration ng/ml	24 ng/ml	12 ng/ml	6 ng/ml	3 ng/ml	1.5ng/ml

Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. Brought all reagents to room temperature before used. The assay is performed at room temperature.

2. The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips should be stored at 2-8°C.

3. fifty microliter of standard was added to standard well.

4. forty microliter of sample was added to sample wells and then 10µl anti-

VEGFR-2/Flk-1 antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealer. Incubated 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. Blotted the plate onto paper towels or other absorbent material.

6. fifty microliter of substrate solution A was added to each well and then 50µl substrate solution B was add to each well. plate was covered incubate with a new sealer for 10 minutes at 37°C in the dark.

7. fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density (OD value) of each well is determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Calculation

A standard curve is constructed by plotting the average Abs for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software.

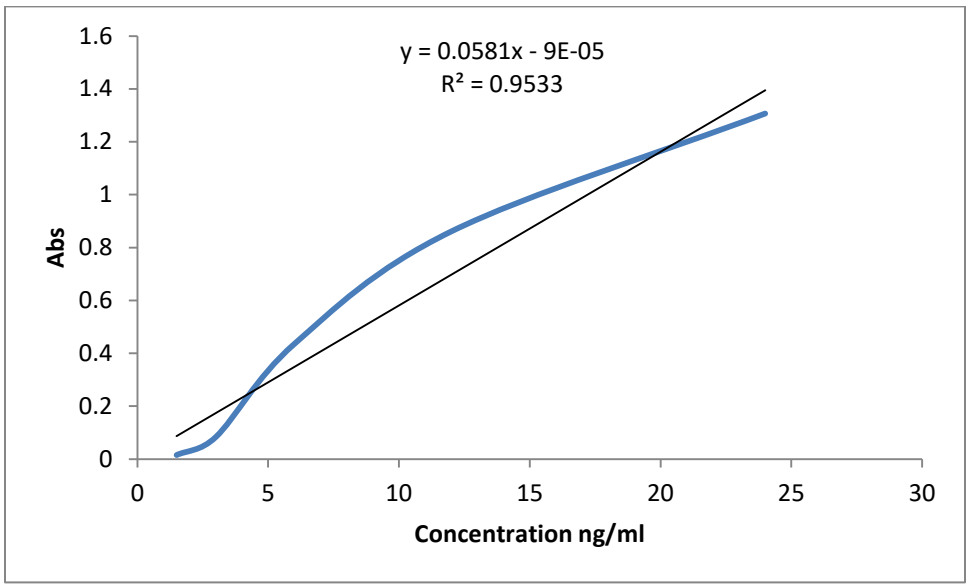


Figure (2-6): Standard Curve of Humam VEGFR-2

3.Result and Discussion

3.1 Host information of the investigated patients

Sixty Prostate cancer and sixty benign prostate hyperplasia specimens were investigated in the current study. The characteristics of the of patients from whom the urological center in Al-Sadder Medical City, and Middle Euphrates Cancer Center in Al-Najaf Al-Ashraf Province were obtained, and analyzed and then their relevance to the expression of SAM 68 gene were evaluated.

3.2. Age distribution

The ages of PCa patients were expressed as mean ± standard deviation according to T-Test shown in table(3-1), with a minimum to maximum of 47-75 years. The age presentation of the recruited PCa patients indicated that (20%) 12 patients were of ages ≤ 50 years and 48 (80%) of ≥ 50 years.

While the age presentation of the recruited BPH patients indicated that 15 (25%) patients were of ages ≤ 50 years and 45 (75 %) of ≥ 50 years.

Table 3-1 Age distribution as mean ± standard deviation

Age distribution	Mean ± Standard Deviation
Prostate cancer patients	61.6 ± 10 years
Benign prostate hyperplasia patients	58.09 ± 7.88 years

The choice of the age of fifty as a cut-off is based on the American Urological Association, which shows that 50 percent of men at the age of fifty years have had a prostate tumor, as it was considered that Age 50 for men who are at average risk of prostate tumor (10)

3.3 Results of SAM 68 Gene Expression

3.3.1 Total RNA Concentration and Purity

3.3.1.1.NanoDrop Spectrophotometer

Total RNA was extracted from blood specimens prepared from patients with malignant prostate tumors as described previously in section 2.3.1. The concentration of extracted RNA was measured by Eppendorf biophotometer. Results (Mean±SD) exhibited a level of 55.5 ± 13.61 ng/µl. The purity of the extracted RNA was estimated by measuring the ratio of A260/A280. It was found to be 1.91 ± 0.04 & A260/A230 was 1.78±0.13 suggesting an appropriate purity (Table 3.2).

Table 3.2: Total RNA level and purity

Total RNA	Mean ± SD
Concentration (ng/ml)	(55.5 ± 13.61)*1000
A260/A280	1.91 ± 0.04
A260/A230	1.78±0.13

3.3.1.2.Quantus Fluorometer

RNA was extracted from blood specimens prepared from patients with prostate tumors as described previously in section 2.3.2. The concentration of extracted RNA was measured by Quantus™ Fluorometer. Results (Mean±SD) showed that the total RNA concentration was a level of (58 ± 4.21)*1000 ng/ml

3.3.2. Validation of Sam68 primer specificity

Extracted RNA from PCa & BPH blood was converted to cDNA . The products were amplified with the use of two levels (5 and 10 pmole) of the

designed primers. Both levels were observed to amplify the cDNA successfully as shown in figure (3-1). A primer level of 10 pmole was considered to be the best.

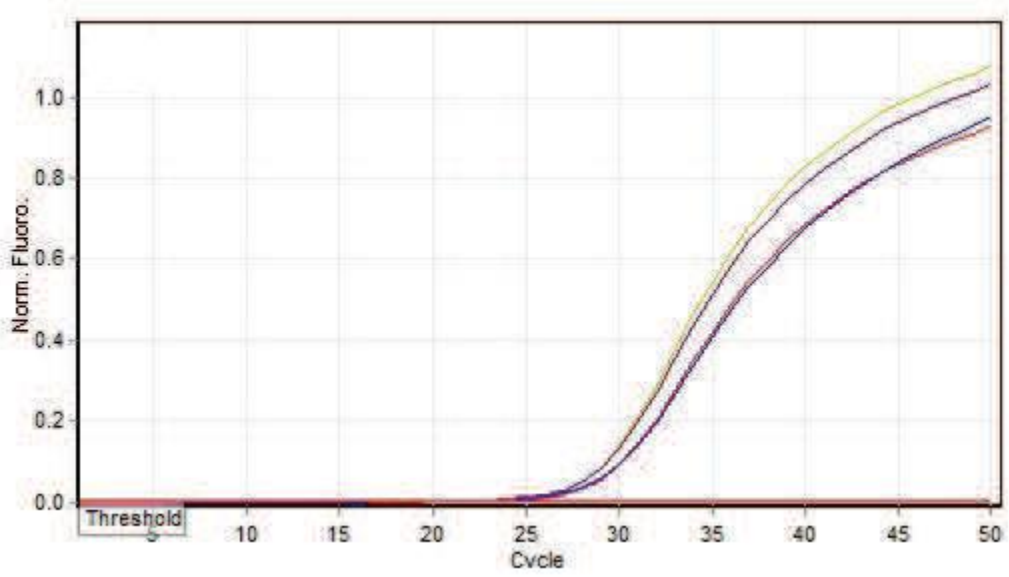


Figure 3-1: Validation of the concentration of the specific primer for Sam 68 gene

3.3.3. SAM 68 gene expression in PCa and BPH

The expression of SAM 68 gene in Prostate tumors was estimated in the 120 specimens of cDNA (60 with PCa and 60 BPH) prepared from RNA of PCa and BPH. In the current investigation, RNAs of Sam 68 gene were extracted from 120 blood patients, converted to cDNA and successfully amplified with appropriate efficiencies.

To our knowledge, did not find a similar study dealing with such extraction and amplification carried out in Iraq. The relative quantification of SAM 68 gene expression in malignant prostate tumors was achieved through the calibration against the expression of the same gene in normal blood (calibrators).

A normalized gene (GAPDH) was used as a control for the experimental variability in this quantification. Thus, the expression folds of SAM 68 gene were calculated with respect to the internal control gene (the housekeeping gene), i.e.,GAPDH.

By using T-Test statistically, gene expressions were found to be significantly raised in prostate cancer as shown in table (3-3)

Table 3-3: P Value, Mean and Standard Division and Mean and Standard Error of SAM 68 Expression

P Value	$p<0.001$
Mean ± SD	1.958 ± 0.493
Mean ± SE	1.958 ± 0.063

In the present study, Prostate cancer patients have showed elevated Sam68 gene expression levels in respect to the normal prostate blood. The elevation is an expected observation and implicates a possible association of Sam68 up-regulation with enhanced proliferation and invasiveness of cancer cells.

On another hand of the study, and as far as the gene is related to BPH, it has been observed that the studied gene did not show an increase in the gene expression of the SAM 68 gene in the blood of BPH patients, gene expressions were found to be significantly decreased, figure(3-2) show that.

The results showed that the significant change was a decrease in gene expression, while the significant change was high in prostate cancer, which may be due to the destruction of prostate cancer cells and their release into the blood. and what proves the validity of the study is that there was no increase in the gene expression in the blood of prostate cancer patients in patients who had their prostate removed, or it may be due to the fact that gene expression is not affected in BPH, where the SAM 68 gene expression is normal and without an elevated in BPH patients, and this reason may be the most accurate because no study has yet

been recorded that shows the gene expression of the sam68 gene is high in BPH patients.

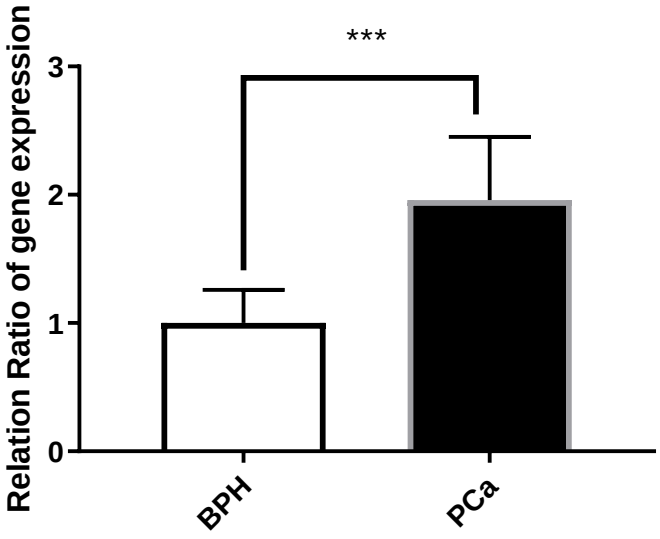


Figure 3-2 SAM 68 gene expression in Prostate Cancer and Benign Prostate hyperplasia Patients

In the current study SAM 68 gene was found to be expressed in blood PCa patients 2 folds relative to those of blood of benign prostate hyperplasia patients. Where the CT values were low in patients with prostate cancer compared with patients with benign prostatic hyperplasia, as shown in the figure (3-3), such results suggested the up regulation of the expression of gene during carcinogenesis.

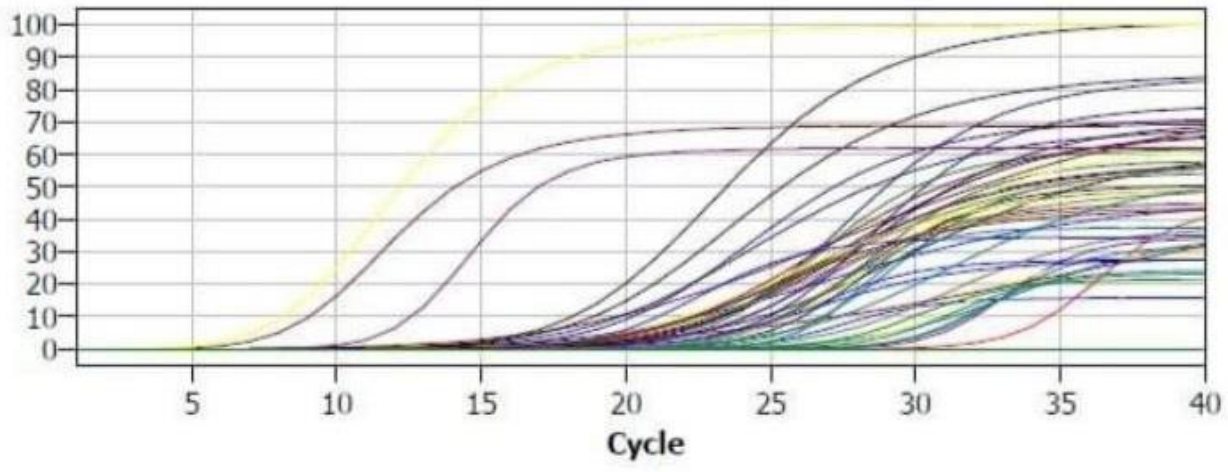


Figure 3-3 Ct cycle of SAM 68 in PCa, BPH Subjects

SAM 68 is upregulated and plays key roles in several human tumors(145), including PCa (74) (146). Sam68 mRNA yield high levels of this protein in PCa cells. Sam68 appears to support the same functions, as its depletion impaired PCa cell proliferation(74) and induced neural stem cell differentiation(147) . It was previously reported that a SAM 68 splice variant lacking a functional KH domain (SAM 68- KH) is generated upon growth arrest (148) and this variant was proposed to participate to cell cycle arrest in fibroblasts.

Also Up-regulation of SAM 68 has been reported in a small number of PCa specimens at both protein and mRNA level(74).

Others studies shown Post-transcriptional regulation of gene expression is often aberrant in cancer cells and changes in both alternative splicing and translational regulation of specific mRNAs have been reported(149)(150).

Remarkably, changes in alternative splicing classify PCa phenotypes more accurately than changes in global transcription (151). These observations indicate that factors influencing pre-mRNA processing could play a crucial role in determining the neoplastic progression of prostate cancer cells. Herein, investigated the role played in human PCas by SAM 68, an RNA-binding protein involved in several aspects of mRNA processing (66). The results demonstrate that SAM 68 is frequently upregulated in human PCas and that down regulation of its expression or activity affects prostate cancer cell proliferation and survival.

SAM 68 also directly interacts with the androgen receptor and binds to androgen responsive elements (AREs) within the promoter region of the prostate-specific antigen (PSA) gene, where SAM 68 seems to have some effect on AR-regulated transcriptional activity independently of its ARN binding capacity and splicing regulatory properties in LNcaP cells (146)

In this study, a significant increase of SAM 68 mRNA expression was observed in blood of prostate cancer patient, which is consistent with previous studies, indicating that the change of SAM 68 expression is related to the

development of prostate cancer, and the abnormal expression of SAM 68 may be a key to the pathogenesis of prostate cancer. it is possible to determine the SAM 68 in the blood or tissue fluid, which may indirectly reflect the malignant degree and metastasis of prostate cancer cells.

3.4. Biochemical Parameters

3.4.1. Prostate-Specific Antigen

By using T-Test statistically the mean concentrations of serum PSA (ng/ml) in prostate cancer (PCa) to benign prostate hyperplasia (BPH) patients shown in table (3-4).

Table 3-4: P Value, Mean and Standard Division and Mean and Standard Error of total PSA (ng/ml)

P Value	$p<0.001$
Mean ± SD	9.55 ± 2.58
Mean ± SE	9.55 ± 0.33

PSA was used as a diagnostic marker for prostate cancer and benign prostatic hyperplasia, the results of which were measured by a diagnostic kit, not a research kit, and the results were as shown in the figure (3-4).

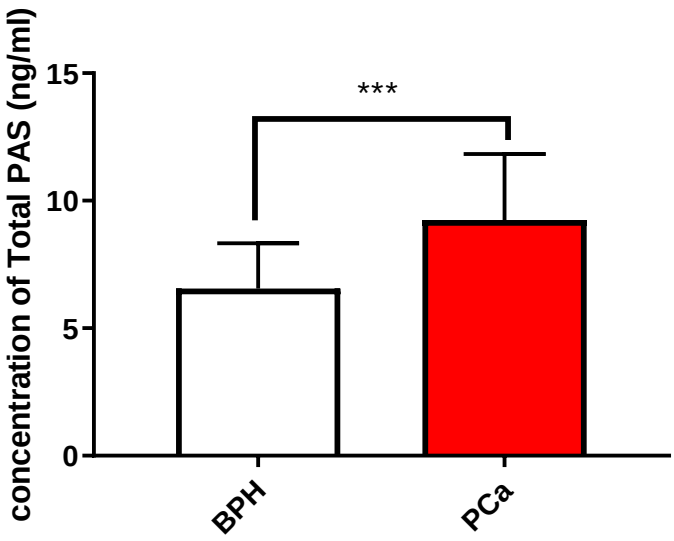


Figure 3-4 : Concentration of Total PSA (ng/ml) in PCa , BPH patient group.

The mean concentrations of serum fPSA (ng/ml) in prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients was shown in table (3-5)
There was a highly significant (P<0.001) increase in the activity of serum (fPSA) in BPH patients compared with prostate cancer group.

Table 3-5: P Value, Mean and Standard Division and Mean and Standard Error of free PSA of BPH (ng/ml)

P Value	p<0.001
Mean ± SD	1.7 ± 0.31
Mean ± SE	1.7 ± 0.085

The ratio of free to total PSA is lower in men with prostatic carcinoma. The PSA index is expressed as the percentage of the total plasma PSA that is free; an index above about 17 per cent is suggestive of BPH and one of less than 17 per cent of prostate carcinoma. Percent free PSA decreased as total PSA increased, so PSA Index in prostate cancer (PCa) patients was shown in table (3-6)

Table (3-6): PSA Index

PAS Index	PCa	BPH
Mean ± SD	13 ± 1.08 (%)	18 ± 1.79 (%)
Mean ± SE	13 ± 0.69 (%)	18 ± 0.94 (%)

The results show that percent free PSA may be used as an aid in distinguishing prostate cancer from benign disease in men with a serum total PSA concentration. Thus, the current study shows that percent free PSA is effective in increasing the specificity of total PSA measurements for prostate cancer detection. Although PSA is one of the most valuable cancer markers, it is far from perfect. PSA screening can lead to unnecessary biopsies, over diagnosis and overtreatment of clinically insignificant prostate cancer.

The diagnostic efficiency of any tumor marker is judged by its specificity and sensitivity (152). Serum PSA played a dominant role, because of its highest sensitivity for prostate carcinoma compared with other modalities (153). The highly elevation of serum PSA concentration is due to advanced malignancy in majority of untreated prostate adenocarcinoma patients. Serum PSA levels may be useful in degree of prostate carcinoma disease (152).

3.4.2- Correlation of Sam68 gene expression with PSA concentration in PCa

The mean correlation in prostate cancer (PCa) between gene expression of SAM 68 with PSA concentration was significantly decreased as shown in table (3-7), P Value > 0.001

Table 3-7 : P value, r Coefficient, and R^2

P Value	0.52

r Coefficient	0.083
R^2	0.0069

There are no correlation between SAM 68 gene expression and PSA concentration (ng/ml), the figure (3-5) prove that.

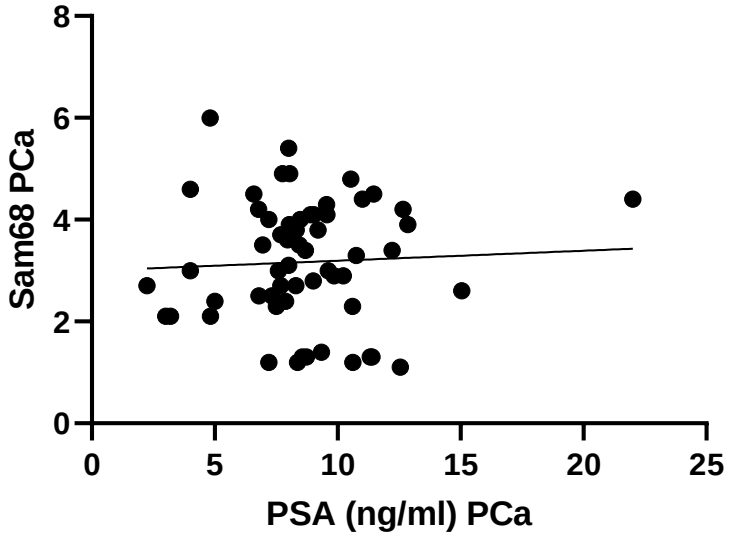


Figure 3-5 Correlation of Sam68 gene expression with PSA concentration in PCa

Our result does not compatible with the study done by Rajam et al where SAM 68 also directly interacts with the androgen receptor and binds to androgen responsive elements (AREs) within the promoter region of the prostate-specific antigen (PSA) gene(146). As the increase in the gene expression of SAM 68 will lead to an increase in the production of the prostate specific antigen.

3.4.3.AKT serine/threonine kinase 1

The mean concentration of serine/threonine kinase (ng/ml) in prostate cancer (PCa)and benign prostate hyperplasia (BPH)patients as shown in table(3-8).

Table 3-8: P Value, Mean and Standard Division and Mean and Standard Error of AKT(ng/ml)

P Value	p>0.001
Mean ± SD	3.88 ± 0.742
Mean ± SE	3.88 ± 0.095

The results obtained prove that the AKT concentration is high in patients with prostate cancer and patients with benign prostatic hyperplasia, p>0.001 figure(3-6) evident that.

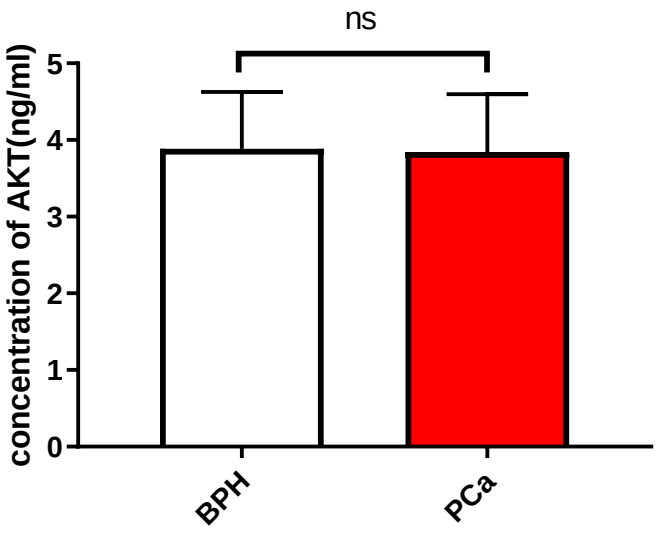


Figure 3-6 concentration of AKT (ng/ml) in PCa, BPH group.

The results obtained prove that the AKT concentration is high in patients with prostate cancer and patients with benign prostatic hyperplasia, but not significant, where p>0.001

These results are consistent with the research conducted with AKT, which showed a high concentration in the blood of patients. Multiple studies demonstrated the significance of Akt as a mediator of cellular proliferation and as an effective target for drug development(93).

Akt plays a crucial role in the pathogenesis of many human cancers. Increased Akt kinase activity has been reported in ~40% of breast, ovarian epithelial, prostate, and gastric cancers (154)(155). Many onco-proteins and tumor suppressors intersect in the Akt pathway that results in cell proliferation, differentiation, inhibition of apoptosis and actin cytoskeletal rearrangements(156). There is growing evidence that these aberrations of Akt, initiate tumor development and confer resistance to the conventional chemotherapy in many types of cancers.

In prostate cancer, RAD9 modulates Akt kinase activation and affects cell migration and invasion (157).

The Phosphoinositide 3 Kinase pathway is the predominant growth factor-activated pathway in LNCaP human prostate carcinoma cells(158)(159). Increased levels of Akt are detected in carcinomas of the breast, ovary and prostate and are associated with a poorer prognosis in comparison with tumors which do not display increased levels of expression(160). Increased AKT activity may play a profound role in the progression of human prostate cancers. AKT regulates many of the processes associated with metastatic progression and the emergence of androgen-independent hPCa cells, such as a diminished apoptotic response(161) as well as a release from the cell cycle control that follows androgen ablation(162).

3.4.4- Correlation of Sam68 gene expression with AKT1 concentration in PCa

In this study the mean correlation in prostate cancer (PCa) between gene expression of SAM 68 with AKT concentration was significantly increase as shown in table (3-9), where the p-value is < 0.001

Table 3-9 : P value, r Coefficient, and R^2

P Value	0.0012
r Coefficient	0.40
R^2	0.16

There are correlation between SAM 68 gene expression and AKT-1 concentration (ng/ml), the figure (3-7) prove that.

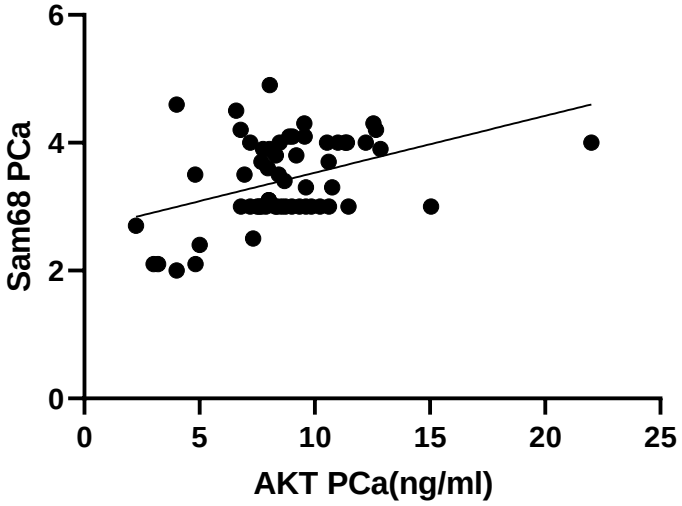


Figure 3-7: Correlation of AKT (ng/ml) in PCa patient with gene expression

The results of our study were in line with other previous studies, which showed the association of gene expression of the sam 68 gene with the high expression and gene expression of AKT1 protein and its high concentration in the serum. Sam68 promoted cell proliferation and stifled caspase-mediated cell death

by enhancing phospho-AKT expression(163). it's antecedently been reportable that Sam68 regulates proliferation of carcinoma via the AKT pathway(164).

3.4.5. β-Catenin

The mean concentration of β-catenin in prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients 9was significantly increased as shown in table (3-10).

Table 3-10: P Value, Mean and Standard Division and Mean and Standard Error of β-cat. (ng/ml)

P Value	p<0.001
Mean ± SD	10.30 ± 2.51
Mean ± SE	10.30 ± 0.29

The results obtained prove that the β-catenin concentration is high in patients with prostate cancer compared to patients with benign prostatic hyperplasia, figure(3-8) evident that

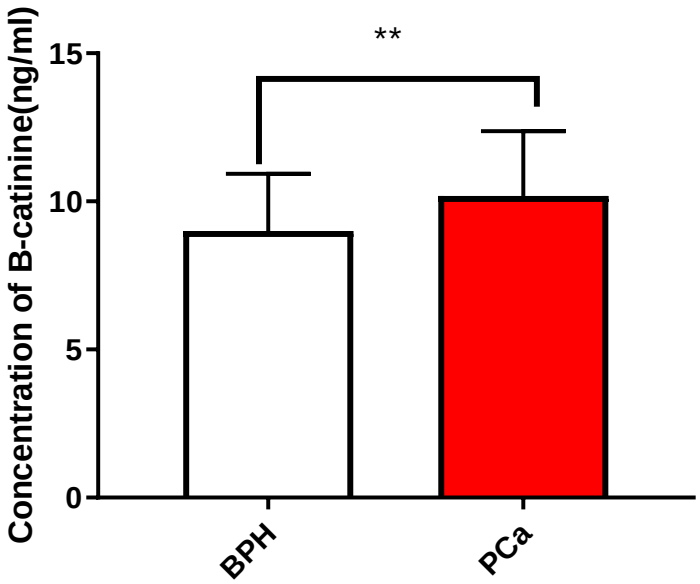


Figure 3-8: concentration of B- catinine (ng/ml) in PCA , BPH group.

The concentration of beta-catenin in patients with prostate cancer and benign prostatic hyperplasia was high in the serum of patients compared with the benign prostate hyperplasia patients, this indicates an increase in its expression in the cell. The results obtained are in agreement with previous studies, many studies investigators have suggested that Wnt/β-catenin signalling and the androgen receptor (AR) play critical roles in prostate cancer progression(165) (108)(166).

β-catenin is ubiquitinated and degraded by the proteasome pathway, resulting in low levels of cytoplasmic β-catenin, β-catenin was expressed in the cytoplasm and membranes of cancer cells(167) .This supports the hypothesis that changes in the Wnt pathway affect the progression of prostate cancer. A more accurate perception of the β-catenin precise regulatory mechanism in prostate cancer carcinogenesis is still needed to conduct in the future.

3.4.6. Correlation of Sam68 gene expression with β-catenin concentration in PCa

In this study the mean correlation in prostate cancer (PCa) between gene expression of sam 68 with β-cat concentration was significantly increase as shown in table (3-11), where the p-value is < 0.001

Table 3-11: P value, r Coefficient, and R^2

P Value	0.0002
r Coefficient	0.46
R^2	0.21

There are correlation between SAM 68 gene expression and β-cat concentration (ng/ml), the figure (3-9) prove that.

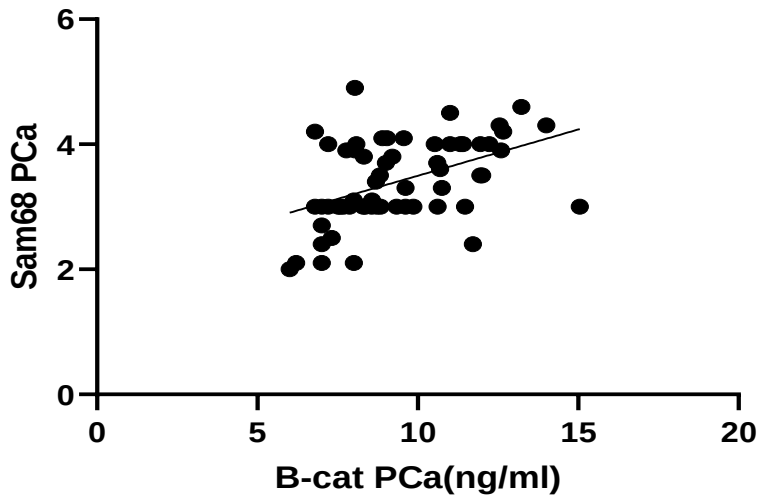


Figure 3-9: Correlation of beta-catenin(ng/ml) in PCa patient with gene expression

The thesis data was in line with other previous studies, which showed the correlation of the gene expression of the Sam68 gene with the high expression and the gene of the protein beta-catenin and its high concentration in the serum. Sam68 may promote the nuclear accumulation of β-catenin, facilitie Wnt/β-catenin signal activation and upregulate TCF/LEF transcription activity in carcinoma cells(168) . Moreover, other studies disclosed that knockdown of Sam68 suppresses cell proliferation via inhibiting Wnt/β-catenin signal. However, the elaborate mechanisms of Sam68 in promoting cell proliferation through activating Wnt/β-catenin signal stay unclear(169)

3.4.7. Interleukin-8

The mean concentration of IL8 in prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients was shown in table (3-12).

Table 3-12: P Value, Mean and Standard Division and Mean and Standard Error of IL8 (ng/l)

P Value	p<0.001

Mean ± SD	114.7 ± 12.39
Mean ± SE	16.17 ± 1.59

The results obtained prove that the IL8 concentration is high in patients with prostate cancer, compared to its concentration in patients with benign prostatic hyperplasia, figure(3-10) evident that

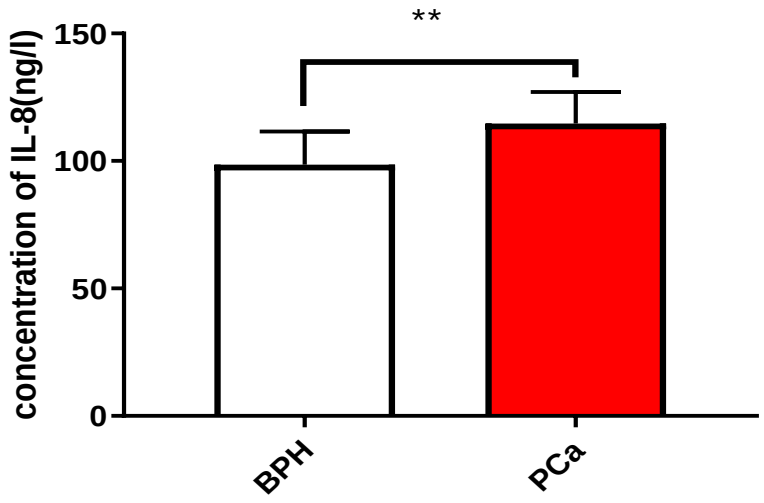


Figure 3-10: concentration of IL-8 (ng/l) in PCa , BPH group.

The results showed that the concentration of interleukin-8 was high in prostate cancer patients compared patients with benign prostatic hyperplasia. These results are consistent with previous studies that showed a high concentration of prostate cancer.

Previous study showed that the concentration of interleukin-8 serum was not elevated in BPH patients compared to its concentration in healthy people(170).

clinical studies have reported an increased expression of IL-8 in the serum of prostate cancer patients over that of normal subjects or patients with benign prostatic hypertrophy(171)

In line with this, high IL-8 concentrations have been detected in the serum of cancer patients and IL-8 levels correlate with cumulative tumor size, stage, and prognosis (172). In addition, IL8 may stimulate tumor-associated macrophages to secrete growth factors that increase the proliferation of tumor cells (173). This finding implies that there may be elevated IL8 expression across broad regions of the prostate in cases with high-grade cancer. Increased expression with grade is consistent with previous reports of IL8 serum and gene expression measurements(170)(174)(175). Further, IL8 protein expression measured in the stroma surrounding prostate tumors is reportedly elevated compared with stroma surrounding normal-appearing prostate epithelium(176). IL-8 is further described as pro-tumorigenic based on its ability to promote cell invasion, cell migration and angiogenesis(173).

While the results of this study did compatible with previous study that showed that the concentration of interleukin-8 was high when compared with the benign prostate hyperplasia patients, previous studies showed that the concentration of interleukin-8 serum was elevated in BPH patients (177)

3.4.8- Correlation of Sam68 gene expression with IL-8 concentration in PCa

The mean correlation in prostate cancer (PCa) between gene expression of sam 68 with IL-8 concentration (ng/ml) was significantly decreased as shown in table (3-13), where the p-value is > 0.001

Table 3-13: P value, r Coefficient, and R^2

P Value	0.1643
r Coefficient	-0.181
R^2	0.033

There are no correlation between SAM 68 gene expression and IL-8 concentration (ng/l), the figure (3-11) prove that

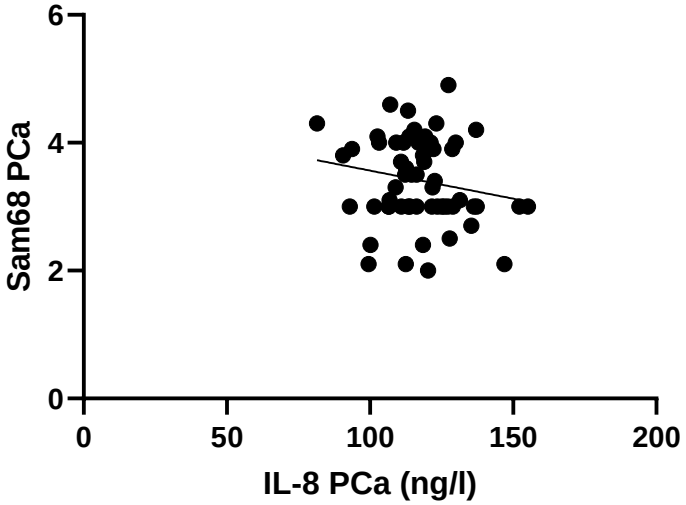


Figure 3-11: Correlation of IL-8 in PCa patient with gene expression

Although there is a relationship between the gene expression of the Sam68 gene and the gene expression of the androgen receptor gene, which in turn, the last (androgen receptor) increases the concentration of interleukin-8 and raises its level in the serum. However, our study did not show any relationship between the gene expression of the Sam68 gene and the concentration of interleukin-8 .

Androgen and estrogen suppress the transcription of IL-8, and the production increases upon androgen withdrawal (178)(179). In addition, circulating levels of IL-8 are increased in advanced prostate cancer at a stage when the tumors no longer respond to antiandrogens (180)(181). Furthermore, the mechanism by which IL-8 contributes to prostate cancer cell growth and metastasis has not been established. endogenous IL-8 production, either constitutive or induced by inflammatory agents, triggers down-regulation of androgen-mediated proliferative pathway, which, in turn, helps prostate cancer cells to overcome androgen-depletion therapy(182).

3.4.9. Vascular Endothelial Growth Factor

The mean concentration of VEGF in prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients was shown in table (3-14).

Table 3-14: P Value, Mean and Standard Division and Mean and Standard Error of VEGF (ng/l)

P Value	p<0.001
Mean ± SD	642.5 ± 76.24
Mean ± SE	642.5 ± 9.84

The results obtained prove that the VEGF concentration is high in patients with prostate cancer compared to its concentration in patients with benign prostatic hyperplasia, figure(3-12) evident that.

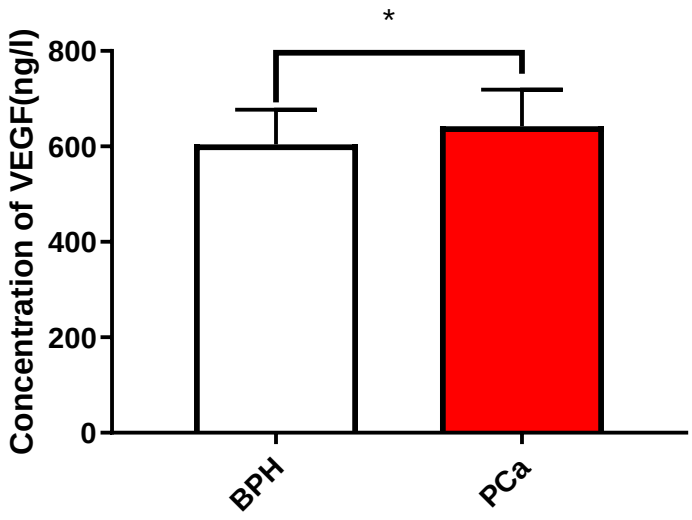


Figure 3-12: Level of VEGF (ng/l) in BPH and PCa group.

Our results revealed that there is a clear increase in the concentration of VEGF, and this was confirmed by previous studies. The behavior simultaneously

of VEGF in benign and malignant prostate environments, both serum and tissue in individuals without comorbidities related to chronic inflammation (9,29).

VEGF is narrowly related to the malignancy grade and metastasis of PCa, suggesting that it has a diagnostic and prognostic value of this illness. Our results and other studies reveal that serum expression of VEGF is not correlated, neither can discriminate a benign form (30-33). We have shown that levels of VEGF in the serum of PCa and BPH are not significantly different, to some extent. Probably the inflammatory response in BPH causes an increase in the VEGF expression leading to stromal hypervascularization, endothelial vessel permeability (34-36), or it might occur through a decrease in the androgen receptors and inhibition of apoptosis in epithelial cells (10).

The two previous studies (11,12) that evaluated participants with high risk of prostate cancer also observed no significant associations between cancer and VEGF levels. compared with subjects with benign prostatic hypertrophy. Such comparisons however, are not clinically relevant since elevated tPSA is the most frequent indication for prostatic biopsy, and reflect limited-challenge-bias (16,17). Study using healthy controls, not representing the whole spectrum of potential diagnosis to prostate cancer which are able to generate false-positive results, namely when prostatitis is present, produce inflated estimates of diagnostic accuracy (18).

Also, in some of these studies (7,10,13,14) whose controls were not suspected of having prostate cancer, the investigators did not perform any biopsy in the individuals that were categorized as healthy or only presenting BPH based on low PSA levels and a negative digital rectal examination. Circulating VEGF in serum from cancer patients may reflect an aggregate of tumor-cell and platelet-stored VEGF (23). To better reflect the disease-related circulating VEGF levels, the use of rapidly processing citrated plasma samples and additional centrifugation

has been recommended (23). This has been disputed, by other authors, suggesting that both plasma and serum levels of VEGF may be equally useful (24).

3.4.10- Correlation of Sam68 gene expression with VEGF concentration in PCa

The mean correlation in prostate cancer (PCa) between gene expression of sam 68 with VEGF concentration (ng/ml) was significantly decreased as shown in table (3-15), where the p-value is > 0.001

Table 3-15: P value, r Coefficient, and R^2

P Value	0.0628
r Coefficient	-0.2417
R^2	0.05841

There are no correlation between SAM 68 gene expression and VEGF concentration (ng/l), the figure (3-13) prove that

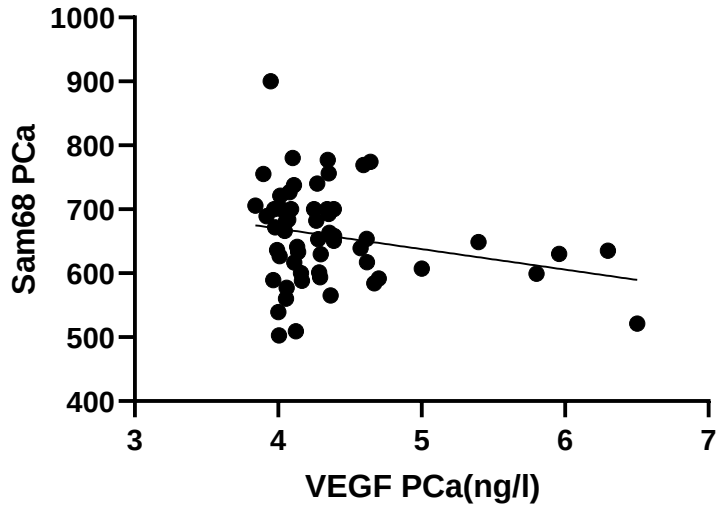


Figure 3-13:Correlation of VEGF in PCa patient with gene expression

Previously, a study had not recorded any relationship between the gene expression of the Sam68 gene and the concentration of VEGF and this is what was proven by our study in this thesis, as it did not find any correlations between the gene expression of the gene Sam68 and its relationship with the concentration of VEGF in the serum.

PCa cells secrete proteic factors such as the vascular endothelial growth factor (VEGF), which is extensively studied and known as the major angiogenic marker. VEGF acts as a direct mediator in endothelial cell proliferation, vascular permeation, tumor growth promotion, and metastasis. Several authors report that there are higher levels of VEGF in biopsies and serum of PCa patients as compared to healthy individuals(183)(184). Although there is a correlation between levels of VEGF in serum and the stages of the disease, its validity as a prognosis marker is still controversial because VEGF is also augmented in BPH and its plasma concentration does not concur with the clinical classification as benign or malignant forms (185)(186).

3.4.11. Vascular endothelial growth factor receptor 2

The mean concentration of VEGF in prostate cancer (PCa) to benign prostate hyperplasia (BPH) patients was shown in table (3-16).

Table 3-16: P Value, Mean and Standard Division and Mean and Standard Error of VEGFR2 (ng/l)

P Value	$p<0.001$
Mean ± SD	4.51 ± 0.60
Mean ± SE	4.51 ± 0.08

The results obtained prove that the VEGFR2 concentration is high in patients with prostate cancer compared to its concentration in patients with benign prostatic hyperplasia, figure(3-14) evident that.

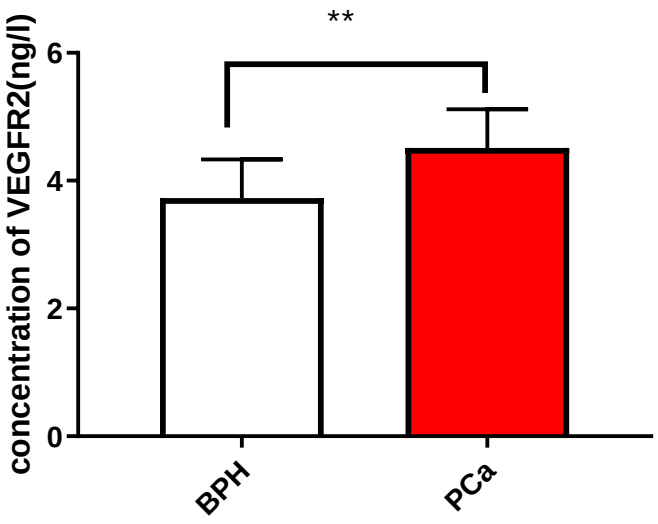


Figure 3-14: Concentration of VEGF receptor2 in BPH, PCa group

The results of measuring the VEGFR2 concentration in the serum showed that there is an expected increase in the level in patients with prostate cancer relative to its concentration in the serum of BPH. The results of the thesis agreed with previous studies in terms of increasing the concentration of the VEGFR2 in the serum of patients.

Recently, it was reported that the soluble vascular endothelial growth factor receptor-2 (sVEGFR-2) is secreted by microvascular endothelial cells from human prostate tumor(187). Further, results of another study show that sVEGFR-2 is able to modify the VEGF effect on the endothelium of BPH(188). Vascular endothelial growth factor receptor 2 (VEGFR2) is highly levels in tumor associated endothelial cells, where it modulates tumor-promoting angiogenesis, and it is also found on the surface of tumor cells(189). Another study found that VEGFR2 is expressed in PC-

3 human prostate cancer cell line and associated with malignancy and metastasis of human prostate cancer.

The prominent effect of VEGFR-2 in cell proliferation, cell differentiation, migration, survival, angiogenesis, and lymph-angiogenesis support the choice of this receptor as a potential target for the discovery of novel inhibitors(189). The tumor vessel was traditionally thought to be an especially attractive target tissue because it is formed from nonmalignant .In addition to the angiogenic actions of VEGFR2 protein in endothelial cells, the receptor is also known to be expressed in various cancer cells, where it is associated with tumor malignancy(190)(191). A previous study reported that the antitumor activity of a VEGFR2 kinase inhibitor is correlated with the expression level of VEGFR2 in human prostate tumor(45)

3.4.12. Correlation of Sam68 gene expression with VEGFR2 concentration in PCa

The mean correlation in prostate cancer (PCa) between gene expression of SAM 68 with VEGF concentration (ng/ml) was significantly decreased as shown in table (3-17), where the p-value is > 0.001

Table 3-17: P value, r Coefficient, and R^2

P Value	0.2872
r Coefficient	-0.1397
R^2	0.01951

There are no correlation between SAM 68 gene expression and VEGF concentration (ng/l), the figure (3-15) prove that

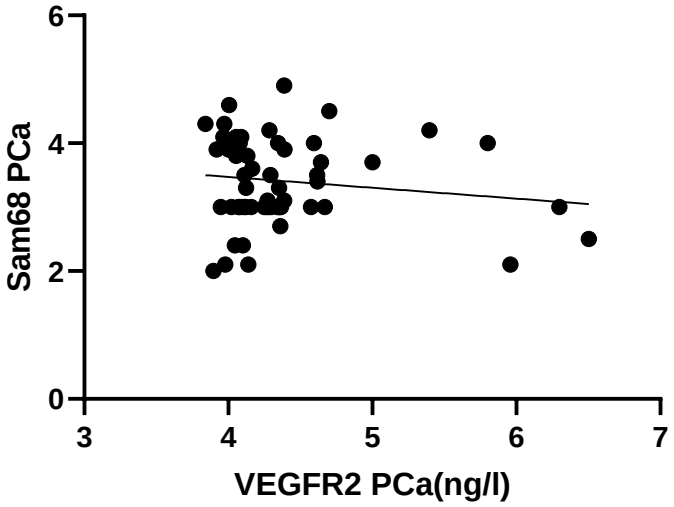


Figure 3-15: Correlation of VEGFR-2 in PCa patient with gene expression

There are no relationship between the gene expression of the SAM 68 gene and the concentration of VEGFR2 according to our study in this thesis, as it did not find any correlations between the gene expression of the gene Sam68 and its relationship with the concentration of VEGFR2 in the serum. The vascular endothelial growth factor receptor-2 (VEGFR-2) plays an important role in angiogenesis, endothelial cell proliferation, migration, and survival, VEGFR-2 was expressed in vascular endothelium in cancerous prostate specimen higher thn normal, as expected from previous studies(192)

3.5.Conclusion

1- Our finding that Sam68 is required for optimal proliferation and survival of prostate cancer cells may demonstrate that Sam68 has separable effects on AR-regulated transcriptional activity and alternative splicing, both of which may affect PCa

2- Serine/threonine kinase-1 activity and substrate phosphorylation in cells, suggesting that active kinase is exclusively associated with cellular membranes, ensures that Akt-1 activity play a role in creating an anti-apoptotic threshold

3- The activation of canonical Wnt signalling and the AR are related in prostate cancer progression, it was associated with PSA progression in prostate tumors patients.

4- Chemokines and cancer are very much intertwined concepts, the surprisingly high production of IL-8 in most tumors, is due to various roles for IL-8 in cancer stem cells, angiogenesis, and inflammatory infiltrates

5- Our results show that VEGF and VEGFR2 levels have no clinical importance in deciding which patients are suspected of having prostatic cancer from those who have benign prostate hyperplasia.

3.6.Recommendations

1- Estimation of the gene expression of the sam68 gene in the tissues of patients with prostate cancer
2- Estimation of the gene expression of the sam68 gene in the tissues of patients with benign prostate hyperplasia
3- Comparison of sam68 gene expression in blood and tissue of patients with prostate cancer
4- Calculation of the AKT-1 gene expression in the blood of patients with prostate cancer
5- Estimation of the gene expression of the gene sam68 in the blood of patients with other types of cancer such as breast cancer

Recommendations

6- Estimation of the gene expression of the sam68 gene in the tissues of patients with prostate cancer

7- Estimation of the gene expression of the sam68 gene in the tissues of patients with benign prostate hyperplasia

8- Comparison of sam68 gene expression in blood and tissue of patients with prostate cancer

9- Calculation of the AKT-1 gene expression in the blood of patients with prostate cancer

10- Estimation of the gene expression of the gene sam68 in the blood of patients with other types of cancer such as breast cancer

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الخلاصة

سرطان البروستات (PCa) هو اضطراب غير متجانس مرتبط بالعمر وهو سبب رئيسي للوفيات المرتبطة بالسرطان بين الرجال. يعتبر سرطان البروستات من أكثر أنواع السرطانات شيوعا في العالم وتحديدا في العراق ، مقارنة بغيره من السرطانات ، حيث يصيب الرجال في سن الخمسين وما فوق ، لكنه نادرا ما يصيب الرجال الأقل من هذه الفئة العمرية. سرطان البروستات هو تراكم متعدد الخطوات للمتغيرات الجينية التي قد تؤدي إلى تكاثر خلوي غير منضبط ، وانخفاض في موت الخلايا أو موت الخلايا المبرمج ، والغزو ، والانتشار النقيلي. تضخم البروستاتا الحميد ليس سرطانًا ولا يتطور إلى سرطان. لكنها يمكن أن تكون مشكلة طبية خطيرة لبعض الرجال.

تضخم البروستاتا الحميد (BPH) هو تشخيص نسيجي يشير إلى تكاثر العضلات الملساء والخلايا الظهارية داخل منطقة انتقال البروستات. ومع ذلك ، فإن التشابه بين تضخم البروستاتا الحميد والتكوين الجنيني للبروستاتا أدى إلى فرضية أن تضخم البروستاتا الحميد قد ينتج عن "إيقاظ" في مرحلة البلوغ لعمليات الحث الجنيني. يتم تنظيم Sam68 في العديد من أنواع السرطان التي تصيب الإنسان ، بما في ذلك سرطان البروستاتا حيث يعزز تكاثر الخلايا والبقاء على قيد الحياة.

تم تقدير التعبير الجيني للجين SAM68 في دم المرضى المصابين بأورام البروستات في 120 عينة (60 مصابين بسرطان البروستات PCa, 60 مصابين بتضخم البروستات الحميد BPH) .كما تم تقدير تركيز الحمض النووي الريبي (RNA) بواسطة Nano Photometer ،حيث كان التركيز 555. ± 13.61

التعبير الجيني للجين المدروس كان عاليًا في سرطان البروستاتا، حيث كان P <0.001 عند مقارنته بالتعبير الجيني لدى الأشخاص المصابين بتضخم البروستات الحميد باستخدام تقنية Real Time –PCR .

كما تم تقدير الدلائل الكيميائية المدروسة في مصل المرضى المصابين بأورام البروستات باستخدام تقنيةELISA , أظهرت الدراسة الحالية أن تركيز سيرين-ثريونين كاينيز كان عاليًا في سرطان البروستاتا ومرضى البروستات الحميد لكن لم يكن هناك قيمة معنوية، حيث سجل P >0.001.

النتائج الإحصائية لبروتين بيتا-كاتينين كانت ذات دلالة عالية في مرضى سرطان البروستات مقانتا مع الاشخاص المصابين بتضخم البروستات الحميد حيث ان قيمة P <0.001 .

كما أظهرت الدراسة الحالية أن إنترلوكين- 8 كانت ذو دلالة معنوية عالية في سرطان البروستات ، حيث سجلت قيمP <0.001 ، مقارنتا لمرضى تضخم البروستات الحميد

تم تعيين دلالة إحصائية لعامل نمو بطانة الأوعية الدموية (P <0.001) ما يشير إلى أن تركيز عامل النمو في سرطان البروستات كان مرتفعًا جدًا مقارنة بالمشاركين المصابين بتضخم البروستاتا الحميد حيث كانت مستوياته أعلى بشكل ملحوظ في سرطان البروستات منها في تضخم البروستات الحميد.

كان تركيز مستقبل عامل نمو بطانة الأوعية الدموية النوع الثاني أعلى إحصائيًا في مرضى سرطان البروستات مقارنة بالمرضى المصابين بتضخم البروستات الحميد P <0.001 .

ان التعبير العالي للجين SAM68 في مرضى سرطان البروستات يثبت ان الجين المدروس له دور في نمو وتطور سرطان البروستات.

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية الطب

2021-2020

علاقة تعبير الجين "سام 68" وبعض قياسات الواسمات الكيموحيوية لمرضى سرطان البروستات

أطروحة

مقدمة الى مجلس كلية الطب/ جامعة بابل كجزء من متطلبات نيل درجة الدكتوراه فلسفة في علوم الكيمياء الحيوية السريرية

كجزء من متطلبات نيل شهادة الدكتوراه في الكيمياء السريرية

مقدمة من قبل الطالب

منير كاظم خضير حمادي

بإشراف

الاستاذ الدكتور مها فاضل سميسم

فرع الكيمياء الحياتية السريرية/كلية الطب/جامعة بابل

الاستاذ الكتور محمد رضا جودي

فرع الجراحة / كلية الطب / جامعة بابل

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