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Study of Some Biochemical Markers and Biochemical Variables in Prostate Cancer Patients with Advanced Bone Metastasis

A Thesis
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Biochemistry

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صدق الله العلي العظيم

سورة يوسف: الآية (76)

Dedication

To my parents

To my brother… (Aamir)

To any one who helped me

in this thesis.....

Certification

We certify that this thesis (Study of Some Biochemical Markers and Biochemical Variables in Prostate Cancer Patients with Advanced Bone Metastasis) was prepared under our supervision at the Department of Chemistry, College of Science, University of Kerbala, as a partial requirement for the degree of master in Biochemistry .

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Aziz Hussein Jasim

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List of abbreviations

Terms	Definitions
®	Registered Trademark
µl	Microliter
A	Absorbance
B.P.H	Benign prostatic hyperplasia
BMD	Body mineral density
BMI	Body mass index
DHT	Dihydrotestosterone
DPD	Deoxypyridinoline
DRE	Digital rectal examination
DW	Distilled water
ELISA	Enzyme-linked immunosorbent assay
Fig	Figure
FSH	Follicle-stimulating hormone
IU/L	International unit / Liter
KDa	Kilo Dalton
L	Liter
L.P.C	Localized prostate cancer
LH	Luteinising hormone
LHR	Luteinising hormone-releasing

M.P.C	Metastasis prostate cancer
mg	Milligram
ml	Milliliter
mmole	Millimole
N.S	Non-Significant
ng	Nanogram
nm	Nanometer
nmole	Nanomole
^{o}C	Degrees Celsius
Pca	Prostate cancer
PSA	Prostate specific antigen
Pyd	Pyridinoline
r	Correlation coefficient
Sd.E	Standard error
T-ALP	Total-Alkaline phosphatase
vs	Versus
www	World Wide Web

Summary

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. The cancer, often metastasis, reaches in advanced stages even up to the bone, which causes an increase in metabolism products (catabolism) of collagen, like Deoxypyridinoline (DPD) level, and an increase of the level of alkaline phosphatase (ALP), and other elements in the bone structure such as calcium (Ca^{2+}) and phosphate (PO_4^{3-}).

This study deals with the verification of cancerous metastasis whether it reaches to the bone or not, through the marker of DPD, as well as to explain the relationship between DPD and other clinical indicators in patients with metastatic prostate cancer, under study. Fifty patients with prostate tumor were selected from AL-Hussein Teaching Hospital of Kerbala and 30 healthy subjects represented as the control group, their ages were identical with the ages of patients.

The clinical characteristics of patients were documented, which included age, smoking, family history, obesity, and chemotherapy drug. Statistical analysis of the results showed that 60% of patients were aged (66-80) year and 40% were between the ages of (50-65) year. The concentrations of PSA, total-alkaline phosphatase (T-ALP), calcium, and phosphate were measured in the sera of both patients with prostate cancer and the healthy group. As well as, the concentrations of deoxypyridinoline, and creatinine were measured in the urine of same patients and healthy group.

The statistical results, by using student`s t-test, showed a highly significant ($P<0.000$) increase in the level of PSA, DPD/Creat., calcium, and phosphate. There also was a highly significant ($P<0.01$) increase in the

activity of serum total-alkaline phosphatase (T-ALP), compared with the healthy group.

In this study, fifty patients with prostate tumor were clinically classified into three groups; benign prostate hyperplasia (B.P.H), localized prostate cancer (L.P.C), and metastasis prostate cancer (M.P.C). The results appeared that urinary excretion of deoxypyridinoline (DPD) showed a highly significant ($P<0.000$) increase in the patients with bone metastasis than in those with B.P.H and those with localized prostate cancer (L.P.C), whereas DPD levels did not show a significant variation between B.P.H and L.P.C groups. In addition, the results of other parameters under study which included PSA, T-ALP, Ca^{2+}, and PO_4^{3-}, showed a significant ($P<0.01$) increase in the concentration of PSA, and a highly significant ($P<0.000$) increase in the concentrations of Ca^{2+} and PO_4^{3-} in M.P.C group, compared with B.P.H and L.P.C groups.

By using Pearson's correlation coefficient between parameters under study in prostate cancer patients with bone metastasis group (M.P.C), the results showed a significant positive correlation between PSA and T-ALP ($P=0.003$, $r=0.577$), DPD and calcium ($P=0.009$, $r=0.520$), DPD and phosphate ($P=0.01$, $r=0.499$), calcium and phosphate ($P<0.000$, $r=0.721$).

The demographic study of patients which included age, smoking, chemotherapy drug, family history, and obesity demonstrated a significant ($P<0.05$) decrease in the level of PSA in smoker patients, when compared with non-smoker patients, whereas there wasn't noticed any significant ($P>0.05$) in the other parameters under study in smoker patients with prostate tumor. In addition, it is shown that a highly significant ($P<0.000$) increase in the levels of PSA and DPD, and significant ($P<0.01$) increase in the levels of calcium and phosphate, in patients who were treated with chemotherapy drug, when compared with patients who were untreated with chemotherapy drug. The results didn`t show any significant variation

(P>0.05) in levels of all parameters that used in the present research, with another clinical characteristics (age, family history, obesity) in patients of prostate tumor.

The results indicate to that Deoxypyridinoline is a good clinical marker. We can monitor through DPD, the stage of cancerous metastasis and for treatment monitoring to the bone, in prostate cancer and all types of cancers. In addition, the results showed a correlation between DPD with other clinical indicators in metastasis prostatic cancer patients. The results also demonstrated a decrease in a therapeutic effect of chemotherapy drug in prostate cancer patients; and increase the progression of cancer, because of the toxicity of bone marrow by chemotherapy drug. Therefore, we recommend using the dependent-dose of radiotherapy with chemotherapy to save the bone.

Introduction

Cancer is one of the most prevalent diseases in Iraq, especially after the wars that suffered in 1990 and 2003. The radiation of weapons that contain depleted uranium was the one of the causes of cancer. Prostate cancer is the first most common cancer in men before of breast cancer (in women) and colon cancer [1]. Prostate cells can begin to mutate and can metastasis into surrounding tissue, such as bone [2], which is a metabolically active tissue being continuously remodeled throughout life [3]. 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 [2].

During the last twenty years, several biochemical markers of both bone formation and resorption have been introduced. Most of these markers were derived from type I collagen. Assays for measuring urinary excretion of smaller breakdown products of type I collagen were introduced somewhat later, first an ELISA method which measured free pyridinoline (Pyr) and deoxypyridinoline (DPD) crosslinks [4].

When the bone matrix is resorbed, the cross-link residues, pyridinoline and deoxypyridinoline (DPD) are released from the collagen molecules and eventually excreted in urine [5]. Several reports suggest that the assay of these collagen cross-link residues may provide valuable markers of bone metastasis in patients with prostate cancer [6] or breast cancer [7].

Prostate specific antigen (PSA) is widely accepted as the most important marker for detecting prostate cancer and for monitoring treatment [8]. However, it has a low positive predictive value for bone metastases [9]. PSA is prostate specific but not prostate cancer specific and is measured most commonly by radioimmunoassay [10].

Also the alkaline phosphatase (ALP) activity was found to be elevated in bone diseases, and for decades it was the only laboratory parameter reflecting bone formation [11]. Elevated skeletal alkaline phosphatase levels may indicate the presence of bony metastasis in 70% of affected patients [12]. Furthermore, the measurement of alkaline phosphatase and PSA at the same time increases clinical effectiveness to approximately 98% [13]. In a prospective study, multiple regression analysis showed the extent of bone disease to be the only variable influencing the serum levels of skeletal alkaline phosphatase and PSA. However, in contrast to serum PSA, skeletal alkaline phosphatase demonstrated a statistical correlation with the extent of bone disease [14].

Bone tissue has three main functions: mechanical support and the site of muscle attachment for locomotion; protective, for vital organs and bone marrow; and metabolic, as a reserve of ions, especially calcium and phosphate [15]. When the cancer cells dissolve bone, calcium is released, this lead to high levels of calcium in the blood [16]. In addition, phosphate is a rise over twice in blood of patients with greater risk of overall prostate cancer and lethal and high grade cancers, compared to patients without cancer; this is due to tumor growth or tumorigenesis, and bone losing [17].

1. Literatures Review

1.1 Prostate gland

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 [18].

The prostate's job is to make some of the fluid that protects and nourishes sperm cells in semen, making the semen more liquid. 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 [19].

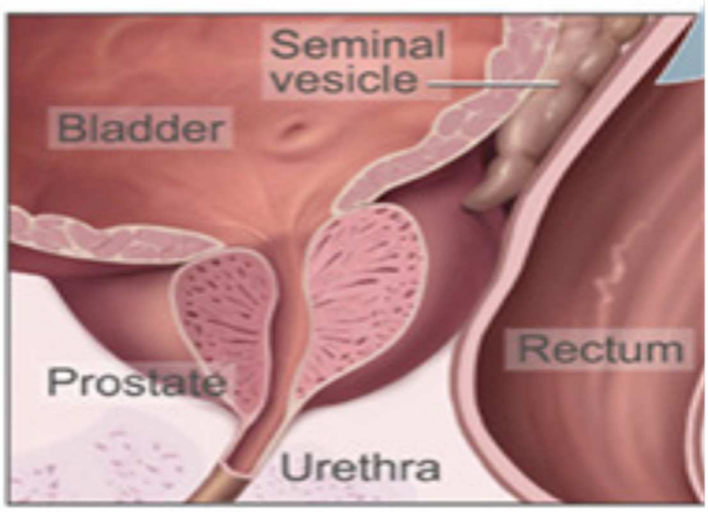


Figure 1.1 Male lower abdominal anatomy [20].

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. The testes are the source of most androgens, with adrenal biosynthesis

providing only 5-10% of androgens (i.e. androstenedione, dihydroepiandrosterone and dihydroepiandrosterone sulphate) [21].

Testosterone secretion is regulated by the hypothalamic-pituitary-gonadal axis. Hypothalamic luteinising hormone-releasing hormone (LHRH) stimulates the anterior pituitary gland to release luteinising 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 [21].

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 [21].

1.1.3 Epidemiology

Prostate cancer (Pca) is now recognized as one of the most important medical problems facing the male population. In Europe, prostate cancer is the most common solid neoplasm, with an incidence rate of 214 cases per 1000 men, outnumbering lung and colorectal cancer [22]. Furthermore, prostate cancer is currently the second most common cause of cancer death in men [23]. In addition, since 1985, there has been a slight increase in most countries in the number of deaths from prostate cancer, even in countries or regions where prostate cancer is not common [24].

Prostate cancer is one of the most common cancer types representing 19% of all cancers diagnosed in 2002 with 679 000 new cases in the western world [25]. Bone metastasis is common in prostate cancer patients

and arises when the primary tumor metastasizes to the bone causing a lesion of high bone remodeling destroying the bone structure. The consequences are devastating symptoms such as severe bone pain, pathologic bone fractures, increased mortality, hypocalcaemia, and spinal cord compression [26, 27].

Bone metastasis occurs in more than 50-60% of patients with advanced cancer disease [28] and is incurable. Osteoblasts and osteoclasts are affected by the invasive tumor cells in the bone metastasis causing increased number, activity and survival of these bone remodeling cells [29] a phenomenon known as the vicious cycle. Prostate cancer is characteristic mainly by sclerotic bone lesions especially in men failing androgen therapy [30].

Prostate cancer affects elderly men more often than young men. It is therefore a bigger health concern in developed countries with their greater proportion of elderly men. Thus, about 15 % of male cancers are prostate cancer in developed countries compared to 4 % of male cancers in developing countries [31].

In Iraq, a statistical study in 2013 revealed that 45 to 50 per 10 thousands person were diagnosed with a cancer in them lifetime, prostate cancer (Pca) is the first most common cancer before all cancers [1].

Benign prostate hyperplasia (B.P.H) affects the quality of life in about 40% of men in their fifth decade and 90% in their ninth decade [32]. It is unusual before the age of 45 and the affects men of Afro-American origin more severely than white men, possibly due to higher testosterone levels, 5-alpha-reductase activity, androgen receptor expression and growth factor activity [33]. One study found some correlation between lower urinary tract symptoms and increased prostate volume [33]. The prostate increases in size with passing years, it`s a double in size every 4.5 years but

this rate reduces subsequently, and it begins a decelerating rate in size between the ages of 31 to 50 [34].

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 [35].

1.2.1 Benign prostate hyperplasia (B.P.H)

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 [18]. Benign prostatic hyperplasia is an increase in size of the prostate gland without malignancy present and it is so common as to be normal with advancing age. 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 [36].

1.2.2 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 [37]. In the prostate, the expression of oncogene is a driven malignant conversion and expression of tumor suppressor genes that inhibit this process [38]. 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 [39]. 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 [39].

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 [40]. Older men tend to have larger tumors of a higher grade than younger patients [41, 42]. Treatment decisions for older men should take into consideration the risk of dying from prostate cancer which depends on the grade and stage of the tumor, potential adverse effects of treatment, and patient preference. Interventions that might decrease health-related quality of life without prolonging survival should be avoided. Evidence suggests that in both the USA [43] and Europe [44] older patients are under-treated: only a minority of older adults with localized prostate cancer receives curative treatment. However, curative treatment should neither be denied where appropriate, nor limited to androgen deprivation therapy [45].

1.2.2.1 Symptoms and signs of prostate cancer [46]

Prostate cancer may not cause signs or symptoms in its early stages. Prostate cancer that is more advanced may cause signs and symptoms such as:

* Trouble urinating
* Decreased force in the stream of urine
* Blood in the urine
* Blood in the semen
* General pain in the lower back, hips or thighs
* Discomfort in the pelvic area
* Bone pain
* Erectile dysfunction

1.2.2.2 Diagnosis of prostate cancer

Evaluating the blood for prostate-specific antigen (PSA) levels and conducting a digital rectal exam (DRE) are two ways to screen for prostate cancer [47]. 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. A cancer's grade is based on 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 [48, 49].The relation between Gleason score and risk of cancer death shown in (Table 1.1).

Table 1.1 Gleason score at diagnosis in prostate cancer patients of ages 55-74 years [21, 50].

Gleason score	% Risk of cancer death	% Cancer-specific mortality
2-4	4-7	8
5	6-11	14
6	18-30	44
7	42-70	76
8-10	60-87	93

1.2.2.2.1 Digital rectal examination (DRE)

Most prostate cancers are located in the peripheral zone (Figure 1.2) of the prostate and may be detected by DRE when the volume is about 0.2 ml or larger. In about 18% of all patients, prostate cancer is detected by a suspect DRE alone ،irrespective of the PSA level. A suspect DRE in patients with a PSA level of up to 2 ng / ml has a positive predictive value of 5-30%. A suspect DRE is a strong indication for prostate biopsy as it is predictive for more aggressive (Gleason score ≥7) prostate cancer [51].

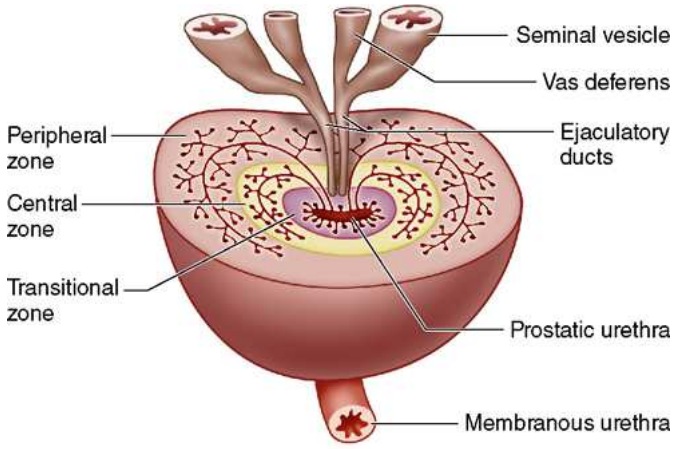


Figure 1.2 Prostate gland zones [52].

1.2.2.3 Treatment of prostate cancer

Hormone 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. The names of some of these GnRH drugs are leuprolide (Lupron®, Eligard®, Viadur®) or goserelin (Zoladex®). In some cases, men who had both testes removed or are receiving a GnRH agonist are also treated with antiandrogens such as bicalutamide (Casodex®) or flutamide (Eulexin®) [53].

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 [54].

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 [53].

External Beam Radiation Therapy (similar to that used to treat early stage prostate cancer) can be aimed at sites of painful bone metastasis. External beam radiation relieves pain in the majority of men and is most useful for treatment of one or two sites of pain [55].

Radiopharmaceuticals are drugs given by intravenous infusion (IV), such as strontium-89 (Metastron®) or samarium-153 (Quadramet®). These drugs target radiation to bone metastasis. They relieve pain in most men. Because radiopharmaceuticals travel throughout the skeleton, this therapy may be most helpful for men with a number of painful bone metastasis.

Bisphosphonates are classes of drugs that keep bone from breaking down or becoming resorbed. Zoledronic acid (Zometa®) is a bisphosphonate given by intravenous infusion. It reduces the risk of bone complications, including pain and fractures, in men with metastatic prostate cancer [56].

Surgery may be needed to treat bone fractures or to relieve pressure on the spinal cord by bone metastasis [57].

Pain medications are important parts of care for most men with metastatic prostate cancer. They are used in combination with other treatments for prostate cancer [53].

1.2.2.4 Risk factors of prostate cancer

Don't yet completely understand the causes of prostate cancer [18], but researchers have found several factors that might change the risk of getting prostate cancer. 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. 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 [58]. Race, black men are at the greatest risk due to a genetic factor [59], African-American men have a higher risk for prostate cancer than men of other races [60]. Diet, a high intake of animal or saturated fat increases the risk [60]. Occupation, people who are regularly exposed to the chemicals (dimethyl acryl nitrate, and metal of cadmium) such as metal increase the rate of prostate cancer. Lack of exercise may increase the risk in those who eat a high fat diet. Obesity, alcohols abuse and cigarette smoking may also be risk factors [61].

1.2.3 Staging of prostate cancer

Clinical stage refers to the extent or severity of a patient’s cancer. Staging is important, because it helps doctor plan patient treatment,

estimate prognosis, and identify suitable clinical trials for specific patients (Figure 1.3).

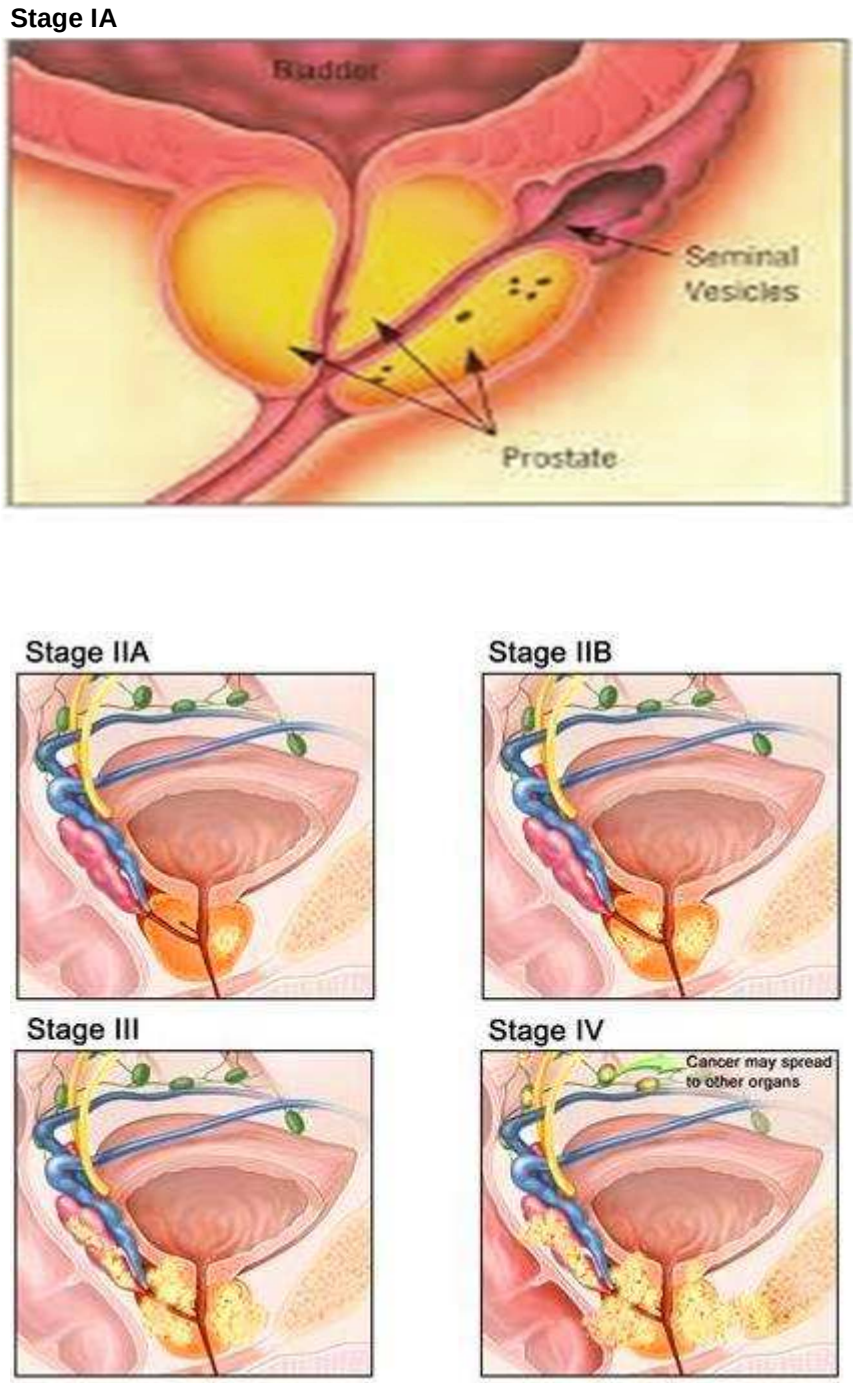


Figure 1.3 Stages of prostate cancer: Stage IA; Tumor incidental histological finding in 5% or less of tissue resected[62], stage IIA; Tumor confined within the prostate, stage IIB; Tumor extends through the prostatic capsule, stage III; Tumor in Regional lymph nodes, stage IV; Tumor is Distant metastasis[63].

1.2.3.1 Localized stage

Localized cancer mean, the cancer cell is stay in the cellular tissue and not metastasis into surrounding tissues of other organs. The first level is the assessment of local tumor stage, where the distinction between intracapsular (T1-T2) and extraprostatic (T3-T4) disease has the most profound impact on treatment decisions [64]. Digital rectal exam (DRE) often underestimates the tumor extension; a positive correlation between DRE and pathological tumor stage was found in a fewer than 50% of localized cases [64].

Between 1967 and 1975, the Veterans Administration Cooperative Urological Research Group randomized 142 patients affected by clinical localized prostate cancer in Europe [65].

Between 1989 and 1999, the Scandinavian Prostate Cancer Group Study Number 4 (SPCG-4) randomized 695 patients with clinical stage T1-T2 (localized prostate cancer) in Europe. This study began after PSA screening was introduced into clinical practice [66]. In recent years, there has been renewed interest in surgery for locally advanced prostate cancer, and several retrospective case series have been published. Although still controversial, it is increasingly evident that surgery has a place in treating locally advanced disease [67, 68].

The Tumor Node Metastasis (TNM) staging system is one of the most commonly used staging system. This system is based on the size and extent of primary tumor (T), presence of distant metastasis (M), and extent of spread to regional lymph nodes (N) [69]. The 2009 Tumor Node Metastasis (TNM) classification for prostate cancer is shown in (Table 1.2).

Table 1.2 Tumor Node Metastasis (TNM) staging system of prostate cancer [70].

T - Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically inapparent tumor not palpable or visible by imaging
T1a	Tumor incidental histological finding in 5% or less of tissue resected
T1b	Tumor incidental histological finding in more than 5% of tissue resected
T1c	Tumor identified by needle biopsy (e.g. because of elevated prostate-Specific antigen [PSA] level)
T2	Tumor confined within the prostate[1]
T2a	Tumor involves one half of one lobe or less
T2b	Tumor involves more than half of one lobe, but not both lobes
T2c	Tumor involves both lobes
T3	Tumor extends through the prostatic capsule[2]
T3a	Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement
T3b	Tumor invades seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall
N - Regional lymph nodes[3]	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M - Distant metastasis[4]	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
M1a	Non-regional lymph node(s)
M1b	Bone(s)
M1c	Other site(s)

1-Tumour found in one or both lobes by needle biopsy, but not palpable or visible by imaging, is classified as T1c. **2-** Invasion into the prostatic apex, or into (but not beyond) the prostate capsule, is not classified as pT3, but as pT2. **3-** Metastasis no larger than 0.2 cm can be designated pN1 mi. **4-** When more than one site of metastasis is present, the most advanced category should be used.

1.2.3.2 Metastasis stage

Bone is the most common site of tumor metastasis in cancer [71]. The incidence of bone metastasis is particular common in breast and prostate cancer patients, and arises when the primary tumor metastasis to the bone causing a lesion where high bone remodeling occurs consequently destroying the bone structure. The most devastating consequence is that once cancer has metastasized to bone it is incurable [72].

In advanced cancer, more than half the patients with bone metastasis develop some form of skeletal complication such as severe pain, fracture, spinal cord compression, marrow failure, or hypercalcemia. Moreover, more than 70 % of all patients are diagnosed between 60 and 80 years of age, making adaptation in bone structure an important element in the pathophysiology of bony complications [73]. Bone metastasis occurs in more than 50% of patients with advanced cancer disease [72, 74].

Patients who have only nodal metastases or pelvic and axial bone metastasis have been classified as having minimal disease, compared to those with visceral metastasis or appendicular bone metastasis [75].

1.2.3.3 The relation between bone and prostate cancer

Bone formation and resorption play a key role in maintaining bone mass volume and bone quality. Bone mineral content or density is increased by bone formation process regulated by osteoblasts, and decreased by bone resorption process regulated by osteoclasts. These two different cell activities are coupled and balanced by cross-talk between these two cellular processes in normal conditions [76].

Sixty five to 75 percent of patients with advanced prostate cancer can eventually develop bone metastasis throughout the course of their disease, in this process, cancer cells travel from the primary tumor to bone tissue, where they settle and grow. The growing cancer cells weaken and destroy the bone around the tumor and can result in a number of serious

complications called skeletal-related events (SREs), which are associated with increased pain, illness and death [77]. Bone metastasis from prostate cancer occurs when prostate cancer cells leave the prostate and enter the bloodstream (Figure 1.4). Then they lodge in the bone where they start to grow and form a secondary cancer within the bone. This may occur at just one site or it may occur at many sites ،so one or many bones might be involved. This process of metastasis usually takes a few years to develop [78]. Bone metastasis from prostate cancer and the increased risk of skeletal-related events remains an important clinical problem [77].

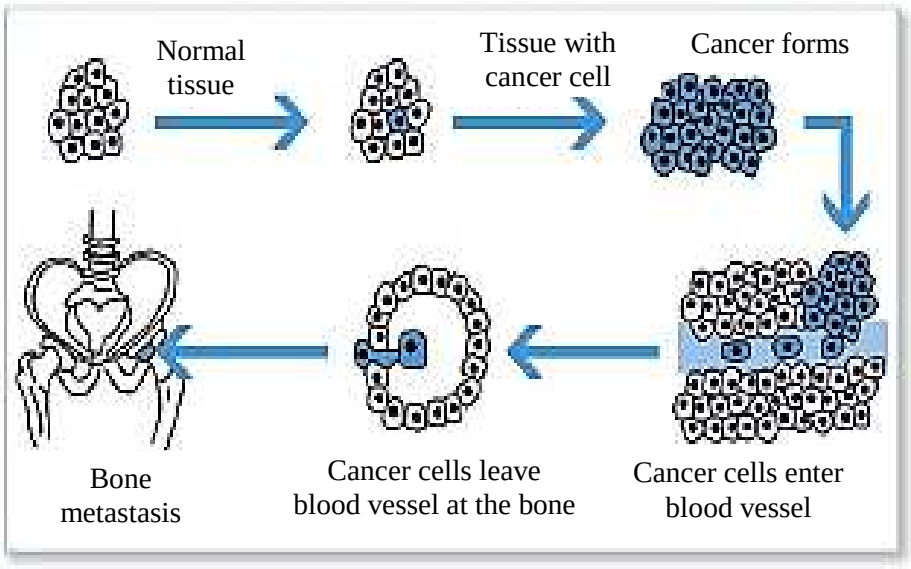


Figure 1.4 Cancer cells are carried from the prostate to the bone via blood vessels [78].

The use of bone turnover markers varies greatly across the UK, in terms of both the test used and the frequency of its measurement. Several factors will need to be considered when choosing the bone turnover marker to be used, not least the availability of the assay methods. Bone turnover markers have a number of potential uses, including: predicting bone loss, identifying people at risk of primary or secondary osteoporosis and fracture, predicting treatment response prior to commencement,

monitoring the response to osteoporosis treatment; identifying non-responders, which will include those not adhering with osteoporosis treatment (including patients not taking the medication or not following the instructions for administration), identifying over suppression of bone turnover in patient on long-term osteoporosis therapy, and monitoring of people who have been on long-term treatment, or shown signs of over suppression, and are taking a 'treatment holiday'[79, 80].

1.2.4 Parameters under study

A limited amount of information is available on markers assessed in prostate cancer patients stratified by lymph node metastasis. Part of the authors group has previously investigated PSA compared to a number of bone markers in which they found the same pattern for PSA [81]. However, they reported that total alkaline phosphatase (T-ALP) was significantly elevated in patients with localized disease and in lymph node positive patients, which was not observed in the present cohort. The reason for this discrepancy is unclear. To further elucidate the role of T-ALP, a study with a higher number of patients should be performed. Another group additionally investigated the ability of a formation marker to diagnose and predict metastatic spread in prostate cancer patients stratified by lymph node and bone metastasis compared to nine other serum markers. They showed that bone formation markers, a bone resorption marker and two osteoclastogenesis markers were elevated in patients with bone metastasis of which the osteoclastogenesis marker had the best discriminating power [82].

1.2.4.1 Prostate specific antigen (PSA)

PSA was first described in 1971 and purified in 1979 in seminal plasma and the prostate. It is a single chain glycoprotein with a molecular weight of about 34 KDa [83]. PSA consists of 237 amino acids and 4 carbohydrates side chains [84], high levels of PSA are found in the seminal fluid, a very little PSA is found in the circulation of healthy men in addition to prostate, PSA is also produced primarily by the epithelial cells of the prostate gland [85].

Prostate specific antigen (PSA) is the optimal tumor marker for prostate cancer, 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 [86].

The measurement of PSA level has revolutionized the diagnosis of prostate cancer [87, 88]. PSA is a kallikrein-like serine protease produced almost exclusively by the epithelial cells of the prostate. 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) [89].There are many different commercial test kits for measuring PSA, but no commonly agreed international standard exists [90]. PSA also has numerous potential clinical applications in breast disease as a predictive indicator for prognosis، diagnosis, and response to treatment [91].

1.2.4.1.1 Limitation of PSA

Detection does not always mean saving lives. Even though the PSA test can detect small tumors, finding a small tumor does not necessarily reduce a man chance 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 [92].

False positive test results (also called false positives) occur when the PSA level is elevated but no cancer is actually present. False positives may lead to additional medical procedures that have potential risks and significant financial costs and can create anxiety for the patient and his family. Most men with an elevated PSA test turn out not to have cancer; only some percent of men who have a biopsy due to elevated PSA levels actually have prostate cancer [93].

False negative test results occur when the PSA level is in the normal range even though prostate cancer is actually present. Most prostate cancers are slow-growing and may exist for decades before they are large enough to cause symptoms. Subsequent PSA tests may indicate a problem before the disease progresses significantly [94].

1.2.4.2 Deoxypyridinoline (DPD)

Deoxypyridinoline is a hydroxypyridinium cross-link (Figure 1.5), which is formed during the extracellular maturation of fibrillar collagen and is released during mature collagen degradation. Measured values of DPD are not affected by the degradation of collagen after being newly synthesized, and are not influenced by meals, and thus the deoxypyridinoline is a highly specific for bone tissue. In urine, DPD is present as a free form (about 40 %) and a peptide-bond form (about 60 %) [95]. Free forms can be detected by direct immunoassays, DPD is found in bone and dentin only [96].

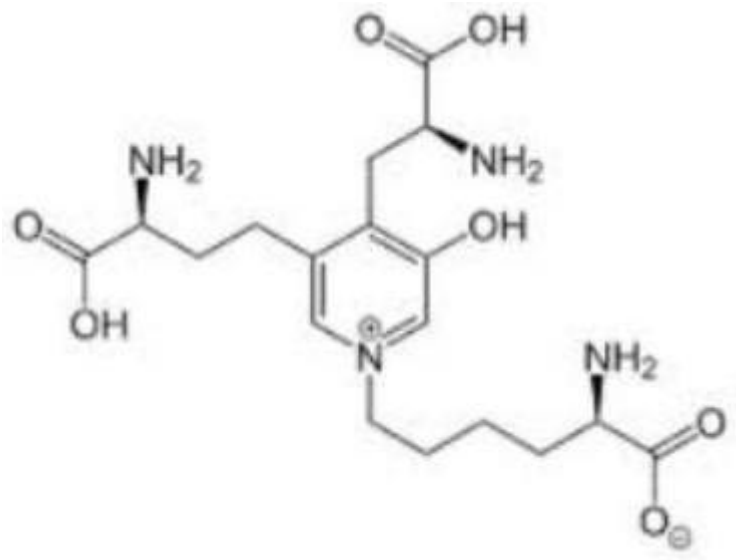


Figure 1.5 Deoxypyridinoline structure`s [97].

Collagen is a triple helical structure that contributes to the strength and integrity of the bony matrix. Degradation products of collagen are excreted in the urine and are not reused in collagen synthesis. Recent evidence suggests that urinary excretion of these cross- links is not affected by dietary habits. Therefore, analysis of collagen metabolites in urine has been used to monitor bone collagen metabolism in physiological and pathological conditions. There was studied which found that patients with bone metastasis had significantly higher mean concentrations of urinary DPD than those of cancer patients without clinical evidence of bone involvement. These results were demonstrated that urinary DPD may be a clinical marker of bone metastasis in prostate cancer. DPD is an analogue of pyridinoline and has a greater specificity for bone than pyridinoline [98].

Excretion of deoxypyridinoline is expressed as ratio to creatinine excretion (Deoxypyridinoline/Creatinine), creatinine is a correction factor. Urine deoxypyridinoline is detected by high-performance liquid chromatography or competitive enzyme-linked immunosorbent assay (ELISA). Increases of between two and three times the upper limits of normal have been reported in people with osteoporosis, primary

hyperparathyroidism, Osteomalacia, thyrotoxicosis and several inflammatory conditions, though the biggest increases (four or more times upper limit of normal) are seen in immobilization, Paget's disease of bone and metastatic cancer. A decrease in the pretreatment value of > 30% has been considered indicative of a good response in osteoporosis [99].

Bone formation markers are substances directly or indirectly produced by osteoblasts at each stage of osteoblast differentiation. They reflect various aspects of osteoblast function and bone formation, and most are measured in the blood. One of these markers is alkaline phosphatase (ALP) [76].

1.2.4.3 Alkaline phosphatase (ALP)

Alkaline Phosphatases are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). The measured in the blood was the total amount of alkaline phosphatases released from these tissues into the blood. As the name implies, this enzyme works best at an alkaline pH (a pH of 10), and thus the enzyme itself is inactive in the blood [100]. Alkaline phosphatases act by splitting off phosphorus (an acidic mineral) creating an alkaline pH. In addition, an elevated serum alkaline phosphatase can be due to rapid growth of bone since it is produced by bone-forming cells called osteoblasts. One would expect that growing children have higher levels than full-grown adults. The relationship of alkalinity to bone development warrants further discussion because it plays a major role in the prevention and reversal of osteoporosis, just as calcium builds up around faucets, so is calcium laid down into bone. The reason of calcium deposits on faucet is because the water is alkaline and calcium comes out of solution and crystallizes in an alkaline environment [100].

The optimal range for alkaline phosphatase depends on age. A growing adolescent will have a much higher alkaline phosphatase than a full grown adult because his/her osteoblasts are laying down bone very rapidly. For an adult, 50-75 mg/dl is considered a reasonable optimal range. An increased serum alkaline phosphatase may be due to; healing fractures, rapid bone growth such as after a fracture, bone cancers like osteogenic sarcoma, Osteomalacia, and Paget's disease [100]. Approximately 40–50 % of the total alkaline phosphatase (T-ALP) activity was arising from the bone as a result of osteoblast activity [101].

Total-ALP has been the most often used marker for detecting increased bone formation in metastatic prostate cancer, being highly elevated as they mainly develop sclerotic bone lesions [102].

Levels of the bone marker total alkaline phosphatase (T-ALP) were high in patients with metastatic disease, indicative of the presence of skeletal metastasis, in prostate cancer, increased levels of T-ALP and bone-specific alkaline phosphatase (B-ALP) appear to be significant predictors of early mortality and have been associated with progression of skeletal metastases [103].

1.2.4.4 Calcium and phosphate

Bone turnover is the process of resorption followed by replacement by new bone with little change in shape, and it occurs throughout a person’s life. Osteoclasts break down bone (bone resorption), releasing the minerals, resulting in a transfer of calcium from bone fluid to the blood. The osteoclast attaches to the osteon (layers of compact bone tissue surrounding a central canal), and secretes collagenase and other enzymes [104]. Calcium (comprises over 40% of bone mass), magnesium, phosphate and products of collagen are released into the extracellular fluid as the osteoclasts tunnel into the mineralized bone. Osteoblasts are mature bone cells responsible for bone formation and ossification. They produce the

organic portion of the matrix of bone tissue, osteoid, which is composed mainly of type I collagen, and are responsible for mineralization of the osteoid matrix. Ossification fixes circulating calcium in its mineral form, removing it from the bloodstream. Repeated stress (such as weight-bearing exercise or bone healing) results in the bone thickening at the points of high stress [104].

Phosphate is an essential mineral that is required by every cell in the body for normal function. Approximately 85% of the body`s phosphate is found in bones and teeth, and it`s a major structural component of bone in the form of a calcium-phosphate salt that called hydroxyapatite. The most serious adverse effect of abnormally elevated blood levels of phosphate (hyperphosphatemia) is calcification of non-skeletal tissue. Calcium-phosphate deposition can lead to organ damage, especially kidney damage, because the kidneys are very efficient at eliminating excess phosphate from the circulation [105].

Remodelling in adults repairs micro-damage to bone and plays a role in the regulation of calcium homeostasis. An imbalance in the bone remodelling processes in adults is thought to impact on bone strength as a result of reductions in bone volume and mineralization, loss of trabecular, deterioration of trabecular connectivity, and the formation of resorption cavities and trabecular perforations [106, 107]. Therefore, an increase in bone turnover where resorption exceeds formation is not only inversely correlated with bone mineral density (BMD), but may also alter bone architecture and porosity, increasing the risk of fracture beyond that due to reduced BMD, and can therefore be an independent predictor of fracture risk [106, 108].

1.3 The aims of study

1- Investigating of the role of deoxypyridinoline (DPD) as a biochemical marker of bone metastasis in prostate cancer patients.

2- Explaining the relationship between DPD and other associated parameters (total-alkaline phosphatase, serum phosphate, and serum calcium) in patients with prostate cancer.

3- Clarifying the stage of prostate cancer, whether the cancer is reached the bone or not.

4- Explaining if the chemotherapy drug is useful for treatment of prostate cancer by measuring PSA, DPD, T-ALP, Ca^{2+}, and PO_4^{3-}.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and kits

The chemicals and kits are summarized in table 2.1

Table 2.1 Chemicals and kits

No.	Material	Company
1.	Prostate specific antigen, (ST AIA-PACK PSAⅡ), TOSOH assay	3-8-2 Shiba, Minato-Ku, Tokyo 105-8623, Japan.
2.	Human Deoxypyridinoline (DPD) ELISA kit.	CSB-E08399h CUSABIO, China.
3.	Creatinine manual kit.	Randox laboratories limited BT29 4QY, United Kingdom.
4.	Calcium manual kit.	Human, Max-Planck-Ring 21.65205 Wiesbaden, Germany.
5.	Phosphate manual kit.	Spinreact 7 E-17176 Sant Estevede bas (GI), Spain.
6.	Alkaline phosphatase strips.	Roche diagnostics GmbH, Sandhofer, Strasse 116 D-68305, Mannheim, Germany.

2.1.2 Apparatus and Equipment

The apparatus and equipment are summarized in table 2.2

Table 2.2 Apparatus and Equipment

No.	Instrument	Company
1.	Enzyme-linked immunosorbent Assays (ELISA)	Bio Tek Instruments 217337, U.S.A.
2.	TOSOH AIA-360 System Analyzer	AIA-360, 13180408, Japan.
3.	Reflotron Plus	Roche diagnostics GmbH 5072 821 Mannheim, Germany.
4.	UV-VIS Spectrophotometer	APLE PD-303 UV, Japan.
5.	Centrifuge	Hettich EBA 20, Germany.
6.	Water bath	Techne junior TE-8J, England.
7.	Deep freeze	ARCTICO 4612033, European Union, Denmark.

2.1.3 Patients and controls

This study included fifty patients with prostate tumor; those patients were enrolled from AL-Hussein Teaching Hospital of Kerbala in the period from October 2013 to April 2014, whose age ranges between (50-80) years. Blood samples of those patients were obtained from the following sources:

Forty samples were taken from oncology unit, diagnosed as prostate cancer by the histopathological examination, and ten samples were taken from urology unit, diagnosed as prostate hyperplasia by elevated serum PSA levels.

The Control group was consisted of 30 healthy subjects who were free from signs and symptoms of cancer, and whose ages were identical with the age of patients.

A questionnaire was designed to obtain the information of prostate cancer patients and control subjects. It contained the name, age, weight, height, smoking, type of treatment, and family history of cancer, especially the prostate cancer.

2.1.4 Collection of samples

Five milliliters of venous blood were drawn from the patients and the control group in the early morning after an overnight fasting to measure PSA, total-alkaline phosphatase (T-ALP), calcium, and phosphate concentrations. In addition, 5 ml of urine specimens were drawn to measure creatinine and DPD concentrations.

Disposable cups were used in the collection of urine specimens, while disposable syringes and needles were used for the collection of blood specimens. The blood and urine samples were centrifuged at 3000 xg for 15 minute. A serum of blood and the urine samples were taken from its tubes and put to freeze at -70 °C until the analysis.

2.2 Methods

2.2.1 Determination of prostate specific antigen (PSA)

PSA was determined by immuno enzymometric assay system, by using TOSOH ST AIA-PACK PSA II (Shiba, Japan).

- **Principle of assay**

This assay is a two-site immuno enzymometric assay which was performed entirely in the assay test cups. PSA present in the test sample was bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the test cups. The

magnetic beads were washed to remove unbound enzyme-labeled monoclonal antibody and then were incubated with a fluorogenic substrate.4-methylumbelliferyl phosphate. The amount of enzyme-labeled monoclonal antibody that binds to the beads was directly proportional to the PSA concentration in the test sample [109].

- **Reagents and materials provided**

Materials provided and reagents were: 5 trays x 20 test cups. Plastic test cups containing lyophilized twelve magnetic beads coated with anti-PSA mouse monoclonal antibody and 100 µl of anti-PSA mouse monoclonal antibody conjugated to bovine alkaline phosphatase with sodium azide as a preservative, ST AIA (Analytical immune assay)-PACK PSAⅡ, calibrator set, sample diluting solution, substrate set Ⅱ (reagent Ⅱ, reconstituent Ⅱ), wash concentrate, diluent concentrate, detector standardization test cup, sample treatment cup.

- **Preparation of reagents**

All reagents were brought to room temperature (18-25 ºC) before preparation of reagents for 30 minute.

1. The entire contents of the substrate reconstituent Ⅱ (100 ml) were added to the lyophilized substrate reagent Ⅱ and were mixed until to dissolve the solid material.
2. The entire contents of wash concentrate (100 ml) were added to approximately 2.0 L of clinical laboratory reagent water, mixed well, and the final volume was adjusted to 2.5 L.
3. The diluent concentrate (100 ml) was added to approximately 4.0 L of clinical laboratory reagent water, mixed well, and the final volume was adjusted to 5.0 L.

- **Procedure**

The calibration curve for the ST analytical immune assay (AIA) - PACK PSA Ⅱ was stable for up 90 days. Calibration stability was monitored by quality control performance and was depended on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer`s instruction. The ST AIA-PACK PSA Ⅱ calibrator set was provided and ready for used. The samples were determined as the following steps:

1. All samples were put in place of samples on the instrument appropriately in the TOSOH AIA system analyzer.
2. The results were read after about 20 minutes by a microplate reader.
3. Some of specimen prostate specific antigen concentrations were found greater than the upper limit of the assay range (100 ng /ml). Therefore, it were diluted with the ST AIA-PACK PSA Ⅱ sample diluting solution and reassayed according to the assay procedure (dilution factor was entered into the software of TOSOH system).

- **Calculations**

The TOSOH analytical immune assay (AIA) system analyzer performed all samples and reagent handling operations automatically. The TOSOH AIA system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to prostate specific antigen concentration in ng/ml.

2.2.2 Determination of urine deoxypyridinoline (DPD)

Urine deoxypyridinoline (DPD) was determined by enzyme-linked immunosorbent assay (ELISA) system, by using human DPD ELISA kit (CUSABIO, China).

- **Principle of assay**

This assay employed the quantitative sandwich enzyme immunoassay technique. Antibody specific for DPD had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any DPD present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for DPD was added to the wells. After washing, avidin conjugated Horseradish peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of DPD bound in the initial step. The color development was stopped and the intensity of the color was measured [110].

- **Reagents and materials provided**

The materials provided and reagents were: Assay plate (12 x 8 coated microwells), standard (freeze dried) (2), biotin-antibody (100 x concentrate), HRP-avidin (100 x concentrate), biotin-antibody diluent, HRP-avidin diluent, sample diluent, wash buffer (25 x concentrate), TMB substrate, stop solution, adhesive strip (for 96 wells) (4), and instruction manual.

- **Preparation of reagents**

All reagents were brought to room temperature (18-25 °C) for 30 minute before used.

1. Biotin-antibody was taken and centrifuged the vial before opening. (Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution was 10 µl of biotin-antibody + 990 µl of biotin-antibody diluent).
2. HRP-avidin was taken and centrifuged the vial before opening. (HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution was 10 µl of HRP-avidin + 990 µl of HRP-avidin diluent).
3. Wash buffer was taken and used when the crystals had formed in the concentrate, warmed up to room temperature and mixed gently until the crystals had completely dissolved. (diluted 20 ml of wash buffer concentrate (25 x) into deionized or distilled water (D.W) to preparation 500 ml of wash buffer (1 x).
4. Standard was taken and centrifuged at 4000 rpm for 3min. (reconstituted the standard with 1 ml of sample diluent. This reconstitution produced a stock solution of 100 ng /ml. The standard was mixed to ensure complete reconstitution and allowed the standard to sit for a minimum of 15min with gentle agitation prior to making dilutions.

- **Procedure**

1. All reagents were prepared, worked standards, and samples as directed in the previous sections.
2. The assay layout sheet was referred to in order to determine the number of wells to be used and were put any remaining wells and the desiccant back into the pouch and sealed the Ziploc, unused wells were stored at 40 °C.
3. 100 µl of standard and sample was added per well, and covered with the adhesive strip provided, then incubated for 2 hours at 37 °C. A plate layout was provided to record standards and samples assayed.

4. The liquid of each well was removed (without wash).
5. 100 µl of biotin-antibody was added to each well, then covered with a new adhesive strip, then incubated for 1 hour at 37 °C (biotin-antibody may be appear cloudy, warmed up to room temperature and mixed gently until solution appeared uniform.
6. Each well was aspirated and washed. The process was repeated two times for a total of three washes. Each well was washed by filling with wash buffer (200 µl) using autowasher, and leaving to stand for 2 minutes. The completed removal of liquid at each step was essential to good performance. After the last wash, any remaining wash buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.
7. 100 µl of HRP-avidin was added to each well, which was covered the microtiter plate with a new adhesive strip, and incubated for 1 hour at 37 °C.
8. The aspiration / wash process were repeated for five times as in step 6.
9. 90 µl of TMB substrate was added to each well, then incubated for 15-30 minat 37 °C, and protected from light.
10. 50 µl of stop solution was added to each well, and gently tap the plate to ensure through mixing.
11. The optical density of each well was determined within 5 minutes by a microplate reader and was set to 450-630 nm.

- **Calculations**

The concentration of DPD was calculated automatically by taken the average for duplicate reading to each standard and sample and subtracted the average zero standard optical density.

A standard curve was created by reducing the data by using computer software capable of generation a four parameter logistic (4-PL) curve-fit automatically. As an alternative, a standard curve was constructed by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and a best fit curve was draw through the point on the graph.

2.2.3 Determination of urine creatinine concentration

Creatinine in urine was determined by spectrophotometric method, by using randox kit (United Kingdom).

- **Principle of assay**

Creatinine in alkaline solution reacts with picric acid to form a colored complex (Figure 2.1). The amount of the complex formed was directly proportional to the creatinine concentration [111] such as following equation.

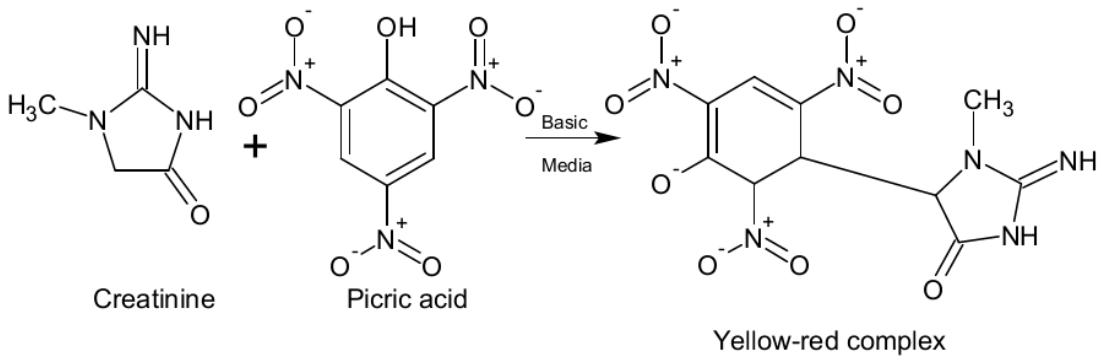


Figure 2.1 Equation of urine creatinine determination [112].

- **Solutions**

Standard, Picric acid is called Reagent 1a (R1a), and sodium hydroxide is called Reagent 1b (R1b) as reagent provided.

- **Preparation of working reagent**

Equal volumes of solution R1a + R1b were mixed (stable for 3 days at 15-25 °C).

- **Procedure**

1. The reagents, samples, and photometer were brought to 37 °C.
2. 1000 µl of working reagent was added in a test tube.
3. 100 µl of urine sample was added.
4. Standard solution was prepared by mixed 1000 µl of working reagent with 100 µl of standard in test tube.
5. The mixture was mixed and incubated for 30 minutes at room temperature.
6. The absorbance was recorded at 490-510 nm after 30 seconds (A1) and after 90 seconds (A2).

- **Calculations**

$$\frac{\Delta A\ sample}{\Delta A\ standard}\ x\ standard\ (conc.\left(\frac{\mu\ mol}{l}\right)x\ 0.05 = mmol/l$$

$\Delta A = A2 - A1$

A1 = absorbance at 30 sec.

A2 = absorbance at 90 sec.

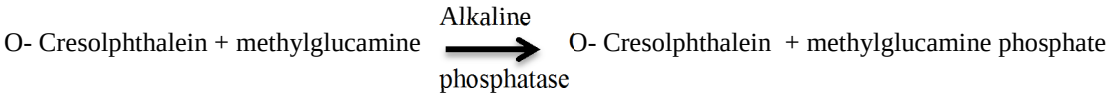
0.05 = Sample dilution factor.

2.2.4 Determination of serum alkaline phosphatase (ALP)

Serum alkaline phosphatase was determined by spectrophotometric method, by using alkaline phosphatase strips (Mannheim, Germany).

- **Principle of assay**

After the application to the test strip, the sample flows into the reaction zone, in the case of blood after separation of the erythrocytes from the plasma. ALP hydrolyzes O-cresolphthalein phosphate to O-cresolphthalein and transfers the phosphate group to the acceptor molecule methylglucamine. The colored hydrolysis product O-cresolphthalein produced per unit of time under alkaline conditions is directly proportional to alkaline phosphatase activity.



Dye formation is determined kinetically at 37 ºC as a measure of the enzyme activity of ALP. The result is displayed after approximately 135 seconds in U/L or µkat/L [113].

- **Procedure**

Required additional materials (not supplied): Reflotron pipette and pipette tips or micro pipette, controls, usual laboratory equipment for collecting blood.

1. The instrument (Reflotron plus) was switched on.
2. A test strip was removed from the container when the display shows (Ready).
3. 50 µl of serum sample was put on a test strip and inside in reflotron.

- **Calculations**

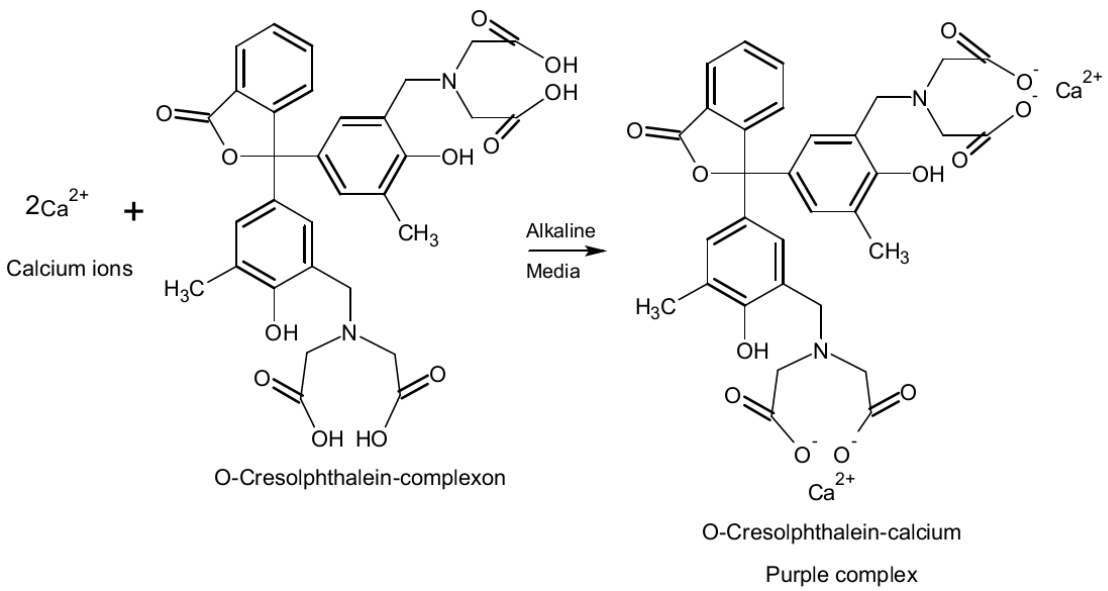
The result was recorded after one minute in auto screen of reflotron system to each strip.

2.2.5 Determination of serum calcium

Calcium was determined by spectrophotometric method, by using human kit (Germany).

- **Principle of assay**

Calcium ions react with O-cresolphthalein-complexone in an alkaline medium to form a purple colored complex. The absorbance of this complex is proportional to the calcium concentration in the sample [114] as in the following.



- **Reagents**

1. Buffer solution (BUF).
2. Color reagent (RGT) consisted of: (8-Hydroxyquinoline, O-cresolphthalein-complexone, and hydrochloric acid).
3. Standard (STD) consisted of: (Calcium (ll) 8 mg/dl, and sodium azide).

- **Preparation of working reagent**

Equal volumes of RGT and BUF were added into test tube then, mixed and allowed to stand for 30 minutes at room temperature before used.

- **Procedure**

1. All reagents, samples, and photometer were brought to 37 °C.
2. 1000 µl of working reagent was added in a test tube.
3. 20 µl of serum sample was added to this test tube.
4. Standard solution was prepared by mixed 1000 µl of working reagent with 20 µl of standard in test tube.
5. The mixture was mixed and inserted into the photometer (Start stopwatch).
6. The absorbance was measured at 546-570 nm of sample (ΔA_{sample}) and standard ($\Delta A_{\text{standard}}$) against the reagent blank (1000 µl of working reagent) within 5 to 30 minutes.

- **Calculations**

$$\frac{\Delta A\ sample}{\Delta A\ standard}\ x\ 8\ x\ standard\ conc. = mg/dl$$

$\Delta A = A2 - A1$

A1 = absorbance at 5 min.

A2 = absorbance at 30 min.

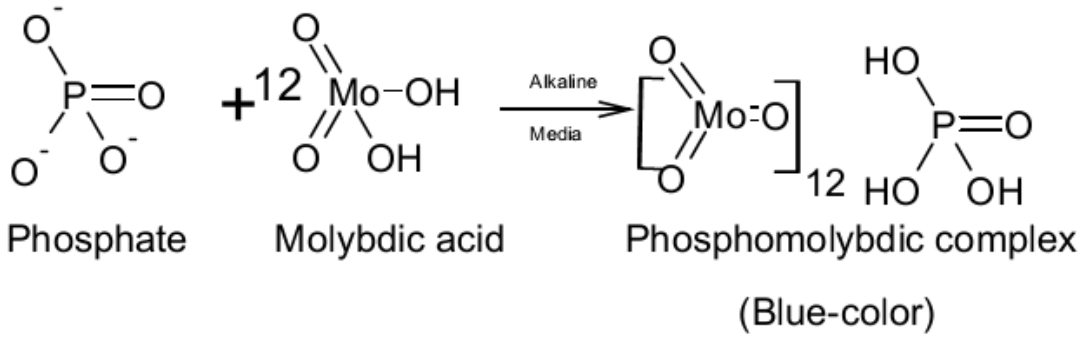
8 = calcium aqueous primary standard 8 mg/dl.

2.2.6 Determination of serum phosphate

Phosphate was determined by spectrophotometric method, by using spinreact kit (Spain).

- **Principle of assay**

Inorganic phosphate reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum color. The intensity of the color formed is proportional to the inorganic phosphate concentration in the sample [115] as the following equation.



- **Reagents**

1. Molbdic (R1) consisted of (Molbdate-borate, and sulphoric acid).
2. Catalyzer (R2) consisted of (1, 2- Phenylenediamine).
3. Phosphate cal. consisted of (phosphate aqueous primary standard 5 mg/ dl).

- **Preparation of working reagent**

Equal volumes of R1 (molybdic) and R2 (catalyzer) were mixed (stability: 10 hours at 2-8 ºC. protected from light).

- **Procedure**

1. The reagents, samples, and photometer were brought to 37 ºC.
2. 1500 µl of working reagent was added in a test tube.
3. 50 µl of serum sample was added.

4. Standard solution was prepared by mixed 1500 µl of working reagent with 50 µl of standard in test tube.
5. The mixture was mixed and incubated for 30 minutes at room temperature.
6. The absorbance (ΔA sample) and (ΔA standard) were measured at 620-750 nm of sample against the blank (1500 µl of working reagent) within 5 to 30 minutes.

- **Calculations**

$$\frac{\Delta A\ sample}{\Delta A\ standard}\ x\ 5\ x\ standard\ conc. = mg/dl$$

$\Delta A = A2 - A1$

A1 = absorbance at 5 min.

A2 = absorbance at 30 min.

5 = phosphate aqueous primary standard 5 mg/dl.

2.2.7 **Biostatistical analysis**

Statistical Package for Social Sciences (SPSS) version 19 was used for statistical analysis. Student t-test and the linear regression analysis were used to analyze the results. All of the data were expressed as mean ± standard error (Sd.E). P-value ≤ 0.05 was considered significant [116].

3. Results and Discussion

3.1 Assessment of prostate specific antigen (PSA), urine deoxypyridinoline/creatinine (DPD/Creat.), total-alkaline phosphatase (T-ALP), and bone minerals (calcium and phosphate)

The present study included 50 patients with prostate tumor and thirty healthy subjects as a control group. Serum PSA, T-ALP, Ca^{2+}, PO_4^{3-} and urine DPD/Creat. were measured.

The results revealed a highly significant ($P<0.000$) increase in the concentrations of serum PSA, calcium, phosphate, and urine DPD compared with the control group. In addition, there was a highly significant ($P<0.01$) increase in the activity of serum total-alkaline phosphatase (T-ALP), compared with healthy group (Table 3.1).

Table 3.1 The levels of parameters under study in patients with prostate tumor and control group.

Parameters	Patients n=50 Mean±Sd.E	Control n=30 Mean±Sd.E	P-value
PSA *(ng/ml)*	**83.67±15.67**	**2.10±0.141**	**0.000**
DPD *(nmole/mmole Cr.)*	**269.03±69.08**	**5.06±0.27**	**0.000**
T-ALP *(IU/L)*	**301.34±62.60**	**85.82±3.62**	**0.008**
Ca^{2+} *(mg/dl)*	**9.41±0.09**	**8.46±0.05**	**0.000**
PO_4^{3-} *(mg/dl)*	**4.95±0.176**	**3.71±0.09**	**0.000**

In this study, fifty patients with prostate tumor were clinically classified into three groups; benign prostate hyperplasia (B.P.H), localized prostate cancer (L.P.C), and metastasis prostate cancer (M.P.C).These groups were diagnosed by histopathologist using Gleason score graduated for each adenocarcinoma specimen (Figure 3.1). The results showed that urinary excretion of deoxypyridinoline (DPD) was a highly significant ($P<0.000$) increase in the patients with bone metastasis than in those with B.P.H and those with localized prostate cancer (L.P.C), whereas DPD levels did not show any significant variation between B.P.H and L.P.C groups (Table 3.2).

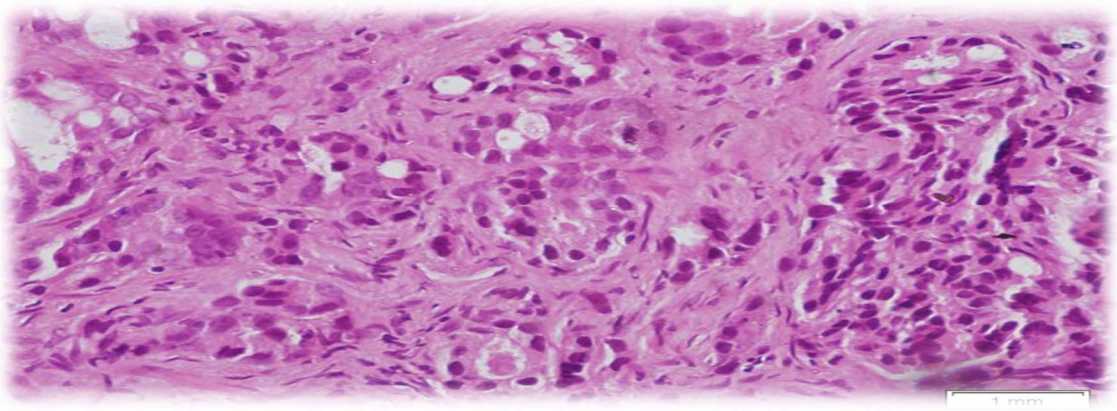


Figure 3.1 Gleason score of prostate cancer.

Table 3.2 Deoxypyridinoline (DPD) value in three groups of prostate tumor patients.

Group	No.	Mean±Sd.E
M.P.C	**25**	**510.48±112.22** *
L.P.C	**15**	**5.48±0.35**
B.P.H	**10**	**5.92±0.45**

* P<0.000: M.P.C versus L.P.C and B.P.H; B.P.H, benign Prostate hyperplasia; L.P.C, localized prostate cancer; M.P.C, metastasis prostate cancer.

In addition, the results showed that Deoxypyridinoline (DPD) values in different groups (M.P.C, L.P.C, and B.P.H) of patients were very greater in metastasis prostate cancer (M.P.C) group than other patient groups (L.P.C, B.P.H) and control group (Figure 3.1).

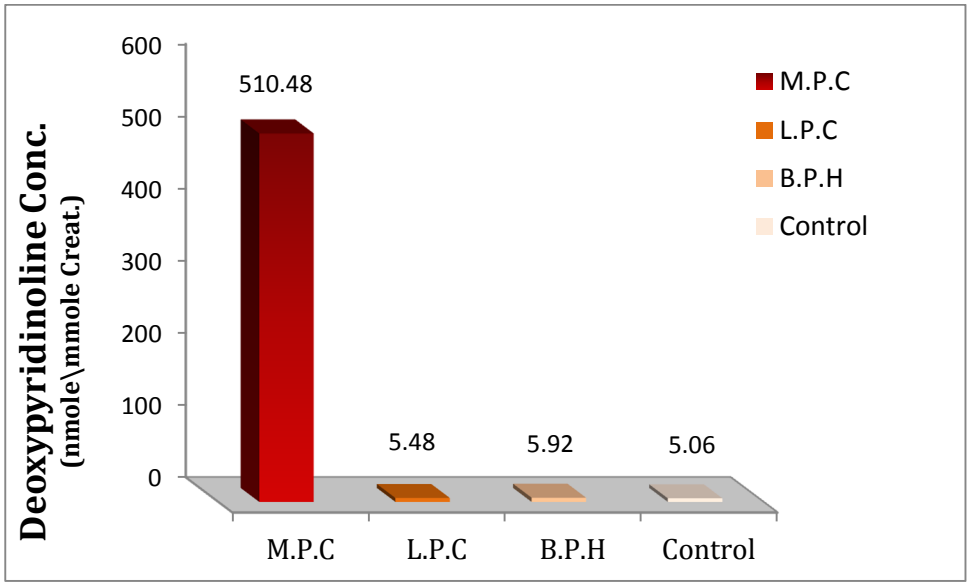


Figure 3.2 Deoxypyridinoline (DPD) level in patients with prostate tumor (B.P.H, L.P.C, M.P.C) and control group.

In this study, the results of other parameters under study which included PSA, T-ALP, Ca^{2+}, and PO_4^{3-}, showed a highly significant ($P<0.01$) increase in the concentration of PSA, in M.P.C group, compared with B.P.H and L.P.C groups. In addition, there was a highly significant ($P<0.000$) increase in the concentrations of Ca^{2+} and PO_4^{3-} in M.P.C group, compared with B.P.H and L.P.C groups (Table 3.3).

Table 3.3 The level of prostate specific antigen (PSA), total alkaline phosphatase (T-ALP), calcium, and phosphate in three groups of prostate tumor patients.

Parameters	B.P.H n=10 Mean±Sd.E	L.P.C n=15 Mean±Sd.E	M.P.C n=25 Mean±Sd.E
PSA *(ng/ml)*	18.37±3.35	62.47±23.04	115.97±24.64 **
T-ALP *(IU/L)*	201.7±18.63	204.99±47.81	390.74±114.37
Ca^{2+} *(mg/dl)*	8.80±0.113	9.09±0.12	9.80±0.11 *
PO_4^{3-} *(mg/dl)*	3.78±0.28	4.06±0.10	5.85±0.18 *

* P<0.000: M.P.C versus L.P.C and B.P.H; ** P<0.01: M.P.C versus B.P.H; B.P.H, benign prostate hyperplasia; L.P.C, localized prostate cancer; M.P.C, Metastasis prostate cancer.

The diagnostic efficiency of any tumor marker is judged by its specificity and sensitivity [117]. Serum PSA played a dominant role, because of its highest sensitivity for prostate carcinoma compared with other modalities [118]. 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 staging of prostate carcinoma disease [117].

The study of Eklund, *et al*. (2009) documented that serum PSA level greater than 100 ng /ml was found to be the single most important indicator of metastatic disease, with a positive predictive value of 100% [119]. Furthermore, Lee, *et al.* (2000) revealed the PSA was helped to reduce the number of patients with newly diagnosed prostate cancer who require a bone scan. Patients with a low serum PSA concentration have only rarely

been found to harbour detectable skeletal metastasis. The correlation between serum PSA and bone scintigraphy in patients with newly diagnosed untreated prostate cancer has been further investigated [120]. The study of Bruwer, *et al.* (1999) suggested that a staging bone scan may be superfluous if the serum PSA concentration is less than 20 ng /mL in a symptomatic patient with well or moderately differentiated tumors. In contrast, in patients with poorly differentiated tumors and locally advanced disease, a staging bone scan should be obtained irrespective of the serum PSA value [121].

During bone turnover, active bone resorption and formation results in the release of bone-associated proteins, protein fragments, or mineral components into the blood and urine, which represents a rich source of potential biomarkers. In metastatic bone disease, the disruption of normal bone turnover leads to abnormally high levels of these biomarkers. Bone biomarkers are usually classified as either “resorption markers such as DPD” or “formation markers such as T-ALP” depending on which side of the process they arise [122].

In present study, the high level of deoxypyridinoline (DPD) was expressed as ratio to creatinine excretion. Increases of deoxypyridinoline level between two and three times the upper limits of normal have been reported in people with osteoporosis, primary hyperparathyroidism, Osteomalacia, thyrotoxicosis and several inflammatory conditions, though the biggest increases (four or more times upper limit of normal) are seen in immobilization, Paget’s disease of bone and metastatic cancer [99]. The high levels of deoxypyridinoline (DPD) excretion due to increasing of bone collagen degradation (type I collagen) because the bone resorption [123]. A decrease in the pretreatment value of > 30% has been considered indicative of a good response in osteoporosis [99].

Type I collagen of bone is strengthened by specific molecular cross-links that provide rigidity to the bone such as pyridinium, pyridinoline and DPD (normal excretion of DPD expected 2.3-7.4 *nmole DPD* / *mmole Creat*.), when the collagen broken down during bone resorption, these collagen cross-links cannot be degraded, therefore released into the blood and subsequently excreted in urine. Urine levels of DPD were elevated in metastatic prostate cancer patients compared to non-metastatic ones [124].

Bone is the most common sites of metastatic prostate cancer; therefor reliable methods to detect bone metastasis are required [125]. There are several recent reports with well-documented need for a biologic marker to allow the early detection of metastatic disease and to follow the efficacy of therapy in patients with hematogenous spread of prostate cancer [126]. The study of Savas, *et al*. (2000) revealed that there are many of bone resorption markers had been studied in prostate and breast cancer metastasis but the urinary DPD and the collagen cross-link associated N-telopeptide were considered to be the most specific ones [126].

Urinary deoxypyridinoline is clearly discriminated between patients with or without bone metastasis, and patients receiving successful endocrine treatment for metastatic prostate cancer had suppressed urinary DPD levels [127]. Joerger, *et al*. (2012) study`s documented the prostate cancer patients with newly diagnosed of bone metastasis had higher urinary DPD levels as compared to patients with localized prostate cancer [127].

The levels of bone resorption markers (such as DPD levels) mainly reflect the overall skeletal change of bone resorption, which can be altered by various factors besides abnormalities of the subchondral bone turnover [128]. In our study, the elevated level of urine DPD in patients with prostate metastasis is similar to the study of Berruti, *et al*. (2000) who found that patients with bone metastasis had a highly significant level of DPD in urine than in those patients with no bone metastasis or with B.P.H [129]. Other

study by Aksoy, *et al.* (2001) found that patients with bone metastasis had highly significant mean concentrations of urinary DPD than those of cancer patients without clinical evidence of bone involvement; the results demonstrate that urinary DPD may be a clinical marker of bone metastasis in prostate cancer. DPD is an analogue of pyridinoline and has a greater specificity for bone than does pyridinoline [98].

In present study, the T-ALP level`s was elevated in prostate tumor patients. Total-ALP has been the most often used marker for detecting increased bone formation in cases of metastatic prostate cancer, being highly elevated as they mainly develop sclerotic bone lesions [102]. In study of Xie, *et al*. (2007) found that ALP was a significant factor in predicting overall survival for men with bone metastatic prostate cancer [130]. Jeremy, *et al.* (2013) revealed an increased serum alkaline phosphatase may be due to; healing fractures, rapid bone growth such as after a fracture, bone cancers like osteogenic sarcoma, Osteomalacia, and Paget's disease. An elevated serum alkaline phosphatase can be due to rapid growth of bone since it is produced by bone-forming cells called osteoblasts. The relationship of alkalinity to bone development plays a major role in the prevention and reversal of osteoporosis [100]. In other study by Coleman, *et al.* (2014) observed that T-ALP and B-ALP can be used in diagnosing advanced prostate cancer. The prostate cancer related increase in serum ALP activity is considered to reflect accelerated bone turnover after bone metastatic prostate cancer [131]. Bone turnover is the process of resorption followed by replacement by new bone with little change in shape, and it occurs throughout a person’s life [131].

However, the results of T-ALP and DPD appeared to be the most powerful predictor of bone metastasis. This result refocuses attention on serum ALP as an important marker of metastatic disease. Urinary DPD may provide a useful marker to supplement ALP and PSA in evaluating

bone scan results and the response to hormonal therapy [132]. In the latter study, bone scans were taken the gold standard for the diagnosis of bone metastasis. Importantly, bone markers were more specific toward pathological bone processes as compared to PSA that is also dependent on extraosseous malignant disease, and is also subjected to hormonal manipulation [133].

The elevated levels of serum $Ca^{2+}$and serum PO_4^{3-} due to release of calcium and phosphate minerals in blood of advanced prostate cancer patients. Bone metastasis can also present as hypercalcemia, because the cancer breaks down the bone, releasing calcium into the blood stream [134]. Tandon, *et al*. (2005) reported that hypocalcemia can be a manifestation of prostate cancer metastatic to bone [135]. In addition, the study by Schwartz (2008) suggested that prostate cancer with bony metastasis might increase parathyroid hormone because calcium is transferred from serum into blastic bone [136].

The osteoclasts break down of bone (bone resorption), releasing the minerals, resulting in a transfer of calcium from bone fluid to the blood [104]. Calcium (comprises over 40% of bone mass), magnesium, phosphate and products of collagen are released into the extracellular fluid as the osteoclasts tunnel into the mineralized bone [104]. In recent study, Heaney, *et al.* (2012) is showed the phosphate is an essential mineral that is required by every cell in the body for normal function. Approximately 85% of the body`s phosphate is found in bones and teeth, and it`s a major structural component of bone in the form of a calcium-phosphate salt called hydroxyapatite [105].

Prostate cancer, breast cancer, and multiple myeloma have a particularly strong association with skeletal metastasis and related bone loss, resulting in fracture, hypercalcemia, pain, and declines in mobility and

performance status [132]. Lipton, *et al*. (2009) study`s has documented skeletal metastasis affect 65% to 75% of men with advanced disease increases bone turnover and decreases bone mineral density (BMD), leading to a 20% to 45% increase in relative fracture risk. By the time patients present with active (symptomatic) disease, 80% have associated with bony destruction [132].

3.2 The correlation between parameters under study

In this study, Pearson's correlation coefficient was used to mean the correlation between parameters in patients group. The results revealed a positive significant correlation between PSA and T-ALP ($P<0.000$, $r=0.591$), PSA and phosphate ($P<0.01$, $r=0.360$), DPD and calcium ($P<0.000$, $r=0.614$), DPD and phosphate ($P<0.000$, $r=0.654$), calcium and phosphate ($P<0.000$, $r=0.797$) (Table 3.4).

Table 3.4 The correlations between parameters under study in prostate tumor patients.

Parameter 1	Parameter 2	n	(r)	P-value
PSA	DPD	50	0.122	0.420
PSA	T-ALP	50	0.591**	0.000
PSA	Ca^{2+}	50	0.258	0.084
PSA	PO_4^{3-}	50	0.360*	0.014
DPD	T-ALP	50	- 0.037	0.809
DPD	Ca^{2+}	50	0.614**	0.000
DPD	PO_4^{3-}	50	0.654**	0.000
T-ALP	Ca^{2+}	50	0.116	0.442
T-ALP	PO_4^{3-}	50	0.161	0.285
Ca^{2+}	PO_4^{3-}	50	0.797**	0.000

** Correlation is significant at the 0.01

* Correlation is significant at the 0.05

In addition, by using Pearson's correlation coefficient between parameters under study in patients with bone metastasis group (M.P.C) the results showed a significant positive correlation between PSA and T-ALP (P=0.003, r=0.577), DPD and calcium (P=0.009, r=0.520), DPD and phosphate (P=0.01, r=0.499), calcium and phosphate (P<0.000, r=0.721) (Table 3.5).

Table 3.5 The correlations between parameters under study in metastasis prostate cancer (M.P.C) patients.

Parameter 1	Parameter 2	n	(r)	P-value
PSA	DPD	25	- 0.077	0.720
PSA	T-ALP	25	0.577**	0.003
PSA	Ca^{2+}	25	0.133	0.536
PSA	PO_4^{3-}	25	0.187	0.381
DPD	T-ALP	25	- 0.199	0.351
DPD	Ca^{2+}	25	0.520**	0.009
DPD	PO_4^{3-}	25	0.499*	0.013
T-ALP	Ca^{2+}	25	- 0.031	0.886
T-ALP	PO_4^{3-}	25	- 0.600	0.781
Ca^{2+}	PO_4^{3-}	25	0.721**	0.000

** Correlation is significant at the 0.01

* Correlation is significant at the 0.05

The correlations between bone markers level during clinical outcomes in patients with locally or metastatic prostate cancer showed strongly correlation [137]. Prostate cancer patients with bone metastasis have higher levels of PSA and ALP than those with localized cancer and benign tumor. Radionuclide bone scan is necessary when the serum PSA level is > 20 ng/ml and/or ALP level > 90 IU/L [138]. In patients with normal ALP, a higher PSA was associated with improved survival [130]. The study of Wymenga, *et al*. (2001) detected that serum ALP and PSA results with the assay of urinary DPD may provide valuable additional indicators of metastasis to the bone in untreated patients, and in monitoring the efficacy of therapy [139].

Serum calcium is bound by ALP, so patients with higher ALP levels might show higher calcium. Therefore, free calcium will be more valuable. These mean, that higher pretreatment serum levels of Ca^{2+} and ALP are good prognostic factors for patients with metastatic prostatic cancer on hormonal treatment, irrespective of tumor grading. [140].

The negatively charged of phosphate groups have strong affinity for cations such as calcium, therefore are easily incorporated into bone. When the calcium is released in highly levels in a bone resorption cases of metastatic prostate cancer patients, the levels of phosphate excretion are increase because these association between phosphate and calcium [141].

3.3 Demographic study

3.3.1 Age factor

In this study, the patients with prostate tumor were categorized into two groups according to their age. Group 1 consists of 20 patients (40%) with ages between 50-65 years. Group 2 consist of 30 patients (60%) with ages between 66-80 years (Figure 3.2). The statistical analysis of results did not show any significant (P>0.05) variation in all parameters under study between the two age groups (Table 3.6).

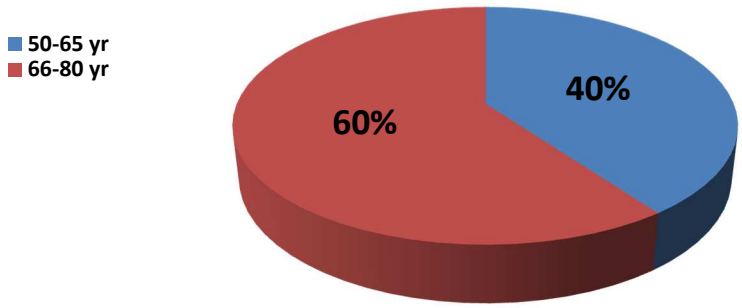


Figure 3.3 The percentage of patients according to their ages.

Table 3.6 The levels of parameters under study in patients with prostate tumor in two age groups.

Parameters	Age(50-65) n=20 Mean±Sd.E	Age(66-80) n=30 Mean±Sd.E	P-value
PSA *(ng/ml)*	74.65±19.08	90.02±23.31	0.612
DPD *(nmole/mmole Cr.)*	318.23±112.53	234.40±88.26	0.561
T-ALP *(IU/L)*	226.50±34.58	354.11±103.54	0.251
Ca^{2+} *(mg/dl)*	9.47±0.16	9.37±0.11	0.629
PO_4^{3-} *(mg/dl)*	4.98±0.27	4.94±0.24	0.908

Depending on Pearson's correlation coefficient between two age groups, the results revealed no any positive significant correlation (P>0.05) between the two age groups (Table 3.7).

Table 3.7 The correlations of parameters under study between age groups (50-65) and (66-80) years in patients with prostate tumor.

Parameters	r	P-value
PSA *(ng/ml)*	-0.063	0.799
DPD *(nmole/mmole Cr.)*	-0.080	0.745
T-ALP *(IU/L)*	-0.052	0.834
Ca^{2+} *(mg/dl)*	0.184	0.450
PO_4^{3-} *(mg/dl)*	-0.066	0.790

In this study, the results revealed that there was a weak linearity between age and PSA, T-ALP; r=0.150, and r=0.235 respectively, in patients with prostate cancer (L.P.C, and M.P.C group), whereas, there wasn't any linearity between age and the other parameters under study (Figure 3.3).

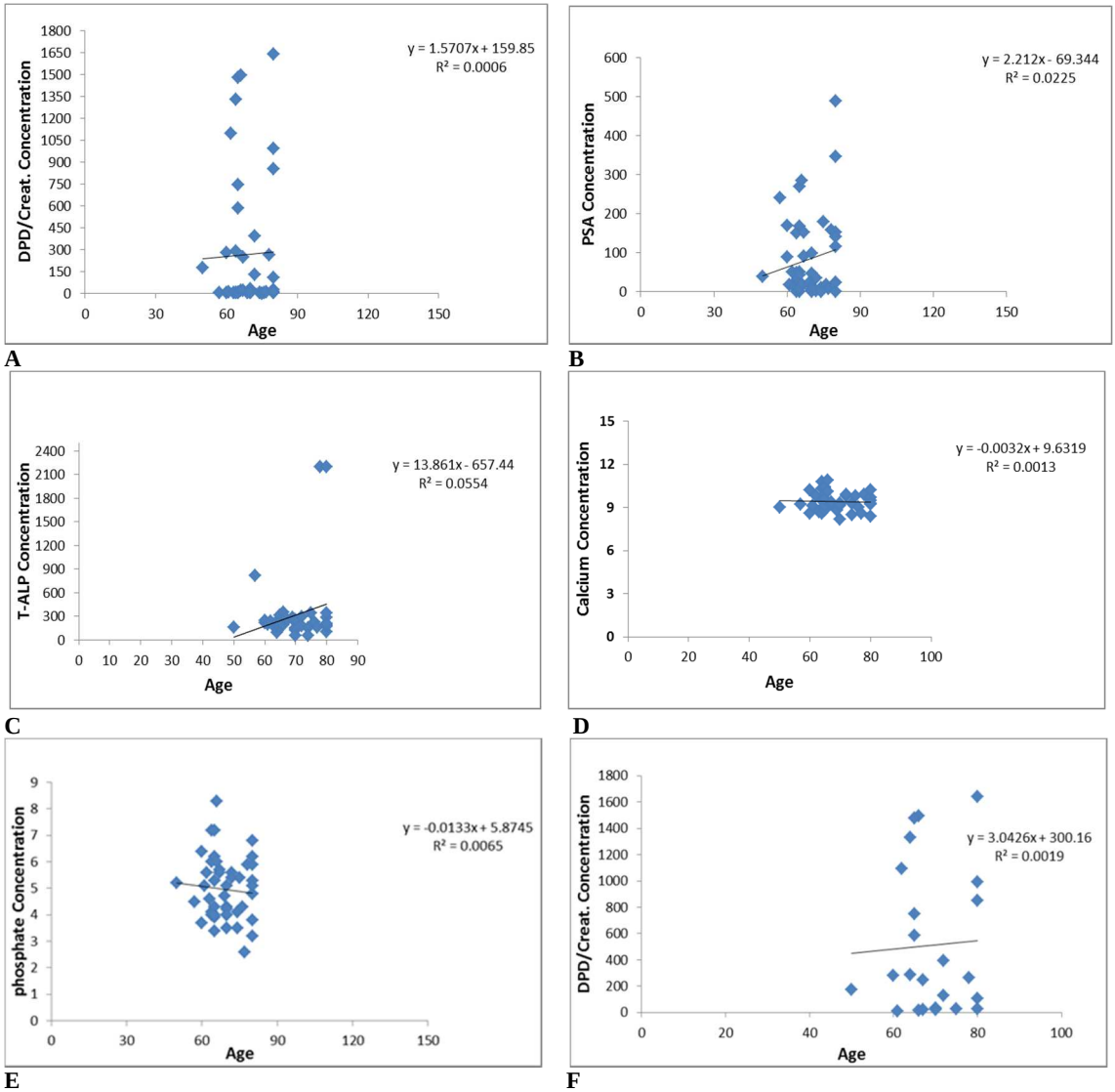


Figure 3.4 The correlation between age and parameters under study in prostate cancer patients. [(A), (B), (C), (D), and (E) in carcinoma group (L.P.C, and M.P.C group)], [(F) only in M.P.C group].

A combination of clinical examination and PSA levels is a better way of attempting to differentiate between a benign and malignant prostate. It is also preferable to relate normal PSA to age [142]. Chang, *et al.* (2012) documented a benign prostate hypertrophy can undergo malignant change. In extreme old age, not only B.P.H almost invariable but areas that seem to have at least carcinoma in situ are very common [142]. Benign prostatic hypertrophy of the prostate is an extremely common disorder in men over age 50. Histologic evidence of nodular hyperplasia (benign prostate hyperplasia) can be seen in approximately 20% of men 40 years of age, which increases to 70% by age 60 and to 90% by age 70 [143]. The Patrick`s study (2012) revealed the older men are more likely to be diagnosed with prostate cancer. Although only 1 in 10,000 men under age 40 will be diagnosed, the rate shoots up to 1 in 38 for ages 40 to 59, and 1 in 14 for ages 60 to 69 [144].

In fact, more than 65% of all prostate cancers are diagnosed in men over the age of 65 year. The average age at diagnosis of prostate cancer in the United States is 69 year. After that age, the chance of developing prostate cancer becomes more common than any other cancer in men or women [144].

3.3.2 Smoking factor

In this study, patients with prostate tumor were classified into two groups, smokers (22%) and non-smokers (78%) (Figure 3.4). The results did not show a significant ($P>0.05$) different in the concentration of all parameters under study between smoker and non-smoker patients, except there was significant ($P<0.05$) decrease in concentration of serum PSA, in smoker patients compared with non-smoker patients (Table 3.8).

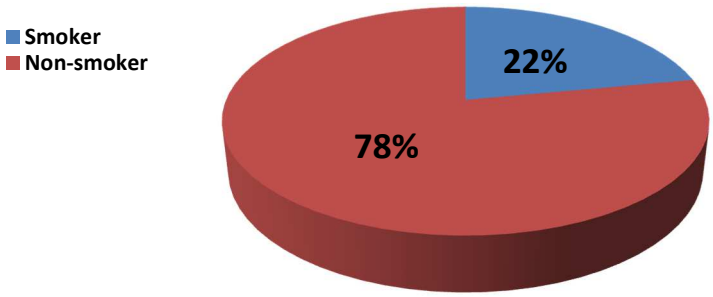


Figure 3.5 The percentage of smokers and non-smokers patients.

Table 3.8 The levels of parameters under study in smoker and non-smoker patients.

Parameters	Smoker n=11 Mean±Sd.E	Non Smoker n=39 Mean±Sd.E	P-value
PSA (ng/ml)	33.33±17.61	95.91±18.54	0.021
DPD (nmole/mmole Cr.)	104.01±81.49	309.17±82.63	0.089
T-ALP (IU/L)	197.47±30.43	326.60±77.14	0.127
Ca^{2+} (mg/dl)	9.36±0.15	9.42±0.11	0.765
PO_4^{3-} (mg/dl)	4.77±0.34	4.99±0.20	0.593

In this study the results of smoking were disagree with the other study, which explained that smoking might directly affect the aggressiveness of cancer and Carcinogens in tobacco smoke may speed tumor growth, for instance, as might the higher levels of testosterone associated with smoking, therefor cause increasing in PSA levels. Heavier smokers and those who smoked for longer periods of time fared the worst, men who had smoked a pack a day for 40 years or equivalently two packs a

day for 20 years, were 82% more likely to succumb to prostate cancer than men who had never smoked [145]. Rosette, *et al.* (2006) revealed that aetiology of B.P.H is multifactorial [146]. Currently, there is no strong evidence that smoking, vasectomy, obesity or high alcohol intakes are risk factors in the development of clinical B.P.H. Results of the different epidemiological studies are controversial, probably because of differences in sampling and methods of analysis. In most cases only insufficient marginal differences can be established [146].

3.3.3 Chemotherapy factor

In present study, patients with prostate cancer were classified into two groups, 30 patients (75%) with prostate cancer were treated with chemotherapy drug, and 10 patients (25%) with prostate cancer were untreated with chemotherapy drug (Figure 3.5). The results revealed a highly significant ($P<0.000$) increase in PSA and DPD levels, also there was a highly significant ($P=0.004$) increase in $Ca^{2+}$and PO_4^{3-} levels in patients who are treated with chemotherapy compared with those patients not treated with chemotherapy, whereas there wasn't any significant difference ($P>0.05$) in the activity of T-ALP in patients who were treated with chemotherapy compared with those patients who were untreated with chemotherapy (Table 3.9).

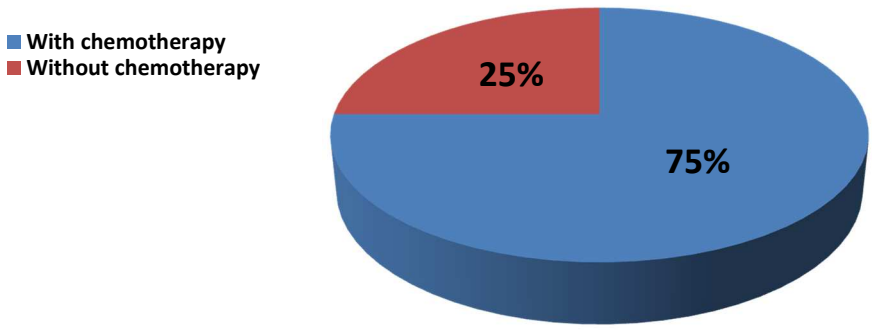


Figure 3.6 The percentage of patients with and without chemotherapy drug.

Table 3.9 The levels of parameters under study in patients of prostate tumor with and without chemotherapy drug.

Parameter	Without Chemotherapy drug n=10	With Chemotherapy drug n=30	P-value
	Mean±Sd.E	Mean±Sd.E	
PSA *(ng/ml)*	18.37±3.35	95.40±17.86	0.000
DPD *(nmole/mmole Cr.)*	5.92±0.45	316.25±79.24	0.000
T-ALP *(IU/L)*	201.71±18.63	319.30±73.56	0.129
Ca^{2+} *(mg/dl)*	8.80±0.11	9.52±0.097	0.004
PO_4^{3-} *(mg/dl)*	3.78±0.28	5.16±0.18	0.004

When the assignment of prostate cancer patients to the Oncology unit in Al-Hussein Teaching Hospital of Kerbala to receive proper treatment, we found that patients who were given initially a hormonal therapy (Zoladex) for about 10-15 times for a period of almost two years to reduce of PSA level, this is in early stages of prostate cancer. If the patient did not response to the hormonal treatment, the drug must be change; the treatment will be done to chemotherapy drug such as (Taxotere (docetaxel) or Zometa) to prevent the cancer from metastasis to the other tissues such as bone tissue. But some cases, especially in advanced prostate cancer stage, are given directly dose of chemotherapy to prohibit the cancer cells from metastasis to other surrounding tissues and to destroy it.

The primary target of chemotherapy is to stop the cancer cells from spreading and the cancer cells from growing, but when we look at whether a drug is working. A rising of PSA during hormonal therapy doesn't mean the patient out of options; it means that patient need to consider the use of other systemic therapies, such as chemotherapy, alternative hormonal

medications, or agents that target the spread of prostate cancer (metastasis) [147].

In preliminary study, Thuret, *et al.* (2008) provided evidence to a significant proportion of patients with prostate cancer have an initial rise in serum PSA during the first 8 weeks following the start of chemotherapy, followed later by a drop in serum PSA, finally reaching the criteria for response or stabilization according to consensus guidelines. Those postchemotherapy PSA surge syndrome was observed both in patients receiving first-line and second-line chemotherapy and with various chemotherapy agents including docetaxel [148]. The explanation most usually proposed is that tumor markers are released in the blood due to acute cell lysis in this extremely chemosensitive disease. The prognostic value of this phenomenon is uncertain, although a negative impact on survival has been reported. In contrast to prostate cancer where the PSA rise may continue up to 8 weeks before a drop occurs, the serum tumor marker rise is usually short lived in germ-cell tumors. The kinetics of tumor marker decline in the latter can therefore be measured as early as 3 weeks after the start of chemotherapy and it was shown to have an independent prognostic value in high-risk disease [148].

In other study, Fizazi, *et al*. (2006) showed that a serum PSA rise was previously reported to be a very common occurrence in patients with prostate cancer receiving consolidation docetaxel after a response or stabilization following induction chemotherapy [149].

In previous study, Banfi, *et al*. (2001) measured bone mineral density (BMD) in 26 patients and revealed a high-dose chemotherapy caused a 10% loss in cortical bone and 20% in trabecular bone and increase in bone resorption rate (pyridinoline (PYD), deoxypyridinoline (DPD), Ca^{2+}, $PO_4^{3-}$and other bone contents). This has been attributed to chemotherapy

without a dose-dependent of irradiation are shows toxicity to bone marrow stromal osteoprogenitors and can cause osteopenia by direct damage of the osteoblastic compartment, as a mechanism distinct from and summable to hypogonadism [150].

In a recent study, Zhang, *et al.* (2012) documented that patient after treatment with zoledronic acid (Zometa) at week 2, the mean of bone formation marker as bone-specific alkaline phosphatase (ALP) level was decreased by 12.9% from a baseline level. Prostate cancer, lung cancer, multiple myeloma, and breast cancer patients with bone metastasis had elevated serum bone-specific alkaline phosphatase (ALP) levels, and zoledronic acid significantly was decreased these levels, which continued to drop steadily with continuation of zoledronic acid use [151].

3.3.4 Family history factor

In this study, patients with prostate tumor were categorized into two groups according to family history of prostate tumor. Group 1 included patients (30%) with history of prostate tumor. Group 2 included patients (70%) without history of prostate tumor (Figure 3.6). The results didn't show any significant variation (P>0.05) in all parameters under study in patients with history family of prostate tumor compared with those patients without history of prostate tumor (Table 3.10).

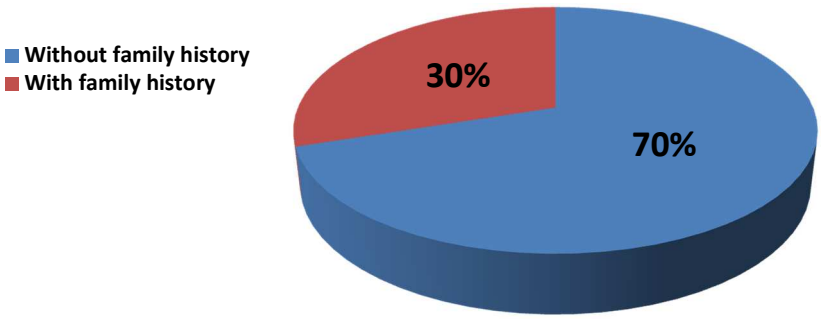


Figure 3.7 The percentage of patients with and without family history.

Table 3.10 The levels of parameters under study in patients with and without family history of prostate tumor.

Parameter	Without history of prostate tumor n=35	With history of prostate tumor n=15	P-value
	Mean±Sd.E	Mean±Sd.E	
PSA (ng/ml)	84.42±20.94	81.95±20.13	N.S
DPD (nmole/mmole Cr.)	236.36±81.81	343.70±131.06	N.S
T-ALP (IU/L)	381.49±87.96	262.36±47.43	N.S
Ca^{2+} (mg/dl)	9.40±0.13	9.42±0.10	N.S
PO_4^{3-} (mg/dl)	5.20±0.23	5.20±0.23	N.S

N.S (no significant P > 0.05

The risk of genotypes for several currently known genes is not strongly correlated with family history [152], but the recent study revealed the patients with breast and colon cancer, familial clustering of prostate cancer was frequently reported. From 5% to 10% of prostate cancer cases are believed to be primarily caused by high-risk inherited genetic factors or prostate cancer susceptibility genes. Results from several large case-control studies and cohort studies representing various populations suggest that family history is a major risk factor in prostate cancer [153].

A family history of a brother or father with prostate cancer increases the risk of prostate cancer, and the risk is inversely related to the age of the affected relative. However, at least some familial aggregation is due to increased prostate cancer screening in families thought to be at high risk [153].

Several reports have suggested an elevated risk of various other cancers among relatives within multiple-case prostate cancer families, but

none of these associations have been established definitively [154]. The study of Pakkanen, *et al.* (2009) detected in a population-based finish study, that no excess risk of all cancers combined in 5,523 family members, and no difference in familial cancer risk was observed when families affected by clinically aggressive prostate cancer when compared with those having non-aggressive prostate cancer. Those data suggested that familial prostate cancer is a cancer site–specific disorder [155].

3.3.5 Obesity factor

The other demographical study included obesity factor, the patients were classified into two groups according to body mass index (BMI). When the patient was more than 30 kg / m², he was considered obese (Figure 3.7). The results showed no significant (P>0.05) difference in the levels of parameters under study between obese and non-obese patients with prostate tumor (Table 3.11).

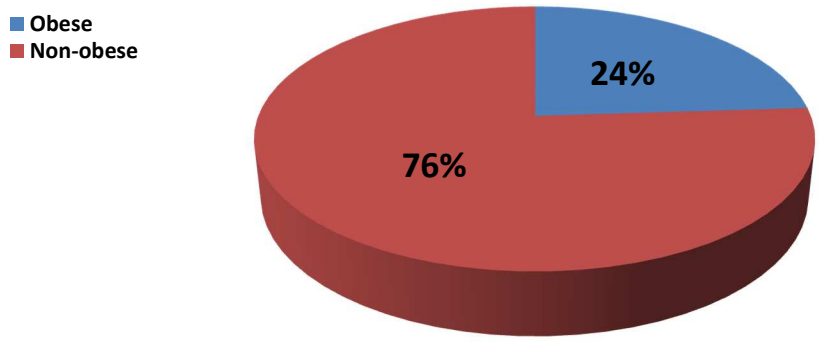


Figure 3.8 The percentage of obese and non-obese patients.

Table 3.11 The levels of parameters under study in obese and non-obese patients with prostate tumor.

Parameter	Obese n=12	non Obese n=38	P-value
	Mean±Sd.E	Mean±Sd.E	
PSA (ng/ml)	99.67±42.18	78.64±16.12	N.S
DPD (nmole/mmole Cr.)	199.12±146.80	291.00±79.05	N.S
T-ALP (IU/L)	383.98±182.25	275.45±60.55	N.S
Ca^{2+} (mg/dl)	9.29±0.19	9.45±0.10	N.S
PO_4^{3-} (mg/dl)	4.99±0.35	4.94±0.21	N.S

N.S (no significant P > 0.05)

By using Pearson`s correlation coefficient, the results demonstrated no significant correlation between BMI and the parameters under study (Table 3.12).

Table 3.12 The correlation between BMI (kg/m^2) and parameters under study in patients with prostate tumor.

Parameters	r	P-value
PSA (ng/ml)	-0.085	N.S
DPD (nmole/mmole Cr.)	0.085	N.S
T-ALP (IU/L)	-0.110	N.S
Ca^{2+} (mg/dl)	0.111	N.S
PO_4^{3-} (mg/dl)	-0.017	N.S

N.S (no significant P > 0.05)

The exact relationship between obesity and prostate cancer remains unclear, but there is no doubt that obesity can have a negative effect on outcomes. Many researches had shown that prostate-specific antigen (PSA) test results in obese men can be lower despite the presence of disease, potentially leading to a delay in diagnosis and treatment. Recovery from surgery tends to be longer and more difficult; and the risk of dying from prostate cancer can be higher [156]. Another study by Patrick (2012) revealed that a measure of obesity wasn't linked to being diagnosed with prostate cancer overall. In fact, obese men may have a relatively lower PSA levels than the non-obese men due to dilution of the PSA in a larger blood volume. However, obese men are more likely to have an aggressive disease [144].

The study of Arner, *et al*. (2014) showed that obesity increases the risk of developing specific cancer forms [157]. The mechanisms are unclear, increased fat cell secretion of specific proteins (adipokines) may promote or facilitate development of malignant tumors in obesity via crosstalk between adipose tissue and the tissues prone to develop cancer among obese [157]. Multiple studies have shown an inverse relationship between serum PSA levels and BMI. A recent population-based study examined the association between BMI and PSA levels in nearly 3,000 men without prostate. The mean PSA level was decreased with the increase of BMI, such that the mean PSA level in men with a normal BMI (18.5–24.9 kg/m^2) was 1.01 ng/ml and the mean PSA level in morbidly obese men (BMI ≥40 kg/m^2) was 0.69 ng/ml ($P<0.0001$). In the multivariate model, age and race did not attenuate the association between PSA levels and BMI. The lower levels of circulating androgens in obese men might be responsible for the observed lower levels of PSA. These findings have been confirmed in other populations. An examination of approximately 300 men in a community-based cancer screening cohort, had found an inverse relationship between

BMI and PSA levels in both Caucasian and African–American groups [158]. Other study by Hotta, *et al.* (2000) measured the association of body weight and bone turnover, which had been performed in more specific populations, such as patients suffering from obesity, and detected the patients with obesity show a negative association between BMI and bone resorption [159]. In addition, the sensitivity of bone markers to measure bone turnover in individuals of any BMI category has been questioned due to their limitations (such as coming from nonskeletal sources), but despite these drawbacks they had been proved to be useful in both research and many clinical practices [160].

Conclusions

1. Prostate cancer is associated with elevated deoxypyridinoline (DPD), PSA, T-ALP, calcium, and phosphate values.
2. We can decide that, if cancer reaches to the bone (metastatic) or not, by measuring the DPD level.
3. Deoxypyridinoline (DPD) can be clinically useful as a marker of metastatic bone tumors and for treatment monitoring.
4. Chemotherapy is not useful in a primary treatment and it is not enough drugs to treat cancer. Therefore, must use dependent-dose of radiotherapy with it to save the bone.
5. Prostate cancer is starts in advanced age in the most cases, that is about 50 years and over.
6. Family history is one of risk factors that associated with prostate cancer.
7. Most patients of prostate cancer have weight loss.

Future Studies and Recommendation

1. Clinical biochemical studies can be done for bone resorption markers (urine deoxypyridinoline (DPD), pyridinoline), bone formation markers (T-ALP, B-ALP), and bone matrix markers (calcium, phosphate) in advanced stage of cancers such as prostate cancer or breast cancer, also in other bone diseases that caused of osteoporosis.

2. Urine deoxypyridinoline (DPD) can be recommended for use in specific detection and sensitivity evaluation of bone metastasis examinations and for treatment monitoring.

3. Comparative study of deoxypyridinoline (DPD) marker with pyridinoline (Pyd) marker in prostate cancer patients can be conducted.

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الخـــلاصة

يعد سرطان البروستات أكثر أنواع السرطانات شيوعا في العالم، ولاسيما في العراق مقارنة ببقية السرطانات، وهذا النوع من السرطانات يصيب الرجال الذين تتجاوز اعمارهم 50 سنة، ونادرا ما يصيب الرجال الذين تقل اعمارهم عن هذه الفئة العمرية. غالبا ما ينتشر هذا السرطان ليصل الى العظم في مرحلة متقدمة من مراحل انتشاره، مما يسبب زيادة في طرح نواتج ايض (هدم) الكولاجين مثل الدايوكسي بايريدينولين(Deoxypyridinoline)، فضلا عن الزيادة في طرح انزيم الفوسفاتيز القاعدي (ALP) والعناصر المهمة الأخرى في بناء العظم مثل الكالسيوم (Ca^{2+}) والفوسفات(PO_4^{3-}).

لقد تضمنت هذه الدراسة التحقق من مدى الانتشار السرطاني عند مرض سرطان البروستات، للوقوف عند مرحلة من مراحله المتقدمة وهي هل انه قد وصل الى العظم ام لا، ولهذا فقد اعتمد المؤشر السريري (الدايوكسي بايريدينولين)، وأيضا لمعرفة العلاقة التي تربط بينه وبين المؤشرات السريرية الاخرى في المرضى المصابين بسرطان البروستات المنتشر الى العظم، حيث تمت دراسة (50) مصابا بورم البروستات، تم جمعهم من (مستشفى الحسين ع التعليمي/ كربلاء)، بالإضافة الى (30) شخصا من الأصحاء اعمارهم متطابقة مع المرضى.

وتم تدوين الخواص السريرية للمرضى والمتضمنة العمر، التدخين، التاريخ العائلي، السمنة، والعلاج الكيميائي المستعمل للمريض. وأظهر التحليل الاحصائي للنتائج أن 60 % من المرضى كانت تتراوح اعمارهم بين (66-80) سنة و 40 % منهم كانت تتراوح اعمارهم بين (50-65) سنة. بعد ذلك تم قياس تراكيز كل من: PSA، انزيم الفوسفاتيز القاعدي، الكالسيوم، والفوسفات في مصول المرضى المصابين بسرطان البروستات ومجموعة الاصحاء. وكذلك تم قياس تراكيز كل من الدايوكسي بايريدينولين والكرياتينين في ادرار نفس المرضى والاصحاء.

وقد اظهرت النتائج الاحصائية باستعمال اختبار t-test عن وجود ارتفاع معنوي ($P<0.000$) في تراكيز كل من: PSA، الدايوكسي بايريدينولين (DPD/Creat)، الكالسيوم، والفوسفات فضلا عن ارتفاع معنوي ($P<0.01$) في تركيز انزيم الفوسفاتيز القاعدي في مرضى ورم البروستات مقارنة مع المجموعة القياسية.

تم تصنيف الخمسين مصابا بورم البروستات سريريا الى ثلاثة مجاميع; المجموعة الاولى تضم المرضى ذوي تضخم البروستات الحميد، والمجموعة الثانية تضم مرضى سرطان البروستات الموضعي، اما المجموعة الثالثة فتضم مرضى سرطان البروستات المنتشر الى العظم. وقد اظهرت النتائج ارتفاعا معنويا ($P<0.000$) في مستوى طرح الدايوكسي بايريدينولين

(DPD) في ادرار مرضى سرطان البروستات المنتشر الى العظم عند مقارنتهم مع مرضى مجموعة تضخم البروستات الحميد، ومرضى مجموعة سرطان البروستات الموضعي، في حين لم يظهر أي ارتفاع متباين لتركيز الدايوكسي بايريدينولين (DPD) عندما قورن بين مرضى مجموعتي تضخم البروستات الحميد، وسرطان البروستات الموضعي. كذلك وجد ارتفاع معنوي (P<0.01) في تركيز PSA وارتفاع معنوي (P<0.000) في تراكيز كل من الكالسيوم، والفوسفات لمرضى سرطان البروستات المنتشر الى العظم عند مقارنتهم مع مرضى مجموعة تضخم البروستات الحميد، ومرضى مجموعة سرطان البروستات الموضعي.

وباستعمال معامل ارتباط بيرسون لمرضى سرطان البروستات المنتشر الى العظم أظهرت النتائج وجود ارتباط موجب (P=0.003, r=0.577) بين PSA وانزيم الفوسفاتيز القاعدي، (P=0.009, r=0.520) بين الدايوكسي بايريدينولين والكالسيوم، (P=0.01, r=0.499) بين الدايوكسي بايريدينولين والفوسفات، (P<0.000, r=0.721) بين الكالسيوم والفوسفات.

اظهرت الدراسة الديموغرافية للمرضى والتي تضمنت العمر، التدخين، العلاج الكيميائي، التاريخ العائلي، والسمنة، عن وجود زيادة معنوية (P<0.05) في مستوى PSA لدى المرضى المدخنون في حين لم يلاحظ هنالك اي ارتفاع معنوي (P>0.05) في مستوى بقية المؤشرات السريرية المستخدمة لدى مرضى ورم البروستات المدخنين. كذلك لوحظ وجود ارتفاع معنوي (P<0.000) في مستوى PSA والدايوكسي بايريدينولين، ووجود ارتفاع معنوي (P<0.01) في مستوى الكالسيوم والفوسفات لدى المرضى الذين يتعالجوا كيميائيا عند مقارنتهم مع المرضى الذين لم يتعالجوا كيميائيا. في حين لم تلاحظ هنالك اية زيادة معنوية (P>0.05) في مستويات جميع المؤشرات السريرية المستخدمة في مجال بحثنا بالنسبة للخواص السريرية الاخرى (العمر، التاريخ العائلي، السمنة) لمرضى ورم البروستات.

تشير النتائج الى ان الدايوكسي بايريدينولين هو مؤشر سريري جيد يمكن ان يستدل به لمرحلة تقدم السرطان وانتشاره الى العظم وايضا لمتابعة العلاج، وليس لسرطان البروستات فحسب بل لجميع انواع السرطانات عند انتشارها ووصولها الى العظم. كذلك اظهرت النتائج مدى ارتباط المؤشرات السريرية الاخرى مع الدايوكسي بايريدينولين في مرضى السرطان البروستاتي المنتشر. ومن الجدير بالذكر ومما اثبتته النتائج خلال البحث، ان العلاج الكيميائي المستعمل للمرضى المصابين بسرطان البروستات يعمل بصورة عكسية، فهو يقوم بزيادة نسبة تقدم السرطان كونه يسبب تسمم نخاع العظم، من هنا فإننا ننصح باستعمال جرعه معتمدة من العلاج الاشعاعي مع العلاج الكيميائي لوقاية العظم.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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كلية العلوم

دراسة بعض الدلائل والمتغيرات الكيمياحيوية لمرضى سرطان البروستات المنتشر الى العظم

رسالة مقدمة الى
مجلس كلية العلوم جامعة كربلاء
وهي جزء من متطلبات نيل درجة الماجستير في
الكيمياء الحياتية

من قبل

عزيز حسين جاسم

بكالوريوس علوم كيمياء / جامعة كربلاء (٢٠١٢)

بإشراف

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