



Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Prostate Specific Membrane  
Antigen in Prostate Tumors

الكشف المناعي الكيميائي النسيجي لبروتين غشاء البروستاتا المحدد في أورام  
البروستاتا

A dissertation submitted for partial fulfillment of the degree of M.Sc. in  
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## الآية

قال تعالى :

﴿ قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ  
تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا (109) ﴾

صدق الله العظيم

(الكهف، 109)

## Dedication

*To my dear parents, no words are enough*

*To my dear brothers and sister*

*To my precious husband for supporting me and believing in me*

*To my little angels, Judy and Mohammed*

*I dedicate this effort*

## Acknowledgment

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## Abstract

This retrospective analytical case control study was carried out in Omdurman Teaching Hospital and Sudan University of Science and Technology- College of Medical Laboratory Science during the period from October 2017 to January 2018. It aimed to detect the expression of prostate specific membrane antigen in prostate tumors by immunohistochemistry. Forty previously diagnosed formalin-fixed paraffin-embedded prostate tissue blocks were collected, 20 (50%) of them were diagnosed benign prostatic hyperplasia as control group and the other 20 (50%) were prostatic adenocarcinoma with different grades and Gleason score as case group. Blocks were cut by a microtome and 4  $\mu$ m sections were obtained. Sections were stained by enhanced polymer one step method and examined under the microscope for PSMA expression. Then results were analyzed by SPSS program version 14.0. The age of the study group ranged between 55-95 years and the mean age was 72 years. Of the 20 prostatic adenocarcinomas, 5 (25%) were well differentiated adenocarcinoma, 5 (25%) were moderately differentiated and 10 (50%) were poorly differentiated. Eighteen (45%) out of the 20 benign prostatic hyperplasia samples were negative for PSMA and 2 (5%) were positive, while 17 (42.5%) out of the 20 prostatic adenocarcinoma were positive and 3 (7.5%) showed negative expression. The study revealed a significant difference in the expression of PSMA between benign prostatic hyperplasia and prostatic adenocarcinoma as it is overexpressed in the latter (P value=0.000). It also revealed that the relation between the expression of PSMA and grade of cancer was statistically significant; as the expression increases with higher grades (P value=0.005). These findings conclude that PSMA is an important diagnostic and prognostic marker for prostate cancer.

## المستخلص

أجريت هذه الدراسة الاسترجاعية التحليلية الحالة والحالة الضابطة في مستشفى أمدمان التعليمي و جامعة السودان للعلوم والتكنولوجيا- كلية علوم المختبرات الطبية في الفترة من أكتوبر 2017 الى يناير 2018. هدفت هذه الدراسة لتحديد الظهور المناعي لبروتين غشاء البروستاتا النوعي في أورام البروستاتا. جمع 40 قالب لعينات بروستاتا نسيجية مثبتة بالفورمالين ومغمورة بشمع البارافين مشخصة مسبقا بأورام البروستاتا. 20 (50%) عينة كانت فرط تنسج البروستاتا الحميد و 20 (50%) عينة كانت سرطان البروستاتا الخبيث. قطعت قوالب العينات بالمايكروتوم وأخذت شرائح نسيجية بسمك 4 ميكرون. صبغت الشرائح ثم تمت معاينتها تحت المجهر للكشف عن الظهور المناعي لبروتين غشاء البروستاتا النوعي. حلت النتائج ببرنامج الحزمة الإحصائية للعلوم الإجتماعية (النسخة 14.0). تراوحت أعمار مجموعة الدراسة بين 55-95 سنة وكان متوسط الأعمار 72 سنة. 5 (25%) عينات من عينات السرطان الخبيث كانت جيدة التمايز، 5 (25%) عينات كانت متوسطة التمايز و 10 (50%) عينات كانت سيئة التمايز. 18 (45%) عينة من العينات الحميدة لم تظهر أي تعبير مناعي نسيجي لبروتين غشاء البروستاتا النوعي و عينتان (5%) أظهرت تعبيراً إيجابياً، بينما 17 (42.5%) عينة من عينات السرطان الخبيث أظهرت تعبيراً إيجابياً و 3 (7.5%) عينات فقط لم تظهر تعبيراً للبروتين. أظهرت هذه الدراسة وجود اختلاف ذو دلالة إحصائية في التعبير المناعي النسيجي للبروتين الغشائي البروستاتي النوعي بين أورام البروستاتا (القيمة الاحتمالية = 0.000) ، حيث انه أكثر في السرطان الخبيث منه في فرط التنسج الحميد. كما أظهرت الدراسة أنه توجد علاقة ذات دلالة إحصائية بين التعبير المناعي للبروتين الغشائي البروستاتي النوعي ودرجة تمايز السرطان؛ حيث ان التعبير المناعي للبروتين يزيد مع زيادة درجة التمايز (القيمة الاحتمالية = 0.005). هذه النتائج تؤكد أن بروتين غشاء البروستاتا النوعي ذو أهمية في تشخيص أورام البروستاتا المختلفة.

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### List of abbreviations

| Abbreviation | Full meaning                                   |
|--------------|--|
| BPH          | Benign prostatic hyperplasia                   |
| DAB          | 3,3 diaminobenzidine tetra hydrochloride       |
| DHT          | Dihydrotestosterone                            |
| DPX          | Distyrene, a plasticizer xylene                |
| DRE          | Digital rectal examination                     |
| EPOS         | Enhanced polymer one step method               |
| GnRH         | Gonadotropin releasing hormone                 |
| HRP          | Horseradish peroxidase                         |
| LHRH         | Luteinizing hormone releasing hormone          |
| mAb          | Monoclonal antibody                            |
| NCI-UG       | National center institute-University of Gezira |
| PBS          | Phosphate buffer saline                        |
| PIN          | Prostatic intraepithelial neoplasia            |
| PSA          | Prostate specific antigen                      |
| PSMA         | Prostate specific membrane antigen             |
| RICK         | Radiation and isotope center Khartoum          |
| TRUS         | Transrectal ultrasound                         |

# Chapter One

## Introduction

## Chapter one

### Introduction

#### 1.1 Introduction:

Cancer of the prostate is the second most common form of cancer in males, followed by lung cancer. It affects men above the age of 50 years and its prevalence increases with increasing age, so that more than 50% of men 80 years old have asymptomatic (latent) carcinoma of the prostate (Mohan. 2015). Based on autopsy studies, its incidence increases from 20% in men in their 50s to approximately 70% in men between the ages of 70 and 80 years (Kumar *et al.* 2015). In the United States the estimated new prostate cancer cases were 161,360 and the estimated deaths were 26,730 in 2017 (Siegel *et al.* 2017). It is tied with colorectal cancer in terms of cancer mortality, causing 9% of cancer deaths in the United States in 2012. The highest frequency of prostate cancer occurs in the United States and Scandinavia (Hansel and Dintzis. 2006).

There are some remarkable and puzzling national and racial differences in its incidence. It is uncommon in Asians and most frequent among blacks. In addition to hereditary factors, environment plays a role, as evidenced by the rise in its incidence in Japanese immigrants to the United States, though not nearly to the level of that of native-born Americans. Also, as the diet in Asia becomes more westernized, the incidence of the disease in this region of the world seems to be increasing. Whether due to dietary factors or other lifestyle changes is not clear (Kumar *et al.* 2015).

The etiology of prostate cancer is not fully understood. Many risk factors contribute in development of prostate cancer. These include age, hormones, family history, race and geographical regions, diet, occupation and others. Diagnosis of prostate cancer is reached following a combination of history taking, physical examination, PSA test and prostate biopsy (Turner and Drudge-Coates. 2010).

Prostate cancer is the most common cancer in Sudanese men. The age-standardized rate is 10.3 and mortality is 8.7 per 100,000 population. It was the most diagnosed cancer among men accounting for 7.6% of all cancer types in men at both RICK and NCI-UG during year 2000–2006, and the most common cancer among male patients treated at the NCI-UG, ranking first among cancer male patients treated in the NCI, central Sudan (2006–2009). The disease was found equally distributed among different tribes and most cases (85.4%) presented with stage III and IV. The mean age of patients was  $72.2 \pm 9.25$  (Elamin *et al.* 2015).

Prostate specific membrane antigen (PSMA) is a type II integral membrane glycoprotein that was initially characterized by the monoclonal antibody (mAb) 7E11 (Chang *et al.* 1999). It is produced by the prostatic epithelium and has been found to be expressed in extraprostatic tissue such as brain, kidney tubules, duodenum and colon (Silver *et al.* 1997). Despite the expression of PSMA by various types of normal and malignant tissue, it is considered to be fairly sensitive and highly specific for prostatic adenocarcinoma (Fauceglia *et al.* 2007). PSMA was found to be a sensitive and specific marker for detecting metastatic prostate carcinoma in cytology specimens as the sensitivity of PSMA immunohistochemistry is higher in detecting metastatic prostate carcinoma than that of PSA immunohistochemistry (Bernacki *et al.* 2013).

## **1.2 Objectives:**

### **1.2.1 General objective:**

To detect the expression of prostate specific membrane antigen (PSMA) in prostate tumors.

### **1.2.2 Specific objectives:**

- 1- To detect the expression of prostate specific membrane antigen in prostate tumors using immunohistochemistry.
- 2- To correlate the expression of prostate specific membrane antigen with histopathological diagnosis.
- 3- To correlate the expression of prostate specific membrane antigen with cancer grade.



# Chapter Two

## Literature Review

## **Chapter two**

### **Literature review**

#### **2.1 Anatomy and histology of the prostate:**

The prostate gland is a walnut-sized fibromuscular and glandular organ that encircles the urethra just inferior to the bladder. The segment of urethra that travels through the prostate gland is called the prostatic urethra. It is lined by a thin layer of smooth muscle that is continuous with the bladder wall. This smooth muscle represents the true involuntary sphincter of the male posterior urethra. Because the prostate surrounds the urethra, it can produce urinary obstruction when it becomes enlarged (Porth. 2011).

The prostate gland has a base and an apex, and anterior, posterior and inferolateral surfaces. The base is the upper surface adjacent to the preprostatic urethra and bladder neck, the blunt apex is the lowest part. The ejaculatory ducts pierce the posterior surface just below the bladder and pass obliquely through the gland for about 2 cm to open separately into the prostatic urethra about halfway along its length on the verumontanum. A thin connective tissue layer at the periphery of the prostate forms a true capsule, outside of which is a condensation of pelvic fascia forming a false capsule. A plexus of veins lies between these two capsules (Dixon. 1999).

Some of the venous drainage passes to the plexus of veins lying in front of the vertebral bodies and within the neural canal. This communication may explain the readiness with which carcinoma of the prostate spreads to the pelvic bones and vertebrae (Ellis. 2006).

Histologically, the prostate is composed of tubular alveoli (acini) embedded in fibromuscular tissue mass. The glandular epithelium forms infoldings and consists of 2 layers: a basal layer of low cuboidal cells and an inner layer of mucus secreting tall columnar cells. The alveoli are separated by thick fibromuscular septa containing abundant smooth muscle fibres (Mohan. 2015).

#### **2.2 Prostate pathology:**

Three pathologic processes affect the prostate gland: inflammation, benign nodular hyperplasia, and carcinoma. Of these three, the benign nodular hyperplasia are the most common in advanced age and can be considered as a normal aging process. Prostatic carcinoma is also an extremely common lesion in men (Kumar *et al.* 2015).

### **2.2.1 Inflammation:**

Prostatitis may be divided into:

#### **2.2.1.1 Acute bacterial prostatitis:**

It is an acute infection of the prostate gland that causes pelvic pain and urinary tract symptoms such as dysuria, urinary frequency and urinary retention. It may lead to systemic symptoms such as fever, chills, nausea and malaise. It comprises up to 10% of all prostatitis cases. It is most frequently caused by *E.coli* followed by *P.aeruginosa* and *Klebsiella*, *Enterococcus*, *Enterobacter* and *Proteus* species. Diagnosis can be based on history and physical examination and aided by urinalysis (Coker and Dierfeldt. 2016)

#### **2.2.1.2 Chronic bacterial prostatitis:**

This is caused by chronic bacterial infection of the prostate with or without prostatitis symptoms and usually with recurrent urinary tract infections by the same bacterial strain. It is usually associated with mild to moderate pelvic pain. Long term antimicrobial therapy is curative in 60% to 80% of patients (Nickel. 2011).

#### **2.2.1.3 Chronic abacterial prostatitis:**

It is the most common type of prostatitis. It is indistinguishable from chronic bacterial prostatitis in terms of signs and symptoms, but there is no history of recurrent urinary tract infections and the bacterial cultures are uniformly negative although the expressed prostatic secretions show leukocytosis (Kumar *et al.* 2015).

#### **2.2.1.4 Granulomatous prostatitis:**

Granulomatous prostatitis is a variety of chronic prostatitis, probably caused by leakage of prostatic secretions into the tissue, or could be of autoimmune origin. Histologically, the inflammatory reaction consists of macrophages, lymphocytes, plasma cells and some multinucleate giant cells. The condition may be confused with tuberculous prostatitis (Mohan. 2015).

### **2.2.2 Benign prostatic hyperplasia (BPH):**

BPH is the most common benign prostatic disease in men older than age 50 years. It results from nodular hyperplasia of prostatic stromal and epithelial cells and often leads to urinary obstruction. It is characterized by the formation of large, fairly discrete nodules in the periurethral region of the prostate, which, when sufficiently large, compress and narrow the urethral canal to cause partial, or sometimes virtually complete, obstruction of the urethra. Nodular hyperplasia is not considered to be a premalignant lesion (Kumar *et al.* 2015).

### **2.2.3 Prostatic intraepithelial neoplasia (PIN):**

In many cases, prostatic adenocarcinoma appears to arise from intraductal dysplastic foci termed prostatic intraepithelial neoplasia (PIN). PIN describes prostatic acini lined by atypical epithelial cells with hyperchromatic nuclei and prominent nucleoli. The basal cell layer is maintained in PIN in contrast to invasive carcinoma. Several lines of evidence support the argument that PIN is a precursor of invasive cancer; these include the cytological similarity, the peripheral distribution of both lesions, the close topographical proximity of high-grade PIN and invasive cancer, and similar molecular changes between the two lesions. PIN is classified as low-grade PIN or high-grade PIN. Low-grade PIN demonstrates cellular crowding and overlap, variation in nuclear size, and the presence of nucleoli. High-grade PIN describes epithelial cells with more prominent cellular crowding, nuclear enlargement, prominent enlarged nucleoli, and decreased numbers of basal cells which can be identified by high molecular weight cytokeratin and p63 immunostains (Hansel and Dintzis. 2006).

### **2.2.4 Prostate cancer:**

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of deaths in males worldwide. It is associated with urinary dysfunction and the advanced disease can spread to other parts of the body such as vertebrae, pelvic or ribs (Mustafa *et al.* 2016). It is a heterogeneous disease ranging from asymptomatic to a rapidly fatal systemic malignancy. The prevalence of prostate cancer is so high that it could be considered a normal age related phenomenon (Hughes *et al.* 2005)

### **2.3 Histological features of prostatic adenocarcinoma:**

Histologically, adenocarcinoma of prostate is characterized by many features, these include architectural disturbance in which there is loss of intra-acinar papillary convolutions. The groups of acini are either closely packed in back-to-back arrangement without intervening stroma or are haphazardly distributed. Also the malignant acini have little or no stroma between them, the malignant cells penetrate or replace the fibromuscular stroma. The gland pattern also show some differences; in well-differentiated adenocarcinoma the glands are small or medium and lined by a single layer of cuboidal cells. Moderately-differentiated tumors have cribriform glandular pattern while poorly-differentiated tumors have little or no glandular arrangement instead they show solid or trabecular pattern. One of the important features of prostatic adenocarcinoma is that the outer basal layer seen in the normal or benign acini is lost. In addition to that, the early and frequent invasion of the intra-prostatic perineural spaces (Mohan. 2015)

## **2.4 Risk factors of prostate cancer:**

### **2.4.1 Age:**

Prostate cancer is associated with aging. It is rarely diagnosed before the age of 50, but after this age incidence and mortality rates both increase almost exponentially (Haas and Sakr. 1997). The probability of developing prostate cancer increases from 0.005% among individuals aged less than 39 years to 2.2% (1 in 45) for those aged 40 to 59 years and 13.7% (1 in 7) for those aged 60 to 79 years (Crawford. 2003).

### **2.4.2 Race:**

The highest incidence rates for prostate cancer in the world are among African-American men. It is nearly 60% higher in African Americans than in whites, which, in turn, is higher than the rates for Hispanics and Asians/Pacific Islanders (Crawford. 2003).

### **2.4.3 Family history:**

Prostate cancer has a hereditary component. Male relatives of prostate cancer patients have an increased risk of developing the disease (Haas and Sakr. 1997). Epidemiologic studies indicate that men with a positive family history are diagnosed at an earlier age (on average 6 to 7 years earlier) than those without affected first-degree relatives (Crawford. 2003).

### **2.4.4 Diet:**

Many dietary factors have contributed in development of prostate cancer. For example fat consumption, especially polyunsaturated fat, shows a strong, positive correlation with prostate cancer incidence and mortality. Retinoids, including vitamin A, help regulate epithelial cell differentiation and proliferation, with a positive association with prostate cancer risk. Vitamin D deficiency may be a risk factor for prostate cancer as its hormonal form 1-25- dihydroxyvitamin D, inhibits invasiveness and has anti-proliferative and anti-differentiative effects on prostate cancer (Bostwick *et al.* 2004).

### **2.4.5 Benign prostatic hyperplasia:**

Because of numerous similarities in pathophysiology, it has been suggested that BPH may predispose to cancer or that a common factor influences the development of both diseases. Men diagnosed with BPH have been reported to be more likely (by as much as fivefold) to develop prostate cancer than are age-matched controls (Haas and Sakr. 1997).

### **2.4.6 Hormones:**

Androgens significantly alter prostate cancer growth rates, and progression of prostate cancer from preclinical to clinically significant forms may result in part from altered androgen

metabolism. Elevated concentrations of testosterone and its metabolite, dihydrotestosterone (DHT), over many decades may increase prostate cancer risk (Bostwick *et al.* 2004).

#### **2.4.7 Occupation:**

Occupation is directly related to socioeconomic status and thus is difficult to study as an independent variable. Most studies focus on exposure to occupational hazards that may affect the biology of the disease. Farmers and city dwellers have similar incidences of disease. Those engaged in heavy physical labor may be at an increased risk. Workers in heavy industry, rubber manufacturing, and newspaper printing may have a slightly higher incidence (Haas and Sakr. 1997).

#### **2.5 Clinical features of prostate cancer:**

Early prostate cancer usually has no clear symptoms, yet sometimes it causes symptoms similar to those caused by benign prostatic hyperplasia. These include frequent urination, nocturia, difficulty starting and maintaining a steady stream of urine, hematuria and dysuria. Prostate cancer is associated with urinary dysfunction as the prostate gland surrounds the prostatic urethra. Because the vas deferense deposits seminal fluid into the prostatic urethra and secretion from the prostate itself are included in the semen content, prostate cancer may also cause problems with sexual function such as difficulty achieving erection or painful ejaculation. Metastatic prostate cancer can cause additional symptoms, most commonly is bone pain often in the vertebrae, pelvis or ribs. Its spread to the spine can also compress the spinal cord causing tingling leg, weakness and urinary and fecal incontinence (Mustafa *et al.* 2016).

#### **2.6 Grading and staging of prostate cancer:**

The preferred histological grading system of prostate cancer is the Gleason system which was developed by Dr. Donald Gleason in the early 1970s. It is based upon the degree of loss of the normal glandular tissue architecture. Biopsies are graded from 1-5 and then an aggregate score incorporating the principal and secondary score ids produced (e.g., 3+4=7). Scores conventionally tend to be grouped into three categories; 1-5: low-grade prostate cancer, 6-7: intermediate-grade cancer (most cancers fall into this category) and 8-10: high-grade cancers. Staging is done using the American Joint Committee on Cancer (AJCC) TNM classification system. T indicates the extent of the primary tumor, N is for status of regional lymph nodes, and M is to indicate if there is distant metastases. The use of additional staging/diagnostic tests should match the severity of disease determined by the biopsy and prostate imaging (TRUS or MRI) (James. 2014).

## **2.7 Diagnosis of prostate cancer:**

### **2.7.1 Digital rectal examination (DRE):**

The DRE test examines the prostate via the rectum for any bumps, enlargements or any suspicious hard areas. As most prostate cancers are located in the peripheral zone, a DRE may detect cancers in this zone when its volume is 0.2 mL or larger (James. 2014). The American Cancer Society recommends annual prostate-specific antigen (PSA) and digital rectal examination (DRE) screening starting at the age of 50 years for all men with a life expectancy of  $\geq 10$  years (Smith *et al.* 2003).

### **2.7.2 Biopsy methods:**

Biopsies can be performed by transrectal, perineal or transurethral methods. It can be guided by transrectal ultrasound (TRUS) to give clinician a visual location of the tumors. Once the suspicious tissue is extracted, the possible cancer can be examined pathologically (James. 2014)

### **2.7.3 Prostate specific antigen (PSA) testing:**

PSA is a glycoprotein secreted into the cytoplasm of benign and malignant prostatic cells (Porth. 2011). It is organ- but not cancer-specific, therefore it may be elevated in BPH, prostatitis and other non-malignant conditions. As an independent variable, PSA is a better predictor of cancer than DRE or transrectal ultrasound (Mottet *et al.* 2015)

## **2.8 Treatment of prostate cancer:**

Treatment choice in prostate cancer is based on initial PSA level, clinical stage of disease and Gleason score, together with baseline urinary function, comorbidities and patient age.

### **2.8.1 Radical prostatectomy:**

Radical prostatectomy is the most commonly used treatment for clinically localized prostate cancer. Use of PSA-based screening has increased the rate of organ-confined disease at time of diagnosis, increasing the chances of successful outcomes after surgery. Main complications are incontinence and erectile dysfunction due to operative damage to urinary sphincter and erectile nerves. Bilateral nerve-sparing prostatectomy can preserve erectile function in many men with normal preoperative function (Keyes *et al.* 2013)

## **8.2 Radiation therapy:**

Radiotherapies are considered as the second therapeutic modalities for localized high-risk prostate cancers. Brachytherapy and external-beam radiotherapy are the widely used strategies. Brachytherapy refers to the placement of radioactive sources inside or adjacent to the cancer. There are 2 forms of brachytherapy: low-dose brachytherapy where radioactive seeds are permanently

implanted into the prostate, and high-dose brachytherapy where treatment is administered over about 10 minutes through temporary catheters that contain the radioactive source (Keyes *et al.* 2013). External-beam radiotherapy may be effective to patients without distant metastases, and a life expectancy of at least 5-10 years (Chen and Zhao. 2013)

### **2.8.3 Androgen deprivation therapy:**

Androgen deprivation therapy is frequently used as the primary treatment for locally advanced and metastatic prostate cancer. It is also used as neoadjuvant and adjuvant therapy in combination with surgical or radiation therapy. Inhibition of various hormones, receptors, or enzymes along the androgen production pathway is the basis of this treatment. Some of the medications used for androgen deprivation include estrogens, Gonadotropin releasing hormone (GnRH) agonist, GnRH antagonist, androgen receptor blockers, 5-alpha reductase inhibitors, adrenal androgen inhibitors and some others (Chen and Zhao. 2013).

### **2.9 Prostate specific membrane antigen (PSMA):**

Prostate-specific membrane antigen (PSMA) is a 100 kDa type II transmembrane glycoprotein with folate hydrolase and neurocarboxypeptidase activity (Gordon *et al.* 2008). The PSMA gene is located on the short arm of chromosome 11. Two molecular forms of the protein, designated PSMA and PSMA', were identified. It was first identified by the monoclonal antibody 7E11 that recognizes an intracellular epitope of PSMA. It is expressed by the prostatic epithelial cells as well as different extraprostatic tissue (Silver *et al.* 1997). It has also been found that PSMA is expressed in endothelial cells of neovasculature from a variety of tumors such as breast and renal cell carcinoma (Chang *et al.* 1999). In prostate, PSMA is expressed by benign and malignant prostatic epithelial cells with a higher extent of staining in the latter. It's also expressed in high-grade prostatic intraepithelial neoplasia (PIN). The expression in prostatic carcinoma correlates with the stage and Gleason score. PSMA is under investigation as a target of therapy in prostatic carcinoma and other solid tumors, given its expression by the neovasculature of extraprostatic tumors (Dabbs. 2014).

### **2.10 Previous studies:**

Mhaweck-Fauceglia *et al.* 2007, aimed to determine the expression of PSMA in normal tissues and in 3161 benign and malignant tumors and subsequently to define its sensitivity and specificity in prostatic adenocarcinoma. It concluded that despite the expression of PSMA by subsets of various types of malignancies, PSMA is still considered to be fairly sensitive and highly specific for prostatic adenocarcinoma.



Bostwick *et al.* 1998, described PSMA expression in 184 cases of prostatic intraepithelial neoplasia (PIN) and adenocarcinoma. It concluded that PSMA was expressed in all cases of adenocarcinoma with the greatest extent and intensity observed in the highest grades. Expression of PSMA is clinically useful for identification of prostatic epithelium, particularly PIN or adenocarcinoma.

Sweat *et al.* 1998, compared the expression of PSMA in prostate adenocarcinoma and lymph node metastases in a large series of patients with node-positive cancer. They concluded that PSMA expression was greatest in primary cancer for both percentage and intensity of immunoreactive cells and that PSMA expression allows the identification of benign and malignant prostatic epithelium and may be a potentially valuable marker in the treatment of patients with prostate cancer.

Wright *et al.* 1995, assessed PSMA expression in normal, benign and malignant prostate tissue. A staining index was established to provide a quantitative measurement of PSMA expression. PSMA was found to be highly expressed in most of the intraepithelial neoplasia and the primary and metastatic prostate tumors evaluated.

# Chapter Three

## Materials and Methods

## Chapter three

### Materials and methods

#### 3.1 Materials:

Archived paraffin blocks were used in this study.

#### 3.2 Methods:

##### 3.2.1 Study design:

This was a retrospective analytical case control study aimed to detect the expression of prostate specific membrane antigen (PSMA) in prostate tumors using immunohistochemistry.

##### 3.2.2 Study area:

This study was conducted in Sudan University of Science and Technology in histopathology and cytology department and Omdurman Teaching Hospital, histopathology and cytology department during the period from October 2017 to January 2018.

##### 3.2.3 Study samples:

Formalin fixed paraffin embedded tissue blocks of patients' samples previously diagnosed as prostate tumors were used.

##### 3.2.4 Sample size:

Forty prostate tissue blocks were included in this study, 20 blocks were malignant and 20 ones were benign.

##### 3.2.5 Sample processing and immunostaining:

All 40 blocks were sectioned using a microtome and 4  $\mu\text{m}$  sections on coated slides were obtained and incubated in oven at 58 °C overnight. Sections were stained by enhanced polymer one step method (EPOS). They were deparaffinized by 2 changes of xylene for 5 minutes each, then hydrated through descending ethanol concentrations of absolute alcohol, 90%, 70% and 50% for 2 minutes each then washed in distilled water. Antigen retrieval was then performed by heating the slides in a water bath at 97°C in a coplin jar filled with citrate buffer (pH 9.0) for 30 minutes.

Then slides were left to cool at room temperature for 10 minutes. After that sections were incubated for 10 minutes in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity then washed in phosphate buffer saline (PBS, pH 7.2). Sections were then incubated in rabbit anti-PSMA for 30 minutes then washed in PBS for 5 minutes. Then sections were incubated for 30 minutes with polymer- HRP solution then washed in PBS for 5 minutes. After that the sections were incubated in freshly prepared DAB chromogen solution for 7 minutes, washed in buffer then water. Sections were then counterstained with Mayer's hematoxylin for 20 seconds then washed in water. Then blued in running tap water for 5 minutes. Then sections were dehydrated in ascending concentrations of alcohol and cleared with xylene. Finally they were mounted in DPX. All quality control measures were obtained during procedure.

### **3.2.6 Result interpretation:**

Sections were examined by the light microscope. PSMA showed brown membranous expression in prostatic epithelial cells. Positive and negative controls were included and examined first. Concerning positivity, more than 5 cells per field showing brown membranous expression was considered positive while less than 5 cells per field was considered negative.

### **3.2.7 Statistical analysis:**

The obtained results and variables were arranged in a master sheet, then analyzed by the SPSS program version 14.0. Frequencies, means and Chi-square test values were calculated.

### **3.2.8 Ethical consideration:**

All samples and information included in this study were collected after taking the permission from Omdurman Teaching Hospital administration.

# Chapter Four

## Results

## Chapter four

### Results

#### 4. Results:

The age of the study group ranged between 55-95 years. The mean age was 72 years. 15 (37.5%) patients aged under 70 years, while 25 (62.5%) patients aged above 70 years, as shown in table (4.1).

Out of the 40 samples included in this study, 20 (50%) were diagnosed as benign prostatic hyperplasia and the other 20 (50%) were prostatic adenocarcinoma with different grades, as shown in table (4.2).

PSMA showed negative expression in 21 (52.5%) samples while 19 (47.5%) showed positive expression, as shown in table (4.3).

18 out of the 20 benign prostatic hyperplasia were negative for PSMA while two were positive. 17 out of the 20 prostatic adenocarcinoma were positive for PSMA while three showed negative expression. The relation between the expression of PSMA and tumor type was statistically significant; it was overexpressed in prostatic adenocarcinoma than in benign hyperplasia (P value=0.000) as shown in table (4.4).

As different grades of prostate adenocarcinoma were studied, in well differentiated adenocarcinoma: 3 (15%) were negative for PSMA, while 2 (10%) were positive, in moderately differentiated adenocarcinoma: 5 (25%) were positive and none negative, in poorly differentiated adenocarcinoma: 10 (50%) showed positive expression, while none was negative. The relation is between PSMA expression and cancer grade was statistically significant; it increases with higher grades (P value=0.005) as shown in table (4.5).

Table 4.1: Frequency of age among study group

| <b>Age group/year</b> | <b>Frequency</b> | <b>Percentage</b> |
|-----------------------|------------------|-------------------|
| < 70                  | 15               | 37.5 %            |
| $\geq 70$             | 25               | 62.5 %            |
| Total                 | 40               | 100%              |

Table 4.2: Frequency of histopathological diagnosis among study group

| <b>Histopathological diagnosis</b> | <b>Frequency</b> | <b>Percentage</b> |
|------------------------------------|------------------|-------------------|
| Benign prostatic hyperplasia       | 20               | 50%               |
| Prostatic adenocarcinoma           | 20               | 50%               |
| Total                              | 40               | 100%              |



Table 4.3: Frequency of PSMA expression among study group:

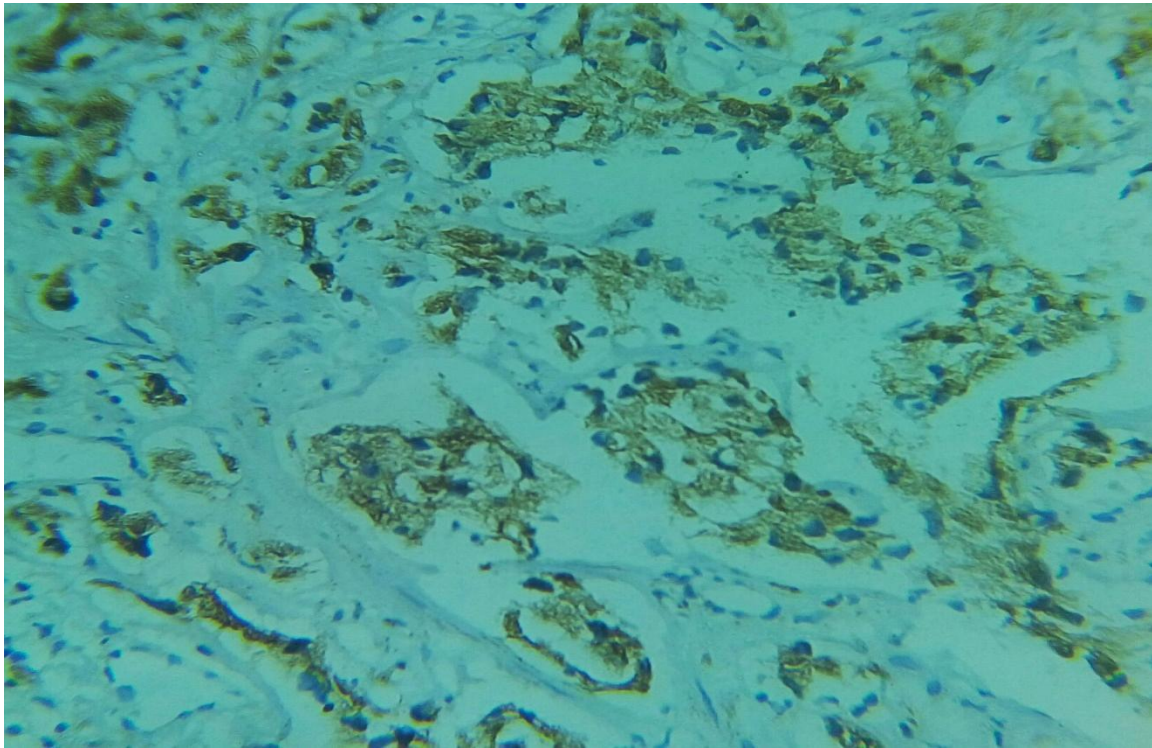
| <b>PSMA expression</b> | <b>Frequency</b> | <b>Percentage</b> |
|------------------------|------------------|-------------------|
| Negative               | 21               | 52.5%             |
| Positive               | 19               | 47.5%             |
| Total                  | 40               | 100%              |

Table 4.4: Relation between PSMA expression and tumor type:

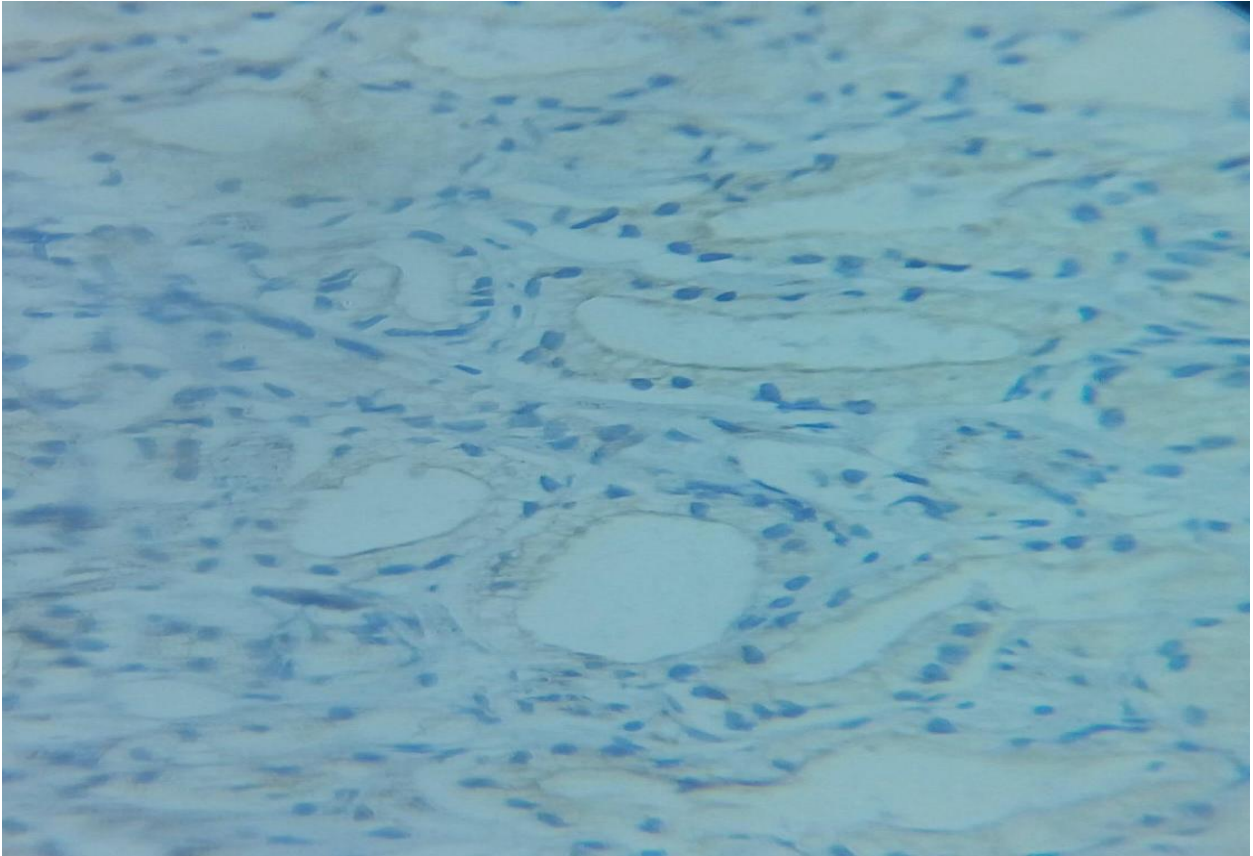
| Tumor type                   | PSMA expression |             | Total      | P. value |
|------------------------------|-----------------|-------------|------------|----------|
|                              | Negative        | Positive    |            |          |
| Benign prostatic hyperplasia | 18<br>45%       | 2<br>5%     | 20<br>50%  | 0.000    |
| Prostatic adenocarcinoma     | 3<br>7.5%       | 17<br>42.5% | 20<br>50%  |          |
| Total                        | 21<br>52.5%     | 19<br>47.5% | 40<br>100% |          |

Table 4.5: Relation between PSMA expression and grade of cancer:

| Cancer grade              | PSMA expression |           | Total      | P. value |
|---------------------------|-----------------|-----------|------------|----------|
|                           | Negative        | Positive  |            |          |
| Well differentiated       | 3<br>15%        | 2<br>10%  | 5<br>25%   | 0.005    |
| Moderately differentiated | 0<br>0%         | 5<br>25%  | 5<br>25%   |          |
| Poorly differentiated     | 0<br>0%         | 10<br>50% | 10<br>50%  |          |
| Total                     | 3<br>15%        | 17<br>85% | 20<br>100% |          |



Microphotography 4.1: Positive PSMA expression in prostatic adenocarcinoma. (x40)



Microphotography 4.2: Negative PSMA expression in benign prostatic hyperplasia. (x40)

# Chapter Five

## Discussion

## Chapter five

### Discussion

#### 5. Discussion:

Prostate cancer is the second most frequently diagnosed cancer in old men worldwide. It is the most common cancer in Sudanese men (Elamin *et al.* 2015). This study aimed to detect the expression of prostate specific membrane antigen in benign and malignant prostate tumors and its usefulness as a marker for differential diagnosis of prostate cancer.

Forty blocks obtained from patient's samples with prostate tumors were used to detect PSMA expression. The age of patients ranged between 55-95 years. The predominant age group was 70 and above representing 62.5%. This study showed that prostate cancer is common among old men which is supported with a study by Carter *et al.* (1990), who showed that 20% of men aged 50 to 60 years and 50% of men aged 70 to 80 years had histologic evidence of malignancy.

Prostate specific membrane antigen (PSMA) was found to be overexpressed in prostatic adenocarcinoma when compared to benign prostatic hyperplasia. These findings agree with a study by Marchal *et al.* (2004), who found that PSMA expression was significantly higher in prostatic carcinoma than benign prostatic epithelium and that overexpression was associated with a higher Gleason score. It also agrees with a study by Bostwick *et al.* (1998), who found an increase in PSMA staining from benign epithelial tissue to high-grade prostatic intraepithelial neoplasia to adenocarcinoma.

This study also found that the expression of PSMA correlates with the grade of cancer; as the expression increases with higher cancer grade. These findings were supported with a study by Ross *et al.* (2003), who described the correlation of primary cancer PSMA expression with disease recurrence and revealed that increased PSMA expression was associated with higher tumor grade.

# Chapter Six

## Conclusion and Recommendations



## **Chapter six**

### **Conclusion and recommendations**

#### **6.1 Conclusion:**

On the basis of this study, we conclude that PSMA is a useful marker for discriminating benignancy from malignancy in prostatic tumors. Its increasing with high grades makes it ideal for application as a prognostic marker.

#### **6.2 Recommendations:**

- Quantitative techniques for measuring expression and intensity of PSMA staining should be implemented.
- PSMA should be used as a differential and prognostic marker for prostatic cancer and for detection of metastases.

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## Appendices:

### Appendix (I): Solutions:

- ❖ Citrate buffer pH 9.0 (10mM citric acid, 0.05% Tween 20):

Citric acid (anhydrous)

Distilled water

Adjust pH with 1M NaOH

- ❖ Phosphate buffered saline (PBS), pH 7.2:

Sodium chloride (NaCl)

0.2 M sodium dihydrogen orthophosphate

0.2 M disodium hydrogen orthophosphate

Adjust pH with HCL

- ❖ 3% Hydrogen peroxide in methanol.
- ❖ Rabbit polyclonal anti-PSMA, 7 ml ready to use. (Zytomed systems).
- ❖ One-step polymer-HRP (BioGenex).
- ❖ DAB chromogen.
- ❖ Mayer's Hematoxylin :

Hematoxylin powder      1 g

Potassium alum            50 g

Sodium iodate             0.2 g

Chloral hydrate            50 g

Citric acid                  1 g

Distilled water             1000 ml

**Materials:**

- Microtome
- Oven
- Water bath
- Sensitive balance
- Light microscope
- Humidity chamber
- Forceps
- Coated slides
- Cover glass
- Gloves
- Safety box
- Cotton
- Gauze
- 20% alcohol
- Coplin jar
- Hydrophobic Pan pen

## Appendix (II): Kit leaflet

**ZYTO MED**  
SYSTEMS

### Rabbit anti-PSMA

**Cat. No. and Size:** **516-17330:** 0.1 ml immunogen affinity purified rabbit polyclonal antibody in PBS/1% BSA buffer pH 7.6 with less than 0.1% sodium azide.  
**516-17332:** 0.5 ml immunogen affinity purified rabbit polyclonal antibody in PBS/1% BSA buffer pH 7.6 with less than 0.1% sodium azide.  
**516-17334:** 1 ml immunogen affinity purified rabbit polyclonal antibody in PBS/1% BSA buffer pH 7.6 with less than 0.1% sodium azide.  
**516-17331:** 7 ml pre-diluted immunogen affinity purified rabbit polyclonal antibody in TBS/1% BSA buffer pH 7.6 with less than 0.1% sodium azide.

**Intended Use:** For research use only. Not for use in diagnostic procedures.  
**Clone:** N/A (polyclonal)  
**Immunogen:** Synthetic peptide derived from C-terminus of human PSMA  
**Ig Isotype:** Rabbit IgG  
**Epitope:** Not determined  
**Molecular Weight:** 84 kDa  
**Species Reactivity:** Human (tested). Bovine, Dog, Mouse, Pig, Rat (by sequence homology). Others not tested.

**Description:** Prostate Specific Membrane Antigen (PSMA) is a type II transmembrane glycoprotein belonging to the M28 peptidase family. Three functionally distinct proteins are encoded, including folylpolygamma-glutamate carboxypeptidase in the intestine, N-acetylated alpha-linked acidic dipeptidase 1 in the brain, and prostate-specific membrane antigen in the prostate. A mutation in the intestinal form may be associated with impaired intestinal absorption of dietary folates, resulting in low blood folate levels and consequent hyperhomocysteinemia. The form expressed in the brain may be involved in a number of pathological conditions associated with glutamate excitotoxicity. The prostate form is up-regulated in cancerous cells. This gene likely arose from a duplication event of a nearby chromosomal region.

**Applications:** Immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tissue sections

**IHC Procedure:** **Antibody Dilution:** If using the concentrate format of this product, dilute the antibody 1:100. The dilutions are estimates; actual results may differ because of variability in methods and protocols.  
**Antigen Retrieval:** Boil tissue sections in citrate buffer, pH 6.0 for 10 min followed by cooling at RT for 20 min.  
**Primary Antibody Incubation:** Incubate for 30 minutes at RT.

**IHC Positive Control:** Prostate carcinoma  
**Cellular Localization:** Membrane



**Storage and Stability**

Store at 2-8°C. Do not freeze. The user must validate any other storage conditions. Every antibody is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use the reagent beyond the expiration date. There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be tested simultaneously with unknown specimens.






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November 10, 2016

Rev: A1116

Doc: 516-17330\_17332\_17334\_17331

Explanation of the symbols on the product label:

|            |  |   |   |   |   |
|------------|--|---|---|---|---|
| <b>REF</b> | Bestellnummer<br>Catalog Number<br>Reference du catalogue  |  | Verwendbar bis<br>Use By<br>Utiliser jusque                             |    | Gebrauchsanweisung beachten<br>Consult Instructions for use<br>Consulter les instructions d'utilisation |
| <b>LOT</b> | Chargenbezeichnung<br>Batch Code<br>Code du lot  |  | Lagerungstemperatur<br>Temperature Limitation<br>Limites de temperature | <b>RUO</b>  | Nur für Forschungszwecke<br>For Research Use Only<br>Pour la recherche uniquement                       |
| <b>IVD</b> | In vitro Diagnostikum<br>In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro |  | Achtung<br>Warning<br>Attention   |  Hersteller / Manufactureur / Fabricant<br>Zytomed Systems GmbH • Anhalterstraße 16<br>14163 Berlin, Germany • Tel: (+49) 30-904 984 990<br><a href="http://www.zytomed-systems.de">www.zytomed-systems.de</a> |   |