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Detection of Bcl-2 among Sudanese Patients with Prostatic Cancer and Benign Hyperplasia by Immunohistochemistry Technique

A Thesis Submitted for Partial Fulfilment for Requirement of M.Sc. in Medical Laboratory Sciences, (Histopathology & Cytology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى:(الحمد لله رب العالمين (2) الرحمن الرحيم (3) مالك يوم الدين (4) إياك نعبد وإياك نستعين (5) أهدنا الصراط المستقيم (6) صراط الذين أنعمت عليهم غير المغضوب عليهم ولا الضالين (7)**)**

صدق الله العظيم

سورة الفاتحة

Dedication

I dedicate this research to my father, my mother, my wife, my sister, my children and my brothers.

Acknowledgement

First and foremost, I thank Allah for letting me live to see this dissertation through. I am forever indebted to Allah who support and give me power to do this dissertation.

I would like to thank my supervisor Dr. Ahmed Mohamed Ahmed, for his patience, continuous guidance throughout my dissertation with his knowledge.

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Abstract

This is retrospective analytical laboratory based study aimed to detect the expression of Bcl-2 marker in prostatic adenocarcinoma and benign prostatic hyperplasia among Sudanese patients using immunohistochemistry technique. 80 paraffin blocks of prostatic tumor collected for this study. Tissue Microarray of 40 samples were selected from paraffin blocks previously diagnosed as prostatic adenocarcinoma and other forty 40 benign prostatic hyperplasia were selected from Elrahama medical center. Patient data (age, diagnosis and tumor grading) were obtained from patients records. Age of study cases ranged from 50 to 80 years with mean age of 64.4 years, STD.D 7.6. Concerning Bcl-2 expression, the study revealed the marker expression was 0/40 sample of BPH, but it was positive in all samples of prostatic adenocercinoma 40/40. Strong positive in 16/40 representing (40%) of prostatic adenocarcinoma samples, moderate expressed in 9/40 representing (22.5%) and weak expressed in 15/40 representing (37.5%), and the study found a significant correlation between the histological grade of prostatic adenocarcinoma and Bcl-2 expression (p-value = 0.000), indicating that high expression of Bcl-2 increased with high grade of prostatic adenocarcinoma. The study found that, there is no significant correlation between age and Bcl-2 expression (P-value = 0.547).

The study found that, there is no correlation between prostatic adenocarcinoma and BPH in Bcl.2 immuno-expression, because the expression was negative in all BPH samples and positive in all prostatic adenocarcinoma samples.

المستخلص

أجريت هذه الدراسة التحليلية الوصفية المخبرية في مركز الرحمة الطبي شعبة الأنسجة والخلايا المريضة بولاية الخرطوم في الفترة من ابريل وحتى يوليو 2018.

تهدف الدراسة للكشف عن ظهور مستضد (ليمفوما الخلايا البائية 2) في أورام غدة البروستاتا باستخدام كيمياء الأنسجة المناعية.

جمع ثمانون قالب شمعي من عينات مرضي كانوا مشخصين مسبقا علي أنهم مصابون بأورام غدة البروستاتا,(40/80-50%) منهم كانوا مشخصين بأورام البروستاتا الخبيثة و(40/80-50%) كانوا مشخصين بأورام البروستاتا الحميدة.

جمعت الأربعين عينة الحميدة في قالب شمعي واحد باستخدام مصفوفات الأنسجة الصغيرة وكذلك الأربعين عينة الخبيثة, ثم قطعت المقاطع بسمك 3 مايكروميتر بواسطة المشراح الدوار وصبغت بطريقة كيمياء الأنسجة المناعية واستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة (22) لتحليل البيانات.

تراوحت أعمار المرضي في هذه الدراسة من 50 إلي 80 سنة بمتوسط عمر 64.3 سنة.

أظهرت الدراسة أن مستضد (ليمفوما الخلايا البائية 2) كان سالب الظهور في كل عينات أورام غدة البروستاتا الحميدة بينما كان موجب الظهور بنسب متفاوتة علي حسب المرحلة المرضية, فكان شديد الايجابية في 40/16 وتمثل 40% من عينات الورم الخبيث وكان متوسط الايجابية في 40/9 وتمثل 22.5% من عينات الورم الخبيث بينما كان ضعيف الايجابية في 40/15 وتمثل 375% من العينات.

أظهرت الدراسة علاقة ذات أهمية بين مستضد(ليمفوما الخلايا البائية 2) وأورام غدة البروستاتا الخبيثة وذلك علي حسب المرحلة المرضية أي انه كلما زادت شدة المرض زادت معها شدة ايجابية مستضد (ليمفوما الخلايا البائية 2) (قيمة P = 0.000).

أظهرت الدراسة عدم وجود علاقة مباشرة ذات أهمية بين أعمار المرضي وايجابية مستضد (ليمفوما الخلايا البائية 2) في أورام غدة البروستاتا، (قيمة P = 0.547).

أظهرت الدراسة أن العلاقة بين أورام غدة البروستاتا الحميدة والخبيثة في ظهور ايجابية مستضد (ليمفوما الخلايا البائية 2) كانت ثابتة وذلك لان مستضد (ليمفوما الخلايا البائية 2) كان سالب الظهور في كل عينات أورام غدة البروستاتا الحميدة.

أظهرت الدراسة إمكانية استخدام مستضد (ليمفوما الخلايا البائية 2) في أورام غدة البروستاتا الخبيثة لتحديد انذارية المرض.

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List of abbreviations

(BPH)	Benign Prostatic Hyperplasia.
(PC)	Prostate Cancer.
(IARC)	International Agency for Research on Cancer.
(RICK)	Radiation and Isotope Cancer at Khartoum.
(NCI-UG)	National Cancer Institute at University of Gezira.
(NCI)	National Cancer Institute.
(LUTS)	Lower Urinary Tract Symptoms.
(AI)	Androgen Independent.
(AIPC)	Androgen Independent Prostate Cancer.
(Bcl-2)	B-cell Lymphoma2
(DHT)	Dihydrotestosterone.
(BOO)	Bladder Outlet Obstruction.
(AUR)	Acute Urinary Retention.
(TURP)	Transurethral Resection of the Prostate.
(PSA)	Prostate Specific Antigen.
(DRE)	Digital Rectal Examination.
(TRUS)	Trans-Rectal Ultrasound Scan.
(BH)	Bcl-2 Homology.

(GS) Gleason Score.

(TMA) Tissue Microarray

(PBS) Phosphate Buffer Saline.

(DAB) 3,3 di amino benzidine tetrahydrochloride.

(DPX) Distyrene A plasticizer and Xylene

(SPSS) Statistical Package of Social Science.

Chapter One

Introduction

I. Introduction

Benign Prostatic hyperplasia (BPH) and prostate cancer (PC) is an escalating health burden in the western world [1]. According to world health organization, the lifetime risk of a man developing histologically confirmed (BPH) has been reported to be 50% in those in the 51-60 years age group and increasing to 70% in the 61-70 years age group [2]. With the exclusion of non-melanomatous skin malignancy, prostate cancer (PCa) is the second most prevalent cancer in men globally. The International Agency for Research on Cancer reported that PCa accounted for 14% of cancers diagnosed in men and over 900, 000 cases were diagnosed throughout the world in 2008 alone [1-2]. Prostate cancer has no national boundaries and may be found on all continents is adapted from the database of the International Agency for Research on Cancer (IARC), and represents the most up to date information on the incidence of prostate cancer around the world. The highest rates are from the United States, particularly among African American men. China has some of the lowest incidence rates. Among European countries, the incidence in Austria is notable, because there is wide variation within the country. Incidence rates are very high in the region of Tyrol compared to those reported from the eastern region. Tyrol has an organized, very thoroughly conducted screening program for prostate cancer. Incidence rates in the United States fluctuated during the last decade . We postulate that the great increase in incidence between the late 1980s and the mid 1990s were due to the large number of cases detected once Prostate Specific Antigen (PSA) became available and widely utilized. This increase was followed by a dip in the curves as most detectable tumors were identified. The current slow rise in

incidence, during the first half of the new century may be due to increased detection efforts with lower PSA thresholds and increased numbers of biopsy cores taken [3]. 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 ranked second among all cancers in both sexes after breast in 2012. Three decades ago, prostate cancer ranked tenth among all men cancers diagnosed at the Sudan Cancer Registry in 1978, less frequent than skin cancers and non Hodgkin lymphoma (n = 1036) . Moreover, prostate cancer represented only 0.8% of all male cancers (n = 10410) investigated at Radiation and Isotope Cancer at Khartoum (RICK) (1967–84) . This recent increase in comparison to decades ago could have been due to progress in diagnostic techniques introduced lately in the country. As a result, prostate cancer was the most diagnosed cancer among men accounting for 7.6% of all cancer types in men (n = 10911) at both (RICK) and National Cancer Institute at Gezira University (NCI-UG) during year 2000–2006 . Recently, prostate cancer was the most common cancer among male patients treated at the (NCI-UG) . It ranked first among cancer male patients (n = 268) treated in the National Cancer Institute (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 ± 9.25 [3]. Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate gland, which results from the progressive hyperplasia of stromal and glandular prostatic cells. BPH causes increased resistance to urine flow, leading to lower urinary tract symptoms (LUTS) including urinary hesitancy, frequent urination, urgency, thin urine flow and urinary retention, which are known to significantly affect the physical and mental health of patients as well as their quality of life [4]. Delayed treatment results in numerous severe

complications, such as bleeding from the prostate, recurrent infections, renal stones and even kidney failure [4].

Concerning prostate cancer, a large number of patients still present with extra-prostatic and therefore incurable disease. Since the work of Huggins in 1940, there have been no major therapeutic advances and androgen ablation remains the best treatment option for extra-prostatic androgen-responsive PC [5]. Eighty to ninety percent of PC patients respond well to this form of treatment initially. After a median time of approximately 2 years, however, relapse to an androgen-independent (AI) state occurs, followed by death after a further median 6 months. Androgen ablation is rarely curative [5]. The major molecular defect in extra-prostatic and (AI PC) is the inability of PC cells to initiate apoptosis in response to a variety of stimuli, including different forms of androgen ablation and Cytotoxic agents. The balance between cellular proliferation and cell death is regulated by multiple genes or families of genes through the cell cycle [5]. The exact mechanisms governing this intricate and complex process are as yet not fully understood. One family of genes involved in cell survival/death control is the Bcl-2 gene family, which consists of homologous proteins that function to regulate distal and crucial commitment steps of the apoptotic pathway [6]. The Bcl-2 family constitutes both agonists and antagonists of apoptosis that function at least in part through protein-protein interactions between various members of the family. The final outcome depends on the relative ratio of death agonists and antagonists. Bcl-2 expression has been closely associated with the AI phenotype of PC [6-7]. Cytotoxic chemotherapy may be used as palliative therapy in AI PC but has not been found effective. Most chemotherapeutic Cytotoxic agents induce apoptosis in cancer cells by direct and indirect action on the cell cycle. In vitro and in vivo studies have established that Bcl-2 expression confers an

antiapoptotic activity against androgen withdrawal and Cytotoxic chemotherapy. It thus offers a tempting potential target for therapeutic manipulations of PC [7]. Throughout this context, this study is an attempt to detect the expression of BCL-2 among patients diagnosed with benign prostatic hypertrophy and prostatic cancer in Sudan, 2018.

1.3 General objective:

To evaluate the expression of BCL-2 among Sudanese Patients with Prostate Cancer and BPH.

1.4 Specific objectives:

- To compare between prostatic cancer and BPH in Bcl-2 Immuno-expression.

To correlate the expression of Bcl-2 with tumor grade.

- To correlate between Bcl-2 immuno-expression and patients age with prostate cancer and BPH.

Chapter Two

Literature Review

2. LITERATURE REVIEW

2.1 THE PROSTATE GLAND

2.1.1 Background

A gland in the male reproductive system. The prostate surrounds the part of the urethra (the tube that empties the bladder) just below the bladder, and produces a fluid that forms part of the semen [8].

The prostate is a compound tubuloalveolar exocrine gland of the male reproductive system in most mammals. It differs considerably among species anatomically, chemically, and physiologically. The function of the prostate is to secrete a slightly alkaline fluid, milky or white in appearance, that in humans usually constitutes roughly 30% of the volume of the semen along with spermatozoa and seminal vesicle fluid [9]. Semen is made alkaline overall with the secretions from the other contributing glands, including, at least, the seminal vesicle fluid. The alkalinity of semen helps neutralize the acidity of the vaginal tract, prolonging the lifespan of sperm. The prostatic fluid is expelled in the first ejaculate fractions, together with most of the spermatozoa. In comparison with the few spermatozoa expelled together with mainly seminal vesicular fluid, those expelled in prostatic fluid have better motility, longer survival and better protection of the genetic material [8-9].

1.1.2 Anatomy of prostatic gland

The normal prostate gland is a part of the male reproductive system. It is a quite small, squishy gland and has nearly the same size and shape as a walnut. The normal gland is volume is about 20-30 cm3. It is located in front of the rectum, or the lower end of the bowel, and between the bladder and urogenital diaphragm .The urethra, the narrow tube that runs the length of the penis and that carries both urine and semen out of the body, runs directly through the prostate. Sitting just above the prostate are the seminal vesicles, two little

glands that secrete about 60% of the substances that makes up a thick white fluid called semen; running alongside and attached to the sides of the prostate are the nerves that control erectile function. The prostate gland is surrounded by a sheet of smooth muscle that helps expel semen during ejaculation**.** The prostate is divided into five histologically distinct lobes (anterior, posterior, median and two laterals) and three zones, a central, a peripheral and a transitional zone (10).

2.1.3 Prostate Function

During male seminal emission, sperm is transmitted from the vas deferens into the male urethra via the ejaculatory ducts, which lie within the prostate gland. Ejaculation is the expulsion of semen from the urethra. It is possible for some men to achieve orgasm solely through stimulation of the prostate gland, such as prostate massage or anal intercourse [11]. Prostatic secretions vary among species. They are generally composed of simple sugars and are often slightly alkaline. In human prostatic secretions, the protein content is less than 1% and includes proteolytic enzymes, prostatic acid phosphatase, beta-microseminoprotein, and prostate-specific antigen. The secretions also contain zinc with a concentration 500–1,000 times the concentration in blood [11]. To function properly, the prostate needs male hormones (androgens), which are responsible for male sex characteristics. The main male hormone is testosterone, which is produced mainly by the testicles. It is dihydrotestosterone (DHT), a metabolite of testosterone that predominantly regulates the prostate [12].

2.1.4 Benign prostatic hyperplasia

Benign prostatic hyperplasia (BPH), also known as benign prostatic hypertrophy, is a histologic diagnosis characterized by proliferation of the cellular elements of the prostate [13]. Cellular accumulation and gland

enlargement may result from epithelial and stromal proliferation, impaired preprogrammed cell death (apoptosis), or both. BPH involves the stromal and epithelial elements of the prostate arising in the periurethral and transition zones of the gland. The hyperplasia presumably results in enlargement of the prostate that may restrict the flow of urine from the bladder [14].

BPH is considered a normal part of the aging process in men and is hormonally dependent on testosterone and dihydrotestosterone (DHT) production. An estimated 50% of men demonstrate histopathologic BPH by age 60 years. This number increases to 90% by age 85 years [15]. The voiding dysfunction that results from prostate gland enlargement and bladder outlet obstruction (BOO) is termed lower urinary tract symptoms (LUTS). It has also been commonly referred to as prostatism, although this term has decreased in popularity [15]. These entities overlap; not all men with BPH have LUTS, and likewise, not all men with LUTS have BPH. Approximately half of men diagnosed with histopathologic BPH report moderate-to-severe LUTS [16]. Clinical manifestations of LUTS include urinary frequency, urgency, nocturia (awakening at night to urinate), decreased or intermittent force of stream, or a sensation of incomplete emptying. Complications occur less commonly but may include acute urinary retention (AUR), impaired bladder emptying, the need for corrective surgery, renal failure, recurrent urinary tract infections, bladder stones, or gross hematouria [17]. Prostate volume may increase over time in men with BPH. In addition, peak urinary flow, voided volume, and symptoms may worsen over time in men with untreated BPH. The risk of AUR and the need for corrective surgery increases with age [16-17].

Patients who are not bothered by their symptoms and are not experiencing complications of BPH should be managed with a strategy of watchful waiting. Patients with mild LUTS can be treated initially with medical therapy.

Transurethral resection of the prostate (TURP) is considered the criterion standard for relieving bladder outlet obstruction (BOO) secondary to BPH. However, there is considerable interest in the development of minimally invasive therapies to accomplish the goal of TURP while avoiding its adverse effects [18].

2.2.1 Pathology of Benign prostatic hyperplasia

Prostatic enlargement depends on the potent androgen dihydrotestosterone (DHT). In the prostate gland, type II 5-alpha-reductase metabolizes circulating testosterone into DHT, which works locally, not systemically. DHT binds to androgen receptors in the cell nuclei, potentially resulting in BPH. In vitro studies have shown that large numbers of alpha-1-adrenergic receptors are located in the smooth muscle of the stroma and capsule of the prostate, as well as in the bladder neck [19]. Stimulation of these receptors causes an increase in smooth-muscle tone, which can worsen LUTS. Conversely, blockade of these receptors can reversibly relax these muscles, with subsequent relief of LUTS [19]. Microscopically, BPH is characterized as a hyperplastic process. The hyperplasia results in enlargement of the prostate that may restrict the flow of urine from the bladder, resulting in clinical manifestations of BPH. The prostate enlarges with age in a hormonally dependent manner. Notably, castrated males (ie, who are unable to make testosterone) do not develop BPH [20]. The traditional theory behind BPH is that, as the prostate enlarges, the surrounding capsule prevents it from radially expanding, potentially resulting in urethral compression. However, obstruction-induced bladder dysfunction contributes significantly to LUTS [21]. The bladder wall becomes thickened, trabeculated, and irritable when it is forced to hypertrophy and increase its own contractile force. This increased sensitivity (detrusor over-activity), even with small volumes of urine in the bladder, is believed to contribute to urinary

frequency and LUTS. The bladder may gradually weaken and lose the ability to empty completely, leading to increased residual urine volume and, possibly, acute or chronic urinary retention [22]. In the bladder, obstruction leads to smooth-muscle-cell hypertrophy. Biopsy specimens of trabeculated bladders demonstrate evidence of scarce smooth-muscle fibers with an increase in collagen. The collagen fibers limit compliance, leading to higher bladder pressures upon filling [23]. In addition, their presence limits shortening of adjacent smooth muscle cells, leading to impaired emptying and the development of residual urine. The main function of the prostate gland is to secrete an alkaline fluid that comprises approximately 70% of the seminal volume. The secretions produce lubrication and nutrition for the sperm. The alkaline fluid in the ejaculate results in liquefaction of the seminal plug and helps to neutralize the acidic vaginal environment [24].

The prostatic urethra is a conduit for semen and prevents retrograde ejaculation (ie, ejaculation resulting in semen being forced backwards into the bladder) by closing off the bladder neck during sexual climax. Ejaculation involves a coordinated contraction of many different components, including the smooth muscles of the seminal vesicles, vasa deferentia, ejaculatory ducts, and the ischiocavernosus and bulbocavernosus muscles [25].

2.3 PROSTATIC CANCER

Prostate cancer is the most common noncutaneous cancer in men. Although prostate cancer can be a slow-growing cancer, thousands of men die of the disease each year [26]. It is the second most common cause of cancer death in males. Marked variation in rates of prostate cancer among populations in different parts of the world suggests the involvement of genetic factors. Familial predisposition also occurs. Environmental factors, notably diet, are also important [26-27].

At present, only three risk factors for prostate cancer have been firmly established; these are all nonmodifiable: age, race, and a positive family history of prostate cancer. However, numerous modifiable factors have also been implicated in the development of prostate cancer [27].

Currently, the majority of prostate cancers are identified in patients who are asymptomatic. Diagnosis in such cases is based on abnormalities in a screening prostate-specific antigen (PSA) level or findings on digital rectal examination [27]. Screening for prostate cancer is a controversial topic, in large part because of the conflicting findings from prospective, randomized studies. Education about the risks and benefits is important to help men make informed decisions regarding screening and, in those diagnosed with prostate cancer, the various treatment options [28].

2.3.1 Pathology of Prostatic cancer

Prostate cancer develops when the rates of cell division and cell death are no longer equal, leading to uncontrolled tumor growth. Following the initial transformation event, further mutations of a multitude of genes, including the genes for p53 and retinoblastoma, can lead to tumor progression and metastasis. Most prostate cancers (95%) are adenocarcinomas [29].

Approximately 4% of cases of prostate cancer have transitional cell morphology and are thought to arise from the urothelial lining of the prostatic urethra. The few cases that have neuroendocrine morphology are believed to arise from the neuroendocrine stem cells normally present in the prostate or from aberrant differentiation programs during cell transformation [30].

Squamous cell carcinomas constitute less than 1% of all prostate carcinomas. In many cases, prostate carcinomas with squamous differentiation arise after radiation or hormone treatment [30]. Of prostate cancer cases, 70% arise in the peripheral zone, 15-20% arise in the central zone, and 10-15% arise in the

transitional zone. Most prostate cancers are multifocal, with synchronous involvement of multiple zones of the prostate, which may be due to clonal and nonclonal tumors [31].

When these cancers are locally invasive, the transitional-zone tumors spread to the bladder neck, while the peripheral-zone tumors extend into the ejaculatory ducts and seminal vesicles. Penetration through the prostatic capsule and along the perineural or vascular spaces occurs relatively late. The mechanism for distant metastasis is poorly understood [32]. The cancer spreads to bone early, often without significant lymphadenopathy. Currently, 2 predominant theories have been proposed for spread: the mechanical theory and the seed-and-soil theory [33].

The mechanical theory attributes metastasis to direct spread through the lymphatics and venous spaces into the lower lumbar spine. Advocates of the seed-and-soil theory, however, believe that tissue factors that allow for preferential growth in certain tissues, such as bone, must be present [34]. Lung, liver, and adrenal metastases have also been documented. Specific tissue growth factors and extracellular matrices are possible examples. The doubling time in early stage disease is variable. In the majority of cases, doubling time is longer than 4 years. Only a small percentage of prostate cancers double in less than 2 years. Doubling time tends to accelerate as the tumor grows and becomes more aggressive. Larger tumors usually have a higher Gleason grade and a faster doubling time [35].

The natural history of clinically localized disease varies, with lower-grade tumors having a more indolent course and some high-grade lesions progressing to metastatic disease with relative rapidity. Given the typically slow progression of localized disease, several studies have examined the strategy of active surveillance in selected groups of patients [36].

2.3.2 Gleason score:

The Gleason score is used to help evaluate the prognosis of men with prostate cancer. Together with other parameters, the Gleason score is incorporated into a strategy of prostate cancer staging which predicts prognosis and helps guide therapy. The scoring system is named after Donald F. Gleason, M.D., a pathologist at the Minneapolis Veterans Affairs Hospital who developed it with other colleagues at that facility in the 1960s **[10].**

2.3.3 Gleason score remains an important tool.

A Gleason score is given to prostate cancer and is based exclusively on the architectural pattern of the glands of the prostate tumour. It evaluates how effectively the cells of any particular cancer are able to structure themselves into glands resembling those of the normal prostate. The ability of a tumour to mimic normal gland architecture is called its differentiation, and experience has shown that a tumour whose structure is nearly normal (well differentiated) will probably have a biological behavior relatively close to normal that is not very aggressively malignant. In addition, higher Gleason scores are given to cancer which is more aggressive. To assign a Gleason score, a piece of prostatic tissue must be obtained (a biopsy). This is done either by removing the entire prostate gland (prostatectomy) or by sampling the gland with a needle introduced through the rectum. A pathologist examines the biopsy specimen and attempts to give a score to the two patterns. G1: The cancerous prostate closely resembles normal prostate tissue. The glands are small, well-formed, and closely packed G2: The tissue still has well-formed glands, but they are larger and have more tissue between them. G3: The tissue still has recognizable glands, but the cells are darker. At high magnification, some of these cells have left the glands and are beginning to invade the surrounding tissue. G4: The tissue has few recognizable glands. Many cells are invading

the surrounding tissue. G5: The tissue does not have recognizable glands. There are often just sheets of cells throughout the surrounding tissue **[10].**

2.3.4 Diagnosis of prostate cancer

2.3.4.1 Digital Rectal Examination (DRE)

As the rectum (back passage) is close to the prostate gland, the doctor can feel for any abnormalities in the prostate by inserting a gloved finger into the rectum. If cancer is present in the prostate gland it may feel hard and knobbly, whereas with benign prostatic hyperplasia it is usually enlarged, firm and smooth. However, often the prostate may feel normal, even when cancer cells are present. The DRE cannot diagnose prostate cancer [9].

2.3.4.2 PSA test

The pronounced increase in incidence of prostate cancer in the last 20 years is probably primarily due to the widespread use of PSA testing [9]. The presence of prostate cancer may be indicated by an elevated PSA (prostate specific antigen) noticed during a routine checkup. PSA is a glycoprotein produced by the epithelial cells of the prostate gland. A blood test measures the amount of PSA circulating in the blood [9], expressed in nanograms per milliliter.

2.3.4.3 Trans-rectal ultrasounds scan (TRUS)

Ultrasound scans use sound waves to build up a picture of part of the inside of the body. In this test, a small cylindrical tube about the size of a finger is inserted into the anus. This is an ultrasound probe which emits high-frequency sound waves. These waves bounce back from internal structures and are computed. The computations allow an image to be produced on a screen. This image gives the radiologist an idea as to the outline of the prostate and the look of the internal structure of the gland. This type of scan is used to measure the size and density of the prostate [9]. A sample of cells (biopsy) can be

taken at the same time for examination under the microscope by a pathologist. The scan may be uncomfortable but it only takes a few minutes.

2.3.4.4 Biopsy

If the initial tests (rectal examination, PSA or ultrasound) show that there is a possibility of cancer, the patient may be offered a biopsy, in which several samples of tissue (usually around 10) are taken from the prostate to be looked at under a microscope. The biopsy is normally done at the same time as the ultrasound. A needle is passed through the wall of the back passage (rectum) and into the prostate. The specimens that are taken from the prostate are processed in the pathology department of the hospital [9].

2.3.4.5 CT and MRI scaning:

A CT (Computerized Tomography) scan can show whether the cancer has spread to the lymph nodes near the prostate. The patient may have this scan if there is a risk of his cancer spreading and he is considering active treatment options such as radiotherapy or radical prostatectomy. As with CT, the patient can have an MRI if there is a risk of his cancer spreading and he is considering active treatment options such as radiotherapy or radical prostatectomy. MRI (Magnetic Resonance Imaging) scans can also create a clear picture of the prostate gland. MRI uses magnetic fields rather than X-rays to create a detailed picture of the prostate and surrounding tissues. In addition, a substantial advantage of MRI, is that the use of non ionizing radiation for the creation of the images, minimizes the potential risk for the patient compared to CT scan. It also provides images in multiple imaging planes (not only in the transverse like CT) (9)

2.4 Bcl-2 proteins

2.4.1 Background

The Bcl-2 proteins are a family of structurally related proteins that serve as central regulators of intrinsic programmed cell death [37]. Bcl-2 proteins can be grouped into three subfamilies. Bcl-2 protectors protect cells against apoptosis. Bcl-2 killers (eg, Bax and Bak) are proapoptotic proteins that actively kill cells. Bcl-2 regulators (widely known as BH3-only proteins) promote cell killing by either interfering with the protectors or activating the killers. Bcl-2 proteins primarily regulate the release of death-promoting factors from mitochondria when cells receive signals that activate the intrinsic pathway [38].

C. elegans genetics identified a gene, ced-9, that protects cells against apoptosis and another gene, egl-1, that inactivates ced-9 protein and triggers apoptosis. In ced-9 mutants, many cells die during development that normally survive into the adulthood. A Ced-9 mutation kills the worm. Human Bcl-2 is functionally and structurally homologous to C. elegans Ced-9 and can substitute for it in living worms. This ability of a human gene to protect nematodes reveals that the fundamental machinery of apoptotic cell death has been conserved over great evolutionary distances [39].

Bcl-2 family members are defined by the presence of one to four short blocks of conserved protein sequence called BH (Bcl-2 homology) domains. Antiapoptotic Bcl-2 protectors typically have four of the domains. Proapoptotic Bcl-2 killers typically have three of these domains, while the Bcl-2 regulators have only the BH3 domain [39-40].

The BH3 domain is a short helix that fits into a groove on the surface of both Bcl-2 protectors and killers, forming complexes that regulate their activity. It is believed that the Bcl-2 protectors regulate the behavior of Bcl-2 killers by a

similar interaction. For example, the Bcl-2 protein forms a complex with a proapoptotic Bcl-2 killer called Bax, thereby interfering with the ability of Bax to kill cells. Binding of BH3-only proteins to Bcl-2 protectors can inactivate their antiapoptotic functions. Egl-1 is a BH3-only protein and this is how it triggers apoptosis. A new generation of BH3 mimetic drugs induces apoptosis of cancer cells by mimicking this second mechanism [41]. Genetic experiments in mice revealed several different functions for Bcl-2 family members. Mice born without Bcl-2 have deficiencies of the immune system that are best understood if one role of this protein in vivo is to render lymphocytes resistant to proapoptotic signals during immune system maturation [40]. Mice lacking another pro-life family member, Bcl-xL, die during embryogenesis, apparently as a result of widespread death of neurons in the central and peripheral nervous systems and hematopoietic cells in the liver. In contrast, loss of the killers Bax plus Bak makes cells highly resistant to apoptosis by a wide variety of intrinsic pathway stimuli [39-40].

2.4.2 The BCL-2 family of proteins

The BCL-2 family of proteins is known as an important gatekeeper to the apoptotic response. This group of structurally related proteins comprises pro-apoptotic and anti-apoptotic members that interact with one another [41]. Short sequences of amino acids common to BCL-2 and other members of this protein family are known as BCL-2 homology (BH) motifs. At least 1 BH motif is contained in each of the BCL-2 family members. These motifs, in part, contribute to the function of each member [42].

The BCL-2 family members can be classified into 3 functional groups: anti-apoptotic proteins such as BCL-2, pro-apoptotic effectors, and pro-apoptotic activators. Preclinical data suggest that activators, which contain only a single BH3 motif, are important mediators in the cellular response to stresses such as

DNA damage [43]. Effectors are those BCL-2 proteins closely associated with the mitochondrial membrane, and when stimulated by BH3-only activators, promote the formation of pores in the mitochondrial membrane, initiating the apoptotic program [44]. Apoptosis-promoting effects from both effectors and activators are inhibited by direct interaction with anti-apoptotic BCL-2 family members. In preclinical models, BCL-2 binds and sequesters BH3-only activators and prevents them from interacting with the pore-forming effectors [44-45]. Likewise, BCL-2 can directly influence effectors to prevent mitochondrial pore formation. The dynamic balance that occurs between anti-apoptotic members, such as BCL-2, and pro-apoptotic members helps determine whether the cell initiates apoptosis [45].

2.4.3 BCL-2 and cancers

Similar to oncogene addiction, in which tumor cells rely on a single dominant gene for survival, tumor cells may also become dependent on BCL-2 in order to survive. In response to stress signals, malignant cells may express pro-apoptotic activators. Some cancer cells overexpress BCL-2 which can dampen this pro-apoptotic response [46]. The result is in many cases an abundance of pro-apoptotic activators bound and sequestered by BCL-2. In this scenario, cancer cells are thought to be “primed” for apoptosis, in that they may contain sufficient amounts of the pro-apoptotic activators, if displaced from BCL-2, to induce programmed cell death. Cancers that depend on BCL-2 for survival in this way are likely to be sensitive to BCL-2 modulation [46].

2.5 BCL-2 expression in BPH and prostate cancer

2.5.1 Approach

The bcl-2 gene family encodes a group of homologous proteins forming two functionally antagonistic groups that regulate distal and crucial commitment steps of the apoptotic pathway, often through protein-protein interaction. The

bcl-2 gene encodes a 26 kDa protein, a potent blocker of apoptosis, expressed in several epithelial tissues [47]. In the normal prostate, bcl-2 expression is limited to basal epithelial cells, which are resistant to the effects of androgen deprivation. In prostate tumors, Overexpression of the bcl-2 protein has been associated with the development of hormone-refractory advanced disease, increased tumor stage and poor outcome [48]. The pro-apoptotic bax protein also belongs to the bcl-2 family and resides in the cytosol or is loosely attached to cell membranes. In response to Cytotoxic signals, bax translocates into the mitochondria, where it triggers cytochrome c release which can be blocked by bcl-2. Released cytochrome c then activates the pathway of capiases which eventually cause DNA fragmentation. While not dependent on each other for their individual functions, bcl-2 and bax do share homology and each may heterodimerise to antagonise their effect on each other [48].

2.5.2 Benign prostatic hyperplasia

Although the pathogenesis of BPH remains unclear, the reduction of cell apoptosis, which leads to the increase in the total number of stromal and epithelial cells, has been strongly associated with the development of BPH. Mitochondrial-dependent pathway is the most common apoptotic pathway in vertebrate animal cells (7–9) [49]. The mitochondrial membrane permeabilization, accompanied by the collapse of electrochemical gradient across the mitochondrial membrane, is one of the key events during cellular apoptosis [49]. This results in the release of numerous apoptogenic proteins from the mitochondria triggering the activation of aspartate-directed cysteine proteases (caspases) and eventually inducing apoptosis. Bcl-2 family proteins are key regulators of mitochondria-mediated apoptosis, including anti-apoptotic members such as Bcl-2 [50].

2.5.3 Prostate cancer

Prostate cancer progression and the development of androgen-independent prostate cancer have been largely related to a number of genetic abnormality that affect not only the androgen receptor but also crucial molecules involved in the regulation of survival or apoptotic pathways [51]. One of these molecules, the pro-survival protein BCL-2, has been associated with the development of androgen-independent prostate cancer due to its high levels of expression in androgen-independent tumors in advanced stages of the pathology. The upregulation of BCL-2 after androgen ablation in prostate carcinoma cell lines and in a castrated-male rat model further established a connection between BCL-2 expression and prostate cancer progression [52]. The way in which hormone ablation influences this survival pathway and the potential application of novel therapeutic strategies to overcome this anti-apoptotic mechanism is examined [52].

2.6 RELEVANT STUDIES

Ahmed H. Abdel-Rahman et al conducted an immunohistochemical study of Bcl-2 Protein and Estrogen Receptor-Alpha expression in benign prostatic hyperplasia and prostatic carcinoma - in Egypt [53]. This study was carried out on one hundred and twenty (120) specimens divided into two groups; group 1: Included forty cases of benign prostatic hyperplasia (BPH) and group 2: Included sixty cases of prostatic adenocarcinoma (PC) (22 were low grade; GS: 2-6 and 38 were high grade; GS: 7- 10),in addition to twenty cases of histologically normal prostates taken as controls. Immunohistochemical technique was applied to detect Bcl-2 as well as ER α positivity in all specimens. Group 1 showed the following profile: ER α (+) in all cases (100%), Bcl-2 (-) in 95%, ERα (+) / Bcl-2 (+) in 95%, ERα (-) / Bcl-2 (+) in 0%, ERα (+) / Bcl-2 (-) in 5% and ERα (-) / Bcl-2 (-) in 0% of cases while

group 2 showed the following profile: ERα (+) in 30%, Bcl-2 (+) in 21.7%, ERα (+) / Bcl-2 (+) in 15%, ERα (-) / Bcl-2 (+) in 6.7%, ERα (+) / Bcl-2 (-) in 15% and ERα (-) / Bcl-2 (-) in 70% of cases. The mean epithelial ER α - immunolabeling was, however, significantly increased in group 2 than in group 1 ($P < 0.05$) which, in turn, being higher than the normal cases ($P< 0.05$), however, the mean ER α immunolabeling revealed no significant correlation with T-stage ($P = 0.219$) or with the clinical stage ($P = 0.391$). In contrast, the Bcl-2 immunostaining was statistically higher in group 1 than in group 2 ($P < 0.05$) and showed a significant correlation with T stage ($P < 0.05$) although the study displayed no significant correlation between Bcl-2 immunopositivity and either Gleason score ($P = 0.125$) or the histological grade ($P = 0.146$). In addition, combined ER α (+)/ Bcl- 2 (+) immunoreactivity demonstrated the aggressive subgroup of PC cases more accurately than either ER α (+) or Bcl-2 (+) alone. Finally, multivariate analysis showed that the Bcl-2, proved to be an independent prognostic indicator ($P < 0.05$). Thus, the immunohistochemical expression of ER α and Bcl-2 protein in prostatic tissue may aid in better understanding the biology and genesis of both prostatic hyperplasia and carcinoma [53].

Wang L et al conducted a study in China assessed the expression of Bcl-2, in benign prostatic hyperplasia and its significances [54]. In this study, all specimens were obtained from patients undergoing surgical resection of the prostate. The study found that the expression of and Bcl-2 was significantly higher in BPH group ($P<0.05$). The study concluded that BPH can up-regulate Bcl-2 expression. Prostatitis appeared to play an important role in the development of BPH by affecting the proliferation and apoptosis of the prostatic cells [54].

Other Japanese study by Matsushima H. et al, addressed the role of Bcl-2 in prediction of in prostatic carcinoma [55]. The study found that Bcl-2 positivity was found in 20% of 146 prostatic carcinomas. Bcl-2 positivity was found only in 5%. They were expressed almost reciprocally in the tumors. Bcl-2 positivity correlated with high T stage. Bcl-2 positivity correlated with poor survival and short progression-free period. Multivariate analysis revealed that bcl-2 positivity was an independent prognostic indicator ($p = 0.001$). The study concluded that Bcl-2 was almost independently expressed in prostatic cancer. It is correlated with malignant phenotypes of prostatic cancer. The data from this staining further improved the ability to predict the patient prognosis [55].

Rocz Akad et al conducted a study in Poland to investigate the Bcl-2 immunohistochemical detection in prostate cancer [56]. In this study, tumors from 28 patients were assessed by immunohistochemistry. Tissue sections were fixed in 10% buffered formaldehyde solution, embedded in paraffin and stained immunohistochemically with the anti-human Bcl-2 antibody (Dako/Clon124). The immunolocalization of Bcl-2 was performed using the Labeled Streptavidin Biotin (LSAB) method. No correlation was found between Bcl-2 protein expression and pT stage, lymph node metastases, Gleason score, seminal vesicles invasion, positive or negative resection margins as well as capsular penetration and preoperative PSA serum level [56].

A study from Switzerland investigated the prognostic significance of Bcl-2 in clinically localized prostate cancer by L. Bubendorf et al [57]. To study the prognostic significance of Bcl-2 overexpression in prostate cancer, 137 consecutive radical prostatectomy specimens were examined by immunohistochemistry. Bcl-2 was associated with malignant phenotype. Bcl-

2 expression was more frequent in pT3 tumors (31% positive) in pT2 tumors (5% positive, P = 0.001). Survival analysis showed that Bcl-2 expression (P = 0.03), high Ki67 LI (P = 0.018), high grade (P = 0.0037), advanced local stage (P = 0.0005), and positive lymph nodes (P = 0.026) were predictors of progression. Prognosis was best in Bcl-2-negative tumors with low Ki67 LI, worst in Bcl-2-positive tumors with high Ki67 LI, and intermediate in the remaining tumors (P = 0.03). These data suggest that altered expression of Bcl-2 play a role in prostate cancer progression. Analysis of factors regulating both apoptosis and cell proliferation may be relevant in prostate cancer [57].

A study from France by Colombel et al. aimed to assess the expression of the anti-death oncoprotein bcl-2 among BPH patients [58]. Ten prostate glands from normal men (mean age 43.7 years) were sampled according to McNeal's zonal anatomy, in addition to 30 prostate adenomas obtained from prostatectomy specimens from symptomatic patients (mean age 61.4 years). Tissue samples were fixed in formalin and embedded in paraffin. Proliferation and bcl-2 expression were assessed by immunostaining using Mib-1 and anti-bcl-2 antibodies, while apoptotic bodies were specifically stained using in situ nick translation. The percentage of positive cells was determined by optical microscopy. In normal epithelium, the rates of proliferation and apoptosis were increased in the peripheral zone (Mib-1 1.7%, apoptotic bodies 3.3%) compared with the central (0.2% vs 1.4%) and transition (0.1% vs 1.8%) zones. Proliferation was significantly greater in BPH than in normal prostate tissue (3.7%), contrasting with a stable rate of apoptosis (1.4%). In the normal prostate, bcl-2 was expressed by glandular and basal cells in the peripheral zone. In the central zone, bcl-2 was overexpressed in basal cells and in most glandular cells of the intraluminal ridges. Bcl-2 expression in the transition zone was limited to disperse basal cells. In BPH, bcl-2 was strongly expressed

by basal cells in mature glandular formations and in most cells of young small nodules. BPH may result from both an increase of proliferation within the basal compartment and a failure of apoptosis to counterbalance basal cell proliferation. Increased expression of bcl-2 may participate in this process by blocking apoptosis [58].

Other review by Marc C. et al surveyed a series of 62 human tissues to evaluate whether bcl-2 might have a role in the developing prostate gland or in prostate oncogenesis [58]. While a primordial epithelial cells in prostate gland immune-stained for bcl-2, normal and hypertrophic prostate glands of the adult show bcl-2 expression restricted to the basal cells. AU epithelial cells in areas of prostatic intraepithelial neoplasia were stained by this antibody, as were most (62%) localized invasive prostatic carcinomas. In contrast, primary prostatic carcinomas and metastases obtained from metastatic prostate cancer patients after hormone treatment (hormone-refractory tumors) stained positive for bcl-2. This study demonstrates that the oncoprotein encoded by bcl-2 can be detected at sequential stages in the natural history of human prostate cancer. Since the bcl-2 oncoprotein is known to suppress the cellular response to apoptotic stimuli, it will be important to determine whether bcl-2 expression is a factor in the development of prostate cancers and in the survival of hormone-refractory prostate cancer cells [59].

Other study assessed the apoptosis-related gene expression in benign prostatic hyperplasia and prostate carcinoma by Iacopino F et al in Italy [59]. This study found that in BPH, bcl-2 gave the weakest signals ($p < 0.001$). In CaP, bcl-2 was the least expressed gene ($p < 0.001$). Bcl-2 were expressed at higher levels in CaP than in BPH ($p < 0.02$). Bcl-2 was more highly expressed in high Gleason grade (> 7) tumours ($p < 0.05$). The study concluded that all

the apoptosis-related genes were expressed in BPH. The stronger expression the higher bcl-2 levels appear to counterbalance the tendency to cell death [60].

Other study in US by Saker Z etal, aimed for evaluation of the activity of these markers in different cells of BPH, PCa and hormonally treated prostate cancer (CRPCa) tissues [60]. Activity of the markers has been evaluated in: 39 BPH, 28 prostate cancer (PCa) and 10 castration resistant PCa (CRPCa) tissues. Possible association of intensity of the expression with the disease clinical parameters has been assessed. This difference has been detected in epithelial and vascular prostatic cells. Epithelial activity of Bcl-2 was significantly lower in BPH as compared with PCa and CRPCa. This pilot study has shown importance of apoptosis markers in BPH and PCa. It is the first study showing complex interrelation between apoptosis and cell cycle regulating proteins in BPH and PCa [61].

A study from US by investigated expression of surviving and apoptotic biomarkers in benign prostatic hyperplasia including bcl-2 by Shahrokh f., et al. [62]. The study found that the Surviving and Bcl-2 expression increased incrementally from normal prostate to epithelial BPH to stromal BPH. Caspase-3 expression was higher in BPH epithelium than in BPH stroma, which in turn was higher than that in normal prostate. Ki-67 was significantly over expressed in BPH stroma and epithelium. Survivin expression in BPH tissue correlated with International Prostate Symptom Score, quality of life, post-void residual urine volume, maximum urine flow rate and transforming growth factor-β1 expression. They concluded that the surviving is over expressed in BPH and it correlates with BPH parameters. Increases in proliferation and inhibition of apoptosis have a role in BPH [62].

A study by Leitzmann M., et al. showed that Prostate cancer incidence strongly increases with age. Based on US Surveillance, Epidemiology and End Results Program4 statistics from 2000–2008, the incidence rate of prostate cancer is 9.2/100,000 for men aged 40–44 years. That rate increases sharply to 984.8/100,000 in men aged 70–74 years, after which it slightly decreases (63).

A study by Chakravarthi, S., et al. aimed to examine the expression of apoptosis-regulating genes bcl-2 and p53 using immunohistochemistry, and the Gleason score in core needle biopsy specimens of prostate adenocarcinoma. Overexpression of bcl-2 was determined in 54 of the 80 cases (67.5%), of which 18 were in grade III, 16 in grade II and 20 in grade I. The relationship between increased levels of Bcl-2 and Gleason score was statistically significant ($p<0.0014$) (64).

Chapter Three
Material and Methods

3. Materials and Methods

3.1 Materials:

Archived tissue block obtained from prostatic tissue samples previously diagnosed as prostatic adenocarcinoma and benign prostatic hyperplasia were selected for this study.

3.2. Methods:

3.2.1 Study design:

This is retrospective analytical laboratory based study aimed to detect the expression of Bcl-2 marker in prostatic adenocarcinoma and Benign prostatic hyperplasia among Sudanese patients using immunohistochemistry technique.

3.2.2 Study samples:

80 samples of paraffin blocks of prostatic tumor were selected for this study. Tissue Microarray of 40 samples were selected from paraffin blocks previously diagnosed as prostatic adenocarcinoma and other TMA of 40 benign prostatic hyperplasia were selected from Elrahama medical center according to my facilities. Patient identification (age, grad and diagnosis) were obtained from patients records.

3.2.3 Study area:

This study was conducted at Elrahama medical center department of histopathology and cytology in Khartoum state.

3.2.4 Immunohistochemistry staining:

The immunohistochemical procedure was done as follows: TMAs sections were cut at (3µm) from formalin-fixed, paraffin-embedded tumors, mounted onto positively charged slides (Thermo). Following deparaffinization in Xylene, slides were rehydrated through a graded series of alcohol and were placed in distilled water. Samples were steamed for antigen retrieval for Bcl-2 using high PH (9) by water bath at 95C for 40 min. After washing with

phosphate buffer saline (PBS) for 3 min. Endogenous peroxides activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and After washing with PBS for 3 min then Slides were incubated with (100 µ L) of (mouse monoclonal antibody (Bcl-2 Dako), for 30 min at room temperature in a moisture chamber. After washing with (PBS) for 3 min, binding of antibodies will be detected by incubating for 20 min with dextrin labeled polymer (Dako). Finally, the sections washed in three changes of (PBS), followed by adding 3, 3 di amino benzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distilled water for 3 min Slides were counter stained with haematoxylin (MAYER'S) for one min and then washed in running tap water for several minutes 7-10 (bluing), then dehydrate ,cleaned and mounted in DPX.
Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

3.2.5 Result interpretation:

Detection of more than 5 cells with brown cytoplasm per one field considered as positive result.

3.2.6 Data analysis:

The obtained results and variables arranged in standard master sheet, then analyzed using statistical package for social science (SPSS) program. Frequencies, means and Chi squire tests were calculated.

3.2.7 Ethical consideration:

The sample were collected after permission according to the laboratory guidelines and regulation.

Chapter Four

Result

4. Result

The study includes 80 samples, 40 samples were previously diagnosed as prostatic adenocarcinoma and another 40 samples were diagnosed as BPH. The age of the study cases ranged between 50 to 80 years with mean age of 64.3 years, S.D 7.6.

Table (4.1)**:** Distribution of patients according to age group.

Age	Frequency	Percent
50-60 years	29	36
61-70 years	35	44
71-80 years	16	20
Total	80	100

The above table showed more frequency in patients age group (61-70) years was represented 44% from the total.

Table (4.2)**:** Distribution of Bcl-2 expression in prostatic adenocarcinoma samples:

Bcl-2 expression	Frequency	Percent
Strong positive	16	40.0
Moderate positive	9	22.5
Weak positive	15	37.5
Total	40	100

The above table showed the expression of Bcl-2 was positive in all prostatic adenocarcinoma samples. Strong positive in 16/40 representing (40%) of prostatic adenocarcinoma samples, moderate expressed in 9/40 representing (22.5%) and weak expressed in 15/40 representing (37.5%).

Table (4.3)**:** Correlation between Bcl-2 expression and histological grading in prostatic carcinoma:

Bcl.2 expression	Grade			Total	P.Value
	Grade I	Grade II	Grade III		
Strong positive	0(0.0%)	0(0.0%)	16(40.0%)	16(40.0%)	0.000
Moderate positive	0(0.0%)	9(22.5%)	0(0.0%)	9(22.5%)	
Weak positive	15(37.5%)	0(0.0%)	0(0.0%)	15(37.5%)	
Total	15(37.5%)	9(22.5%)	16(40.0%)	40(100.0%)	

The above table showed a significant correlation between histological grade of prostatic adenocarcinoma and Bcl-2 expression (P-value = 0.000), indicating that high expression of Bcl-2 increased with high grade of prostatic adenocarcinoma.

Table (4.4) Correlation between Bcl.2 Expression and age among the study cases with prostatic adenocarcinoma:

Bcl-2 expression	Age			Total	P.Value
	50-60 years	61-70 years	71-80 years		
Strong positive	4(10.0%)	7(17.5%)	5(12.5%)	16(40.0%)	0.547
Moderate positive	3(7.5%)	4(10.0%)	2(5.0%)	9(22.5%)	
Weak positive	1(2.5%)	9(22.5%)	5(12.5%)	15(37.5%)	
Total	8(20.0%)	20(50.0%)	12(30.0%)	40(100.0%)	

The above table showed there was insignificant correlation between patients age and Bcl-2 expression prostatic adenocercinoma samples (P-value =0.547).

Table (4.5): Correlation between Bcl-2 expression in BPH and prostatic adenocarcinoma in the study cases:

Bcl-2 / BPH	Bcl.2 / Adenocarcinoma			Total
	Strongly positive	Positive	Weakly positive	
Negative	16 (40.0%)	9 (22.5%)	15 (37.5%)	40(100.0%)
Total	16 (40.0%)	9 (22.5%)	15 (37.5%)	40 (100.0%)

The above table showed no correlation between prostatic adenocarcinoma and BPH in Bcl-2 expression, because the expression was negative in all BPH samples and positive in all prostatic adenocarcinoma samples.

Chapter Five
Discussion

5. DISCUSSION:

The present study included 80 samples of prostatic tissue, 40 were previously diagnosed as prostatic adenocarcinoma and another 40 diagnosed as BPH, stained by immunohistochemistry for Bcl-2 marker.

Concerning age, the study revealed that the majority of patients age lie between (61-80) years which represent 80% of samples, indicating that the prostate cancer most common in elderly more than young men**.** This result agrees with Leitzmann M., et al. (2012), they report that the prostate cancer incidence strongly increases with age.

Concerning Bcl-2 expression, the study revealed the marker expression was 0/40 sample of BPH, but it was strongly positive in 16/40 representing (40%) of prostatic adenocarcinoma samples, moderately expressed in 9/40 representing (22.5%) and weakly expressed in 15/40 representing (37.5%), and the study found a significant correlation between the histological grade of prostatic adenocarcinoma and Bcl-2 expression (p-value = 0.000), indicating that high expression of Bcl-2 increased with high grade of prostatic adenocarcinoma. This result agrees with Iacopino F., et al (2012), they reported that Bcl-2 was more highly expressed in high Gleason grade tumors ($p < 0.05$). Also agrees with Chakravarthi, S., et al. (2010), they reported that there a significant correlation between Bcl-2 expression and Gleason score (p-value = 0.0014).

The study found that, there is no significant correlation between patients age and Bcl-2 expression (P-value = 0.547).

The study found that, the correlation between Bcl-2 expression with prostatic adenocarcinoma and BPH is constant.

Chapter Six

Conclusion and Recommendations

6. Conclusion and Recommendation

6.1 Conclusion:

The study concluded that:

There was no correlation between Bcl-2 expression with prostatic adenocarcinoma and BPH, because the expression of Bcl-2 were negative in all BPH samples and positive in all prostatic adenocarcinoma samples.

Majority of patients age with prostatic adenocarcinoma were more than 60 years old.

There was a significant correlation between Bcl-2 expression and prostatic adenocarcinoma histological grade, but there was no correlation with patients age.

6.2 Recommendation:

Further studies should be carried in larger sample size.

Bcl-2 marker recommended to used as prognostic marker in prostatic adenocarcinoma.

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Appendices

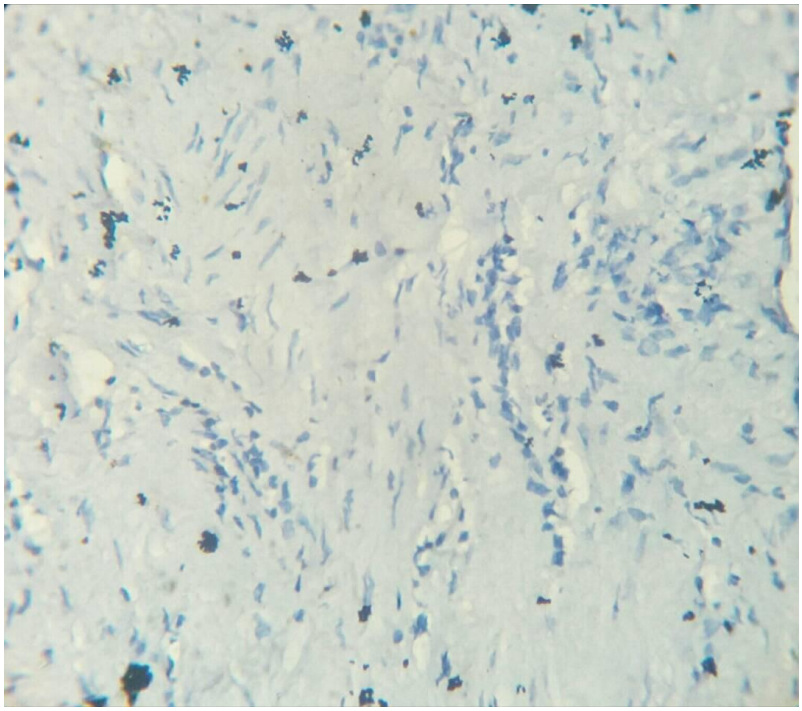
Appendix 1:

Materials and instrument used for processing and staining of the specimens include:

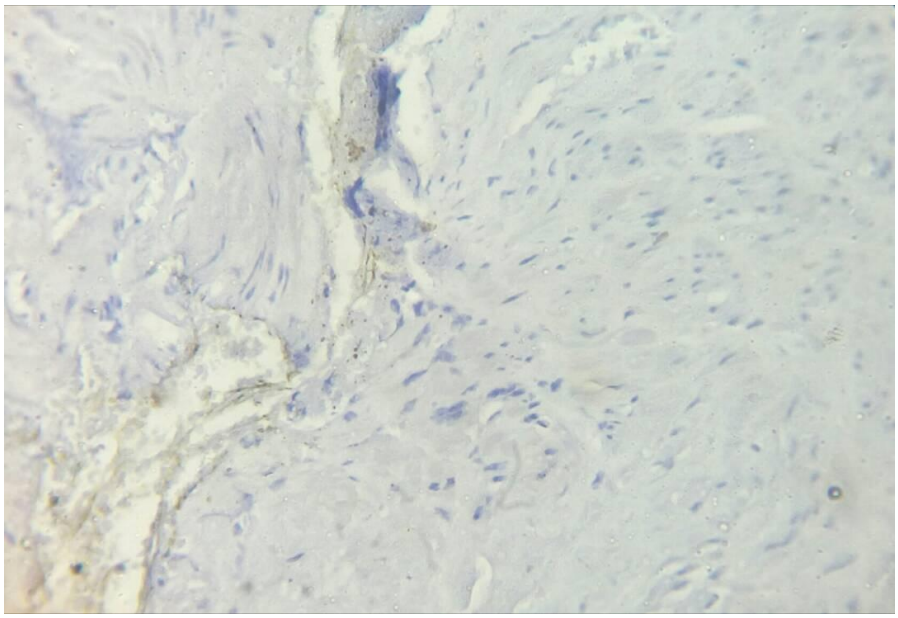
- Disposable gloves
- Microtome knife
- Rotary microtome
- Positively charged slide
- Cover glass
- Dry oven
- Water bath
- Embedding center
- Coplin jars
- Humidity chamber
- Ethanol (100%, 90%, 70%, 50%)
- Mayer s haematoxylin (haematoxylin , DW,K or ammonium alum ,sodium iodated ,citric acid ,chloral hydrate)
- Reaction buffer
- Primary antibody (Bcl-2)
- Tris EDTA buffer (PH9)
- Phosphate buffer saline (PH7.4)
- Peroxides blocker (3% hydrogen peroxide in methanol)
- Secondary antibody (dextran polymer conjugated secondary – HRP)
- DAB (3,3 di amino benzidin tetra hydrochloride) substrate solution

- Bluing Reagent (0.1MLi2 CO3 , 0.5 M Na2CO3)
- Xylene
- DPX mounting media

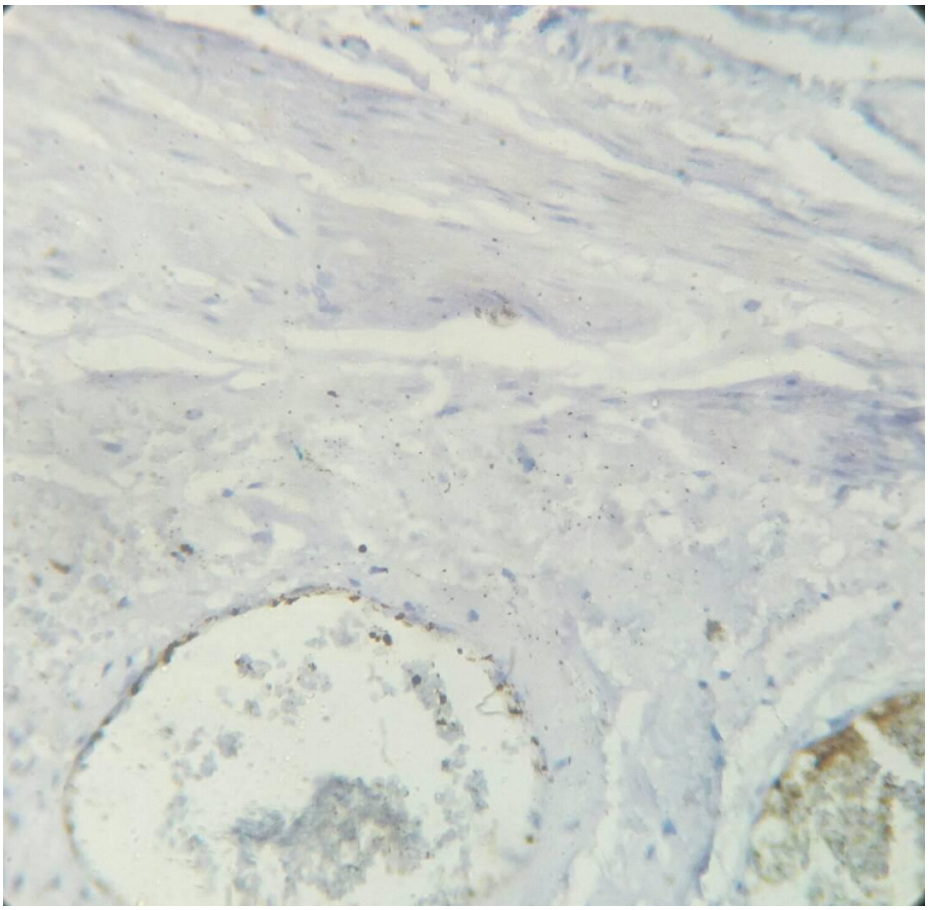
Microphotograph:(1) Shows the expression of Bcl-2 in BPH tissue are negative.



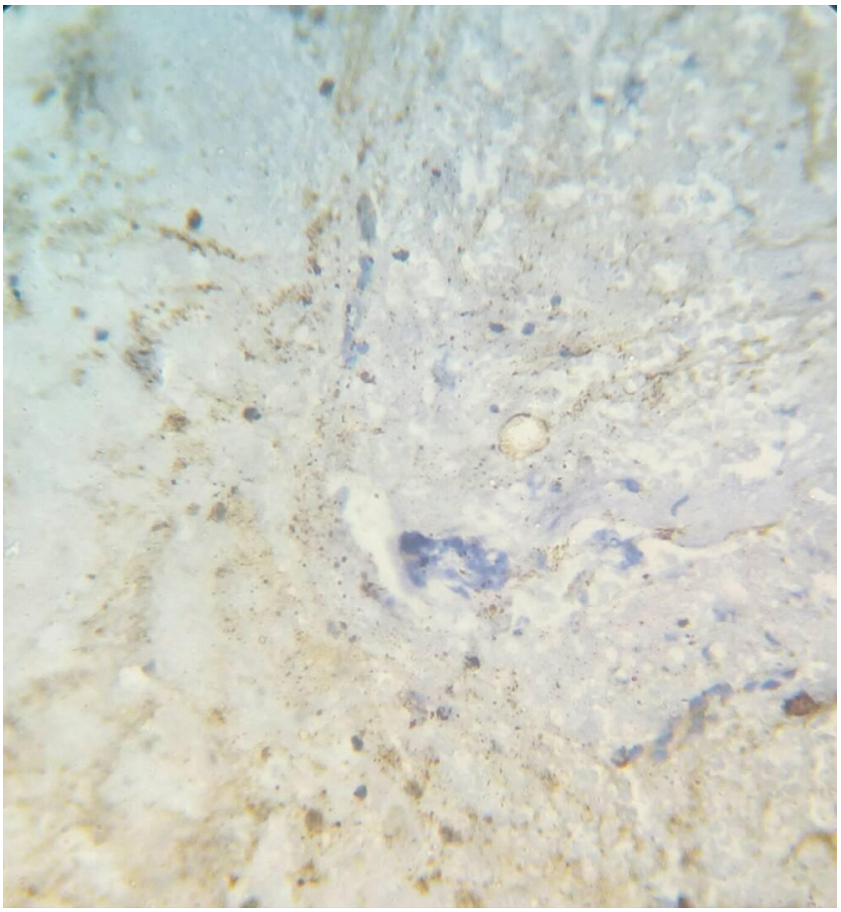
Microphotograph (2): Shows weak positive of Bcl-2 expression in prostatic adenocarcinoma grade I.



Microphotograph (3): Shows moderate positive of Bcl-2 expression in prostatic adenocarcinoma grade II.



Microphotograph (4): Shows strong positive of Bcl-2 expression in prostatic adenocarcinoma grade III.



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