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Histopathological study of prostate cancer in Libyan patients in Eastern region between years (2015 to 2018)

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This project submitted to Libyan International Medical University in partial fulfillment of the requirement for the Bachelor degree of Basic Medical Sience.

This project is dedicated to my Father, for believing I could do whatever I set out to accomplish.

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CONTENTS

LIST OF ABBREVIATIONS

AR	Androgens
GSTP1	Gutathione S-transferase
PSA	Prostate-specific antigen.
EZH-2	Enhancer of zeste-2
AMACR	Alpha-methylacyl-CoA racemase
PIN	Prostatic intraepithelial neoplasia
TURP	Transurethral resection of prostate
BPH	Benign Prostatic Hyperplasia
GnRH	Gonadotropin releasing hormone
LH	luteinizing hormone
FSH	follicle-stimulating hormone
BMC	Benghazi medical center
PAP	Prostatic acid phosphate

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Prostate cancer is more common in men over the age of 65 years and the second leading cause of death among men in the United States. We studied prostate cancer (n=239) with regards to age, histopathological types, grade, and stages between years (2015 to 2018) using H&E stained section. All cases were histologically adenocarcinoma with different grades. Most of the cases were ≥65 (90%), grade 1 (35.6%), stage IV (89.8%) and most of them presented with back pain (47.1%). Majority of cases show gradual decrease in PSA level after the patient underwent surgery, hormonal and chemotherapy (36.4%).

Chapter 1: Introduction

1.1 Anatomical structures of the prostate

The human prostate at the base of the urinary bladder is a walnut-sized organ. The prostatic urethra is enveloped by a fibromuscular glandular organ (Figures 1.1 and 1.2). The inverted coin, as it is located just below the urinary bladder, measures approximately 3cm in diameter and is located superiorly between the bladder neck and the urogenital diaphragm. (Figure 1.2).[1]

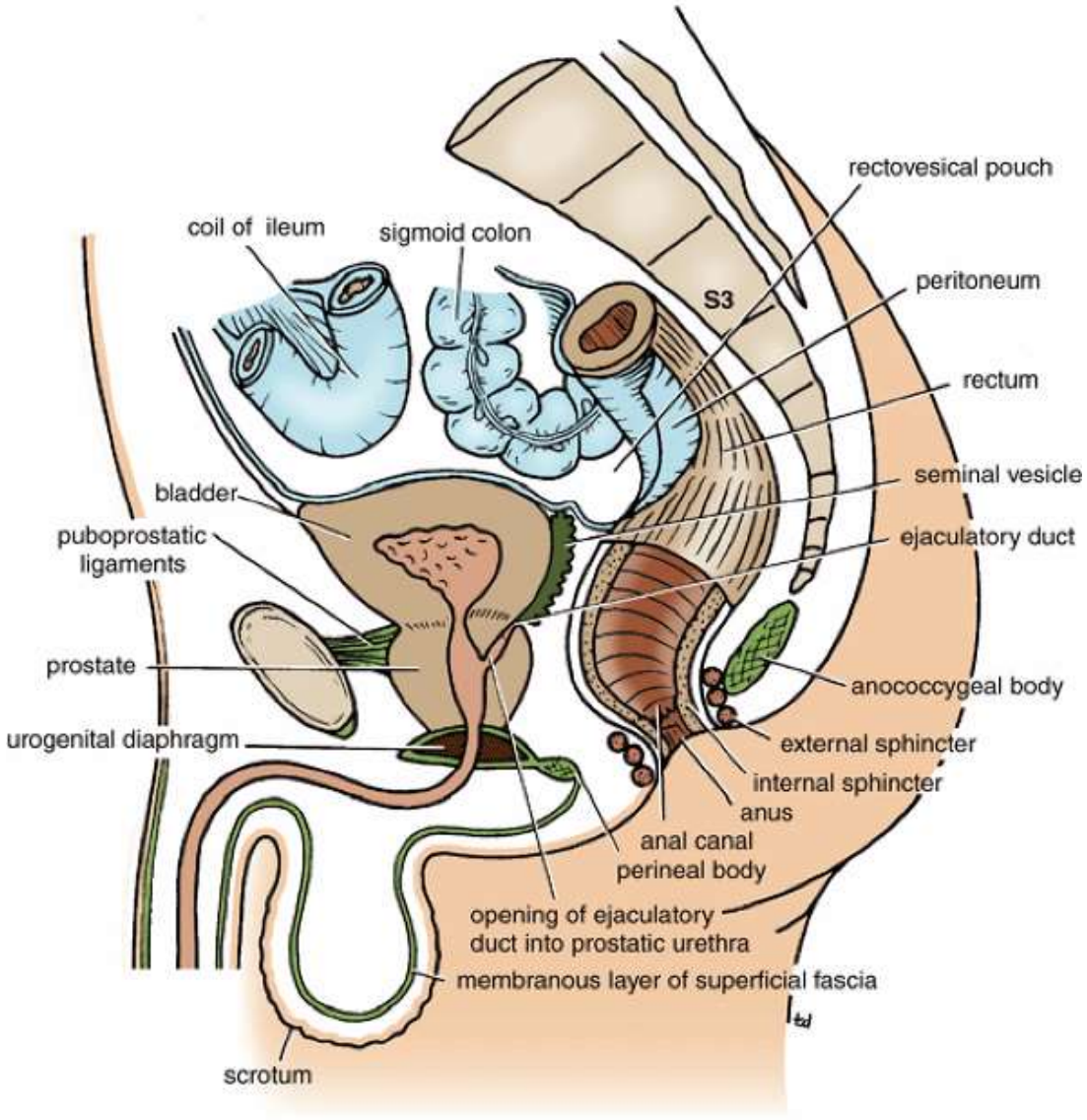

Figure 1.1: Sagittal section of the male pelvis.

1.1.1 Relations

Apex is associated with the bladder's neck (Figure 1.1). The base is linked to the urogenital diaphragm (Figure 1.2). Ventrally connected to the pubis symphony but separated by extraperitoneal fat and its dorsally associated rectum and separated from it by fascia denonvilliers Laterally, the prostate is connected to the dorsal aspect. (Figures 1.1 and 1.2).[2]

1.1.2 Structure of the prostate

Plentiful glands of the prostate are planted into a mixture of smooth muscle and connective tissue the ducts opens towards the prostatic urethra. The prostate is inadequately branched into five lobes (Figure 1.2). The ventral one lies towards the urethra and its bare from glandular tissue, the middle lobe is the chunk of the gland locates between the urethra and ejaculatory duct, its superior surface is linked to bladder trigon, dorsal lobe is located behind the urethra. Right and left lateral lobes are located on each side of urethra and they are detached from one another by a flat vertical groove.[1,2]

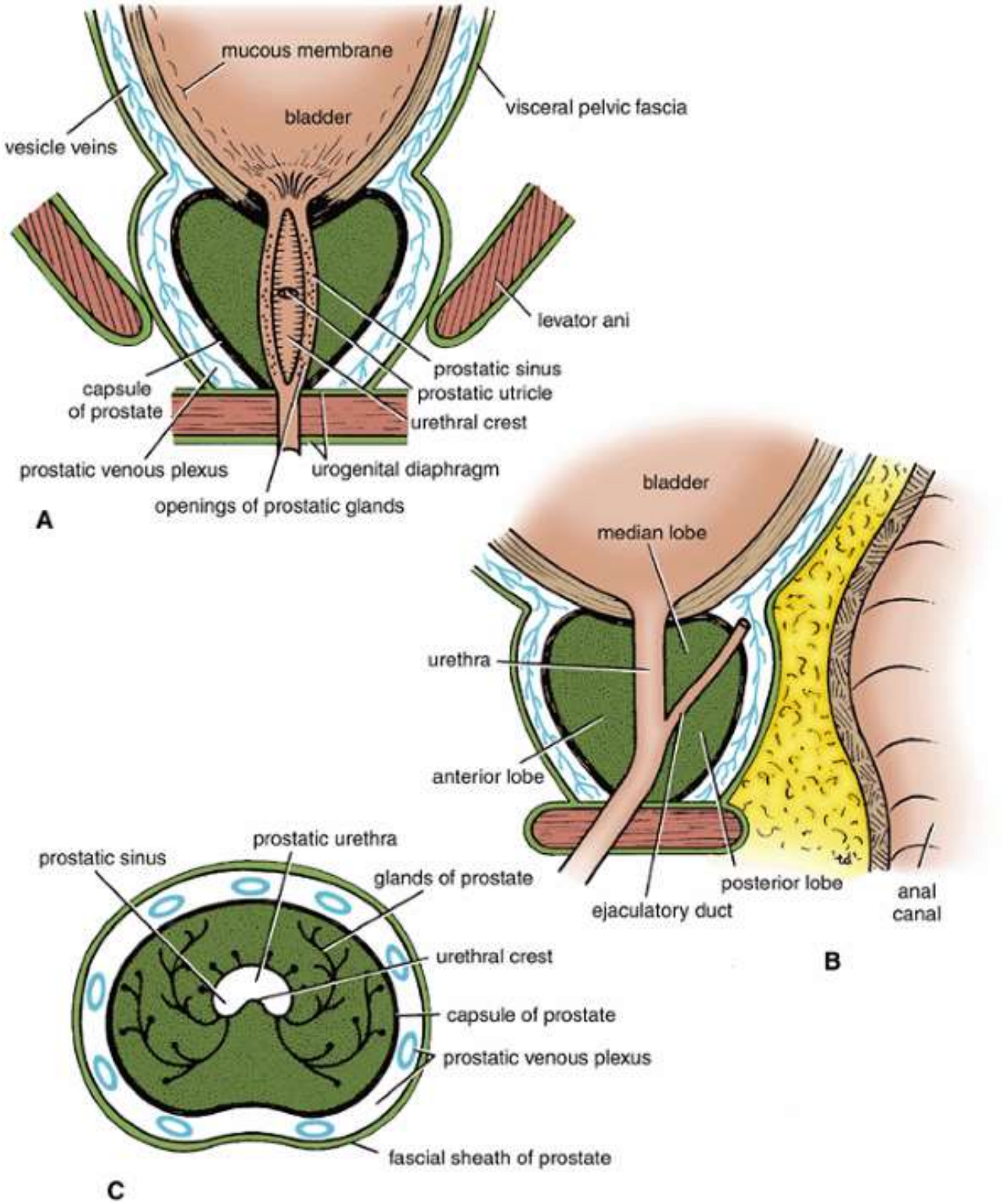

Figure 1.2: Prostate in coronal section (A), sagittal section (B), and horizontal section (C). In the coronal section, note the openings of the ejaculatory ducts on the margin of the prostatic utricle.

1.1.3 Functions

The prostate crops a thin, milky fluid consist of citric acid accompanied by acid phosphatase that is combined to seminal fluid at time of ejaculation the smooth muscle which envelopes the gland and transfer secretions towards the prostatic urethra ,the prostatic suppuration is alkaline so it helps in neutralizing vaginal acidity.[2]

1.1.4 Blood supply

The arterial supply is subsidiary of the inferior vesical and middle rectal arteries. The veins form the prostatic venous plexus, which is located outside the gland capsule (Figure 1.2). consumed into internal iliac veins and internal iliac nodes.[2]

1.1.5 Nerve supply

The nerve supply of prostate gland is Inferior hypogastric plexuses. The sympathetic nervous system which arouse the smooth muscle of prostate during ejaculation.[2]

1.2 Histological structures of the prostate

It's a collection of numerous tubo-alveolar glands all arranged between two layers a mucosal layer and a submucosal layer, each of these glands have its own single duct that bears the secretory product towards the prostatic urethra (Figure 1.3). The slender capsule of the gland is consists of a richly vascularized, dense, irregular collagenous connective tissue interspersed with smooth muscle cells. The connective tissue stroma of the gland is derived from the capsule and is, therefore, also enriched by smooth muscle fibers in addition to their normal connective tissue cells.[3,4]

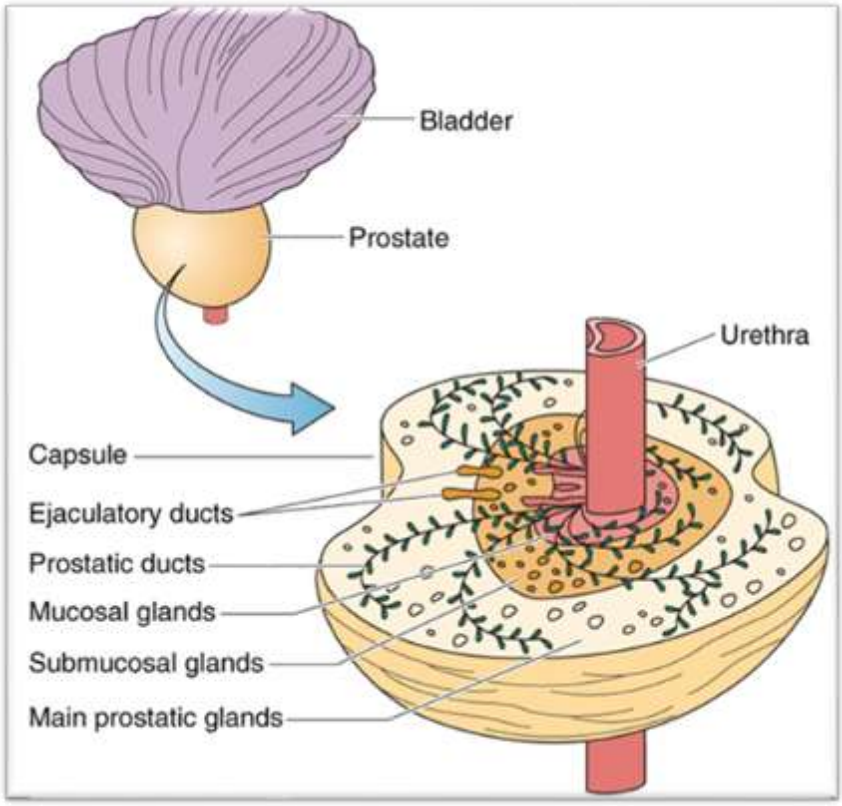

Figure 1.3: Human prostate gland.

Each tubuloalveolar gland has its own duct that delivers the secretory product into the prostatic urethra. The mucosal glands are the shortest and the most closest glands to the urethra on the other side the; submucosal gland are located laterally to the mucosal ones and they are the largest ones they, also represent the bulk of the gland. these glands are lined by a simple to pseudostratified columnar epithelium (Figure 1.4), the cells of which are well endowed with organelles responsible for the synthesis and packaging of proteins. Hence, these cells have an abundant RER, a large Golgi apparatus, numerous secretory granules (Figure 1-5), and many lysosomes.[3,4] which is responsible for synthesis and packaging of proteins Corpora amylacea which is an egg-shaped calcification which increases with a age (Figure 1.4).[3]

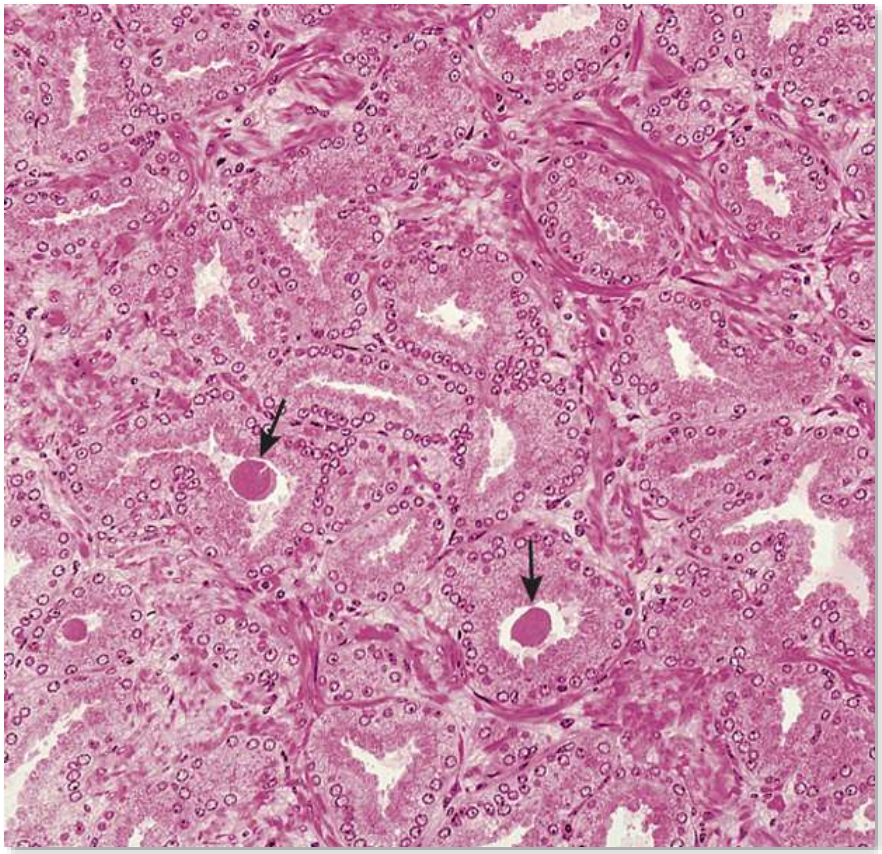

Figure 1.4: Light micrograph of the prostate gland (×132). Note areas of prostatic concretion (arrows).

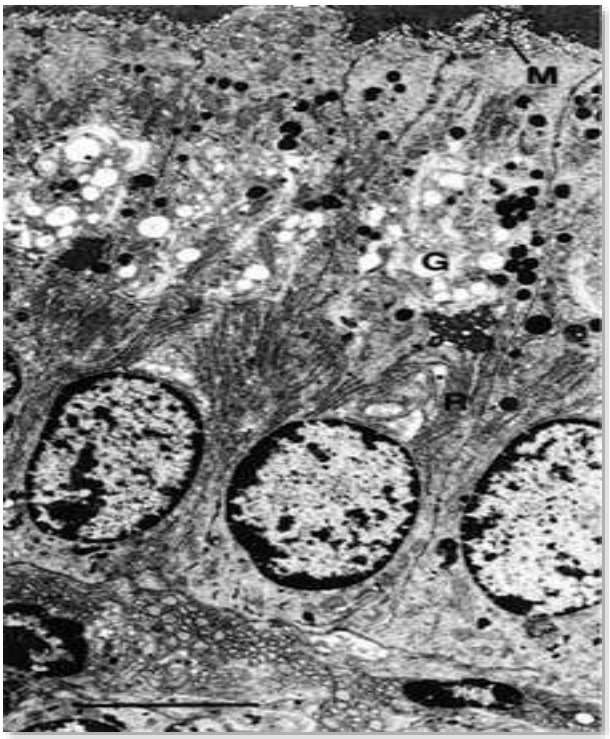

Figure: 1.5 Electron micrograph of the prostate gland. G, Golgi apparatus; M, microvilli; R, rough endoplasmic reticulum.

1.3 What is a tumor

It is an abnormal cell aggregation that will eventually lead to swelling, but not all swellings are cancerous. If the tumor is either benign or malignant, it all depends on the cell growth pattern, so logical if it has normal cell growth is called benign and if there is an abnormal development pattern, it is called malignant.[5]

1.3.1 Benign tumors

The tumor is benign if the cells are not cancerous. It will not invade neighboring tissues or spread (metastasize) to other regions of the body. Unless it presses on neighboring tissues, nerves, or blood vessels and causes harm, a benign tumor is less worrisome.

Examples of benign tumors are uterine fibroids or lipomas. Operation may require the removal of benign tumors. They can grow very large, weighing pounds at times. They can be dangerous, such as when the ordinary structures in the enclosed space of the skull happen in the brain and crowd. They can press or block essential organs. Some types of benign tumors, such as intestinal polyps, are also regarded as precancerous and removed to avoid malignancy. Usually, benign tumors do not recur once removed, but it is generally in the same location if they do so. [5]

1.3.2 Malignant tumors

Malignant means that the tumor is made up of cancer cells and can invade nearby tissues. Some cancer cells can move into the bloodstream or lymph nodes, where they can spread to other tissues within the body, this is called metastasis. Cancer can occur anywhere in the body, including the breast, bowel, lungs, reproductive organs, blood and skin. [5]

1.4 What is cancer?

Cancer can begin anywhere in the body. It begins when cells grow out of control and the normal cells are crowded out. This makes working the way it should be difficult for the body. Many people can treat cancer very well. Indeed, after cancer treatment, more people than ever before lead full lives.[6]

1.5 What is prostate cancer?

Prostate cancer begins when cells begin to grow uncontrollably in the prostate gland. Prostate adenocarcinoma is the most common form of cancer in men, ranked the second most common cause of cancer-related deaths after lung carcinoma in excess of 50 years of age. The probability of being diagnosed with prostate cancer is one in six lifetimes. There has been a significant drop in mortality from prostate cancer over the past 20 years. It is one of the most remarkable tumors, exhibiting a wide range of clinical behaviors from very aggressive lethal cancers to clinically insignificant cancers that have incidentally been found. 7.8

1.6 Types of prostate cancer

Nearly all cancers of the prostate are adenocarcinomas. These cancers develop from the cells of the gland (the cells that make the semen-added prostate fluid). Other prostate cancer types are including sarcomas, carcinomas of small cells, tumors of neuroendocrine, and carcinomas of transitional cells.[8]

1.7 Etiology and pathogenesis

The exact cause for prostatic cancer is unknown. However, there are several factors such as age, race, family history, hormone levels, and environmental factors are play a role in development of the prostatic cancer. The environmental influences are an important factor to increase the incidence of this disease. For example, high fat diet has been expected as a risk factor. On the other hand, dietary products such as lycopenes (found in tomatoes), selenium, soy products, and vitamin D are suspected of preventing the development of prostatic cancer.[9] It has been demonstrated that, androgens has an important role in the growth and survival of prostatic cancer cells by the binding to the androgen receptor (AR) and induce the expression of pro-growth and pro-survival genes. There are some differences in prostate cancer risk among races.[10] In addition, previous study has suggested that using of anti-androgen treatment is associated with disease regression and that prove the importance of androgens in maintaining the growth and survival of prostate cancer cells. However, tumors can become resistant to androgen blockade by escaping through a variety of mechanisms such as, acquisition of hypersensitivity to low levels of androgen (e.g., through AR gene amplification); mutations in AR that allow it to be activated by non-androgen ligands, and other mutations or epigenetic changes that activate alternative signaling pathways, which may bypass the need for AR altogether. Among the latter are changes that lead to increased activation of the P1-3 kinase/AKT signaling pathway, which is observed most often in tumors that have become resistant to anti-androgen therapy.[11] The X-linked AR gene contains a polymorphic sequence which composed of repeats of the codon CAG (which codes for glutamine). Very large expansions of this stretch of CAGs cause a rare neurodegenerative disorder and Kennedy disease. However, even in normal individuals, there is sufficient variation in the length of the CAG repeats to affect AR function. It

has been shown that, there is a high sensitivity to the androgen by ARs with the shortest stretches of polyglutamine. There are several variation among the races, the shortest polyglutamine repeats are found in African Americans, while Caucasians have an intermediate length and Asians have the longest. Morover, the length of the repeats is inversely related to rate at which prostate cancer develops in mouse models.

Several studies have shown a familial predisposition for prostate cancer. Men with one first-degree relative with prostate cancer have twice the risk compared with men with no family history and those with two first-degree relatives have five times the risk of developing prostate cancer. In addition, the disease tend to develop at an earlier age in patient with strong family history. Moreover, there is a 20-fold increased risk of prostate cancer in men with germline mutations of the tumor suppressor BRCA2 , but the vast majority of familial prostate cancers are due to variation in other loci that confer a small increase in cancer risk. It has demonstrated that, a number of risk-associated loci, including one at 8q24 that appears to increase the risk among African American men.[12] In addition, a number of those genes are involved in innate immunity and that lead to contribute the inflammation to several forms of prostatic cancer. Studies have demonstrated that tumor-specific acquired somatic mutations and epigenetic changes have a role in development of prostatic cancer. One very common type of somatic mutation in prostate cancer gives rise to chromosomal rearrangements that juxtapose the coding sequence of an ETS family transcription factor gene (most commonly ERG or ETV1) next to the androgen-regulated TMPRSS2 promoter.[13] That leads to place the involved ETS gene under the control of the TMPRSS2 promoter and lead to their over-expression in an androgen-dependent fashion. As a result of the over-expression of ETS transcription factors, the normal prostate epithelial cells become more invasive, possibly through the upregulation of matrix metalloproteases. It has been demonstrated that, the

presence of rearranged ETS genes in the tumors have certain distinctive morphologic features and a different gene expression signature than those lacking ETS gene rearrangements. Moreover, rearranged ETS genes play an important role in prostate cancer screening and early diagnosis.[14]

The epigenetic changes in prostate cancer include hypermethylation of glutathione S-transferase (GSTP1) gene which down-regualtes GSTP1 expression.[15] Other epigenetic changes that play a role in development of prostate cancer are a number of tumor suppressor genes, including PTEN, RB, p16/INK4a, MLH1, MSH2, and APC. Moreover, there is a down regulation of E-cadherin and that associated with expression of high levels of EZH-2, a transcriptional repressor which contribute to progression of prostate cancer. In addition, AMACR, an enzyme involved in the beta-oxidation of branched chain amino acids, is selectively up-regulated in prostate cancer.[16]

1.8 Morphology

Seventy to eighty percent of prostate cancers occur in the outer (peripheral) glands and can be palpable by rectal digital examination as irregular difficult nodules. Prostate cancer is less probable to cause urethral obstruction in its initial stages because of its peripheral place than is nodular hyperplasia. Typically, early lesions appear as undefined masses just below the prostate capsule. The carcinoma foci appear as firm, gray-white to yellow lesions on the cut surface, infiltrating the neighboring gland with undefined edges. (Figure 1.6). Metastasis may occur early to regional pelvic lymph nodes. Locally developed cancers often infiltrate the prostate's seminal vesicles and periurethral zones and may invade the neighboring soft tissue and urinary bladder wall. Denonvilliers fascia, the connective tissue layer that separates the lower genitourinary structures from the rectum, usually prevents growth of the tumor posteriorly. Invasion

of the rectum therefore is less common than is invasion of other contiguous structures.[7,17]

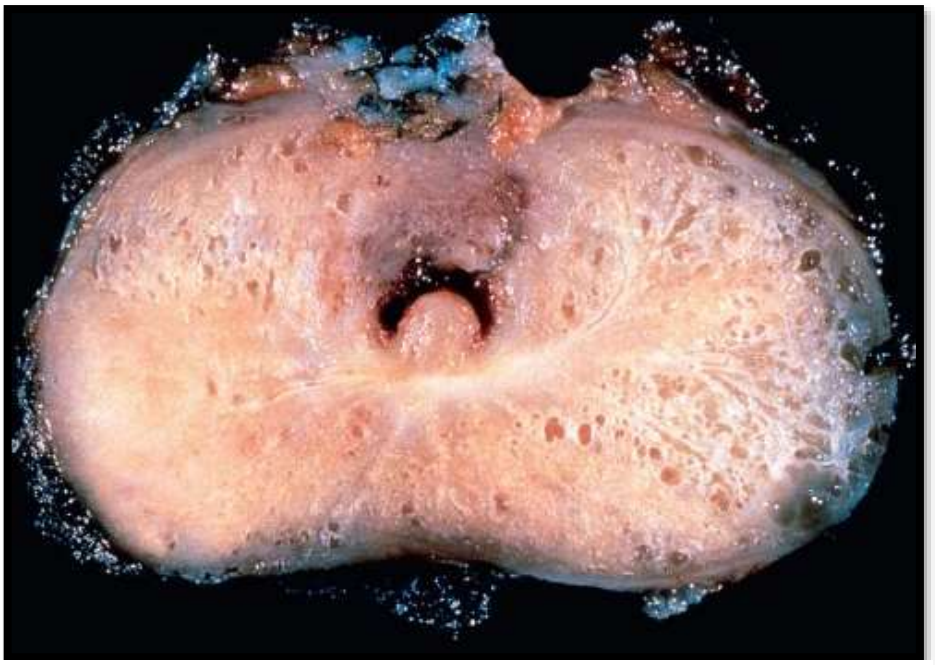

Figure 1.6: Adenocarcinoma of the prostate. Carcinomatous tissue is seen on the posterior aspect (lower left). Note the solid whiter tissue of cancer in contrast to the spongy appearance of the benign peripheral zone on the contralateral side.

Microscopically, the majority of prostatic carcinomas are adenocarcinomas with variable differentiation degrees. Small cells that invade the neighboring stroma in an irregular, haphazard fashion are the better differentiated lesions. In contrast to normal and hyperplastic prostate, the carcinoma glands lie "back to back" and seem to dissect sharply through the native stroma. (Figure 1.7). A single layer of cuboidal cells with conspicuous nuclei lines the neoplastic glands; the basal cell layer seen in normal or hyperplastic gland is absent. With anaplasia, irregular, ragged glandular structures, papillary or cribriform epithelial structures, and sheets of poorly differentiated cells are present in extreme cases. Glands adjacent to areas of invasive prostate carcinoma often contain epithelial atypia foci, or PIN due to their frequent occurrence.

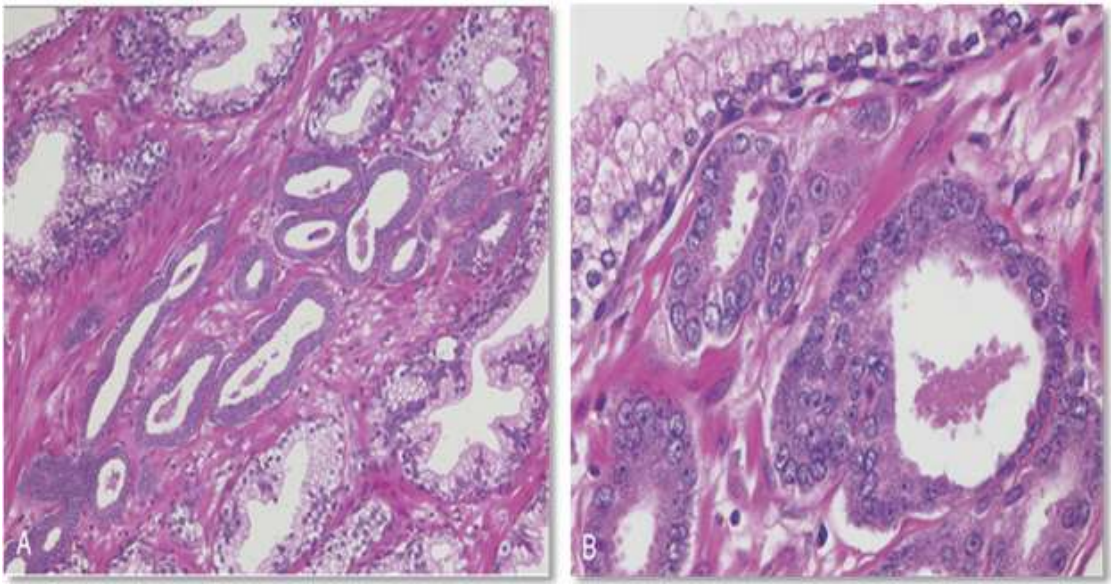

Figure 1.7: A, Photomicrograph of a small focus of adenocarcinoma of the prostate demonstrating small glands crowded in between larger benign glands. B, Higher magnification shows several small malignant glands with enlarged nuclei, prominent nucleoli, and dark cytoplasm, as compared with the larger benign gland.

Coexistence with carcinoma infiltration was suggested as a likely precursor to prostate carcinoma. Depending on the degree of atypia, PIN has been subdivided into high grade and low grade patterns. Importantly, high-grade PIN shares with invasive carcinoma molecular modifications, providing assistance for the argument that PIN is an intermediate between normal and frankly malignant tissue. Several histological grading systems for prostate carcinoma have been suggested. They are based on characteristics such as glandular differentiation, neoplastic gland architecture, nuclear anaplasia, and mitotic activity. The Gleason system is a widely used technique of grading. Despite the potential difficulties involved with incomplete sampling in biopsy material and the subjectivity inherent in histological assessment, Gleason grade has been shown to correlate reasonably well with both prostatic carcinoma anatomy and prognosis.7

1.9 Grading

The Gleason system is the grading scheme used for prostate cancer. Based on glandular patterns of differentiation, prostate cancers are stratified into five grades according to this scheme. Grade 1 The tissue looks very much like normal prostate cells. Grades 2-4 Cells that score lower look closest to normal and represent a less aggressive cancer. Those that score higher look the furthest from normal and will probably grow faster. Grade 5 Most cells look very different from normal. Most tumors contain more than one pattern where the dominant pattern is assigned a primary grade and the second most common pattern is assigned a secondary grade. The two numeric grades are then added to obtain a combined Gleason grade or score. Thus, for example, a tumor with a dominant grade 3 and a secondary grade 4 would achieve a Gleason score of 7. Tumors with only one pattern will be treated as if their primary and secondary grades are the same, thus doubling the number. An exception to the rule is if there are three patterns on biopsy, to reach the Gleason score, the most common and highest grades will be added together. Thus, the most well-differentiated tumors under this scheme have a Gleason score of 2 (1 + 1), and the least-differentiated tumors merit a score of 10 (5 + 5). Gleason scores are often divided into groups of comparable biological conduct with well-differentiated cancer grades 2 through 4, 5 and 6 (Figure 1.8 A). Tumor intermediate, 7 (Figure 1.8 B). moderate to poorly differentiated cancer, and 8 through 10 high-grade tumor (Figure 1.8 C), (Figure 1.8 D). Gleason scores of 2 to 4 are typically discovered within the transition area in tiny tumors. Such low-grade cancer in surgical samples is typically an incidental finding on prostate transurethral resection (TURP) for Benign Prostatic Hyperplasia (BPH) symptoms. Most cancers identified as a consequence of testing on needle biopsy have Gleason ratings of 5 to 7. Gleason scores tumors 8 through 10 tend to be developed, unlikely to be cured cancers.

Although there is some evidence that prostate cancers may become more aggressive over time, the Gleason score stays stable over several years, most frequently. In prostatic cancer, grading is particularly important because grade and stage are the best predictors for prognosis.[17]

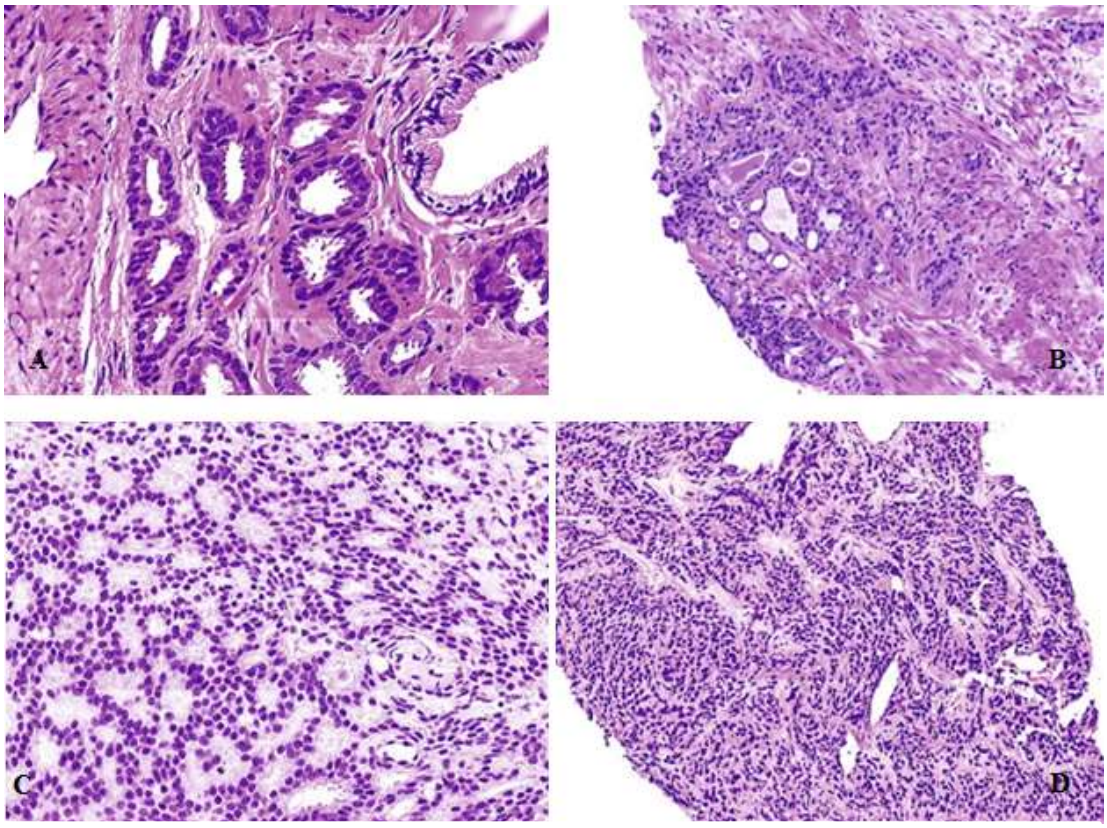

Figure: 1.8: Prostatic adenocarcinoma, A: Gleason 3 + 3 = 6/10. B: Gleason 3 + 4 = 7/10. C: Gleason 4 + 4 = 8/10. The tumor has a cribriform pattern of growth. D: Gleason 5 + 5 = 10/10.

1.10 Staging

In selecting the suitable type of treatment, the staging of prostatic cancer is also essential (Table 1.1). Stage T1 relates to incidentally discovered cancer, either on BPH symptom TURP (T1a and T1b depending on extent and grade) or on needle biopsy typically conducted for high serum prostate-specific antigen (PSA) concentrations (stage T1c). Stage T2 is cancer that is confined to organs. Stage T3a and T3b tumors with and without seminal vesicle invasion demonstrate extra-prostatic extension, respectively. Stage T4 represents immediate organs invasion. Any tumor spread to the

lymph nodes regardless of extent is eventually associated with a fatal outcome, so the staging system only documents the existence or lack of this finding (N0/N1). [7]

Table 1.1: Staging of prostatic adenocarcinoma using the TNM system

TNM Designation	**Anatomic Findings**
Extent of Primary Tumor (T)	
T1	**CLINICALLY INAPPARENT LESION (BY PALPATION/IMAGING STUDIES)**
T1a	Involvement of ≤5% of resected tissue
T1b	Involvement of >5% of resected tissue
T1c	Carcinoma present on needle biopsy (following elevated PSA)
T2	**PALPABLE OR VISIBLE CANCER CONFINED TO PROSTATE**
T2a	Involvement of ≤50% of one lobe
T2b	Involvement of >50% of one lobe, but unilateral
T2c	Involvement of both lobes, but still inside prostate gland
T3	**LOCAL EXTRAPROSTATIC EXTENSION**
T3a	Extracapsular extension
T3b	Seminal vesical invasion
T4	**INVASION OF CONTIGUOUS ORGANS AND/OR SUPPORTING STRUCTURES INCLUDING BLADDER NECK, RECTUM, EXTERNAL SPHINCTER, LEVATOR MUSCLES, OR PELVIC FLOOR**
Status of Regional Lymph Nodes (N)	

N0	**NO REGIONAL NODAL METASTASES**
N1	**METASTASIS IN REGIONAL LYMPH NODES**
Distant Metastases (M)	
M0	**NO DISTANT METASTASES**
M1	**DISTANT METASTASES PRESENT**
M1a	Metastases to distant lymph nodes
M1b	Bone metastases
M1c	Other distant sites

1.11 Prostatic specific antigen

It is a protein produced by the prostate gland's normal and malignant cells. The test measures the blood PSA amount. There is no specific normal or abnormal PSA level in the blood, and levels in the same man may vary over time. In the past, PSA concentrations were regarded as normal at 4.0 ng/mL and lower. Therefore, prostate biopsy is recommended to determine the presence of prostate cancer in patients with a PSA level of 4.0 ng/ml. However, more recent studies have shown that some men with PSA levels below 4.0 ng/ml have prostate cancer and that there is no prostate cancer in many men with higher levels. Furthermore, different variables can cause the PSA level of a patient to fluctuate. For instance, if the person has prostatitis or an infection with the urinary tract, the PSA rate often increases. The amount of PSA is also increased by prostate biopsies and prostate surgery. In contrast, some drugs, including finasteride and dutasteride, used to treat benign prostatic hyperplasia (BPH) decrease the amount of PSA in patients.[18]

1.12 Prognosis

Many parameters have been evaluated for their ability to predict outcome in patients with prostatic carcinoma, because of the usually long natural history of the disease, the PSA-free survival and PSA recurrence have been used in some of the studies as surrogates for actual survival and recurrence, respectively.

- Clinical stage: This is a very significant prognostic determinant, and with the introduction of new technology it has become even more so.
- Pathological stage: This is the ultimate measure of the extent of the tumor and, as such, the most accurate predictor of the available prognosis. Of course, there is also a relationship between the prognosis and the status of the individual factors determining the stage, such as the prostatic capsule, the seminal vesicles, and the lymph nodes.[19] Thus, the level of tumor invasion in or through the prostatic capsule is strongly associated with the grade, volume, and rate of tumor recurrence. There is also a relation between the extraprostatic extension radial distance (as measured by an ocular micrometer) and the recurrence of PSA. Microscopic participation of the bladder neck, on the other hand, is not an important prognostic factor. The prognosis is worse in cases with nodal metastases when they are multiple rather than solitary, when they are grossly detectable rather than microscopically, when their general volume is large, and when accompanied by extracapsular extension. Their prognostic significance seems to be the same, whether in the usual pelvic location or around the prostate seminal vesicles.[20]
- Microscopic grading: There is a direct correlation between clinical or pathological staging and microscopic grading irrespective of the grading system used. Moreover, there is convincing evidence that as an independent prognostic

variable, microscopic grading using the Gleason score system is superior to the others.[21] Gleason score was by far the best predictor of progression in a particularly impressive multivariate analysis of 185 cases of clinical stage B prostatic carcinoma treated with radical prostatectomy. Gleason tumor grade was found to be the only significant predictor of disease outcome in another study involving 1143 consecutive patients with radical prostatectomy for localized prostatic carcinoma. There has been no significant prognostic difference between 3 + 4 and 4 + 3 tumors among Gleason score 7 cases. A combined Gleason score of 7 is a clinically significant disease indicator even if the disease is limited to a single microscopic focus.[22] There is some suggestion that by taking into account the tertiary pattern whenever present, the predictive power of the Gleason scheme could be further increased.

- Surgical margins: Positive margins strongly correlated with progression in a multivariate analysis of over 500 retropubic prostatectomy specimens performed in clinical stages A and B of prostatic carcinomas. Several other studies confirmed the margin status value as an indicator of increased tumor progression risk. However, the extent of the positive surgical margin does not appear to affect the rate of recurrence of PSA.[23] Similarly, the distance between the tumor and the surgical margin does not have any prognostic significance in margin-negative tumors. Positive margins of extraprostatic extension should be distinguished from capsular incision margins in which the surgeon transects either benign or malignant prostatic tissue and leaves the prostate edge within the patient; a not particularly easy determination.[24]
- Tumor volume: Tumor volume has been shown to correlate with Gleason score, capsular penetration, capsular resection margins, seminal vesicle invasion, and

lymph node metastases as measured in whole sections of prostatectomy specimens using morphometric techniques.[25] However, there is some question as to whether tumor volume measurement provides additional prognostic information beyond that given by the parameters already listed (especially the Gleason score), and it is therefore difficult in routine practice to justify the use of a technique involving the performance of step sections of the entire specimen and the measurement of the tumor at 3 mm intervals. with computer-assisted image analysis. To make the determination more acceptable to the overwhelmed general surgeon pathologist, some reasonable compromises have been proposed. One is based on the ratio of tumor-positive tissue blocks to the total number of blocks ('positive-block ratio') submitted. Indeed, Vollmer has shown that a plain visual estimate of tumor volume ('eyeballing') is more closely associated with overall survival than PSA or Gleason score. Parenthetically, by evaluating a combination of morphological and laboratory data, a reasonably good prediction of tumor volume can be made from needle biopsy specimens. It was even suggested that the following formula could obtain a good estimate of the tumor volume (calculated tumor volume): cancer-specific serum PSA / amount of PSA leaking into the serum per cm3 of cancerWhile it may be mentioned on the subject of tumor volume or surface, even the roughest estimates of tumor amount in core needle biopsy material – such as cancer percentage, number of positive cores, and disease bilaterality – have prognostic significance. It is therefore important for the pathologist to provide a quantitative estimate of the amount of tumor in the biopsy of the prostate needle. [26]

- Age: The age of the patient in general is not an important determinant of the prognosis. True, the few reported cases of prostatic carcinoma in men under the

age of 35 have typically been characterized by poor differentiation and very aggressive behaviour. Statistical analysis of prostatic carcinomas after 40 years of age (the overwhelming majority) did not show a definite relationship between age and survival, however.[27]

- Race: Black males have a death rate of almost twice that of white males from prostatic carcinoma. This is because they are more likely to have a more advanced stage at presentation, at least in part. In both races, survival is similar when the disease is stratified for grade and stage.[28]
- Method of initial diagnosis: Patients in whom TUR has been diagnosed with prostatic carcinoma have a greater incidence of tumor dissemination than those diagnosed with needle biopsy; whether this is the consequence of the TUR operation itself (unlikely) or a reflection of the reality that TUR-diagnosable tumors are generally more advanced is not yet apparent. By contrast, the vast majority of prostatic carcinomas discovered in radical cystoprostatectomy specimens for bladder carcinomas (over 80%) are clinically insignificant.[29]
- PSA serum levels: The PSA serum level is associated with prostatic carcinoma prognosis as an indirect indicator of tumor volume, tumor extension, and therapy response.[30]
- PSA and PAP immunoreactivity: Prostatic carcinomas with areas of weak or negative reactivity for PSA or PAP behave as a group more aggressively than the others.[24]
- Perineurial invasion: Perineurial invasion is a time-honored clue to carcinoma diagnosis, but it remains contentious for its prognostic significance. Some studies showed correlation with extraprostatic resection expansion and tumor development after radical prostatectomy (especially if the perineurial invasion

diameter is quantitated), but other writers failed to discover such an association.[31]

- Lymphovascular invasion: Vascular channel permeation as identified in whole-mount radical prostatectomy samples was discovered to correlate with Gleason score, extraprostatic expansion, seminal vesicle participation, and tumor progression probability. In addition, an enhanced probability of regional lymph node metastases is correlated with peritumoral lymph vessel invasion.[32]
- Androgen-receptor status: High androgen receptor levels are associated as immunohistochemically measured and decreased PSA-free survival with aggressive clinicopathological features. Mutations of the androgen receptor gene in metastatic prostatic carcinoma were recognized and the reason for the androgen independence of these tumors was postulated.[33]

1.13 Treatment

Surgical, radiation therapy, and hormonal manipulations treat prostate cancer. Chemotherapy in the therapy of prostate cancer has shown restricted efficacy. Treatment choices are based on the grade and stage of the tumor and the man's age and health. Expectant therapy (watchful waiting) may be used if the tumor does not cause symptoms, is anticipated to grow slowly, is tiny, and is contained within one prostate region.[34] This strategy is particularly suitable for males who are older or have other issues with their health. For surgical or radiation therapy, most males with an expected survival time of more than 10 years are regarded. Radical prostatectomy includes removing seminal vesicles, prostate and vas deferens ampullae completely. Refinements in surgical methods (nerve-sparing prostatectomy) have enabled most males to maintain continence and, in chosen instances, erectile function. Radiation treatment can be carried out using a range of methods, including internal radiation treatment and

radioisotope transperineal implantation.[35] Metastatic disease is often handled with deprivation treatment for androgen. A range of techniques or agents can be used to induce androgen deprivation at several concentrations along the pituitary-gonadal axis. Orchiectomy or estrogen therapy often works to reduce symptoms and extend survival. The analogs of gonadotropin-releasing hormone (GnRH) (e.g. leuprolide, buserelin, nafarelin, triptorelin, and goserelin) block the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary and decrease testosterone concentrations without orchiectomy or estrogen treatment.[36] These medications desensitize GnRH receptors in the pituitary when administered continually (as opposed to pulsatile, which is the standard physiological secretory rhythm) and in therapeutic doses, thus stopping the release of LH. Since these agents are GnRH agonists, however, LH and FSH originally rise and boost the concentrations of testosterone.[37] This can cause a clinical flare, which in certain conditions can be particularly essential, such as compression of the spinal cord owing to metastatic disease.[36] his clinical flare may be reduced by antiandrogens pretreatment. Nonsteroidal antiandrogens (i.e., flutamide, nilutamide, and bicalutamide) block anrogens in the target tissues from taking and acting. It is possible to achieve complete androgen blockage by combining an antiandrogen with a GnRH agent or orchidectomy. Treatment with a mixture of a GnRH agonist and flutamide appears to boost survival in males with metastatic prostate cancer, especially in those with minimal disease. While testosterone is the primary androgen circulating, the adrenal gland also secretes androgens. Adrenal androgen synthesis inhibitors (i.e., ketoconazole and aminoglutethimide) may be used to treat people with developed prostate disease who have compression of the spinal cord, bilateral ureteral obstruction, or intravascular coagulation. This is because these males need to reduce their testosterone Palliative care involves proper control of pain and focal irradiation of

symptomatic or unstable bone illness. Bisphosphonates (e.g., etidronate, pamidronate, zoledronate, alendronate, risedronate), which primarily operate by inhibiting osteoclastic activity, have multiple prospective uses in prostate cancer in males with developed prostate cancer. These include (1) preventing osteopenia that accompanies the use of androgen depravation therapy, (2) preventing and delaying skeletal complications (e.g., the need for local radiation treatment, fractures) in patients with metastatic bone involvement, (3) decreasing bone pain, and (4) treating hypercalcemia of malignancy.[38]

1.14 Aim of the study

To determine the histopathological characteristics of prostate cancer in male Libyan patients in eastern part of Libya in years between (2015-2018) through analysis clinicopathological features and to correlate these features with other clinical data to better understand the biological behavior, epidemiology and provide awareness about prostate cancer.

Chapter 2: Materials and methods

2.1 Patients and tumor

The data were extracted from the archives of the Benghazi university clinic and the oncology department of Benghazi medical center (BMC). The files of cases and histopathological reports were arranged according to years and histopathology number. All the cases of patient with prostatic carcinoma that were diagnosed between year (2015 to 2018) were selected (n= 239). The clinicopathological data of patients for each year were found in several files. The patients files were listed from year (2015 to 2018), the files were checked and all the necessary data were extracted, such as: patients name, age, nationality, symptoms, PSA level, type and mode of biopsy and diagnosis, grades, scores, stages, treatment, date of diagnosis, date of registration and date of the last visit.

2.2 Tissue preparation

The available paraffin blocks of cases were cut into (3 to 4 mm) by using a rotatory microtome with a steal sharp knife. The paraffin blocks were sectioned into ribbons, and painted by fine brushes. The sections were floated on a 37°C water bath then picked up on a surface of clean glass slide. The slides with the paraffin sections were placed in a 65°C oven to bound the staining steps: Deparafinize and hydrate to water then adding Mayer's hematoxyllin for 15 minutes after that they were washed in running tap water for 20 minutes, then we counterstain them with eosin from 15 seconds to 2 minutes. For even staining results the slides are dipped several times before allowing them to set in the eosin for the desired time, after that we dehydrate them in 95% absolute alcohols, two changes of 2 minutes each or until excess eosin is removed. All of the slides are cleaned in xylene, two changes of 2 minutes each and mounted in in DPX (distyrene, plasticiser and xylen) and finally coverslip applied. The slides are read under light

microscope by pathologist (Dr. Ahmed Benhasouna) and all the missed histopathological data were completed.

2.3 Statistical analysis

The collected data were fed to computer using Statistical Package for Social Sciences (SPSS) version 22.0. Revision of data for any missing was done followed by analysis. Categorical data were presented in proportion for; numerical data were presented as mean, standard deviation, minimum & maximum values. Relationship between different variables were studied by applying chi square, using P value is considered as significant when it is < 0.05. The biostatistics is the branch of applied statistics directed toward applications in the health sciences and biology. Biostatistics is sometimes distinguished from the field of biometry, based upon whether applications are in the health sciences (biostatistics) or in broader biology (biometry eg: agriculture, ecology, wildlife biology) Other branches of (applied) statistics: psychometrics, econometrics, chemometrics, astrostatistics, environmetrics etc. biostatics are divided into two parts, descriptive statistic and Statistical inference.[39]

2.3.1 Descriptive statistics

It is the best way of summarizing large sets of data (information). divided into central tendency, dispersion and graphical representation. In graphical representation the data are presented in the form of graphs, this done by grouping the data into a set of classes, using frequencies, theses frequencies can be displayed graphically by:

- Histogram: is a plot that lets you discover, and show the underlying frequency distribute\on (shape) of a set of continuous data.

- Pie chart: are useful for displaying the relative frequency distribution of a nominal variable.

2.3.2 Test of hypothesis

Testing the researcher believe (hypothesis) whether it is correct or false. The null hypothesis H_0 is the statement being tested. Usually the null hypothesis is a statement of "no effect" or "no difference". The alternative hypothesis H_1 is the statement or belief that going to be accepted in case of rejecting H_0. The decision of rejection or acceptance H_0 depends on the comparison of p-value with the level of test significance α.[40]

NOTE (α = 0.05, 0.01, 0.1)

Chapter 3: Results

3.1 Clinicopathological results

According to the data available; the most age affected were 70 and above, and the least age affected were 40 (Figure 3.1). all cases were histologically adenocarcinoma with different grades (Figure 3.2). Most of the patients were grade 1 (Figure 3.3). With regards to the stage; stage IV was the commonest stage in our cases (Figure 3.4). Most of the patients with prostate cancer were presented with back pain 48 (47.1%), then urinary retention 29 (28.4%) and urinary incontinence {14 (13.7%, Figure 3.5)}. The majority of cases were from Benghazi {120 (61.5%, Figure 3.6)}. The majority of cases were diagnosed using transurethral resection of the prostatic tissue {179 (86.9%, Figure 3.7)}. Most patients with grade 5 were 70 years old and above {17 (9.4%, Table 3.1)}. The level of prostate-specific antigen were high (≥100 ng/ml) in most cases with grade 3 {24 (15.7%, Table 3.2)}. The Majority of cases of prostate cancer with advanced stage were 70 years old and above 48 (48.10%, Table 3.3). Most patients with Gleason's score 7 were stage IV {34 (34.7%, Table 3.4)}. The majority of cases with Gleason's score 7, show a high level of PSA {≥100 (12%, Table 3.5)}. The majority of cases with stage IV, show a high level of PSA {≥100 (54.5%, Table 3.6)}. Surgical removal of the tumor with chemotherapy and hormonal therapy was the most common mode of treatment {47 (36.4%, Table 3.7)}.

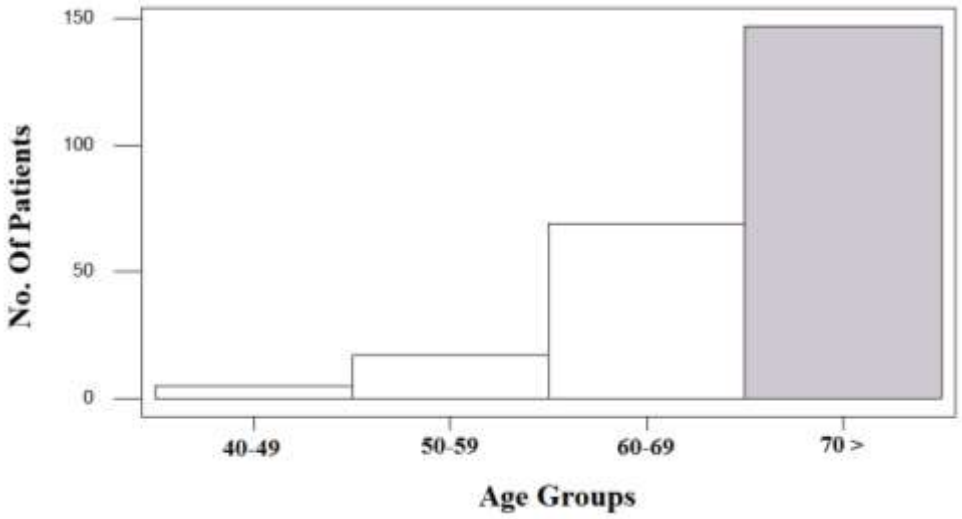

Figure 3.1: Age affected of prostate cancer.

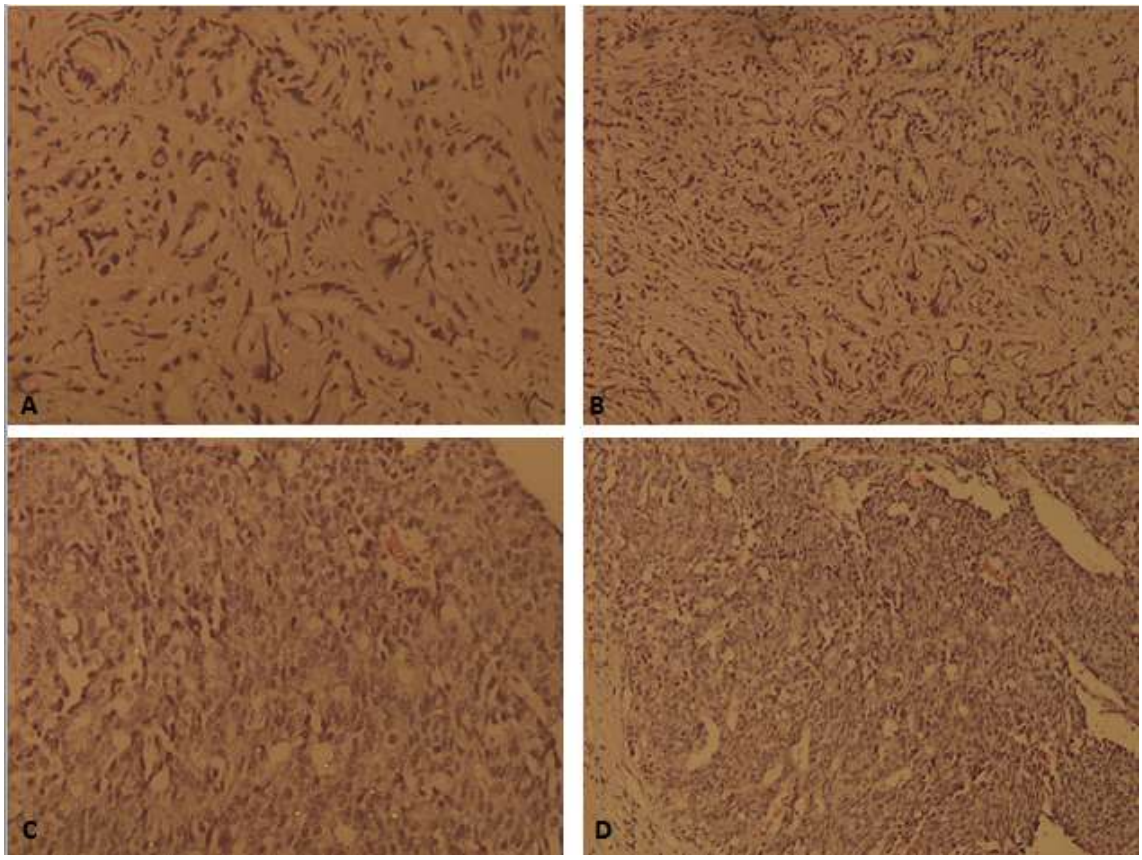

Figure: 3.2: Prostatic adenocarcinoma, A: Gleason 3 + 2 = 5/10 (200x). B: Gleason 3 + 2 = 5/10 (100x). C: Gleason 5 + 4 = 9/10 (200x). D: Gleason 5 + 4 = 9/10 (100x).

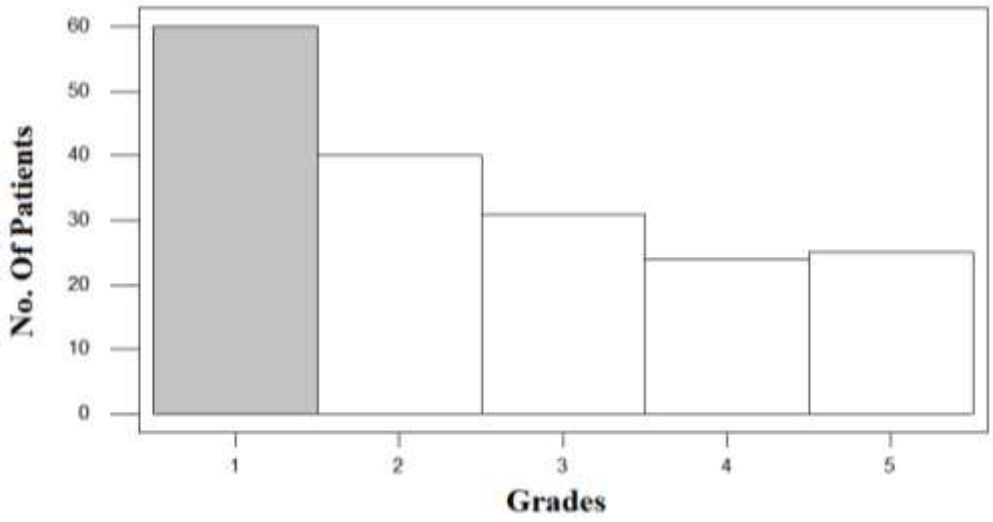

Figure 3.3: Grade of prostate cancer.

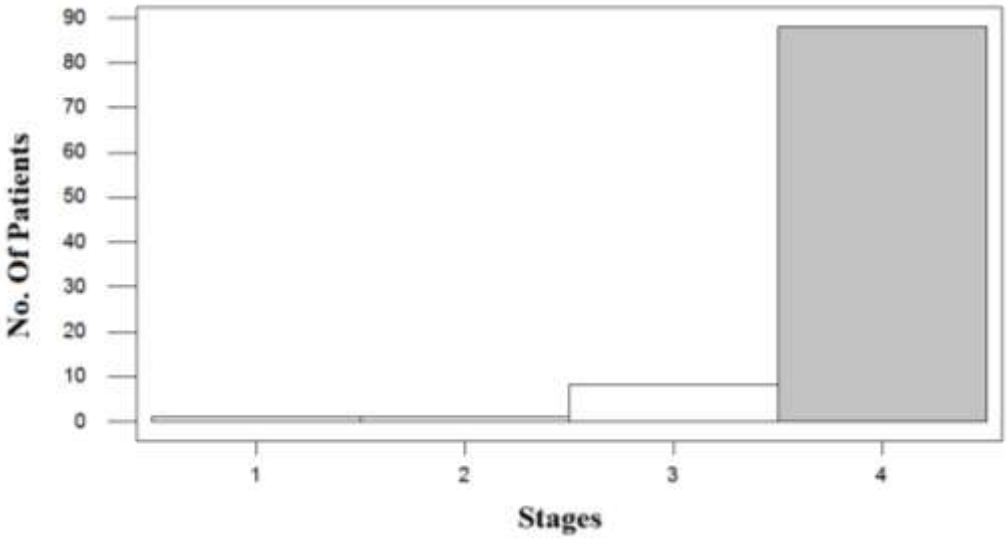

Figure 3.4: Stages of prostate cancer.

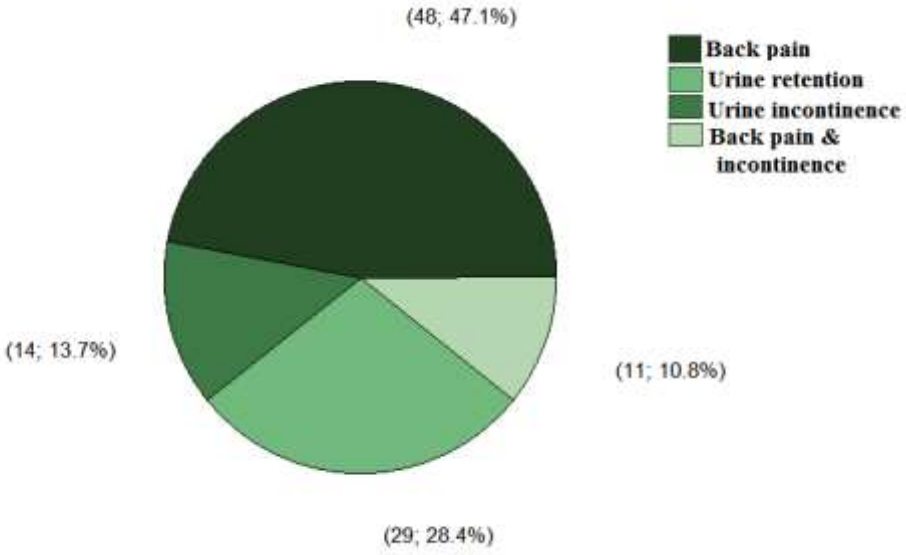

Figure 3.5: Most common symptoms of prostate cancer.

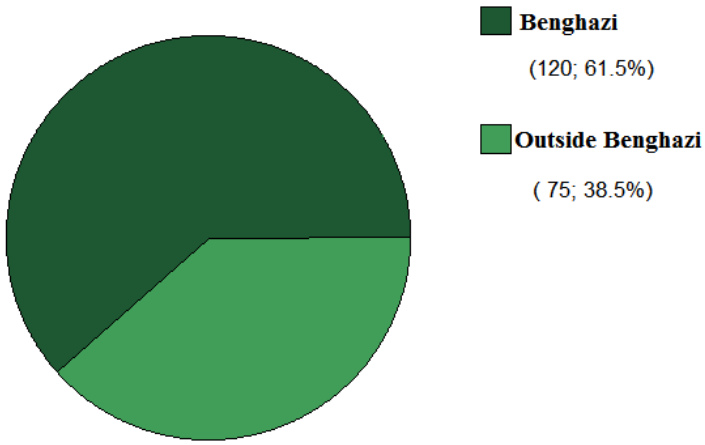

Figure 3.6: Number and percentage of patients according to residence.

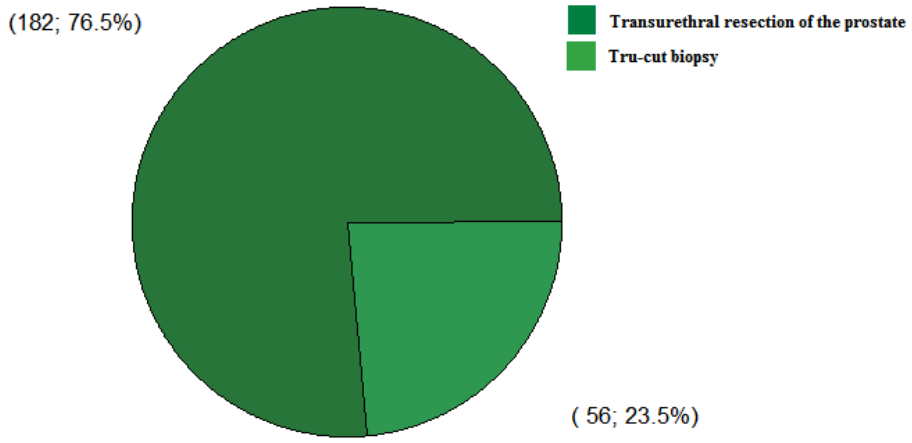

Figure 3.7: Number and percentage of patients according to nature of specimen.

Table 3.1: Bivariate frequency distribution of age groups and grades of prostate cancer .

Age	Grades				
	1	**2**	**3**	**4**	**5**
40-49	2 (1.1%)	0 (0%)	1 (0.6%)	1 (0.6%)	1 (0.6%)
50-59	6 (3.3%)	3 (1.7%)	1 (0.6%)	2 (1.1%)	1 (0.6%)
60-69	14 (7.8%)	13 (7.2%)	11 (6.1%)	8 (4.4%)	6 (3.3%)
≥70	38 (21.1%)	24 (13.3%)	18 (10%)	13 (7.2%)	17 (9.4%)

Table 3.2: Bivariate frequency distribution of grade and prostatic specific antigen.

Grade	Prostatic specific antigen level				
	< 4.0	**4.0-9.9**	**10.0-19.9**	**20.0-100.0**	**≥100**
1	5 (3.3%)	1 (0.7%)	3 (1.10%)	17 (11.1%)	21 (13.7%)
2	9 (5.9%)	3 (1.10%)	6 (3.10%)	21 (13.7%)	18 (11.8%)
3	2 (1.3%)	4 (2.6%)	2 (1.3%)	17 (11.1%)	24 (15.7%)

Table 3.3: Correlations between age and stages of prostate cancer.

Age	Stages			
	Stage I	**Stage II**	**Stage III**	**Stage IV**
40-49	0 (0%)	0 (0%)	0 (0%)	5 (5.1%)
50-59	0 (0%)	0 (0%)	0 (0%)	5 (5.1%)
60-69	1 (1.0%)	0 (0%)	3 (3.2%)	30 (30.7%)
≥70	0 (0%)	1 (1.0%)	5 (5.1%)	48 (48.10%)

Table 3.4: Correlations between Gleason's score and stages of prostate cancer.

Gleason's Score	Stages			
	Stage I	**Stage II**	**Stage III**	**Stage IV**
2	0 (0%)	0 (0%)	0 (0%)	1 (1%)
3	0 (0%)	0 (0%)	0 (0%)	4 (4.1%)
4	0 (0%)	0 (0%)	0 (0%)	2 (2%)
5	0 (0%)	0 (0%)	0 (0%)	9 (9.1%)
6	0 (0%)	0 (0%)	1 (1%)	5 (5.1%)
7	0 (0%)	0 (0%)	4 (4.1%)	34 (34.7%)
8	0 (0%)	1 (1%)	2 (2%)	16 (16.3%)
9	1 (1%)	0 (0%)	0 (0%)	14 (14.2%)
10	0 (0%)	0 (0%)	1 (1%)	3 (3%)

Table 3.5: Correlations between Gleason's score and prostatic specific antigen level.

Gleason's Score	Prostatic Specific antigen level				
	<4.0	**4.0-9.9**	**10.0-19.9**	**20.0-100.0**	**≥100**
2	0 (0%)	0 (0%)	0 (0%)	2 (1.3%)	2 (1.3%)
3	0 (0%)	1 (0.6%)	0 (0%)	1 (0.6%)	5 (3.3%)
4	1 (0.6%)	0 (0%)	1 (0.6%)	3 (2%)	2 (1.3%)
5	4 (2.6%)	0 (0%)	1 (0.6%)	6 (4%)	6 (4%)
6	0 (0%)	0 (0%)	1 (0.6%)	5 (3.3%)	3 (2%)
7	9 (6%)	3 (2%)	6 (4%)	22 (14.6)	18 (12%)
8	0 (0%)	2 (1.3%)	0 (0%)	13 (8.6%)	10 (6.6%)
9	2 (1.3%)	1 (0.6%)	1 (0.6%)	4 (2.6%)	11 (7.3%)
10	0 (0%)	1 (0.6%)	1 (0.6%)	0 (0%)	3 (2%)

Table 3.6: Correlations between stages and prostatic specific antigen level.

Stages	Prostatic Specific antigen level				
	<4.0	**4.0-9.9**	**10.0-19.9**	**20.0-100.0**	**≥100**
Stage II	0 (0%)	0 (0%)	0 (0%)	1 (1.29%)	0 (0%)
Stage III	0 (0%)	0 (0%)	2 (2.59%)	3 (3.89%)	1 (1.29%)
Stage IV	6 (7.8%)	3 (3.89%)	3 (3.89%)	16 (20.8%)	42 (54.5%)

Table 3.7: Correlations between mode of treatment and prostatic specific antigen level.

Treatment	Prostate Specific antigen				
	<4.0	**4.0-9.9**	**10.0-19.9**	**20.0-100.0**	**≥100**
Surgical	0 (0%)	0 (0%)	0 (0%)	2 (1.5%)	0 (1.3%)
Hormonal	1 (0.8%)	0 (0%)	1 (0.8%)	1 (0.8%)	0 (0%)
Chemotherapy	4 (3.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Radiotherapy	1 (0.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hormonal & Chemotherapy	16 (12.4%)	1 (0.8%)	0 (0%)	5 (3.9%)	3 (2.3%)
Surgical, hormonal & chemotherapy	47 (36.4%)	7 (5.4%)	8 (6.2%)	20 (15.5%)	12 (9.3%)

Chapter 4: Discussion

Prostate cancer is one of the most common cancers in men worldwide with a rising incidence trend in many countries. clinical stage, Gleason grade, and PSA are three main indicators of prognosis and deciding for therapy. Almost 75% of the men diagnosed with prostatic cancer were age 65 or older,[41] this is consistent with our study in which 90% of men with prostate cancer are age 65 or older. Berney DM, Beltran L, Fisher G, et al. found that the most common grade was grade 1.[42] this is in agreement with our study in which 35.6% of men are grade 1.

Chapter 5: Conclusion

Men with prostate cancer are old age, histopathologically; all cases were adenocarcinoma and are well-differentiated (grade 1). More patients are diagnosed at a late stage (stage IV**),** and most of them show gradual decrease in PSA level after the patient underwent the surgery, hormonal and chemotherapy.

Chapter 6: Limitation and recommendation

Although the material of this study originally accounts 239 prostate cancer cases, there is a problem related to the accessibility of the selective and relevant medical information. Some of this information missed in the archive of oncology department in BMC. Therefore, there is a need for an electronic version for the preservation of data and to become more accessible. It is essential to improve prostate cancer awareness as well as to train general practitioners to reduce prostate cancer mortality by promoting early detection. Further study including large number of cases with complete clincopathological data is recommended with immunohistochemical study for tumor marker such as NKX3.1, p63, thrombomodulin, and GATA3. The study support the establishment of National Cancer Institute in the city of Benghazi, which will accommodate all the clinical and pathological information regarding patients to facilitate the research and studies on cancer, to result in better understanding of cancer indicators in Libya. With more cases discovered every year; it is recommended to know more about the genomic characterization and behavior of prostate cancer.

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