"EVALUATION OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKERS HIGH MOLECULAR WEIGHT CYTOKERATIN (HMWCK) AND ALPHA-METHYLACYL CoA RACEMOSE (AMACR) IN PROSTATIC NEEDLE BIOPSIES AND TRANSURETHRAL RESECTION OF PROSTATE (TURP) SPECIMEN – A ONE YEAR OBSERVATIONAL STUDY"

By

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LIST OF ABBREVIATIONS USED

S.No.	Abbreviations	Expanded Form
1	BPH	Benign Prostatic Hyperplasia
2	DHT	Dihydrotestosterone
3	FGF	Fibroblast Growth Factor
4	TGF – b	Transforming Growth Factor – beta
5	BCH	Basal Cell Hyperplasia
6	PIN	Prostatic Intraepithelial Neoplasia
7	LGPIN	Low Grade Prostatic Intraepithelial Neoplasia
8	HGPIN	High Grade Prostatic Intraepithelial Neoplasia
9	DRE	Digital Rectal Examination
10	WHO	World Health Organisation
11	PAS	Periodic Acid Schiff
12	TRUS	Transrectal ultrasound
13	PPV	Positive Predictive Value
14	PSA	Prostate Specific Antigen
15	USG	Ultrasound
16	MRI	Magnetic Resonance Imaging
17	mpMRI	multiparametric Magnetic Resonance Imaging

18	PET	Positron Emission Tomography
19	FDG	Fluorodeoxyglucose
20	PSAP	Prostate Specific Acid Phosphatase
21	IHC	Immuohistochemistry
22	H & E	Haematoxylin-Eosin
23	CK	Cytokeratin
24	HMWCK	High Molecular Weight Cytokeratin
25	AMACR	Alpha-methyl-acyl-CoA Racemase
26	TURP	Transurethral Resection of Prostate
27	AAH	Atypical Adenomatous Hyperplasia
28	PNI	Perineural Invasion
29	EDTA	Ethylenediamine tetraacetic acid
30	HRP	Horseradish Peroxidase
31	DAB	Diaminobenzidine
32	DPX	Dibutylphthalate Polystyrene Xylene
33	HP Diagnosis	Histopathological Diagnosis
34	SD	Standard Deviation

ABSTRACT

TITILE: EVALUATION OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKERS HIGH MOLECULAR WEIGHT CYTOKERATIN (HMWCK) AND ALPHA-METHYLACYL CoA RACEMOSE (AMACR) IN PROSTATIC NEEDLE BIOPSIES AND TRANSURETHRAL RESECTION OF PROSTATE (TURP) SPECIMEN – A ONE YEAR OBSERVATIONAL STUDY

BACKGOUND and OBJECTIVES:

In view of increasing incidence of Prostate cancer with age, its early detection and management is of utmost importance. Digital rectal examination, clinical picture and USG findings are non-specific. This study was conducted to resolve the problems a pathologist face in diagnosing cases of prostatic lesions having a suspicious morphology. IHC staining (HMWCK and AMACR) was done to distinguish benign from malignant lesions.

METHODOLOGY:

One-year observational study conducted from January 2020 to December 2020. A total of 30 cases of prostatic lesions were studied. The specimens were fixed in 10% formalin and routinely processed. Haematoxylin-Eosin (H&E) and IHC staining (HMWCK and AMACR) was done in all 30 cases.

RESULTS:

A total of 30 cases including 19 cases of prostatic needle core biopsies and 11 cases of TURP specimens were included in our study. Maximum patients were in the age group of 70-79 years. According to initial histopathological diagnosis, we got 1 case each of Adenosis, Atypical adenomatous hyperplasia (AAH) and Transitional cell metaplasia, 9 cases of BPH with suspicious foci, 4 cases of LGPIN, 3 cases of HGPIN and 11 cases of Prostatic adenocarcinoma. In 5 cases including 3 cases of BPH with suspicious foci and 1 case each of adenosis and AAH, the diagnosis was changed to Prostatic Adenocarcinoma after IHC analysis.

CONCLUSION:

In the present study, we came across difficulties and limitations of routine H&E sections to give a diagnosis in morphologically suspicious and challenging foci under the microscope. Thus, we conclude that IHC staining should be done in cases where routine H&E sections have an ambiguous morphology. HMWCK along with AMACR is a good marker combination to differentiate Benign from Malignant lesions.

KEYWORDS:

Prostatic Adenocarcinoma, PIN (prostatic intraepithelial neoplasia), IHC (immunohistochemistry), AMACR, HMWCK.

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INTRODUCTION

Prostate adenocarcinoma is a leading cause of morbidity and mortality worldwide affecting 1 out of 9 men over 65 years and being the second most common malignancy, after lung cancer causing mortality in men. [1]

The incidence of prostatic carcinoma is increasing with age. It rises from 20% in males in their 50s to 70% in men aged 70 to 80 years. [1]

Clinical presentation of benign prostatic hyperplasia (BPH) and carcinoma prostate are same - retention of urine, dysuria, frequency, urgency, backache, hematuria, etc. Due to their posterior position, a rectal examination can identify some early prostatic carcinomas, however the test has limited sensitivity and specificity.[2]

Ultrasonography has some characteristic findings of BPH and carcinoma prostate, but poor sensitivity and specificity limit its diagnostic utility. The prostate specific antigen (PSA) has proven to be helpful in the detection and treatment of prostate cancer, though PSA is not cancer specific but organ specific. [2]

In view of increasing trend of the occurrence of both neoplastic and non-neoplastic lesions of the prostate in the elderly, the current study aims at evaluating the histomorphological features of Transurethral Resection specimens of prostate (TURP) and prostatic needle core biopsies for a period of one year. Use of immunohistochemical markers – HMWCK and AMACR in this study helps in arriving at diagnosis and to differentiate between benign and malignant lesions of prostate.

AIMS AND OBJECTIVES

1. To evaluate the utility of IHC markers HMWCK and AMACR in resolving morphologically suspicious foci on Prostatic needle core biopsies and TURP specimens.

REVIEW OF LITERATURE

Embryology of the prostate

Interactions between the urogenital sinus mesenchyme and the endoderm of the proximal region of the urethra give rise to the prostate gland in the third month of intrauterine life. Early outgrowths, 14 to 20 in number, emerge from the endoderm along the whole perimeter of the tube, predominantly on its lateral aspects, except the dorsal wall above the utricular plate, giving rise to the prostate's outermost glandular zone.

Later outgrowths of the epithelium of mixed urogenital, mesonephric, and paramesonephric ducts occur at the dorsal wall superior to the mesonephric ducts. They form the internal zone of prostate gland. The outgrowths, which are solid in the beginning, branches out to become tubular invading the surrounding mesenchyme. The latter develops into smooth muscle, blood & lymphatic vessels, and connective tissue which is invaded by autonomic nerves.[3]

Anatomy:

The prostate is an organ having fibromuscular and glandular part surrounding the prostatic urethra. It is around 3 centimeters in length and is located between the bladder neck above and the urogenital diaphragm below. A fibrous capsule surrounds the prostate that is conical in shape having a base and an apex. The base is located above the bladder neck, while the apex is located below the urogenital diaphragm. The two ejaculatory ducts enter the prostatic urethra at the lateral edges of the prostatic utricle after piercing the upper portion of the prostate on the posterior aspect.

Superiorly, prostate’s base is continuous with the bladder neck. The urethra penetrates the centre of the prostate's base. The apex of the prostate is located

inferiorly on the upper portion of the urogenital diaphragm. Anteriorly, the prostate lies posterior to the symphysis pubis and is isolated from it by additional peritoneal fat in the retropubic region known as the Cave of Retzius and connected to the posterior aspect of the pubic bones by puboprostatic ligaments. Posteriorly, the prostate lies close to the anterior aspect of the rectal ampulla and is separated from it by the rectovesical septum known as the fascia of Denonvilliers.[4]

Structure of the prostate:

The plentiful prostatic glands are invested in a mixture of connective tissue and smooth muscle with its ducts opening into the prostatic urethra. The gland is divided into five lobes i.e. Anterior, Posterior, Middle, Right lateral and Left lateral.

The anterior lobe is in front of the urethra which lacks glandular tissue.

The middle lobe is located between the urethra and the ejaculatory ducts; it has numerous glandular elements.

The posterior lobe is positioned at the back of the urethra and beneath the ejaculatory ducts and contains glandular tissue.

The Right and Left lateral lobes lie on each side of the urethra and is rich in glands.[4]

Zones of the prostate:

The prostate consists of four zones:

The peripheral zone amounts to the bulk of the gland (nearly 70%).

The central zone environs the ejaculatory ducts (around 20%).

The transition zone is around the proximal prostatic urethra containing glands. (about 5%)

The anterior fibromuscular stroma is devoid of glandular tissue.

Most often, prostate cancer arises from the peripheral zone whereas the transition zone is the area which harbors most of the cases of benign nodular hyperplasia.[5]

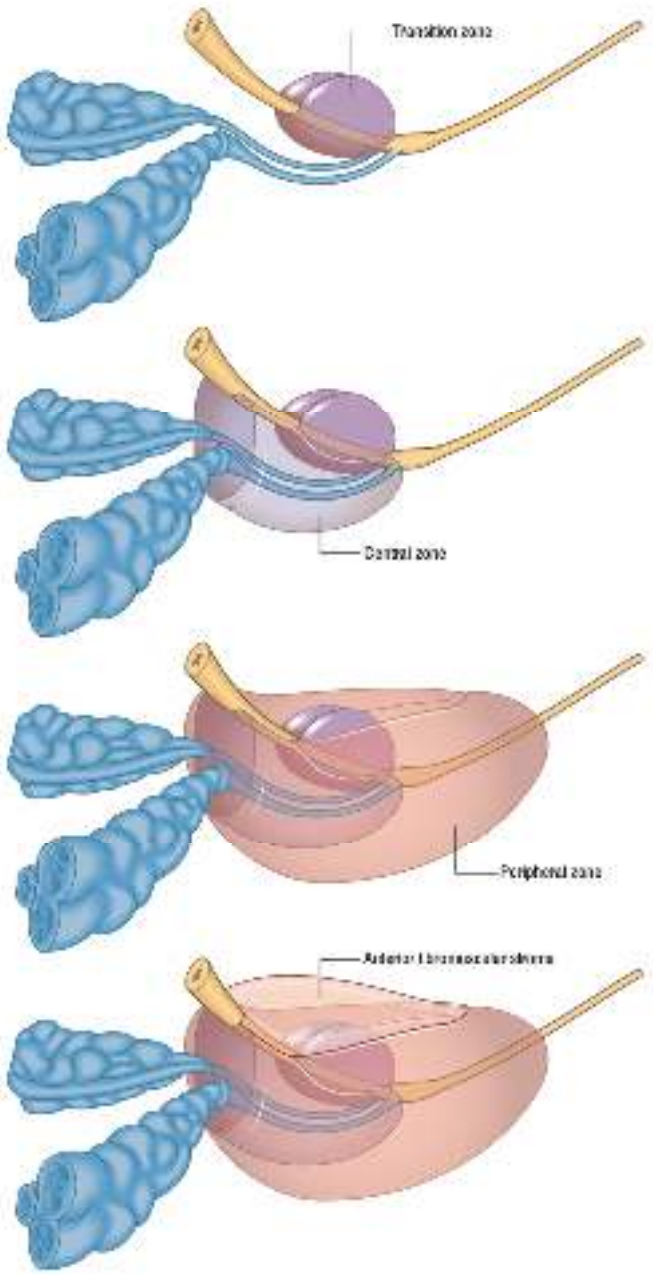


Figure 1 : Zonal Anatomy of Prostate

Blood supply:

The blood supply of the prostate is from the branches of the internal pudendal, middle rectal and inferior vesical arteries.

The base and the sides of the gland has a venous plexus that communicates with the vesical plexus and the internal pudendal vein draining into internal iliac and vesical veins.

Lymphatic drainage:

Lymphatic vessels from the prostate gland drain principally into the internal iliac and sacral nodes and partially into the external iliac nodes.

Nerve supply:

The prostate is supplied by both sympathetic and parasympathetic nerves. The lower part of inferior hypogastric plexus gives rise to prostatic plexus of nerves.[5]

Histology:

The prostate comprises of branched tubulo-acinar glands invested in a fibromuscular stroma. The posterior and lateral parts of the prostate are encased by a partial capsule, whereas the anterior fibromuscular stroma surrounds the anterior and apical surfaces. The epithelium of the glands is thrown up into folds in a convoluted form.[6] They are lined by a bi-layered epithelium; the luminal cells are tall columnar secretory type having conspicuous round basal nuclei and pale eosinophilic cytoplasm. At the base of the gland, is a population of small flat basal cells with ovoid nuclei consistent with outer basal myoepithelial layer.[3]

Inspissated secretions can pool in certain glands and produce spherical concretions (corpora amylacea), which can become calcified over time.[6]

A wide variety of lesions affect the prostate which includes Inflammatory (acute, chronic and granulomatous prostatitis), Benign (nodular hyperplasia, stromal nodule, basal cell hyperplasia, sclerosing adenosis), Pre-malignant (prostatic intraepithelial neoplasia) and Malignant (prostate carcinoma).

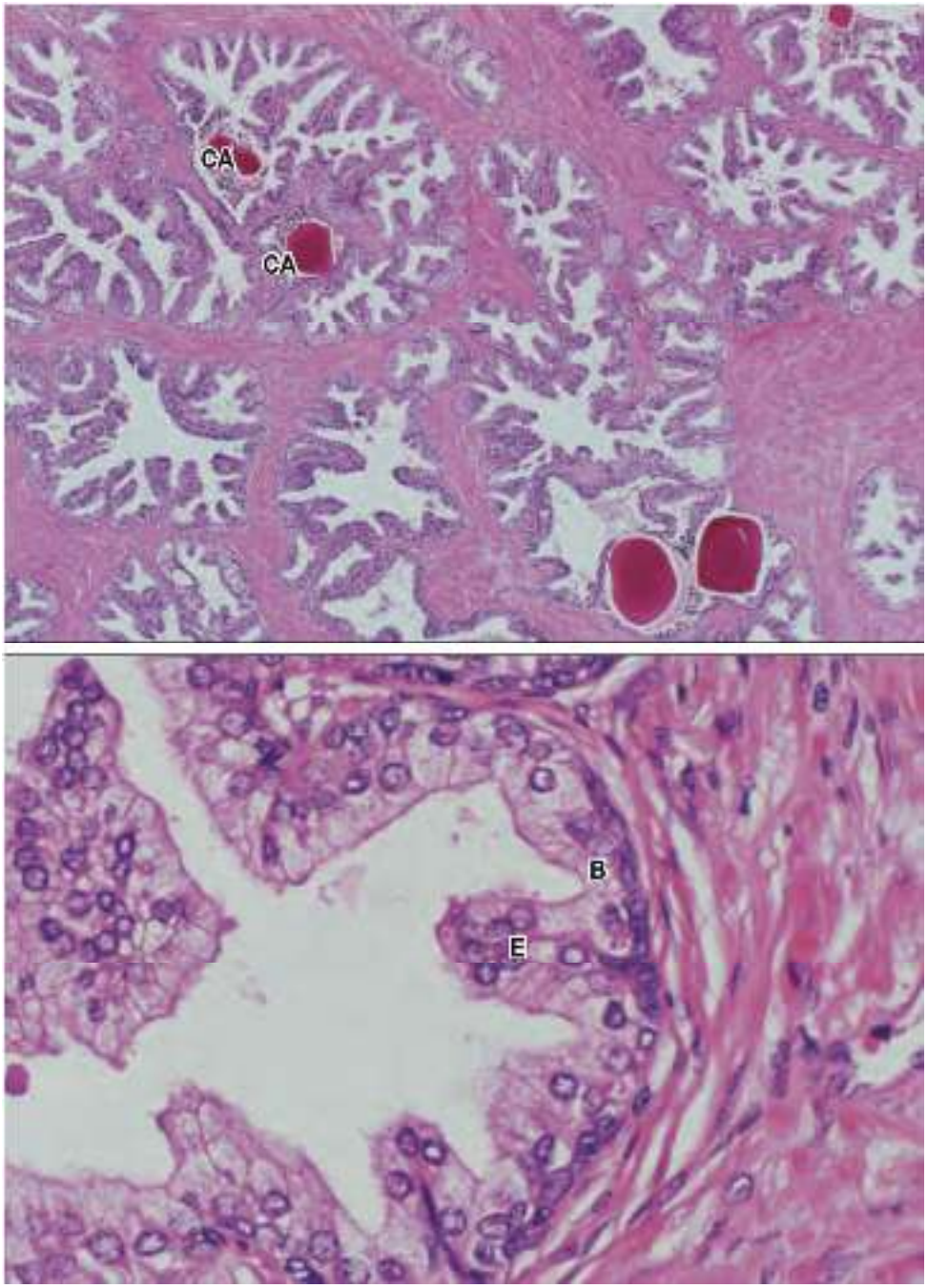


Figure 2 : Histology of Prostate

(CA – Corpora amylacea, E – Epithelial cell, B – Basal cell)

BENIGN PROLIFERATIVE LESIONS:

1. Benign prostatic hyperplasia(BPH):

It has the highest incidence among all the prostatic disease in men older than 50 years characterized by stromal and epithelial proliferation.

BPH is not considered to be premalignant.[7]

Prevalence:

Berry et al[8] reported the prevalence of BPH during fourth, fifth and eighth decades to be 8%, 50% and 75% respectively.

Etio-pathogenesis:

Kumar et al[7] have highlighted the fact that the main component of the BPH is cell death impairment, resulting in the collection of senescent cells in the prostate. The main androgen in the prostate, constituting 90% of total prostatic androgens, is dihydrotestosterone (DHT) produced in the prostate from testosterone by the enzyme type 2,5α reductase. This enzyme is located almost entirely in stromal cells. DHT induced growth factors are members of fibroblast growth factor family, particularly FGF-7, FGFs 1 and 2 and TGF β, which promote fibroblast proliferation. It is believed that these work by augmenting the proliferation of stromal cells and reducing the epithelial cell death.[7]

Gross:

On cross section, the nodules differ in colour and consistency. If the tissue is yellow-pink with a soft consistency, it indicates glandular proliferation is prominent. Nodules comprised chiefly of fibromuscular stroma are pale grey in colour and tough in consistency.[7]

Microscopy:

Stromal proliferation around small sinusoidal spaces in the periurethral regions, occasionally in the periductal and intralobular areas is the first microscopic change followed by hyperplasia of the glandular component. The glands are distended or cystic, with an inspissated glycoprotein secretion (Corpora Amylacea) that is frequently calcified. The epithelium may be flat or columnar having pale cytoplasm and regular, centrally positioned nuclei with inconspicuous nucleoli. Papillary infoldings can be seen. Immediately above a well-developed basement membrane, a continuous basal cell layer is seen. Small clusters of lymphocytes are common in the stroma.[9]

2. Stromal Nodule:

Petersen et al[10] have emphasized that this entity represents one end of the morphologic spectrum of nodular hyperplasia, comprising of fibroblastic, fibromuscular, muscular or immature mesenchymal cells in order of decreasing frequency.

Microscopically, the nodule comprises spindle cells, fibroblasts, and/or smooth muscle cells in a hyalinized or myxoid stroma along with thick hyalinized blood vessels.[10]

3. Basal Cell Hyperplasia(BCH):

BCH is infrequently a component of untreated usual, nodular glandular and stromal hyperplasia, or BPH, which arises in the transition zone of prostate.[11] Devaraj et al[12] recognized BCH as a nodular growth of homogenous glands that are spherical and connected to a cellular stroma. It is either complete type or incomplete type. Complete form has nests of dark-blue cells devoid of secretory (luminal) cell differentiation. In the incomplete form, small lumina is seen lined by secretory cells

having transparent cytoplasm and encircled by several layers of basal cells.

4. Sclerosing Adenosis:

It is described as a microglandular lesion, that can mimic prostate cancer. Microscopically, the lesion may be partly confined and the available borders indicate an infiltrative lesion. Stroma is cellular and myxoid with absence of smooth muscle cells. Other characteristics include a twofold cell population comprising transparent amphophilic cells (secretory) and fusiform cells infiltrating the stroma occasionally. In sclerosing adenosis foci, nucleoli are readily discernible.[13]

PREMALIGNANT LESIONS OF THE PROSTATE:

Prostatic intraepithelial neoplasia (PIN):

In the year 1969, McNeal defined PIN as a neoplastic proliferation of prostatic epithelial cells that is confined to pre- existing prostatic ducts or acini (glands).[14] McNeal and Bostwick coined the term intraductal dysplasia in 1986.[15] In 1987, Bostwick and Brawer coined the term prostatic intraepithelial neoplasia, which is now widely used.[15]

Definition:

PIN is best described as a neoplastic alteration of the lining epithelium of prostatic ducts and acini. It is limited within the epithelium therefore, intraepithelial.[16]

Grading of PIN:

Originally, PIN was categorized into grades 1 to 3, but currently two grades of PIN are described (i.e., low grade and high grade). Grade 1 corresponds to Low-Grade PIN (LGPIN), whereas High-Grade PIN (HGPIN) include grades 2 and 3.[17]

Table 1: Diagnostic Criteria of Prostatic Intraepithelial Neoplasia[18]

Features	Low grade PIN	High grade PIN
Architecture	Epithelial cell crowding and stratification, with irregular spacing.	Similar to low-grade PIN, but more crowding and stratification with four patterns – tufting, flat micro-papillary and cribriform
Nuclear membrane	Thin	Thick
Chromatin	Normal	Increased density and clumping
Nuclei	Enlarged, with marked size variation	Enlarged, some variation in size and shape
Nucleoli	Not discernible	Prominent, similar to those in prostate carcinoma, multiple.
Basal cell layer	Continuous and intact	Discontinuous, may show some disruption
Basement Membrane	Intact	Intact

Incidence and prevalence of PIN:

Mc Neal and Bostwick witnessed HGPIN in 82% of prostates bearing cancer in a study of 400 prostate specimens.[15] In a similar study, Sakr et al[19] concluded that the incidence of PIN is higher in prostates with carcinoma than that without carcinoma. They also established that the peripheral zone of prostate was most

commonly affected by PIN, same as carcinoma.

Troncoso et al[20] after examining 100 cystoprostatectomy specimens found the incidence of HGPIN and carcinoma to be 49% and 61% respectively.

Prevalence of HGPIN in radical prostatectomy specimens as stated by De La Torre et al[21] was remarkably high (>85%) reproducing the strong association between the lesion and prostate cancer.

Desai and Borges, in a study of 110 prostate specimens found that a majority of prostate carcinoma specimens (85.24%) were found to harbour HGPIN. Conversely, none of the benign prostate samples were found to have HGPIN.[22]

MALIGNANT LESIONS OF PROSTATE

Carcinoma Prostate:

Prostate carcinoma affects one out of nine men aged over 65 years and is the leading cause of mortality in men.[1]

In recent years, the number of cases has increased steadily, partially due to the increased life expectancy and partially due Western lifestyle which is characterized by a lack of physical exercise and high calorie diet. Epidemiological evidence indicates that blacks are the most vulnerable, white people follow, while Asians are the least at risk.[7]

Incidence:

Butler et al[23] found one or more foci of carcinoma in 32.2% of 220 cases of prostate and majority of them exhibited involvement of the lateral lobes.

Jasani et al[24] have studied 180 cases of prostate and witnessed adenocarcinoma in 58 (32%) cases.

Jayapradeep D et al[25] gave an incidence of 18.2% of carcinoma prostate in 170 prostatic biopsies studied.

Rullis et al[26] studied 57 cases of prostatic disease in men over 80 yrs of age and observed an incidence of 66.7% of prostate cancer.

Mohammed G et al gave an incidence of 43% of cancer prostate out of the 348 patients studied with mean age of the patient being 68.4 years.[27]

Etiology:

There are three etiologic factors which seem to be closely associated with prostatic cancer: age, race and the endocrine system.[28]

The prostate has alpha and beta oestrogen receptors usually found in stroma and epithelium. The physiological function of these receptors is unclear, however the involvement of oestrogen in prostate cancer has been shown. Studies further show that direct suppression of prostate oestrogen might be an unique approach of treating prostate cancer.[29]

Localization:

In a study of 208 surgically resected prostates for early carcinoma of the prostate, Byar and Mostofi established that 97% were located either peripherally or both peripherally and centrally and 85% were multifocal.[30]

McNeal et al[31] reviewed 104 prostate glands obtained at radical prostatectomy for adenocarcinoma. Among the 88 cancers, peripheral zone entails 68% , transition zone 24% , and 8% in the central zone. Carcinomas in transition zone had usually been diagnosed by transurethral resection.

Definition of terms:

As highlighted by Franks,[32] three types of prostate cancer have been identified.

1. Clinical cancer: Any case in which a firm clinical diagnosis of prostatic cancer is

made and confirmed by histology should be described as a clinical cancer.

2. Latent cancer: These tumors by definition exist but do not become manifest, i.e. they produce no clinical evidence of disease. They are found incidentally.
3. Occult cancer: These tumors manifest themselves by their metastases. The primary tumor remains hidden (occult).

Spread Of Cancer Prostate:

1. Direct spread: This occurs within the gland into the capsule of prostate and in later stages extends to the neck of bladder, trigone and ureteral openings.
2. Metastatic spread:

-Hematogenous spread: It occurs by retrograde spread via the venous plexus of prostate and spreads to lumbar spine and pelvic bones leading to osseous osteoblastic lesions. Metastasis to lung, kidney and brain also occurs through this route.

-Lymphatic spread: By this route metastasis occurs first to the obturator lymph nodes. Sacral, iliac and para-aortic lymph nodes can be involved.[33]

Clinical Features

Majority of the cases are asymptomatic if diagnosed at an early stage.

Symptoms:

1. Weak flow of urine
2. Difficulty in initiating urination
3. A feeling of presence of residual urine
4. Dribbling of urine post urination
5. Increased frequency of urination

In advanced stages, with metastasis to spine, patient presents with back pain or hip pain and unexplained weight loss.[33]

Signs:

On digital rectal examination (DRE), induration and enlargement of the gland can be detected.

With locally advanced disease, regional lymphadenopathy can be seen and in metastatic lesions signs of cord compression are evident.[33]

Morphology:

Prostatic carcinomas are categorized into:

1. Adenocarcinoma of peripheral (secondary) ducts and acini.
2. Carcinoma of large (primary) ducts.

Most of the tumors are included in the first category. Large primary duct carcinomas are normally found in a periurethral location. Outer zone is the site of predilection for the ordinary adenocarcinoma.[9]

Gross:

Peripheral zone in the posterior lobe of the gland is most often involved. The growth is firm, fibrous and ill defined.

Cut section is homogenous with irregular yellow areas. Haemorrhage and necrosis is uncommon.[33]

Microscopy:

Four major cytoarchitectural patterns:

-Small glands,

-Medium glands

-Diffuse individual cell infiltration

-Cribriform

The existence of only one cell type with absence of basal cell layer is characteristic with almost all prostate cancer.[9]

Architectural features:

Prostate cancers contain glands with sharp luminal borders. These glands grow in a disorganized manner; few are perpendicular to each other and irregularly separated by fibromuscular stroma which is indicative of an infiltrative process. The tumor's infiltration is demonstrated by the existence of tiny atypical glands between bigger benign ones. The lack of glandular differentiation leads to cribriform structures, fused glands and poorly shaped glands. Undifferentiated prostate cancer is characterized by solid sheets, cords of cell or isolated individual cells.[16]

Cytoplasmic features:

Prostate adenocarcinoma glands tend to have a sharp, discrete border without undulations or cytoplasmic ruffling. Whereas glands with an irregular luminal surface with convoluted appearance and papillary infoldings are considered as benign. Amphophilic cytoplasm can be seen in neoplastic glands which can be used as a diagnostic criterion for malignancy.[16]

Nuclear features:

The nucleus is enlarged and hyperchromatic with a conspicuous nucleoli. Mitotic figures are frequent in high-grade cancer, but uncommon in low-grade tumors.[9] Aihara et al[34] showed positive correlation between Gleason grade and mitotic figures. Montironi et al[35] discovered that apoptotic bodies are increased from small acinar carcinoma to cribriform carcinoma.

Luminal features:

High grade tumors display Comedonecrosis.

There is lack of corpora amylacea in the lumen.

Granular eosinophilic secretions may also be seen.[36]

Malignant specific features:

- Perineural invasion
- Glomerulations
- Mucinous fibroplasia (collagenous micronodules)

Mucinous fibroplasia is characterized by loose fibrous tissue with fibroblast ingrowth. Glomerulations are glands that have a non-transluminal cribriform proliferation. Rather, these cribriform forms are connected to only one edge of the gland, forming a structure that looks like a glomerulus of kidney.[36]

WHO CLASSIFICATION OF PROSTATIC TUMOURS[16]

1. EPITHELIAL TUMOURS

- Glandular neoplasms
- Acinar adenocarcinoma
  - Atrophic
  - Psedohyperplastic
  - Microcystic
  - Foamy gland
  - Mucinous (colloid)
  - Signet ring like cell
  - Pleomorphic giant cell
  - Sarcomatoid
- Prostatic intraepithelial neoplasm
  - High grade
    - Intraductal carcinoma

- Ductal adenocarcinoma
  - Cribriform
  - Papillary
  - Solid
- Urothelial carcinoma
- Squamous neoplasm
- Adenosquamous carcinoma
- Squamous cell carcinoma
- Basal cell carcinoma

2. *NEUROENDOCRINE TUMOURS*

- Adenocarcinoma with neuroendocrine differentiation
- Well differentiated neuroendocrine tumour
- Small cell and large cell neuroendocrine neoplasm

3. *MESENCHYMAL TUMOURS*

- Stromal tumour of uncertain malignant potential
- Stromal sarcoma
- Leiomyosarcoma
- Rhabdomyosarcoma
- Leioyoma
- Angiosarcoma
- Synovial sarcoma
- Inflammatory myofibroblastic tumour
- Osteosarcoma

- Undifferentiated pleomorphic sarcomaa
- Solitary fibrous tumour
- Solitary fibrous tumour , malignant
- Haemangioma
- Granular cell tumour

4. HEMATOLYMPHOID TUMOURS

5. MISCELLANEOUS TUMOURS

6. METASTATIC TUMOURS

Grading:

Multiple grading systems for prostate adenocarcinoma have been proposed in past years, including the WHO system (1975), the M.D. Anderson system **(**1982), Gleason system (1966, 1977) and Veterans Administration Cooperative Urological Research Group.[37]

The Gleason histological grading system has been widely accepted and is explained according to :

1. Degree of glandular distribution and differentiation
2. Growth pattern of the tumour in respect to the stroma

Grade and stage are the best prognostic predictors in carcinoma prostate.[9]

Gleason's primary and secondary score is calculated based on above two features examined under low power of the microscope. The most predominant pattern is the Primary score which is graded from 1 to 5; and the Secondary score is the other pattern seen. Both the scores are combined to get the Gleason score and grade. If the tumor shows only single pattern, the score is doubled. Sometimes a third pattern can be seen which is the minor pattern.[38]

Prostate specific acid phosphatase (PSAP) and Prostate specific antigen (PSA) levels, clinical and pathologic staging, frequency of apoptotic bodies, p53 overexpression, prevalence of lymph node and bone metastases, survival rate, and responsiveness to treatment all correlate well with microscopic grading of prostatic adenocarcinoma. The association of the Gleason's grading system with the mortality rate is specifically remarkable.[9]

Types of Specimens :

1. Biopsy specimen – the primary score is the most predominant pattern and the secondary score is the worst or highest pattern seen.
2. Prostatectomy specimen - the primary score is the most predominant pattern. The other pattern should involve at least 5% or more of the tumor volume to be considered as secondary pattern.

The score ranges as:

a) Well differentiated tumours - Gleason score 2 to 4
b) Moderately differentiated tumors - Gleason score 5 to 7
c) Poorly differentiated tumors - Gleason score 8 to 10

Gleason grade 1 represents well-differentiated tumours, whereas Gleason grade 5 represents the most poorly differentiated tumours.

In the present scenario the Gleason grade 1 and 2 are not used as their outcome is similar to grade 3.[38]

Patients with a score of 2–4 almost never give rise to aggressive disease, whereas most patients having a score of 8–10 develop prostatic carcinoma. Thus, the best predictive results are achieved by combining staging and grading.[9] WHO has developed a Group Grade system for better labeling with the Group Grade ranging from 1 to 5.

Figure 3 : Schematic diagram of the Gleason grading system.[39]

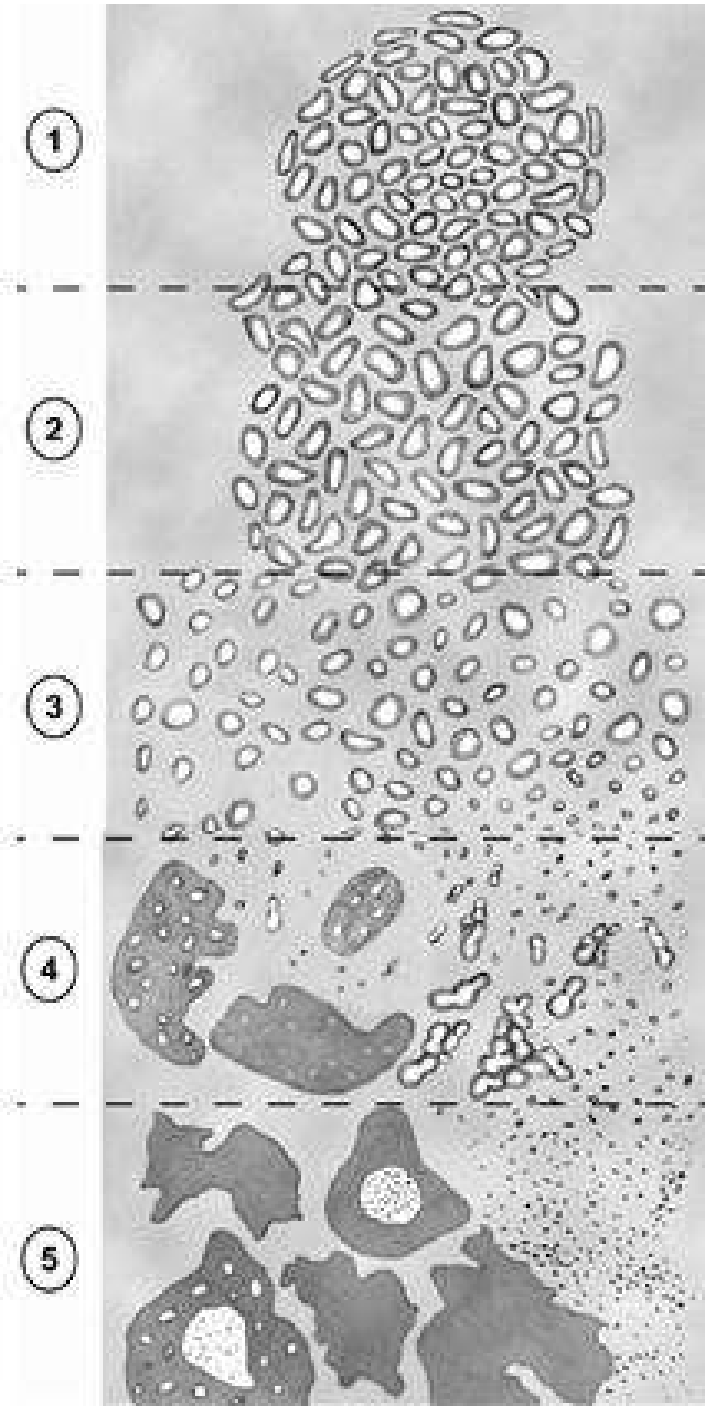


Table 2: Stage description of adenocarcinoma prostate[9]

Stage	Description
1	Single, separate, uniform glands in closely packed masses with a definite, usually rounded, edge limiting the area of tumor.
2	Single, separate, slightly less uniform glands, loosely packed (separated by small amounts of stroma), with less sharp edge.
3a	Single, separate, much more variable glands; may be closely packed but usually irregularly separated; ragged, poorly defined edge
3b	Like 3a, but very small glands or tiny cell clusters
3c	Sharply and smoothly circumscribed rounded masses of papillary or loose cribriform tumor (papillary intraductal tumor).
4a	Raggedly outlined, raggedly infiltrating, fused glandular tumour.
4b	Like 4a, with large pale cells (hypernephroid).
5a	Sharply circumscribed, rounded masses of almost solid cribriform tumor, usually with central necrosis (comedocarcinoma).
5b	Ragged masses of anaplastic carcinoma with only enough gland formation or vacuoles to identify it as adenocarcinoma.

Table 3. Gleason score along with Gleason grade group with histological Features[9]

Grade Group	Gleason Score	HISTOLOGICAL FEATURES
1	≤3 + 3 = 6	Only individual well formed and discrete glands
2	3 + 4 = 7	Predominantly well formed glands with lesser component of poorly formed glands,fused glands, glomerulations or cribriform glands
3	4 + 3 = 7	Predominantly poorly formed glands, fused glands, glomerulations or cribriform glands with lesser component of well formed glands (>5%)
4	4 + 4 = 8	Only poorly formed glands, fused glands, glomerulations or cribriform glands
	3 + 5 = 8	Predominantly well formed glands with lesser component of sheets, cribriform glands with comedonecrosis or single cells
	5 + 3 = 8	Predominantly sheets, cribriform glands with comedonecrosis or Single cells with lesser component of well formed glands (>5%)
5	≥4 + 5 = 9	Only sheets, single cells or cribriform glands with comedonecrosis

Table 4. TNM staging of prostatic adenocarcinoma[7]

TNM DESIGNATAION	ANATOMIC FINDINGS
EXTENT OF PRIMARY TUMOR (T)	
T1	Clinically inapparent lesion on palpation/imaging
T1a	Involvement of ≤ 5% of resected tissue
T1b	Involvement of > 5% of resected tissue
T1c	Carcinoma present on needle biopsy with prior levels of elevated PSA
T2	Palpable or visible cancer confined to prostate
T2a	Involvement of ≤5% of one lobe
T2b	Involvement of >5% of one lobe but unilateral
T2c	Involvement of both the lobes
T3	Local extraprostatic extension
T3a	Extracapsular extension
T3b	Seminal vesical invasion
T4	Invasion of contiguous organs or supporting structures like the bladder neck, rectum,external sphincter, levator muscle and pelvic floor

STATUS OF REGIONAL LYMPH NODES(N)	
N0	No regional nodal metastasis
N1	Metastases in regional lymph nodes
DISTANT METASTASES (M)	
M0	No distant metastases
M1	Distant metastases present
M1a	Metastases to distant lymph nodes
M1b	Bony metastases
M1c	Other distant metastases

Histological Variants Of Prostatic Adenocarcinoma:

a. Foamy gland cancer

The tumor is composed of cuboidal to columnar neoplastic cells with small nucleus and inconspicuous nucleoli; cytoplasm is finely granular, sometimes clear or foamy or xanthogranulomatous due to accumulation of lipids. Most of these cancers have a low Gleason score.[40]

b. Adenocarcinoma with atrophy

The tumour cells have atrophic cytoplasm arranged in an infiltrative pattern and the nucleus occupying almost entire cell. Nuclear enlargement with macronucleoli is also seen.

c. PIN like adenocarcinoma

This is a rare variant which shows crowded glands with stratified or pseudostratified columnar epithelium.

d. Pseudo-hyperplastic type

Hyperplastic glands are seen arranged in a microcystic pattern with papillary infoldings. There is presence of nuclear enlargement, macro-nucleoli, mitosis and crystalloids in the lumen.[41]

e. Mucinous (colloid) carcinoma:

Grossly, the cut surface has a mucoid appearance or sometimes gelatinous. On histological examination, the stroma is made from extravasated mucin pools with suspended nests, cords or clusters of carcinoma cells in acinar pattern. They have a worse prognosis than usual acinar adenocarcinoma.[42]

Saito et al[43] studied 87 cases of prostatic mucinous carcinoma and established the 3-year and 5-year survival rates to be 50% and 25%, respectively.

f. Signet ring cell carcinoma:

It is characterized by a crescent shaped nucleus due to compression by intracytoplasmic vacuole. The cytoplasm has lipid or mucin vacuoles and stain positive with mucicarmine in about 50% of cases, PAS in approximately 60% of cases, and alcian blue in approximately 60% of cases.

Warner et al[44] stated that 2.5% of cases of adenocarcinoma of the prostate are known to have Signet ring cell changes.

Remmele et al[45] highlighted that the presence of more than 50% of the signet ring cells in the tumour as a criterion for the diagnosis.

g. Sarcomatoid carcinoma (carcinosarcoma):

It is a rare tumor. According to Mostofi et al[46] tumor displaying certain sarcomatous, metaplastic cartilaginous or osseous components along with the usual adenocarcinoma can be referred to prostatic carcinosarcoma. These are associated with a very poor prognosis.[47]

Ductal Adenocarcinoma :

It accounts for <1% of total prostate cancers and often occurs in the periurethral zone. Grossly, it has a polypoidal or villous and/or infiltrative component. Microscopically, tumor involves the central ducts of prostate and is arranged in a papillary or cribriform pattern lined by columnar pseudostratified epithelium.[48]

Other microscopic types:

Squamous cell carcinoma :

Malik et al[49] mentioned that primary squamous cell carcinoma of the prostate accounts for only 0.5% to 1% among all prostate carcinomas.

Characteristics of squamous cell carcinoma are:

1) lack of glandular or acinar pattern

2) features of keratinization (squamous pearls and intercellular bridges)

3) invasion, disordered growth, and cellular anaplasia characterizing a malignant neoplasm.

4) no prior estrogen therapy

5) non-appearance of primary squamous cell carcinoma elsewhere, particularly in the bladder.

Adenosquamous carcinoma:

This is an unusual histological variant of prostate cancer. It is most frequently seen in association with hormonal treatment.[50] There are numerous theories to enlighten the histogenesis of adenosquamous carcinoma –

1) Metaplastic Transformation of adenocarcinoma cells

2) it is a Collision Type Tumour resulting after radiation or hormonal therapy

3) Multidirectional Differentiation : Histologically it is composed of malignant squamous elements and a disorderly admixture of adenocarcinomatous elements.[51]

Small-cell (neuroendocrine) carcinoma:

Primary small cell prostate cancer is not frequent, and is generally an incidental finding, as Trotz and his colleagues have emphasised. Three theories of histogenesis have been proposed-

1) originate from local endodermal amine precursor uptake decarboxylation cells
2) arise from dedifferentiation of prostatic adenocarcinoma
3) originate from the prostate's totipotent stem cells. This is the most widely accepted view.[52]

Tetu et al[53] witnessed that small cell prostate cancers have been reported to give rise to paraneoplastic syndromes allied with the formation of

adrenocorticotrophic hormone. Di Sant'agnese et al[54] identified that the tumour cells stain positively for the neuroendocrine markers.

Transitional cell (Urothelial) carcinoma (TCC):

The incidence of primary urothelial carcinoma of prostate ranges from 0.7 to 2.8% among all prostatic tumors in adults. Most patients are in the age group of 45–90 years.[55] The urothelium that lines the prostatic urethra and the proximal parts of the prostatic ducts is the first to be affected. These may arise as a result of a hyperplasia-to-dysplasia progression.[56]

When TCC of the urinary bladder involves the prostate, there are two patterns of involvement:

1) mucosal pagetoid spread though the prostatic urethra, prostatic ducts and acini with or without stromal invasion

2) direct invasion though bladder wall.[57]

Mesenchymal tumours:

Leiomyoma**:**

Leiomyoma is a rare entity described in the prostate. It is probably of embryologic origin.[58] Leiomyomas may be perplexed with stromal nodules as both contain abundant smooth muscle, but well-organized fascicles are commonly seen in leiomyomas. In addition, leiomyomas may have little mitotic activity and little to no nuclear atypia.[59]

Leiomyosarcoma of prostate:

Leiomyosarcoma of the prostate is rare and most repeatedly presents with urinary obstruction.[59] A mild degree of atypia, foci of enhanced cellularity, scattered mitotic

figures, and a focally infiltrative growth pattern surrounding benign prostate glands at the periphery distinguish low-grade leiomyosarcomas from leiomyomas. Leiomyosarcomas commonly express vimentin, actin, and desmin.[60]

LAB DIAGNOSIS

Four tier approach is utilized for the diagnosis of carcinoma prostate with:

1. Digital rectal exam (DRE)
2. Transrectal ultrasound (TRUS) and MRI
3. Serology
4. Transrectal ultrasound guided core needle biopsy

Digital rectal examination or DRE:

DRE is used to detect asymmetry, induration and hard palpable nodules but it is non-specific and subjective. An abnormal DRE has a positive predictive value (PPV) of 22% to 36%.[61]

Transrectal ultrasound or TRUS:

It is a common imaging modality in diagnosis of carcinoma prostate but it has a poor sensitivity and specificity. Hence, is now used for :

- ✓ Estimation of volume of prostate to calculate PSA density
- ✓ USG Guided needle core biopsies.[62]

Two more techniques have recently come up for clinical staging of carcinoma prostate which are endorectal Magnetic Resonance Imaging (MRI) and multiparametric MRI (mpMRI). It has the best sensitivity and specificity compared to

other imaging modalities.

Another important modality is positron emission tomography (PET) tracers, which is useful in early stage detection of cancer prostate. The tracers using FDG and sodium F18-fluoride are used in detection of advanced cases and also for assessing bony metastasis.[63]

Serology:

PSA and (PSAP)

- PSA :

PSA is also called gamma-seminoprotein or kallikrein-3. It is produced by the epithelium of prostatic ducts and acini and is found in normal, hyperplastic and malignant conditions of the protatic gland. The normal prostate releases very little PSA into the bloodstream, whereas the release of PSA is increased in prostatic illness.[64] PSA levels are significantly high in prostate cancer and it is an excellent marker for monitoring response to therapy, which includes residual and recurrent disease and may predict tumor stage.[65] It is considered to be a sensitive marker for early stage disease.[66]

PSA is detected in female ejaculate at amounts comparable to those seen in male semen. Apart from sperm and female ejaculate, breast milk and amniotic fluid have the highest PSA concentrations in biological fluids. PSA has been found in the urethral glands, endometrium, normal breast tissue, and salivary gland tissue at low levels. PSA is also detected in the serum of women with breast, lung, or uterine cancer, as well as certain kidney cancer patients. [67]

PSA is present in the serum in very low concentration in bound and unbound forms with the normal value ranging from 1.0 to 4.0 ng/ml.[66]

PSA SCREENING:

The main aim of screening is to decrease deaths due to cancer. 4ng/ml is the most commonly used cut off for PSA levels. The cancer detection rate on a prostate biopsy when the DRE is normal and the PSA levels are between 4-10 ng/ml is 25% but with levels more than 10ng/ml in a similar setting increases the rate to 67%.[68]

AGE ADJUSTED REFERENCE RANGE OF PSA:

Table 5. Age specific PSA reference ranges[68]

UPPER REFERENCE RANGE(ng/ml)	AGE (in years)
2.5	40 - 49
3.5	50 - 59
4.5	60 - 69
6.5	70 - 79

PSA VELOCITY :

It is the rate of change of serum PSA value. Patients with prostate cancer have an increase rate of change of PSA as compared to those without cancer with the PSA velocity being 0.75 ng/ml per year. For this derivative to be valid atleast three PSA values must be available in a period of two years.

Another derivative used is PSA doubling time which is the amount of time required for PSA to double. It is used along with PSA velocity and the combined term is PSA dynamics.[69]

PSA DENSITY (PSAD):

This derivative gives the amount of PSA produced per gram of prostatic tissue-

PSA density = Total serum PSA/ Estimated gland volume

The gland volume is measured by transrectal ultrasound. A higher value of PSA density suggests an increased risk of carcinoma prostate. The cut off value to perform biopsy is 0.15 ng/ml/cumm. PSA density has been found to be increased in patients with aggressive cancer prostate.[70]

Feedland et al found that PSAD is a better predictor of extracapsular disease, positive surgical margin, seminal vesical invasion as compared to PSA.[39]

CORRELATION OF PSA WITH HISTOPATHOLOGICAL FINDINGS:

Many studies have been done which have revealed that serum PSA levels correlate positively with the Gleason score that is as the Gleason score increases, PSA values also are found to increase.[71]

Compared to the well differentiated cancer prostate the poorly differentiated ones secrete PSA in large quantities and, higher the Gleason group more significantly high amounts of PSA is produced in comparison to low Gleason groups.[72] As there is an increase in Gleason grade and score along with increasing anaplasia , the PSA levels are seen to increase.[73]

- PSAP :

It is a sialoglycoprotein formed by benign and malignant prostatic epithelial cells. Serum levels can be measured by enzymatic or immunological assays and it helps to predict staging and monitoring of patients with prostatic carcinoma.[65] Higher levels have been noticed in the prostatic malignancies.[66]

Core Needle Biopsy:

These biopsies are obtained from :

i. Enlarged Prostate – Studies have shown that there is a decreased detection of cancer in larger prostates.

ii. Transition Zone – it is done in patients if the first biopsy was benign even when

there was a suspicion of cancer.[74,75]

iii. Non-Transition Zone – it is done for accurate pathological stage and gleason score, 14 cores are obtained with 12 cores from the sextant (parasagittal mid-lobe region) and posterolateral biopsy along with 2 additional cores from transition zone.[76]

IMMUNOHISTOCHEMISTRY (IHC)

In cases of prostatic adenocarcinoma (limited) on needle core biopsy, IHC markers are often used as an aid to correct diagnosis.[77]

PSA and PSAP are the oldest IHC markers which can be used to confirm prostatic origin acinar lesions.

In the recent times, there are 2 most important types of markers:

a) Negative Markers for Malignancy

These are Basal cell specific markers – p63, CK5/6, HMWCK. These stain the outer basal cell layer of the prostatic glands.

Absence of basal cells is the diagnostic hallmark of Prostatic adenocarcinoma. For more than 15 years, basal cell markers have been the cornerstone of prostatic malignancy diagnosis.[78]

High Molecular Weight Cytokeratin (HMWCK) Staining

HMWCK is a cytoplasmic staining basal cell IHC marker.[79]

In cases, where morphology is highly suspicious yet not confirmatory, negative immunostaining for basal cell markers (HMWCK and/or p63) provides extra support towards malignant diagnosis. On the other hand, less atypical cases having positive staining for these IHC markers can result in a confident benign diagnosis.[80]

HMWCK shows discontinuous, intact, circumferential staining of basal cells in benign and pre-malignant lesions but absent staining in malignant lesion.[81]

Garg M et al[82] studied 364 prostate specimens and found HMWCK positive in all cases of BPH (100%) while negative in all cases (100%) of prostate carcinoma.

In a study by Shah and colleagues, HMWCK was negative in all cases of prostate cancer (100%).[95]

b) Positive Markers for Malignancy

These markers are AMACR, ERG (ETS related gene), FASN(Fatty acid synthase), GOLM1(Golgi membrane protein 1)

It has long been a desire of surgical pathologists to complement basal cell markers, with a positive marker for malignancy. AMACR was the first such marker.[83]

Alpha-methyl-acyl CoA Racemose (AMACR) Staining

AMACR is a cytoplasmic protein identified by cDNA library as it is substantially upregulated in prostate cancer.[84] The beta-oxidation of branched-chain fatty acids and fatty acid derivatives is thought to be mediated by AMACR.[85] Peroxisomes and mitochondria of human cells contain it.[86] Before beta oxidation, AMACR in peroxisomes and mitochondria converts racemic combinations of branched fatty acids and fatty acid derivatives into (S) isomers.[87] P504S, a monoclonal AMACR antibody, has been produced and is commercially available for use on conventional formalin-fixed, paraffin-embedded tissue specimens.[88] Overexpression of the AMACR gene has been found in a variety of human cancers, including colon and mammary tumours, malignant melanomas, and papillary renal cell carcinomas. When compared to benign prostatic lesions, prostatic

adenocarcinomas showed a 36-times higher expression of P504S (AMACR).[89]

Interpretation of AMACR staining

Dark diffuse or granular, cytoplasmic or luminal, but circumferential staining signifies AMACR positivity.

The positivity is graded from 0 to 3+ as follows:

Table 6. Interpretation of AMACR staining[92]

% of stained cells	Grade	Interpretation
0	0	NEGATIVE
1-10	1+	MILD
11-50	2+	MODERATE
>51	3+	STRONG

Jiang et al[90] in their study found strong immunostaining of AMACR in prostate cancer and HGPIN and, negative staining for AMACR in 254 (91.7%) out of 277 cases of benign prostate diseases.

Molinie et al[91] reported AMACR overexpression in 97% of prostate cancer cases. (252 of 260 cases)

IHC markers (HMWCK and AMACR) play a crucial role in prostate lesions, especially for lesions in grey zone in routine haematoxylin-eosin (H & E) and helps to distinguish malignant glands from benign lesions.

METHODOLOGY

This is a hospital based – observational (prospective) study in which 30 cases of morphologically suspicious TURP specimens and needle core prostatic biopsies were taken. The study was done for a period of one year i.e., from 1st January 2020 to 31st December 2020 at J.N. Medical College and KLE'S Dr. Prabhakar Kore Hospital and MRC, Belagavi.

Sample size: 30

Sampling procedure: Universal Sampling

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human Subjects Research.

Inclusion criteria:

1. Morphologically suspicious cases of TURP specimens and needle core prostatic biopsies.

Exclusion criteria:

1. Frank Benign cases on Histology.
2. Cases of Prostatitis.

Consent from the patients was taken, and the clinical history as well as results of relevant investigations were collected. The specimens were received at Department of Pathology, J.N. Medical College, Belagavi and were fixed in 10% formalin solution for a period of 6-18 hours and were routinely processed. Representative

formalin-fixed-paraffin-embedded blocks were made and consecutive ribbon sections of 3.5-4 µm thickness were prepared and stained with Haematoxylin-Eosin (H&E) and studied for the histopathological features. In cases of prostatic adenocarcinoma, histological type, pattern, Gleason score, Gleason group grade, perineural invasion (PNI) and tumor volume were studied.

In addition, 4 µm sections were cut in similar manner and taken on 2 glass slides coated with Poly-L-Lysine for IHC analysis to observe expression of HMWCK and AMACR. In HMWCK, cytoplasmic staining of the basal layer and with AMACR, cytoplasmic granular staining (0 to 3+) of epithelial cells were studied. The correlation between various clinicopathologic parameters along with serology markers and histopathological features as well as IHC were studied.

Statistical Analysis:

The statistical analysis was done using SPSS 28 version software system. Results were expressed in numbers and percentages along with graphs and charts.

The predictive values (positive and negative) were calculated after analysis of true negatives, true positives, false negatives and false positives. Kappa statistics was done for studying the true agreement of using IHC markers for differentiating benign lesions from malignant ones.

RESULTS

A one-year observational study of 30 male patients with prostatic disease having suspicious morphology on histopathological examination is undertaken and were further subjected to IHC staining. The expression of IHC markers HMWCK and AMACR is studied to arrive to a definitive final diagnosis.

Out of the 30 cases in this study, the initial Histopathological diagnosis revealed one case each of Adenosis, Atypical Adenomatous Hyperplasia (AAH) and Transitional Cell Metaplasia (TCM). Nine cases showed BPH with suspicious focus, 7 cases of PIN (4 cases of LGPIN and 3 cases of HGPIN) and 11 cases of Prostatic Adenocarcinoma. IHC staining was done using HMWCK and AMACR for confirmation of all the histopathological diagnosis.

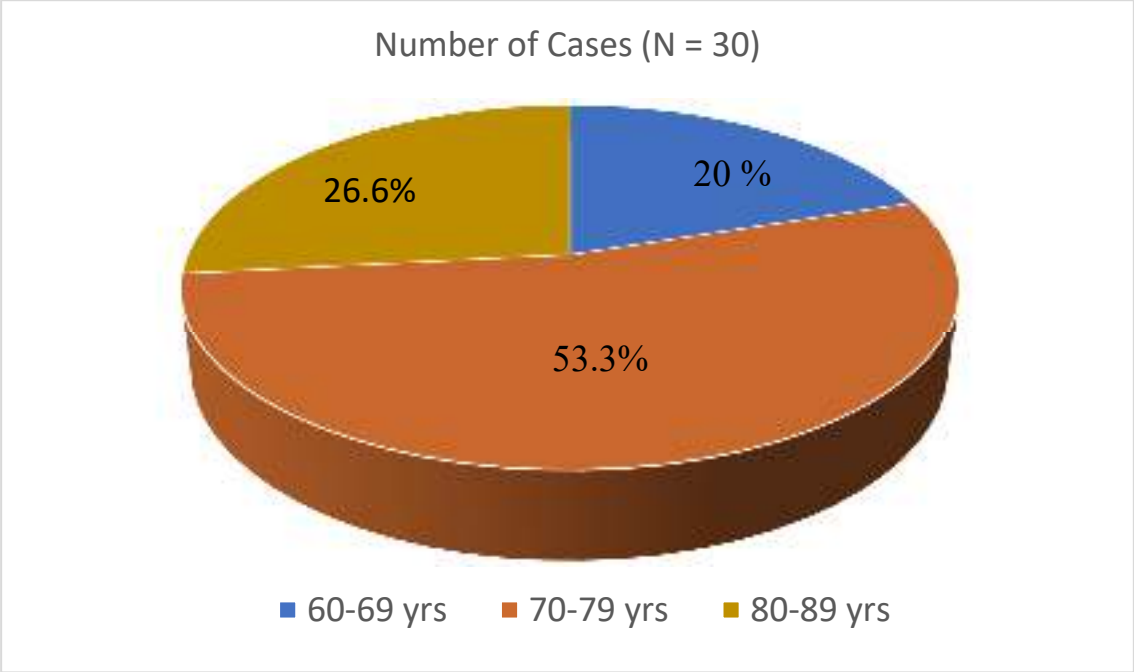
The observation and results were as follows:

In this study, age ranged from 60-88 years and Mean Age ± SD was 74.53±7.41 years. Majority (16 cases, 53.3%) of cases were in the 70-79 years age group. (Table 7; Figure 4)

Table 7 : Distribution of cases according to age group

Age (in years)	Number of Cases	Percentage (%)
60-69	6	20
70-79	16	53.3
80-89	8	26.6
TOTAL	30	100

Figure 4 : Distribution of cases according to age group

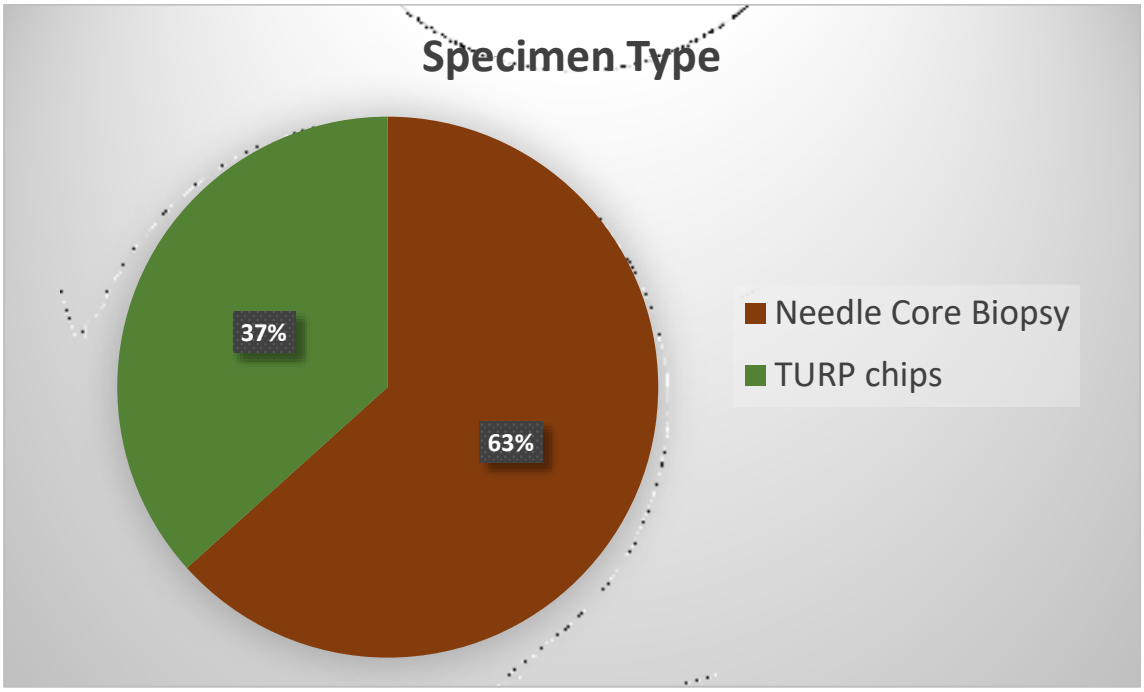


Out of 30 cases, 19 cases (63.3%) were Prostatic needle core biopsy specimens and remaining 11 cases (36.7%) were TURP chips. (Table 8; Figure 5)

Table 8 : Distribution of cases according to type of specimen received

Type of Specimen	Number of Cases	Percentage (%)
Needle core Biopsy	19	63.3
TURP Chips	11	36.7
TOTAL	30	100

Figure 5 : Distribution of cases according to type of specimen received (N=30)

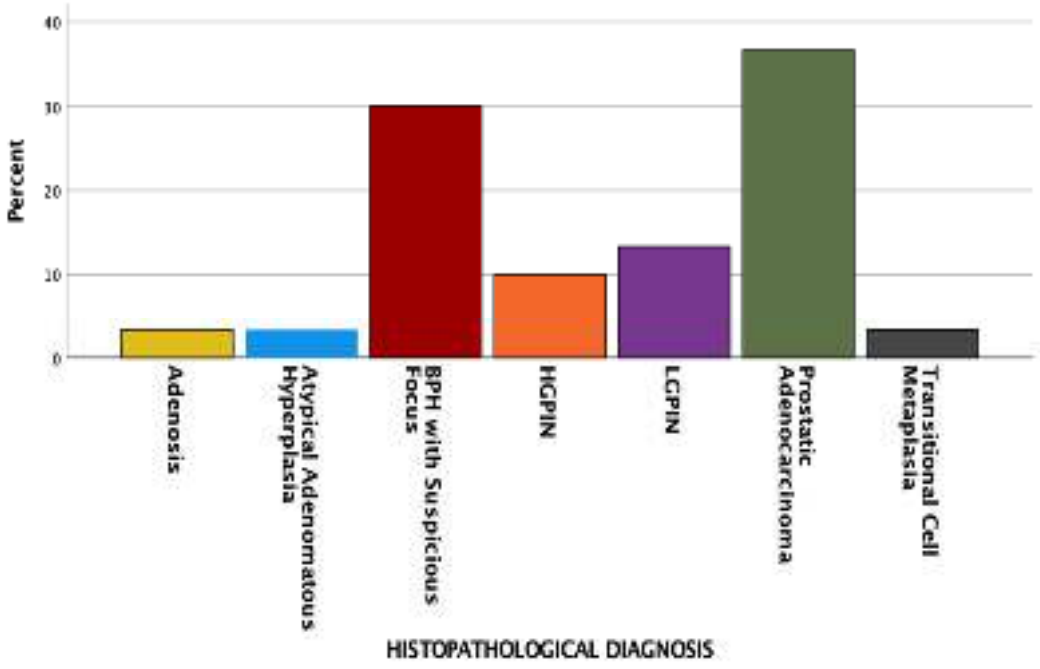


According to the Histopathological diagnosis, one case (3.3%) each of Adenosis, Atypical Adenomatous Hyperplasia (AAH) and Transitional Cell Metaplasia (TCM) were noted. Nine cases (30%) showed BPH with suspicious focus, 7 cases (23.3%) of PIN (4 cases of LGPIN and 3 cases of HGPIN) and 11 cases (36.7%) of Prostatic Adenocarcinoma. (Table 9; Figure 6)

Table 9 : Distribution of cases according to Histopathological Diagnosis

HP Diagnosis	Number of Cases	Percentage (%)
AAH	1	3.3
Adenosis	1	3.3
BPH with Suspicious Focus	9	30
LGPIN	4	13.3
HGPIN	3	10
Transitional Cell Metaplasia	1	3.3
Prostatic Adenocarcinoma	11	36.7
TOTAL	30	100

Figure 6 : Distribution of cases according to Histopathological Diagnosis (N=30)

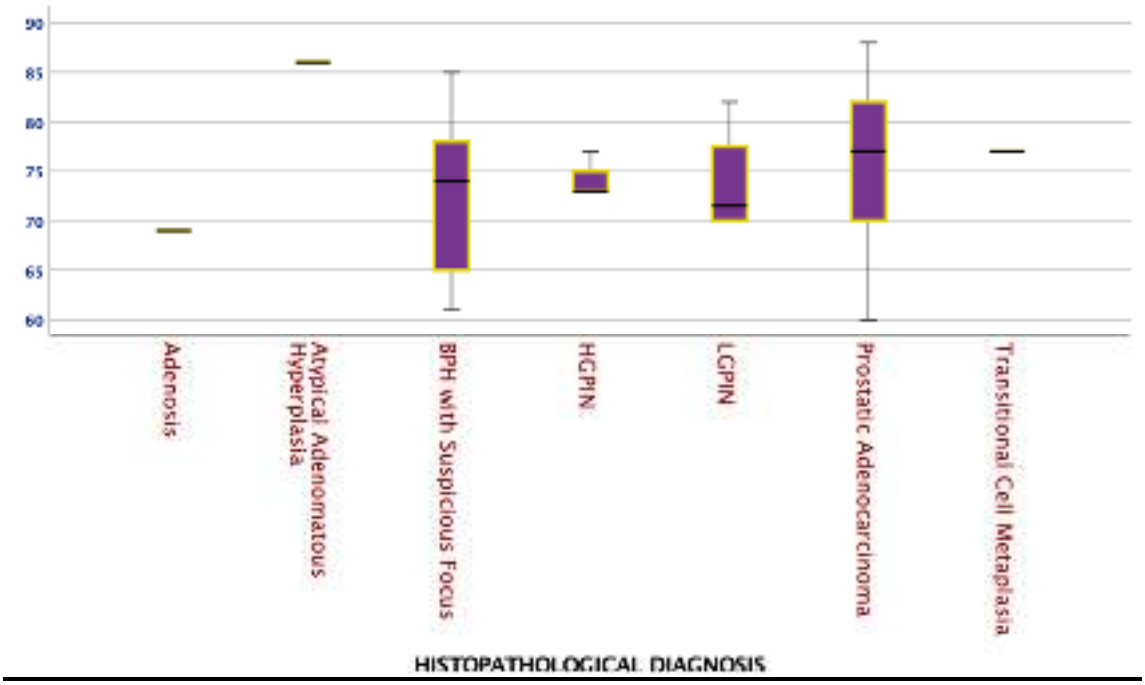


The minimum and maximum age (in years) along with mean age (in years) and standard deviation(SD) according to the histopathological diagnosis were analysed. (Table 10; Figure 7)

Table 10 : Mean Age (years) of cases according to Histopathological Diagnosis

HP Diagnosis	Frequency	Mean Age	SD	Min	Max
AAH	1	86	-	86	86
Adenosis	1	60	-	60	60
BPH with Suspicious Focus	9	72.88	7.865	61	85
LGPIN	4	73.75	5.679	70	82
HGPIN	3	74.33	2.309	73	77
Transitional Cell Metaplasia	1	77	-	77	77
Prostatic Adenocarcinoma	11	75.62	8.870	60	88
TOTAL	30	74.53	7.41	60	88

Figure 7 : Mean Age (years) of cases according to Histopathological Diagnosis



PSA levels were correlated according to the histopathological diagnosis. Mean PSA level was highest in Prostatic adenocarcinoma while TCM has the lowest value followed by AAH. (Table 11)

Table 11 : PSA correlation according to Histopathological Diagnosis

HP Diagnosis	Number of Cases	Minimum PSA (ng/mL)	Maximum PSA (ng/mL)	MEAN PSA (ng/mL)
AAH	1	-	-	6.75
Adenosis	1	-	-	8.63
BPH with Suspicious Focus	9	4.61	66	18.31
LGPIN	4	14.8	48.08	31.03
HGPIN	3	6.13	33.7	16.05
TCM	1	-	-	3.5
Prostatic Adenocarcinoma	11	6.91	180.35	39.4

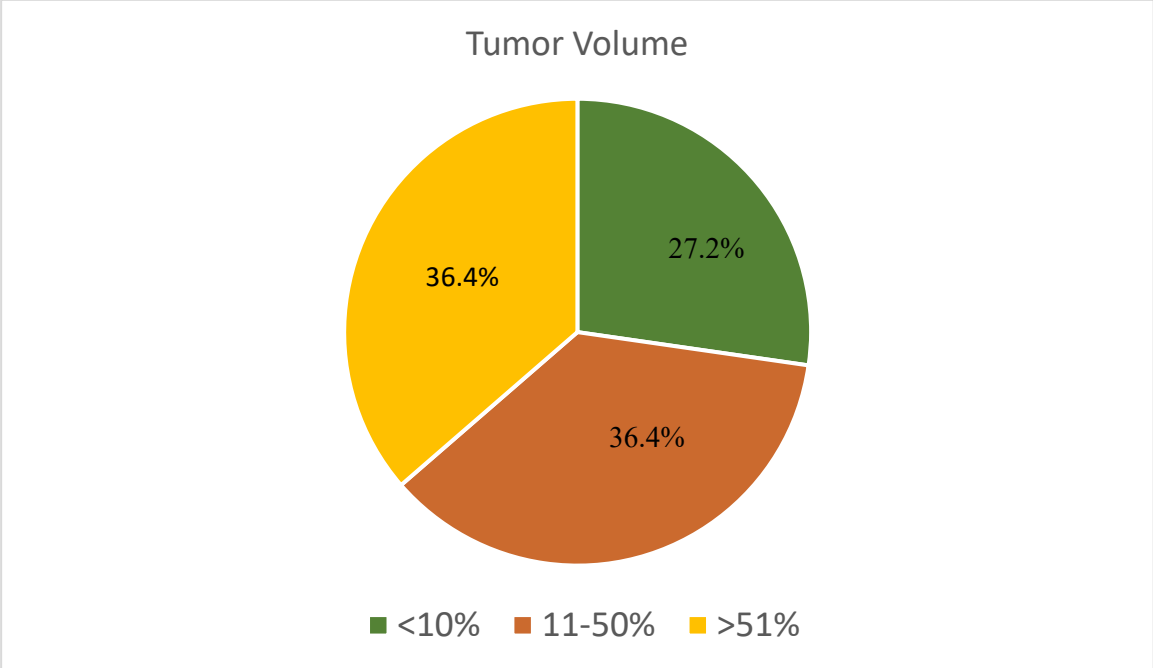
Out of 11 Prostatic Adenocarcinoma cases, 27.2% (3 cases) show approximate tumor volume of <10% while 36.4% (4 cases) of 11-50% and 36.4% (4 cases) >50% tumor volume. (Table 12; Figure 8)

Table 12 : Distribution of Microscopic Feature – Tumor Volume in cases of Prostatic Adenocarcinoma

Tumor Volume	Number of cases	Percentage (%)
<10%	3	27.2
11-50%	4	36.4
>51%	4	36.4
TOTAL	11	100

Figure 8 : Distribution of Microscopic Feature – Tumor Volume in cases of Prostatic Adenocarcinoma

(N=11)



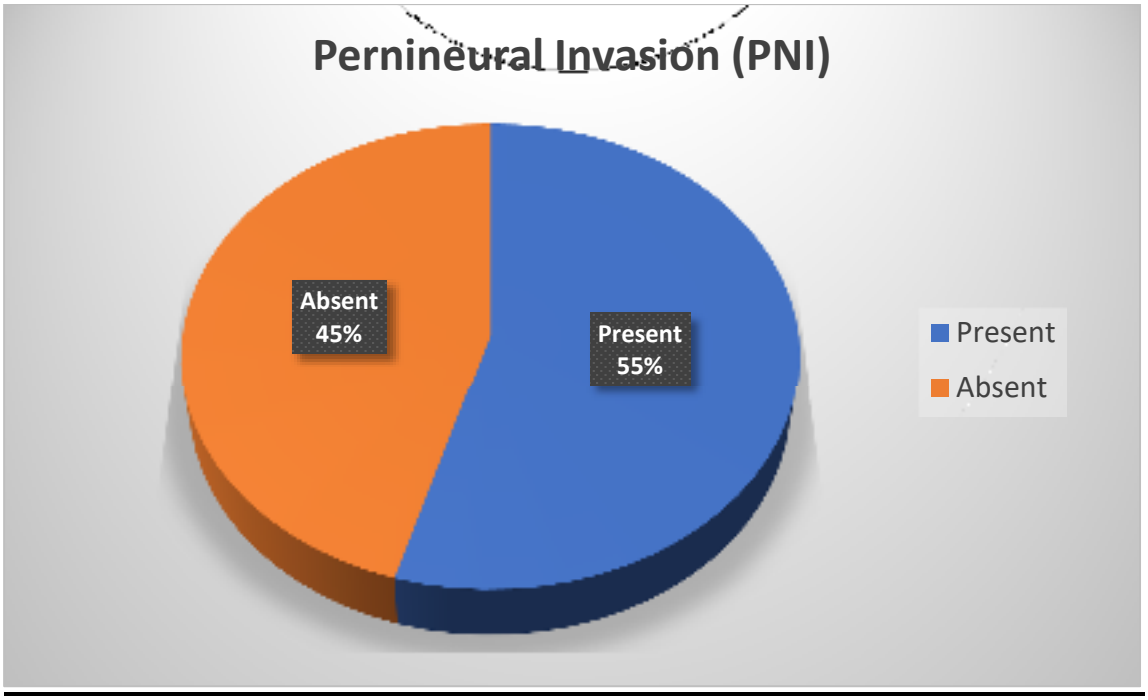
Six cases (55%) had a prominent Perineural Invasion while in remaining 5 cases (45%) perineural invasion was not seen. (Table 13; Figure 9)

Table 13 : Distribution of Microscopic Feature – Perineural Invasion(PNI) in cases of Prostatic Adenocarcinoma

PNI	Number of cases	Percentage (%)
Present	6	55
Negative	5	45
TOTAL	11	100

Figure 9 : Distribution of Microscopic Feature – Perineural Invasion(PNI) in cases of Prostatic Adenocarcinoma

(N=11)



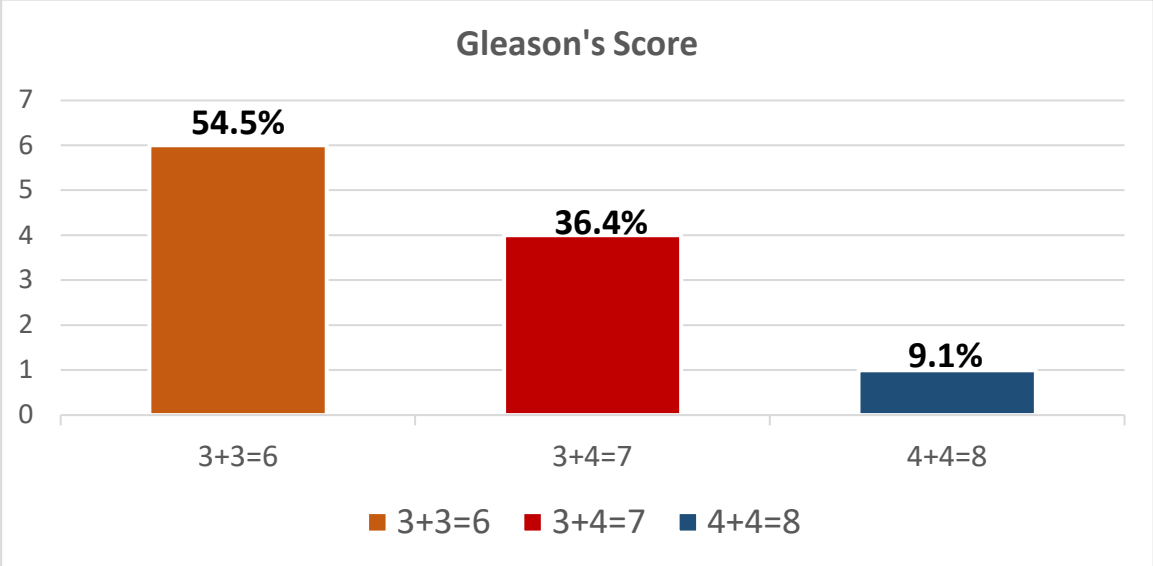
In this study, out of 11 Adenocarcinoma cases, majority (6 cases, 54.5%) had Gleason's Score of 3 + 3 = 6. (Table 14; Figure 10)

Table 14 : Distribution of cases of Prostatic Adenocarcinoma according to Gleason's score

Gleason's Score	Number of cases	Percentage (%)
3+3=6	6	54.5
3+4=7	4	36.4
4+4=8	1	9.1
TOTAL	11	100

Figure 10 : Distribution of cases of Prostatic Adenocarcinoma according to Gleason's score

(N=11)

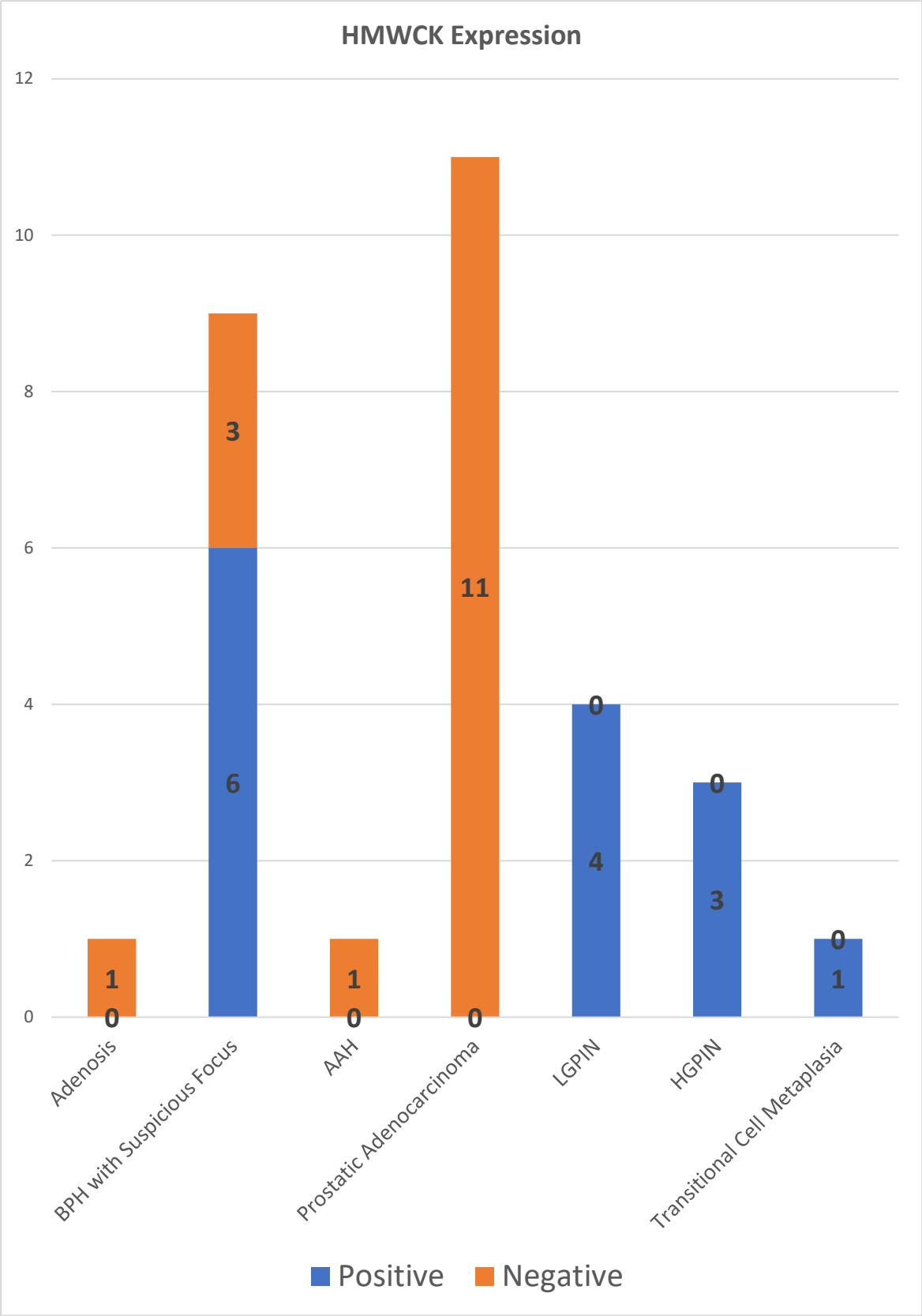


On HMWCK immunostaining, one case each of AAH and Adenosis showed loss of basal cell layer. Out of 9 cases of BPH with Suspicious focus, 6 cases (66.7%) showed positive intact basal layer while 3 cases (33.3%) showed negative HMWCK expression rendering a malignant diagnosis. All 7 PIN cases (100%) had circumferential luminal and cytoplasmic staining of the basal cells. One case of TCM has positive intact basal cell layer. All 11 cases (100%) of Adenocarcinoma prostate shows negative staining of basal cell layer. (Table 15; Figure 11)

Table 15 : Expression of HMWCK immunostaining in different cases

HP Diagnosis	**IHC – HMWCK**			
	Positive cases	**%**	**Negative cases**	**%**
AAH	0	0	1	100
Adenosis	0	0	1	100
BPH with Suspicious Focus	6	66.7	3	33.3
LGPIN	4	100	0	0
HGPIN	3	100	0	0
Transitional Cell Metaplasia	1	100	0	0
Prostatic Adenocarcinoma	0	0	11	100

Figure 11: Expression of HMWCK immunostaining in different cases



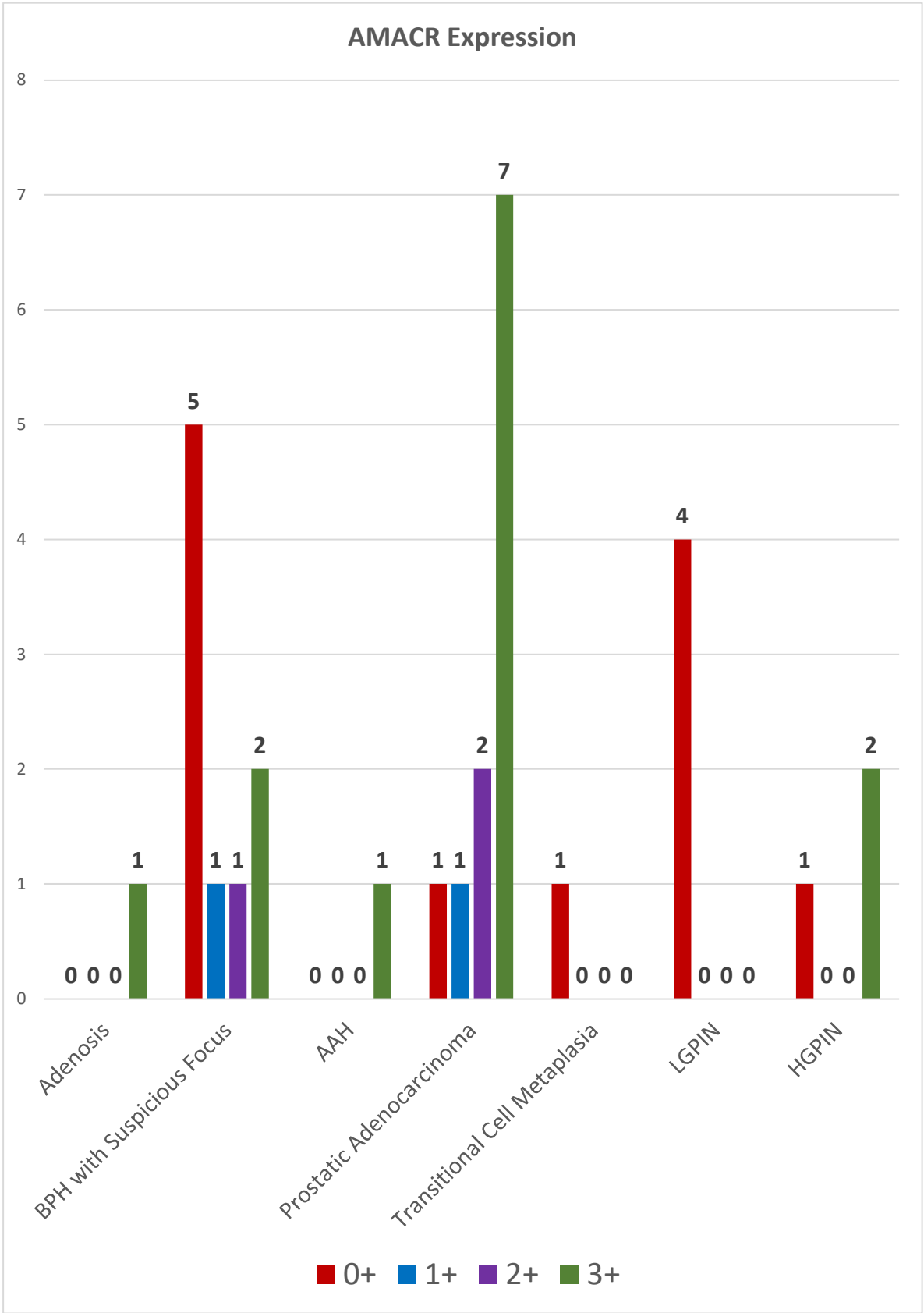
On AMACR immunostaining, one case each of AAH and Adenosis showed strong AMACR overexpression rendering a malignant diagnosis. Out of 9 cases of BPH with Suspicious focus, 3 cases showed moderate to strong positivity for AMACR favouring Adenocarcinoma while 6 cases were AMACR negative pointing to a benign diagnosis. Out of 7 PIN cases, all four cases of LGPIN were negative for AMACR and 2 out of 3 cases of HGPIN showed strong AMACR expression. One case of TCM was negative for AMACR. Out of 11 Adenocarcinoma cases, 7 cases showed strong cytoplasmic granular positivity while 3 cases showed mild to moderate positivity. Only 1 out of 11 cases, was AMACR negative.

(Table 16; Figure 12)

Table 16 : Expression of AMACR immunostaining in different cases

HP Diagnosis	IHC – AMACR			
	Negative	Positive		
	0+ Nil (%)	1+ Mild (%)	2+ Moderate (%)	3+ Strong (%)
AAH	-	-	-	1 (100)
Adenosis	-	-	-	1 (100)
AAH	-	-	-	1 (100)
BPH with Suspicious Focus	5 (55.5)	1 (11.11)	1 (11.11)	2 (2.22)
LGPIN	4 (100)	-	-	-
HGPIN	1 (33.33)	-	-	2 (66.66)
Transitional Cell Metaplasia	1 (100)	-	-	-
Prostatic Adenocarcinoma	1 (9.09)	1 (9.09)	2 (18.18)	7 (63.63)

Figure 12 : Expression of AMACR immunostaining in different cases



In our study, one case each of AAH and Adenosis showed HMWCK negative in basal cells as well as AMACR positivity in the glands; these were given a malignant diagnosis of Prostatic Adenocarcinoma (*False Negatives*). Out of 9 BPH with suspicious focus cases, 3 cases show AMACR overexpression along with loss of basal cell layer (HMWCK negative) at the suspicious focus; these were changed to Prostatic Adenocarcinoma (*False Negatives*). Four cases of LGPIN were consistent with intact basal layer i.e., HMWCK positive while AMACR showed no immunostaining. In all the 3 cases of HGPIN, HMWCK was positive while AMACR was positive in 2 out of 3 cases. Ten out of 11 cases of adenocarcinoma were HMWCK negative and AMACR positive and 1 case showed both HMWCK and AMACR negativity (*True Positives*). (Table 17)

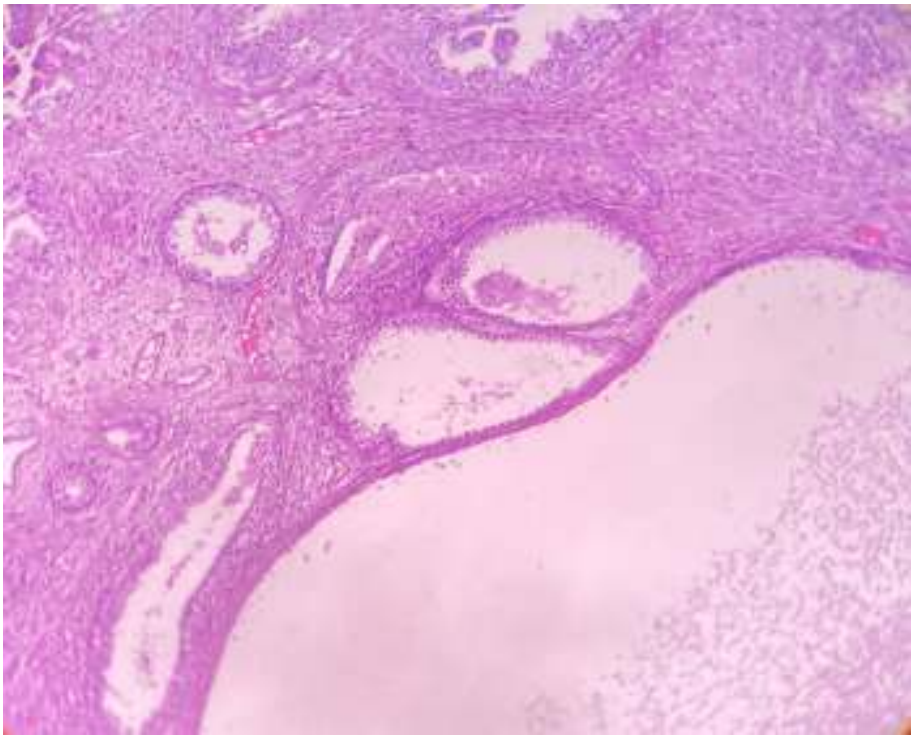
According to our study, after analysis of both the IHC markers (HMWCK and AMACR), the Positive and Negative Predictive value of using these IHC markers for a correct diagnosis is 100% and 73.7% respectively.

Kappa statistics for true agreement was calculated which showed a kappa value (κ) of *0.67*, which signifies Substantial Agreement for using IHC markers (HMWCK and AMACR) to differentiate Benign from Malignant lesions.

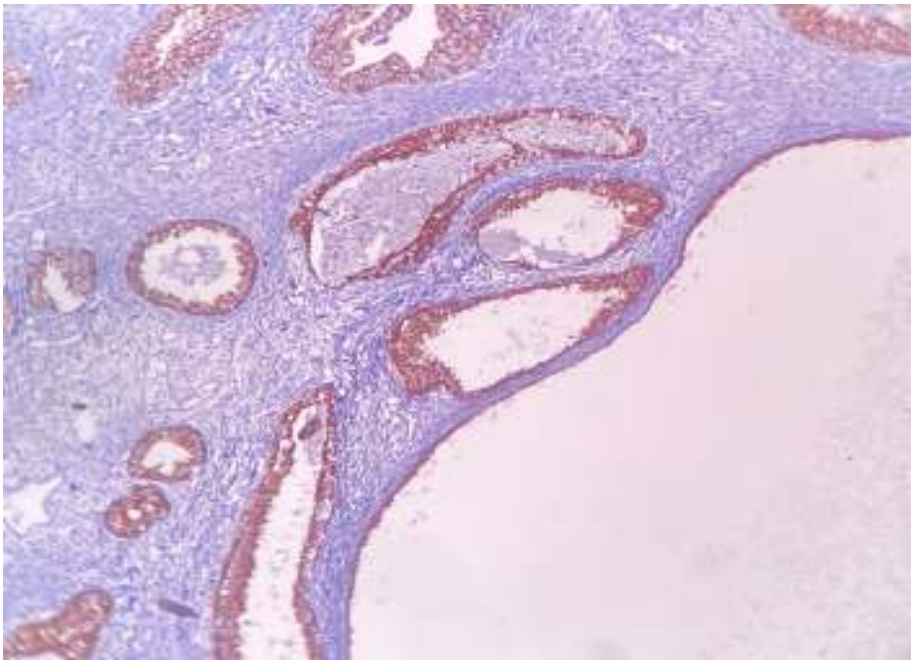
Table 17: Comparison in Change of diagnosis with IHC markers (N=30)

Initial Histopathological Diagnosis	Number of Cases	HMWCK (Basal Cells)	AMACR (Glands)	Final Diagnosis
AAH	1	Negative	Positive	Prostatic Adenocarcinoma
Adenosis	1	Negative	Positive	Prostatic Adenocarcinoma
BPH with Suspicious Focus	6	Positive	Negative	BPH
	3	Negative	Positive	Prostatic Adenocarcinoma
LGPIN	4	Positive	Negative	LGPIN
HGPIN	2	Positive	Positive	HGPIN
	1	Positive	Negative	
Transitional Cell Metaplasia	1	Positive	Negative	TCM
Prostatic Adenocarcinoma	10	Negative	Positive	Prostatic Adenocarcinoma
	1	Negative	Negative	

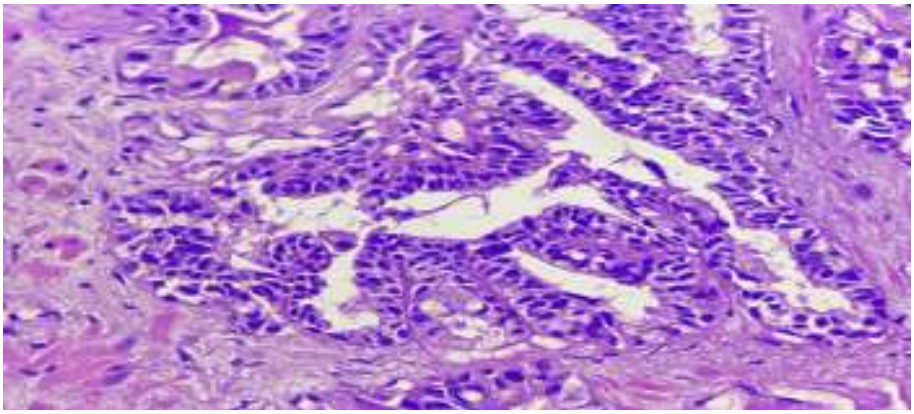
PHOTOMICROGRAPHS



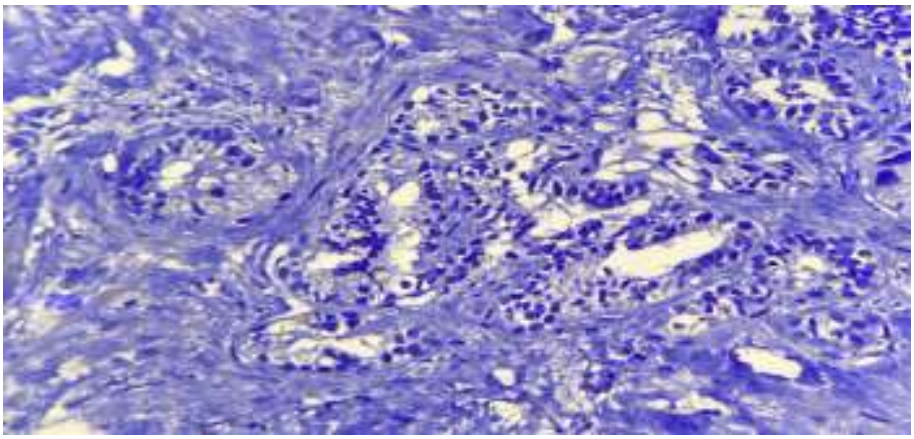
Picture 1 : BPH – H&E (10x)



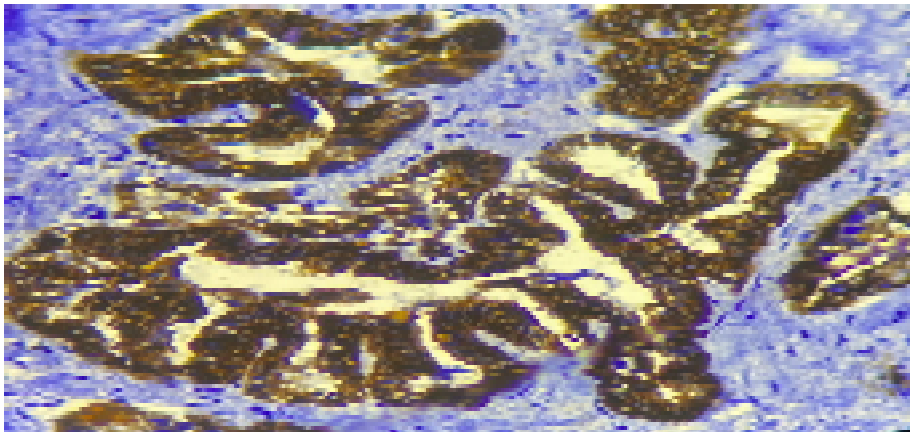
Picture 2 : BPH – HMWCK Positive (10x)



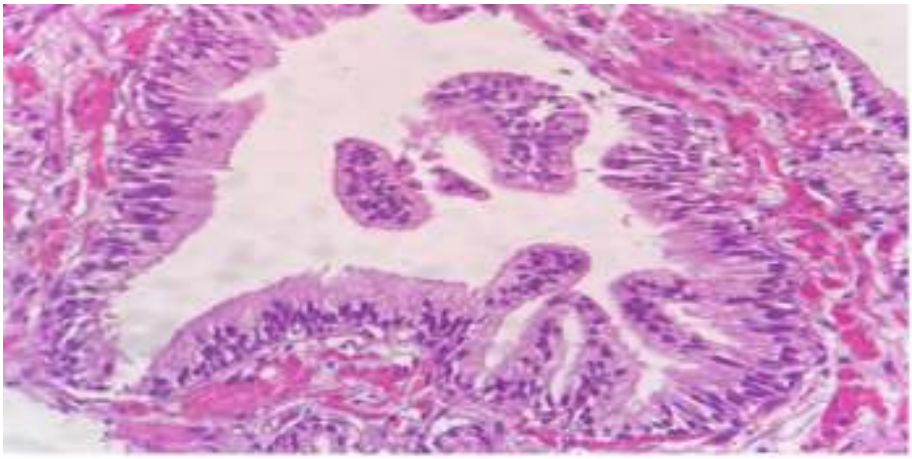
Picture 3 : LGPIN – H&E (40x)



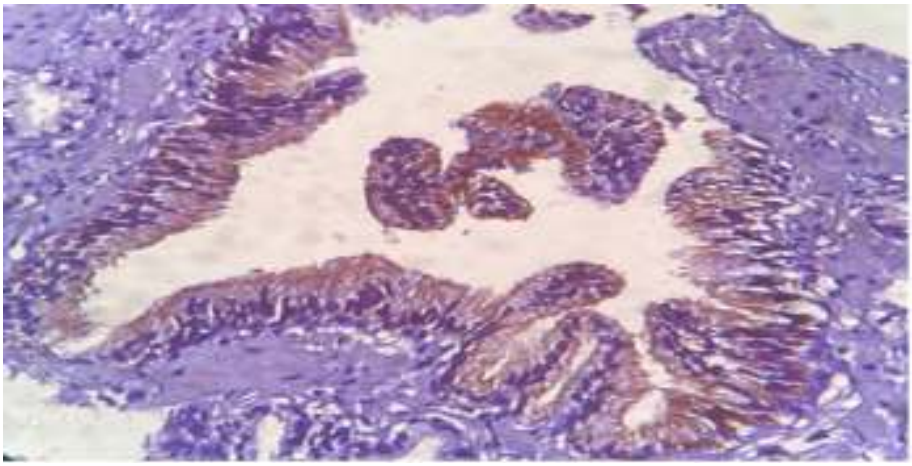
Picture 4 : LGPIN – AMACR Negative (40x)



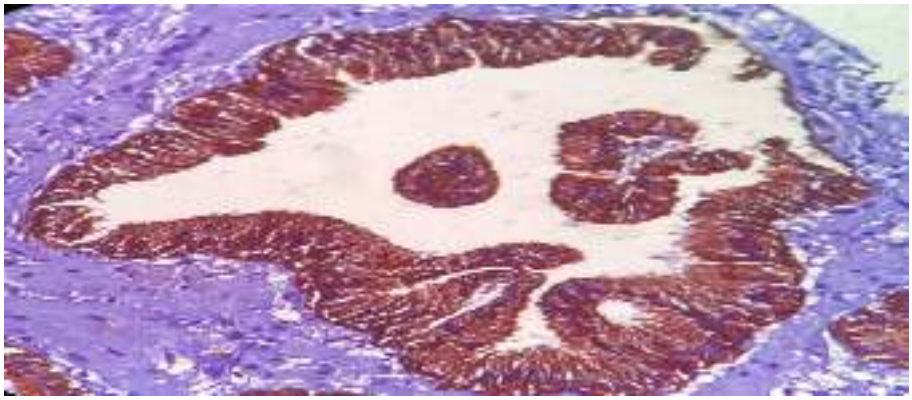
Picture 5 : LGPIN – HMWCK Positive (40x)



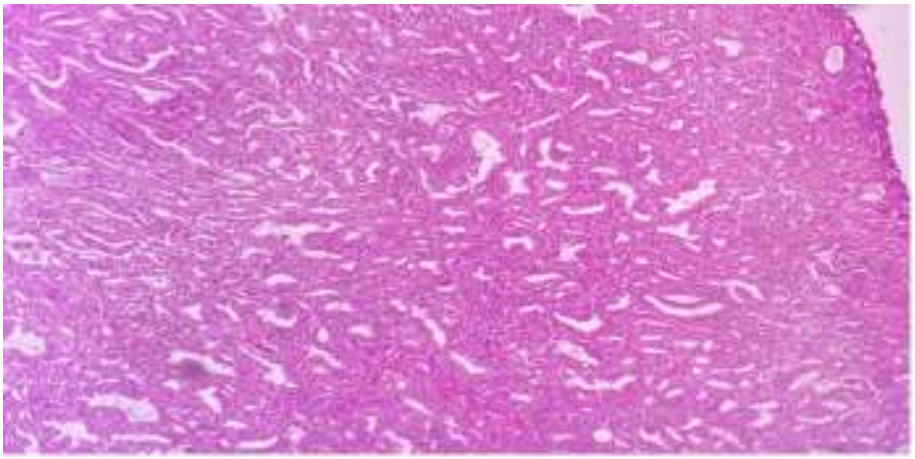
Picture 6 : HGPIN – H&E (40x)



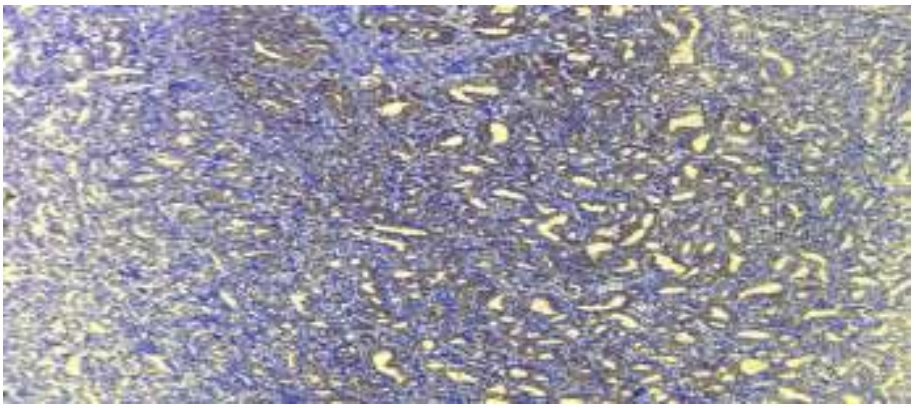
Picture 7 : HGPIN – AMACR 3+ Positive (40x)



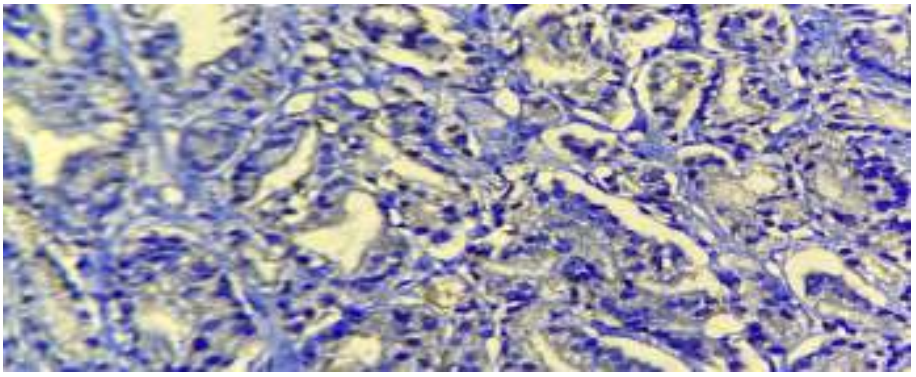
Picture 8 : HGPIN – HMWCK Positive (40x)



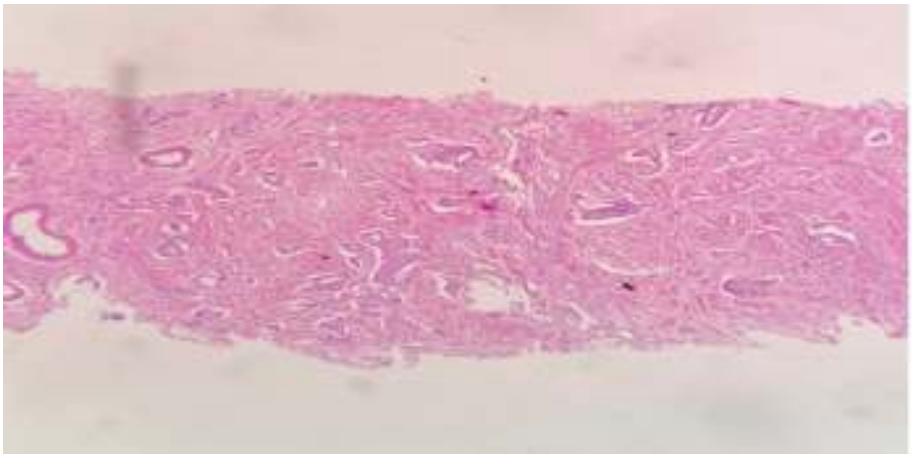
Picture 9 : Adenosis – H&E (10x)



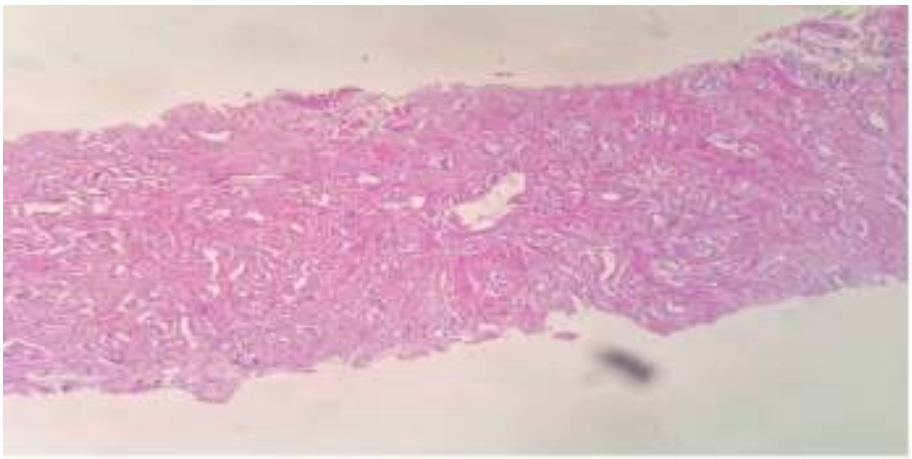
Picture 10: Adenosis – AMACR 3+ Positive (10x)



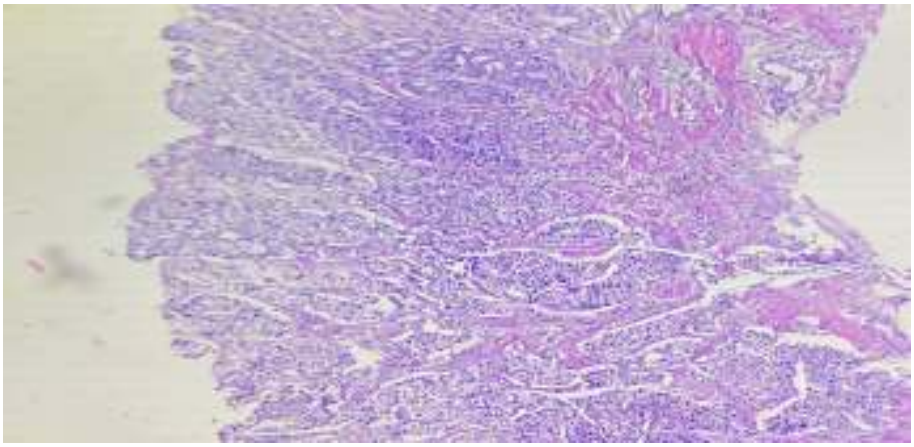
Picture 11: Adenosis – HMWCK Negative (40x)



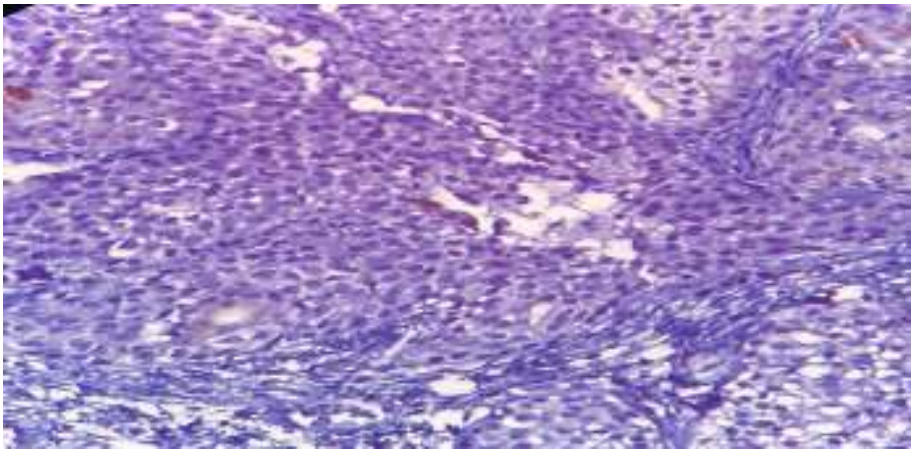
Picture 12 : Prostatic Adenocarcinoma (3+3=6) – H&E (10x)



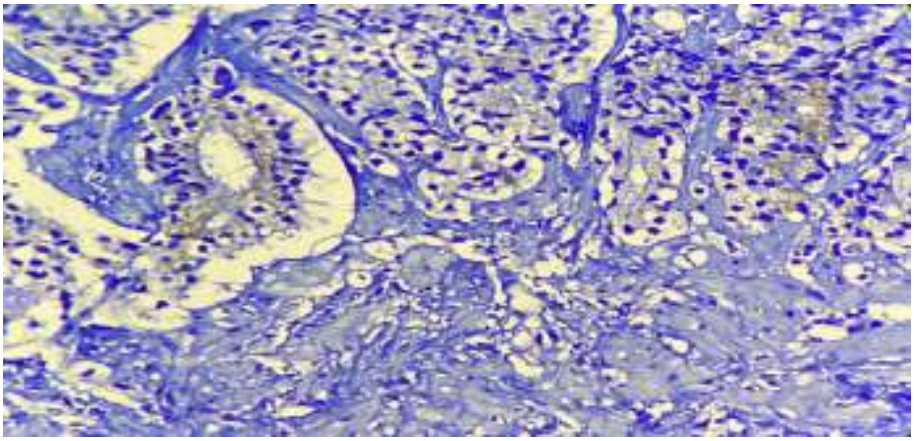
Picture 13 : Prostatic Adenocarcinoma (3+4=7) – H&E (10x)



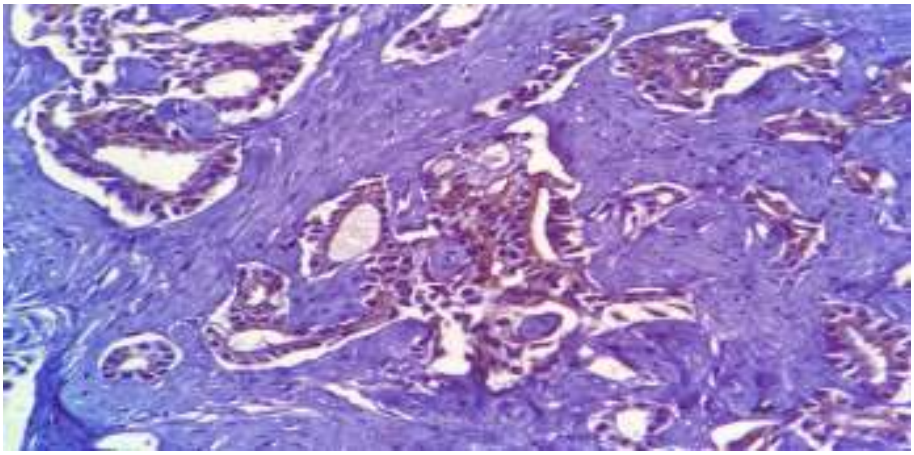
Picture 14 : Prostatic Adenocarcinoma (4+4=8) – H&E (20x)



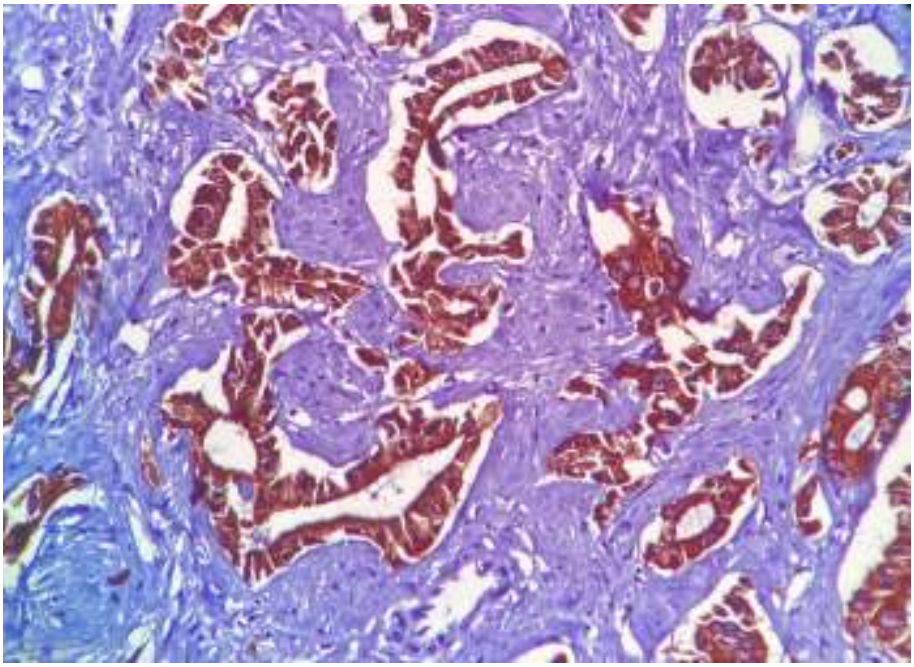
Picture 15 : Prostatic Adenocarcinoma – AMACR 1+ (40x)



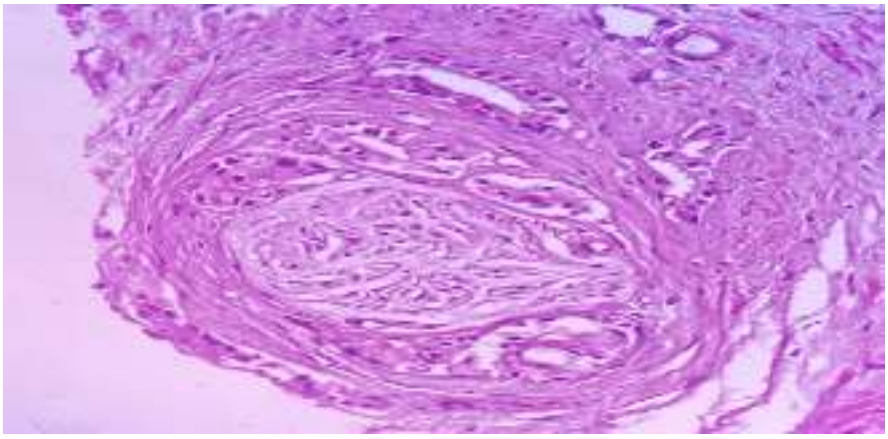
Picture 16 : Prostatic Adenocarcinoma – AMACR 2+ (40x)



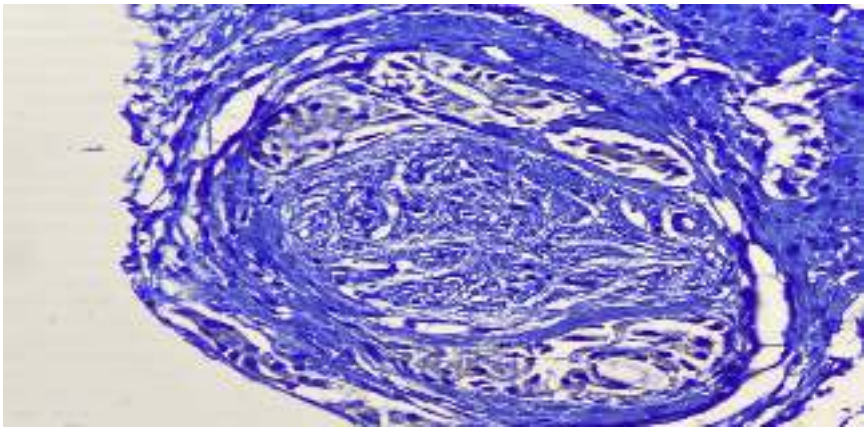
Picture 17 : Prostatic Adenocarcinoma – AMACR 3+ (40x)



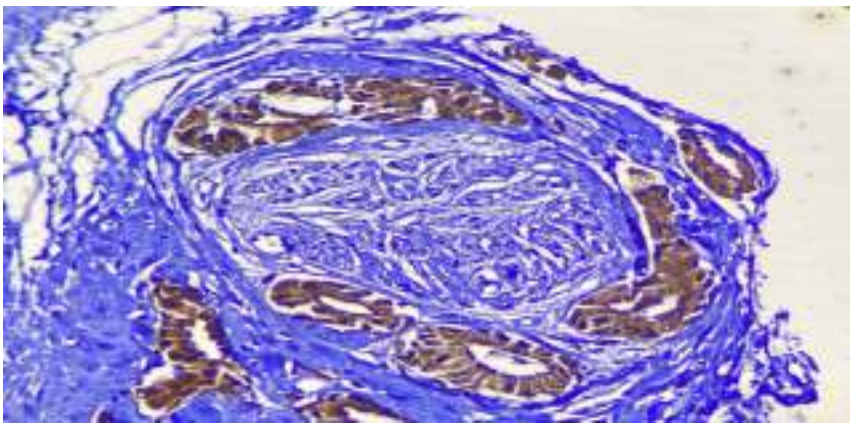
Picture 18 : Prostatic Adenocarcinoma – HMWCK Negative (40x)



Picture 19 : Perineural Invasion in a case of Prostatic Adenocarcinoma – H&E (40x)



Picture 20 : Perineural Invasion in a case of Prostatic Adenocarcinoma – AMACR Positive (40x)



Picture 21 : Perineural Invasion in a case of Prostatic Adenocarcinoma – HMWCK Negative (40x)

DISCUSSION

The present study was carried out on 30 cases of Prostatic lesions on needle core biopsies and TURP specimens. These were examined for histomorphological lesions with suspicious morphology and further subjected to IHC marker staining using HMWCK and AMACR for confirmation of diagnosis.

In the present study, majority of cases were in the age group 70-79 years. The mean age for BPH with suspicious focus and PIN was 72.88 years and 74.04 years respectively, which is comparable to a study by Mwakoma HA[94] and, Pacelli A and Bostwick DG.[97] Prostatic adenocarcinoma is a disease of elderly. In our study mean age of adenocarcinoma was 75.62 years which is comparable to previously done studies.[94,97,101] Ten cases out of 11 adenocarcinoma cases (90.9%) were seen in patients aged over 65 years which is also established in earlier studies.[99,100]

In studies done by Kumaresan et al[92] and Shah RB et al,[95] the incidence of BPH with suspicious focus ranged from 26 - 46%; in our study we found an incidence of 30%. Pre-malignant lesions like LGPIN and HGPIN in the present study have an incidence of 13.3% and 10% respectively which is similar to studies done by Rekhi et al[96] (11.2%) and Kumaresan et al[92] (14.2%). The incidence of Prostate Adenocarcinoma was comparable to studies by Jasani et al[24], Haroun et al[93], Xie et al[99] and Shimada et al.[100]

A correlation between serum PSA levels and histopathological diagnosis was done in the present study which showed all the lesions have a raised (>4ng/mL) PSA levels with mean PSA value being higher in the Prostate Adenocarcinoma patients as compared to BPH and other pre-malignant entities. These results are consistent with the previously done studies as well.[72,73,98]

In the present study, all 30 cases were stained using IHC markers HMWCK and AMACR.

In the 9 cases of BPH with suspicious foci, 6 cases (66.7%) showed intact continuous basal cell layer as highlighted by HMWCK and lack of AMACR staining in the glands; while 3 cases (33.3%) had HMWCK negative staining in the suspicious focus along with AMACR expression in the glands which led to the change in diagnosis to prostatic adenocarcinoma. Comparison is given below in Table 18.

Table 18 : Comparison of Immunostaining HMWCK and AMACR in cases of BPH with suspicious focus

Authors	% of HMWCK Positivity	% of AMACR Negativity
Garg et al[82]	50	64.3
Kumaresan et al[92]	69.2	50
Present study	**66.7**	**66.7**

In the present study, all 7 cases (100%) of PIN (LGPIN and HGPIN) showed HMWCK positivity of basal myoepithelial cell layer. AMACR was negative in all 4 cases of LGPIN and 1 case of HGPIN; while AMACR expression was observed in 2 cases (66.7%) of HGPIN.

These results correlate with previously done studies with similar findings as highlighted by Kunju et al[88], Jiang et al[90], Molinie et al[91], Kumaresan et al[92] and Kruslin et al.[98]

In the present study, out of 11 cases of carcinoma prostate, 10 cases(90.9%) showed positive AMACR overexpression (7 cases showing strong positivity, 2 moderate positivity while 1 case mild positivity) and, all cases were HMWCK negative. One case of prostatic adenocarcinoma showed both HMWCK and AMACR negativity. Similar results were observed in previously done studies by various authors. Comparison with other studies is given in the Table 19.

Table 19: Comparison of Immunostaining HMWCK and AMACR in Adenocarcinoma Prostate cases

Authors	% of HMWCK Negativity	% of AMACR Positivity
Molinie et al[91]	86	97
Kumaresan et al[92]	84	92
Garg et al[82]	100	100
Present study	**100**	**90.9**

CONCLUSION

Our study was an attempt to evaluate the expression of IHC markers HMWCK and AMACR in cases of prostatic lesions having suspicious or inconclusive morphological focus on routine H&E sections; resolving such dilemmas and arriving at a definitive diagnosis with the aid of IHC staining.

In this study, we examined lesions like BPH with suspicious focus, PIN (LGPIN and HGPIN), cancer mimickers like AAH and Adenosis, as well as Prostatic Adenocarcinoma cases. All these cases were subjected to IHC analysis using HMWCK and AMACR.

HMWCK was used to highlight the presence and intactness of basal myoepithelial cell layer giving a luminal and cytoplasmic staining; absence of HMWCK staining was suggestive of malignant diagnosis. AMACR positivity was noted in neoplastic glands as circumferential cytoplasmic granular staining and was graded (0 to 3+) based on the percentage of cells stained.

One case each of AAH and Adenosis show HMWCK negativity and AMACR positivity favoring a malignant diagnosis.

Out of 9 cases of BPH with suspicious focus, AMACR positivity was noted in 3 cases (33.33%) along with absence of basal cell layer (HMWCK negative) changing its diagnosis to Focal Adenocarcinoma.

Out of 7 cases of PIN (4 cases of LGPIN and 3 cases of HGPIN), all the 4 cases of LGPIN showed intact basal cell layer as highlighted by HMWCK positivity with AMACR negativity. Two cases of HGPIN were AMACR positive while all cases showed basal cell HMWCK positivity rendering the initial diagnosis of HGPIN unchanged.

There was one case of transitional cell metaplasia which was AMACR negative and HMWCK positive.

Ten cases out of 11 cases (90.9%) of cancer prostate showed moderate to strong positivity for AMACR while basal cell marker HMWCK was negative in all cases which was consistent with the diagnosis of Prostate Adenocarcinoma.

Thus, we conclude that IHC staining should be done in such cases where routine H&E sections have an ambiguous morphology. HMWCK and along with it, AMACR is a good IHC pair to differentiate Benign from Malignant lesions especially when it comes to distinguishing HGPIN from adenocarcinoma.

IHC is an aid to resolve such dilemmas and come to a confirmatory diagnosis so that early detection of cancer can be done and the patient is benefitted by appropriate management from the treating clinicians.

SUMMARY

This was a one-year observational (prospective) study from January 2020 to December 2020 done in the Department of Pathology, J N Medical College, Belagavi.

- A total of 30 cases including 19 cases of prostatic needle core biopsies and 11 cases of TURP specimens were included in our study.
- Majority (16 cases, 53.3%) of cases were in the 70-79 years age group with a mean age of 74.53 years.
- On H&E staining, 11cases (36.7%) were of Prostatic Adenocarcinoma; 9 cases (30%) were BPH with Suspicious Focus; 7 cases (23.3%) were PIN; and 1 case (3.3%) each of Transitional cell metaplasia, Adenosis and AAH were diagnosed on H&E.
- One case each of AAH and Adenosis showed strong AMACR overexpression with HMWCK negativity rendering a malignant diagnosis.
- Six (66.7%) out of 9 cases of BPH with Suspicious focus showed HMWCK positivity along with AMACR negativity with final diagnosis retained as on H&E; while 3 cases (33.3%) showed negative HMWCK expression and AMACR positivity in the glands changing the final diagnosis to prostate adenocarcinoma.
- One case of transitional cell metaplasia showed HMWCK positivity with AMACR negativity.
- All 11 cases (100%) of Adenocarcinoma prostate shows negative staining of basal cell layer (HMWCK negative) while 10 cases (90.9%) showed immunopositivity for AMACR.

- In 25 cases (83.3%) initial histopathological diagnosis was confirmed by IHC staining.
- In 5 cases (16.7%) including 3 cases of BPH with suspicious focus and 1 case each of Adenosis and AAH, the diagnosis was changed to Prostatic Adenocarcinoma after IHC analysis.

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ANNEXURE I

PROFORMA

PATIENT HISTORY

Name : Age: IP no.:

Brief clinical history:

EXAMINATION FINDINGS

General Physical Examination :

Pulse - B.P - Respiratory rate -

Temperature –

Pallor - Cynosis - Edema -

Icterus - Clubbing - Lymphadenopathy –

Systemic Examination :

Per Abdomen -

CVS -

RS -

CNS –

Per Rectal Examination :

CLINICAL DIAGNOSIS:

LAB INVESTIGATIONS:

Haematopathological :

CBC - Urine analysis -

PSA levels –

Radiological :

X-ray - CT scan -

USG - MRI -

HISTOPATHOLOGICAL DIAGNOSIS:

1. Hematoxylin and Eosin staining :

2. IHC staining :

ANNEXURE II

INFORMED CONSENT

EVALUATION OF EXPRESSION OF IMMUNOHISTOCHEMICAL MARKERS HIGH MOLECULAR WEIGHT CYTOKERATIN (HMWCK) AND ALPHA-METHYLACYL CoA RACEMOSE (AMACR) IN PROSTATIC NEEDLE BIOPSIES AND TRANSURETHRAL RESECTION OF PROSTATE(TURP) SPECIMEN – A ONE YEAR OBSERVATIONAL STUDY.

Purpose of the study: You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo Transurethral resection of prostate or Needle core biopsy for a prostate lesion you will be included in this study.

The purpose of this study is to evaluate the expression of HMWCK and AMACR in prostate specimens. It will help in early diagnosis of suspected prostate malignancy.

Procedure: During this study, you will be asked questions regarding the complaints and history of your illness, current symptoms, family history of similar complaints and you are supposed to answer to the best of your knowledge. The principal investigator of the study is **REG. NO: BN0119007** under the guidance of Dr._____ (guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know a better way to diagnose prostatic cancer which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and

withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KLE University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. **REG. NO: BN0119007,** Department of Pathology, J.N. Medical College.
2. Dr. _____, Department of Pathology, J.N. Medical College.
3. If you have any queries about your rights as a study subject, you may call Dr. Roopa Bellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Principal Investigator : REG. NO: BN0119007

Guide **:** Dr. _____

Name of the participant:

(signature/thumbprint)

Name of the witness : (signature)

Name of the investigator:

(signature)

Date:

Address:

Phone no:

ANNEXURE III

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed - to-be University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 240 **Date: 24/12/2019**

To.
REG. NO: BN0119007
PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EVALUATION OF EXPRESSION OF IMMUNOHISTOCHEMICAL (IHC) MARKERS HIGH MOLECULAR WEIGHT CYTOKERATIN (HMWCK) AND ALPH-METHYLACYL CoA RACEMOSE (AMACR) IN PROSTATIC NEEDLE BIOPSIES AND TRANSURETHRAL RESECTION OF PROSTATE (TURP) SPECIMEN – A ONE YEAR OBSERVATIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE IV

H&E STAINING

1. De-paraffinised sections on the slides dipped in 100% ethanol for 2 mins.
2. Slides dipped in 70%ethanol for 2 mins.
3. Slides taken down to water for 2 mins.
4. Thereafter, stained with Harris' Hematoxylin for 10-15 mins.
5. Down to water for 2 min.
6. Dip in 1%acid alcohol
7. Down to water 2min
8. One dip in lithium carbonate
9. Down to water
10. Stain with eosin two to three min
11. Down to water 2 min
12. Dehydrate in 100% alcohol one dip
13. Blotting, flaming and clearing in xylene.
14. Thereafter, mount using DPX and coverslip.

ANNEXURE V

IHC STAINING

1. Cut 3mm sections using microtome and incubate sections on polylysine coated slides marked AMACR and HMWCK overnight at 56ºC.

2. Before staining, bake at 60 ºC for 1 hour.

3. De paraffinise sections in xylene, for 10 minutes, two cycles.

4. Dehydrate in alcohol, 100% for 10 minutes, 70% for 10 minutes.

5. Rinse in water for 5 minutes.

6. Prepare Antigen retrieval solution (Tris Buffer + EDTA) in the required amount.

7. Place the slides with above solution in the racks and place in the pressure cooker and cook for 3 whistles (approx. 1 hour), taking care that the rack is correctly aligned.

8. Allow it to cool for 15 minutes and bring slides to room temperature.

9. Wash with wash buffer twice with a gap of 30 seconds each.

10. Blot out excess moisture from sides of the sections and put 3% Peroxide block and place in humidified cabinet for 30 min.

11. Add Primary antibody (AMACR or HMWCK), and incubate in a closed chamber at room temperature for 45 to 60 minutes.

12. Wash with wash buffer thrice with a gap of 30 seconds each.

13. Apply Polymer HRP for 25 to 30 minutes in a closed chamber at room temperature.

14. Wash with wash buffer thrice with a gap of 30 seconds each.

15. Apply DAB substrate chromogen for 10 minutes.

16. Wash in water for 2 minutes.

17. Counterstain with 3-4 dips in Harris Hematoxylin followed by blueing in warm water for 1 minute.

18. Serially dehydrate in 70% alcohol followed by 100% alcohol for 2 minutes each.

19. Blot, flame and place in Xylene for clearing.

20. Mount using DPX and cover slip.

Solution Preparation:

1) *Antigen Retrieval*

Tris buffer - 0.605 grams

EDTA - 0.185 grams

Distilled Water - 1000 mL

pH - 8.5 to 9

2) *Wash Buffer*

Tris buffer - 4.3 grams

NaCl - 4.8 grams

Distilled Water - 1000 mL

pH - 7.4 to 7.6

3) *Hydrogen Peroxide*

H_2O_2 - 3 mL

Distilled Water - 97 mL

4) *DAB solution*

Substrate Sol. - 1mL

Chromogen - 20 microL

ANNEXURE VI

MASTERCHART

CASE No.	HP No.	AGE (in years)	HISTOPATHOLOGICAL DIAGNOSIS	PSA (ng/mL)	HMWCK	AMACR	FINAL DIAGNOSIS
1	17/20	85	BPH with Suspicious Focus	66	Negative	3+	Prostatic Adenocarcinoma
2	138/20	88	Prostatic Adenocarcinoma	6.91	Negative	3+	Prostatic Adenocarcinoma
3	324/20	73	LGPIN	14.8	Positive	0	LGPIN
4	342/20	70	Prostatic Adenocarcinoma	180.35	Negative	3+	Prostatic Adenocarcinoma
5	403/20	74	BPH with Suspicious Focus	6.9	Positive	1+	BPH
6	570/20	77	Transitional Cell Metaplasia	3.5	Positive	0	Transitional Cell Metaplasia
7	614/20	78	BPH with Suspicious Focus	10.46	Negative	3+	Prostatic Adenocarcinoma
8	708/20	70	LGPIN	23.1	Positive	0	LGPIN

9	726/20	70	Prostatic Adenocarcinoma	19.4	Negative	1+	Prostatic Adenocarcinoma
10	827/20	77	Prostatic Adenocarcinoma	48.13	Negative	3+	Prostatic Adenocarcinoma
11	895/20	70	Prostatic Adenocarcinoma	18.61	Negative	2+	Prostatic Adenocarcinoma
12	983/20	60	Prostatic Adenocarcinoma	13.99	Negative	3+	Prostatic Adenocarcinoma
13	1086/20	70	LGPIN	47.08	Positive	0	LGPIN
14	1301/20	66	Prostatic Adenocarcinoma	13.85	Negative	3+	Prostatic Adenocarcinoma
15	1462/20	82	Prostatic Adenocarcinoma	29.1	Negative	3+	Prostatic Adenocarcinoma
16	1514/20	82	LGPIN	39.15	Positive	0	LGPIN
17	1516/20	79	Prostatic Adenocarcinoma	56.29	Negative	2+	Prostatic Adenocarcinoma
18	1607/20	86	Prostatic Adenocarcinoma	38.11	Negative	3+	Prostatic Adenocarcinoma
19	1639/20	73	HGPIN	8.34	Positive	3+	HGPIN
20	1761/20	86	Atypical Adenomatous Hyperplasia	6.75	Negative	3+	Prostatic Adenocarcinoma
21	1804/20	82	Prostatic Adenocarcinoma	8.23	Negative	0	Prostatic Adenocarcinoma

22	1805/20	73	HGPIN	33.7	Positive	3+	HGPIN
23	1916/20	66	BPH with Suspicious Focus	5.93	Positive	0	BPH
24	1989/20	65	BPH with Suspicious Focus	30.14	Negative	2+	Prostatic Adenocarcinoma
25	2023/20	75	BPH with Suspicious Focus	7.53	Positive	0	BPH
26	2046/20	77	HGPIN	6.13	Positive	0	HGPIN
27	2084/20	61	BPH with Suspicious Focus	4.61	Positive	0	BPH
28	2111/20	65	BPH with Suspicious Focus	11.5	Positive	0	BPH
29	2260/20	69	Adenosis	8.63	Negative	3+	Prostatic Adenocarcinoma
30	2471/20	80	BPH with Suspicious Focus	21.7	Positive	0	BPH

ANNEXURE VII

KEY TO MASTER CHART

HP No.	-	Histopathology Number
BPH	-	Benign Prostatic Hyperplasia
LGPIN	-	Low grade Prostatic Intraepithelial Neoplasia
HGPIN	-	High grade Prostatic Intraepithelial Neoplasia
0	-	0% cells (NEGATIVE)
1+	-	1-10% cells (MILD)
2+	-	11-50% cells (MODERATE)
3+	-	>51% cells (STRONG)