HISTOPATHOLOGICAL STUDY OF PROSTATIC BIOPSIES WITH REFERENCE TO IMMUNOHISTOCHEMISTRY ON PREMALIGNANT AND MALIGNANT LESIONS

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CERTIFICATE

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DECLARATION

In the following pages is presented a consolidated report of the study **"HISTOPATHOLOGICAL STUDY OF PROSTATIC BIOPSIES WITH REFERENCE TO IMMUNOHISTOCHEMISTRY ON PREMALIGNANT AND MALIGNANT LESIONS"** on cases studied and followed up by me at Sree Mookambika Institute of Medical Sciences, Kulasekharam from 2016-2019. This thesis is submitted to the Dr. M.G.R. Medical University, Chennai in partial fulfilment of the rules and regulations for the award of MD Degree examination in Pathology.

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ABSTRACT

ABSTRACT

Prostatism is a common problem in the geriatric age group. Prostatic hyperplasia and Carcinoma of the prostate are increasingly frequent with advancing age. Prostatic lesions on routine H & E staining, especially when malignant tissue is limited and is mixed with benign prostatic glands or due to the presence of benign mimickers of carcinoma or technical problems like crush artifact, can cause a diagnostic dilemma. Therefore it is not uncommon to under diagnose small focus of prostatic adenocarcinoma or over diagnose benign lesions mimicking cancer. In such situation, Immunohistochemical detection of basal cells are widely used. The most commonly used basal cell specific markers are Cytokeratin 34BetaE12 (High molecular weight cytokeratin) and p63.

Aims:

The aim of the present study is to study the spectrum of prostatic lesions among the biopsies received in a tertiary care hospital in south India and to assess the utility of Cytokeratin 34BetaE12 and p63 by Immunohistochemistry in premalignant and malignant lesions of prostate.

Materials and methods:

A total of 50 prostatic specimens (both retrospective and prospective) received in department of pathology over 2 years were evaluated. Routine hematoxylin and eosin staining and immunohistochemical staining against CK 34βE12 were performed in 18 cases (premalignant and malignant lesions.)

Results:

Among the 50 biopsies received, 78% cases were TURP, 32% cases were Trucut needle biopsies. 32 (64%) cases were of Nodular hyperplasia of prostate, one case (2%) was Prostatic intraepithelial neoplasia and 17 cases (34%) were Carcinoma of Prostate. They were common in the sixth to seventh decade and majority presents with urinary symptoms. Pseudo neoplastic lesions like basal cell hyperplasia and squamous metaplasia were noted accounts for 32% and 6% respectively. Adenocarcinoma diagnosed were graded according to Modified Gleason grading, IUSP 2015 guidelines. Gleason grade 2 was seen in 41% cases with score 3+4 = 7.

Immunohistochemistry was done on 18 cases. p63 is expressed in the nuclei of basal cells, whereas 34βE12 stains their cytoplasm. 4 cases showed entrapped benign glands. Compared to p63, four cases stained by CK 34βE12 showed 1+ staining intensity. This false positive reaction may be because of formalin fixation and antigen retrieval.

Conclusion:

Benign prostatic hyperplasia is the most commonly encountered prostatic lesion. Definitive diagnosis of benign and malignant lesions of prostate can be made by histopathological study of prostatic biopsies. Immunohistochemical stains helps in confirmation and in cases with diagnostic dilemma. Ultimately, understanding the potential pitfalls of IHC stains and paying careful attention to morphologic details are crucial to prevent the false-positive and false-negative diagnosis.

Key Words:

Prostatic lesions, Gleason’s grading, CK34BetaE12, p63.

INTRODUCTION

Prostate is a fibro musculo glandular organ. Enlargement of prostate or growth most commonly occurs due to nodular hyperplasia or neoplasm like adenocarcinoma and also prostatic intraepithelial neoplasia (PIN), its precursor lesion[1]. With advancing age frequency increases and are uncommon before the age of 40 yrs.[2]

Diagnosis of malignancy at higher stage carries poor prognosis hence early diagnosis of malignancy is important. More over prostatic cancers are difficult to diagnose as these remain asymptomatic for long. With the primary being silent, these are notorious for metastasis, particularly to bone.

Prostatic lesions are routinely diagnosed by biopsies (TURP, needle biopsies). Specific histological criteria, like infiltrative small glands, absence of basal cell layer and nuclear features can point towards a positive diagnosis of carcinoma. Other pathologies like prostatitis, nodular hyperplasia and high grade intraepithelial lesions can also be seen.

Prostatic lesions on routine Haematoxylin & Eosin (H&E) staining sometimes cause diagnostic dilemma. Benign lesions, other malignant lesions, premalignant lesions like atypical adenomatous hyperplasia (AAH) and prostatic intraepithelial neoplasia (PIN) are mimickers of Prostatic carcinoma (PCa) in needle biopsies and can be challenging particularly when the malignant tissue is limited and is mixed with benign prostatic glands.[3] It is not uncommon to underdiagnose a small focus of carcinoma or over diagnose benign lesions mimicking cancer. An important diagnostic criterion in the differentiation is the loss of basal cell layer in adenocarcinoma and its presence in the benign lesions. Several immunohistochemical stains have been used to stain the basal cells of prostate, e.g. high molecular weight cytokeratin

(CK34BetaE12), p63 etc. To the practicing pathologist, Immunohistology has become an integral part of the diagnostic armamentarium.[4]

This study is aimed to evaluate a complete spectrum of various prostatic lesions in a tertiary care centre in south India during a period of two years and value of Immunohistolochemical stains in definite histopathology diagnosis of suspicious or atypical prostatic lesions.

AIMS & OBJECTIVES

- To study the Histomorphological spectrum of non-neoplastic and neoplastic lesions of prostate in prostatic biopsies.
- To assess the utility of Cytokeratin 34BetaE12 and p63 by Immuno histochemistry in premalignant and malignant lesions of prostate.

REVIEW OF LITERATURE

Prostate is a functional conduit in males that allows urine to pass from urinary bladder to the urethra and adds nutritional secretions to the sperms to form semen. It surrounds the proximal part of urethra, the prostatic urethra. Knowing the normal histology of prostate make us better understand the clinical features and pathology of diseases related to prostate.

ANATOMY

Prostate is a pear-shaped organ, weighs up to 20 g in normal adults and is located within the pelvis. It lies anterior to the rectum, with urethra running through its centre and serves as a reference landmark. The base of the prostate is in continuity with the bladder and becoming the striated external urethral sphincter ends at the apex.

Three distinct layers of fascia covers the prostate on the anterior, posterior and lateral aspects. The anterior and anterolateral fascia is in direct continuity to the true capsule and levator fascia fuses with it laterally. Rectovesical (Denonvilliers) fascia covers posteriorly, which is a connective tissue located between the anterior wall of the rectum and posterior aspect of the prostate. This fascial layer covers the prostate and seminal vesicles posteriorly and extends caudally to terminate as a fibrous plate just below the urethra at the level of the external urethral sphincter, which is known as the median fibrous raphe. (Fig. 14) The puboprostatic ligament supports the gland anteriorly and external urethral sphincter and perineal membrane inferiorly. The puboprostatic ligaments are the pubovesical ligament.[5]

Prostate is divided into anterior fibromuscular stroma and three distinct glandular zones (peripheral zone, transition zone, central zone). Peripheral and central zones are included in outer prostate whereas transition zone and anterior fibromuscular layer are included in the inner prostate.[6]

Peripheral zone of the prostate comprises majority of glandular tissue that accounts for almost 70%. This zone covers the posterior and lateral aspects of the prostate. The peripheral zone is the area that is palpated on digital rectal examination (DRE) and this zone represents the area where 70% of adenocarcinomas are found. This portion is also most commonly affected by chronic prostatitis.

The central zone, the area surrounding the ejaculatory ducts consists of 25% of the glandular tissue. Very few adenocarcinomas are found in this zone, which presents with lower urinary tract symptoms. Urethral crest runs along the posterior midline and disappears at the membranous urethra. A groove is noted on both sides of the urethral crest. Prostatic sinuses exit and drain all the glandular elements in this area.

The transition zone accounts for 10% of the prostatic glandular tissue and 20% of the adenocarcinomas in this region and this zone represent only 1-5% of tumors in the prostate.[5, 7]

The arterial supply to the prostate is from the inferior vesical artery, a branch of anterior division of the internal iliac (hypogastric) artery and then it branches into two main arteries that supply the prostate. This also supplies the base of the bladder and the distal ureters. The prostatic vessels and the autonomic innervations run between the layers of the lateral prostatic fascia and the prostate.

The venous drainage starts with the deep dorsal vein, which leaves the penis under the deep penile (Buck) fascia between the corpora cavernosa and then under the pubic arch. One of the common sites for metastasis of prostatic cancer is vertebral column that occurs as a result of tumor spread via venous plexuses.

The lymphatic drainage of the prostate primarily drains to the obturator and the internal iliac lymphatic channels. There is also lymphatic communication with the external iliac, presacral, and para-aortic lymph nodes.

Parasympathetic, visceral, efferent and preganglionic fibres form the autonomic innervations of the prostate, arises from the pelvic plexuses. Preganglionic fibres arise from the sacral levels (S2-S4) and the sympathetic fibres from the thoracolumbar levels (L1-L2). The parasympathetic nerves ends at the acini and leads to prostatic secretion. The sympathetic nerves are responsible for contraction of the smooth muscle on the capsule and the stroma.

NORMAL HISTOLOGY

Prostate is made up of duct and acini arranged in a complex pattern which is embedded in a dense fibro muscular stroma. The glands show irregular contour with luminal undulation and papillary infolding and are lined by double layered epithelium, luminal secretory cells and peripheral basal cells. Prostate glands of central zone are morphologically distinct from peripheral and transition zones glands. Peripheral zone and transition zone are composed of small simple acini in lobular configuration with loose and dense stroma respectively. The cells lining the acini shows clear cytoplasm. Central zone is composed of large complex acini with cribriform and intraluminal ridges with eosinophilic cytoplasm of the lining cells. A layer of flattened cell is seen at the base called as the basal layer. The lumen contains small luminal secretions, sometimes forms spherical concretions, the corpora amylacea. (Fig. 15) The supporting stroma is a mixture of collagenous fibrous tissue and smooth muscle fibers.[8]

PSA Estimation

In 1979, Wang[9] and associates isolated prostate-specific antigen, Prostate-specific antigen (PSA) is also known as gamma-semino protein or kallikrein-3 (KLK3), a glycoprotein of 34,000 Daltons encoded in humans by the KLK3 gene. Epithelial cells of the prostate gland secrete PSA, a member of the Kallikrein-related peptidase family. PSA is produced for the ejaculate, where it liquefies semen in the seminal coagulum and allows sperm to swim freely.[10]

Methods such as Western blot or enzyme-linked immunosorbent assay (ELISA) detect specific protein via fluorescence or other chemically-induced signals which is used for quantification of PSA. Although highly specific for tissue of prostatic origin, PSA is not cancer-specific as serum PSA levels are commonly elevated in benign conditions like prostatitis, benign nodular hyperplasia, infarct and major trauma such as needle biopsy and transurethral resections. In contemporary series, where most men undergo needle biopsy for PSA >4.0ng⁄ml, fewer than half of biopsies result in a diagnosis of prostate cancer.

PSA density - calculated by dividing the total serum PSA level by the estimated gland volume to estimate the PSA produced per gram of prostate tissue.

PSA velocity - Rapidity of change in PSA level is measured by PSA velocity and a value more than 75ng/ml/year should trigger a biopsy referral. At least three PSA measurements must be performed over a period of 1.5 to 2 years.[11]

Ratio of free and bound PSA in the serum – Immunoreactive PSA exists in two forms: major fraction bound to Alpha 1-antichymotrypsin and minor free fraction. Percentage of free PSA (free PSA/total PSA × 100) is lower in men with prostate cancer than in men with benign prostatic diseases.

Age-specific PSA

- 2.5 ng/mL for men 40 to 49 years
- 3.5 ng/mL for men 50 to 59 years
- 4.5 ng/mL for men 60 to 69 years
- 6.5 ng/mL for men 70 to 79 years

Prostatic cancer gene 3 assay: It is a noncoding RNA which is overexpressed in 95% of prostate cancers. Elevated urine PCA3 scores have been shown to be associated with an increased risk of a positive biopsy

PROSTATIC BIOPSY[12]

Specimens received include prostatic needle biopsies, Trans Urethral Resection of Prostate (TURP), rarely radical and suprapubic prostatectomy.

Prostatic needle biopsy

Prostatic needle biopsy is usually done as an outpatient procedure. Biopsy is done by the urologist in patients suspected of prostatic pathology mostly carcinoma and with hard prostate on digital rectal examination, rarely for prostatitis.

Majority agree that TRUS guided prostate needle biopsy should be performed in men with an abnormal Digital Rectal Examination (DRE), an elevated PSA (>4.0 ng/ml) or PSA velocity (rate of PSA change) >0.4 to 0.75ng/ml/yr. Also, repeat biopsy after 3 to 12 months has to be done for men who were diagnosed with high-grade prostatic intraepithelial neoplasia (PIN) or atypia on a previous prostate needle biopsy.[13]

An 18-gauge biopsy needle loaded in a spring-action automatic biopsy device is commonly used to procure multiple 1.5cm prostate biopsy specimens. It is

done under local infiltration. Hard nodules in prostatic areas are sampled using prostatic biopsy "gun" needles after per rectal examination.

In transrectal ultrasound, hypoechoic lesion (dark compared to normal tissue) in the peripheral zone is the most common appearance for cancer. With PSA based screening and earlier cancer detection, fewer overt abnormal sonographic findings are being detected at the time of transrectal ultrasound.[14] Systematic sextant TRUS-guided prostate biopsies have traditionally been done with no form of anaesthesia and have been relatively well tolerated. Sextant biopsies from six different sites are sampled and labelled separately. Hodge et al. in 1989, directed the biopsies to 6 standard quadrants and also to hypoechogenic areas (Bx6C). This standard procedure identified PCa in 62% of 136 patients.[13]

Biopsies obtained using a "biopsy gun," are usually thin and are processed as single core in individual cassettes. To detect significant lesions, three levels are needed. Additional levels may show PCa if focal glandular atypia is found in the first three slides.

TURP

TURP is usually carried out using a device called a resectoscope. It is a thin metal tube containing a light, camera and loop of wire. Resectoscope is inserted by the surgeon into the urethra before guiding it to the site of prostate with the help of the light and the camera. A catheter is used to pump fluid into the bladder after the procedure and this flushes away pieces of prostate that have been removed. (Fig. 16)

Multiple fragments are curetted from the central and transitional zone of the prostate in order to relieve obstruction. Carcinoma may be found in 7% to 8% of TURPs with limited sampling and 14% to 19% if the entire specimen is examined.

CAP[15] (College of American Pathologist) recommends examining of entire specimen if weighing 12 grams or less. For larger specimens, the first 12 grams should be submitted (in 6 to 8 cassettes – in general 1 to 2 grams of tissue will fit in one cassette), with one more cassette for each additional 5 grams of tissue. The remaining tissue is generally submitted for examination if an unsuspected carcinoma is found involving <5% of tissue. If any firm, yellow or yellow-orange chips are present, they should be submitted, as these chips are more likely to contain carcinoma.

Additional recommendations in the literature have been mentioned to submit the entire specimen in the following situations includes patients <60 years of age (small low grade carcinomas may be more likely to become clinically significant in this group) and patients with elevated PSA.

Suprapubic Prostatectomy

Enucleation procedures are rare and performed when the prostate is much enlarged or if there are other contraindications (e.g., urethral disease, bladder diverticula) for transurethral surgery. The specimen usually looks like a large apple with a wedge cut out of one side, but may come in two or more fragments. There are usually no orienting features. Margins are irrelevant since the entire prostate is not removed. Serial sections to be made at 3 to 4 mm thickness. The parenchyma including color (white/tan, yellow, gray), consistency (firm, hard, soft, indurated), areas of necrosis or hemorrhage. Carcinomas may be more yellow and firmer than hyperplastic nodules. Submit at least eight cassettes from different areas including right and left lobes, urethra (if recognizable), capsule and any areas suspicious for tumor.

Radical Prostatectomy

Radical prostatectomies are not performed in the present era. Numerous protocols for submitting tissue have been proposed ranging from submission of the entire specimen in whole mount specimens to limited sampling using standard slides. Any method used should be designed to evaluate the extent of carcinoma, grade, stage and margin status.

Prostatic biopsies obtained are fixed in 10% formalin, paraffin embedded and stained with Haematoxylin and Eosin (H&E) routinely for assessment of malignancy or other pathology.

COMPLICATIONS OF BIOPSY

Persistent haematuria is the most common complication. Intraoperative complications can also occur including vasovagal episodes. Hematochezia (rectal bleeding) and hematospermia (blood in the semen) are other alarming symptoms that can occur, although most subside spontaneously.

According to International Society of Urologic Pathology (ISUP) 2005 guidelines, Malignancy when detected should be graded according to Modified Gleason score. Review of a few common and benign conditions of prostate are elaborated below, before a discussion of carcinoma.

NONNEOPLASTIC LESIONS OF THE PROSTATE

Prostatitis:

Prostatitis occurs in approximately 10% to 15% of men. The term prostatitis refers to microscopic inflammation of the tissue of the prostate gland. It can be bacterial induced or not.[16] Prostatitis is classified into 4 categories: acute bacterial

(National Institutes of Health [NIH] type I); chronic bacterial (NIH type II); chronic prostatitis/chronic pelvic pain syndrome (NIH type III), which is divided into inflammatory (type IIIA) and noninflammatory (type IIIB); and finally, asymptomatic (NIH type IV). Asymptomatic prostatitis or NIH type IV is the most common type of prostatitis encountered by the surgical pathologist.[17] Most have chronic inflammation (94%) followed by acute inflammation (6%), and rarely, granulomatous inflammation (0.2%). Studies describing inflammation in the prostate have subclassified it by the type of inflammatory cell, location and pattern of inflammation (glandular, stromal and periglandular) and extent or grade.[18]

Acute prostatitis:

Acute prostatitis is due to the inflammation produced by bacteria with the common etiological agent being gram negative organisms. The organisms become implanted in the prostate by intraprostatic reflux of urine from the posterior urethra or urinary bladder, but occasionally from distant foci of infection they seed the prostate by lympho haematogenous routes. Acute prostatitis may appear as minute, disseminated abscesses (Fig.17) to large, coalescent focal areas of necrosis.[19]

Chronic Prostatitis:

Chronic bacterial prostatitis the presentation is that of recurrent urinary tract infection. Bacteria find safe in the parenchyma and constantly seed the urinary tract since antibiotics do not penetrate the prostatic stroma. Pathogenesis of chronic prostatitis involves reflux of infected urine into prostatic ducts, with associated factors contributing to infection such as infected prostatic calculi and local prostatic duct obstruction.

Granulomatous prostatitis:

Granulomatous inflammation can be seen in the prostate but is less common. Mainly consists of nonspecific granulomatous prostatitis and is characterized by granulomatous inflammation arranged concentrically around prostatic ducts or glands and can be accompanied by giant cells and a mixture of inflammatory cells.[20] The most common cause in the west is related to instillation of BCG within the bladder for treatment of superficial bladder cancer.[21](Fig.18)

Xanthogranulomatous Prostatitis:

As the name indicates it is nothing but collections of lipid-laden macrophages in the prostate. Xanthomatous histiocytes have small uniform nuclei with inconspicuous nucleoli and are commonly admixed with other types of inflammatory cells.[22] Multiple inflammatory cytokines have been identified as potential mediators in interplay between prostatic inflammation and prostate carcinogenesis. Macrophage inhibitory cytokine 1 (MIC-1), a member of the transforming growth factor-β (TGF-β) family is one of the cytokine. In prostate cancer, multiple lines of evidence point to a contributory role of IL-6 in cancer initiation and/or progression. Regions of prostatic atrophy, which are generally associated with inflammation, referred to as proliferative inflammatory atrophy (PIA), contain atrophic epithelial cells that appear to be regenerating in response to cellular damage. Wang et al. also documented the morphological transition between PIA and PIN as well as PIA and prostate cancer.[23]

Nodular Hyperplasia of Prostate

Most common disease diagnosed in prostate and affects elderly men. Prevalence increases with age. Microscopic evidence of hyperplasia is seen in 90%

of men above 80 years, only 50% develop clinically detectable prostatic enlargement out of which only 50% develop symptoms.[24] Patients may experience symptoms like increased urinary frequency, nocturia, difficulty in starting and stopping the stream of urine, overflow dribbling and dysuria.

Microscopy is characterized by nodular proliferation. The composition of the nodules may be purely stromal (fibrous), fibromuscular, muscular, fibroadenomatous and fibromyoadenomatous. Glandular proliferation takes the form of aggregations of small, large to cystically dilated glands, lined by two layers, an inner columnar and an outer cuboidal or flattened epithelium. (Fig.19) The histology of glandular or mixed glandular-stromal nodules cannot be appreciated in limited samples hence diagnosis of nodular hyperplasia cannot be made on needle biopsy. Drawback of transrectal needle biopsies is that, it do not typically sample the periurethral transition zone where nodular hyperplasia commonly occurs. [25]

Diagnosis of nodular hyperplasia from needle core biopsies of the prostate is discouraged, since no correlation was found between the histologic findings on needle core biopsy and clinical or symptomatic nodular hyperplasia. Stromal nodules can be confidently identified on needle core biopsies; however, they are not unique to nodular hyperplasia of prostate, since they have been identified in small-sized prostates (15.0 g)[26]

PSEUDO NEOPLASTIC LESIONS OF PROSTATE

The diagnosis of prostatic adenocarcinoma, especially when present in small amounts, is often challenging. Before making a diagnosis of carcinoma, pathologist must consider the various benign patterns and processes that can simulate prostatic

adenocarcinoma. Most of these lesions are readily recognized and easily separated from malignancy but can become problematic when dealing with limited sampling especially in thin core needle biopsies. Most benign mimickers enter the differential diagnosis of small acinar adenocarcinoma which is the predominant pattern of adenocarcinoma that corresponds to Gleason patterns 1, 2 and 3. Awareness of the differential diagnosis of prostatic adenocarcinoma is especially important in the context of diagnosing limited carcinoma in small biopsy samples.[27,28]

Seminal vesicle

Seminal vesicle tissue may be present in needle biopsies or in transurethral resectates usually unexpectedly. Microscopically it shows central lumen with branching glands surrounded by smooth muscle. End branching is complex with numerous small glands, resulting in the so-called adenotic pattern of seminal vesicle. When the overall gland structure and central lumen are not recognized this latter pattern can present problems. Presence of pleomorphism and nuclear hyperchromasia which at times striking is helpful in differentiating adenotic seminal vesicle from carcinoma.[29,30]

Cowper's gland

Bulbourethral glands also referred to as Cowper's glands, are paired periurethral structures located near the prostatic apex. They are rarely sampled in prostatic specimens. Cowper's glands have a lobular configuration with tightly packed round acini composed surrounding a central duct. Small acinar carcinoma is rarely confused with these glands. The duct-acinar architecture, lack of cellular atypia and cytoplasmic mucin distinguish adenocarcinoma from Cowper's glands.[31]

Atrophy

Older patients have atrophied prostatic glands but not exclusively. Atrophy is also seen in young adult prostate and is commonly admixed with areas of nodular prostatic hyperplasia.[32] It is also seen in the central and transition zones eventhough common in the peripheral zones. Glandular atrophy is commonly associated with chronic prostatitis which may have an active component characterized by intraglandular neutrophils. Diagnosis may be particularly challenging and one should be cautious in diagnosing carcinoma in inflamed small gland foci when atrophy is associated with inflammation, especially active inflammation. Stromal fibrosis may be seen in association with radiation therapy and use of antiandrogens, which result in architectural distortion. When coupled with radiation effects like cytological atypia can cause considerable diagnostic confusion.

Four main patterns of atrophy are recognized that includes sclerotic, cystic, lobular (simple) and linear or streaming. Combined patterns are common. Occasionally, atrophy may have a linear, streaming pattern in which small dark acini are lined up in a row permeating through stroma. This pattern is prone to be overinterpreted as carcinoma.[33]

Cells are small, dark and shrunken, have high nuclear to cytoplasmic ratio but the nuclei are uniform and lack nuclear membrane irregularity and chromatin abnormalities. Maintenance of lobular architecture in low power at least in part, uniform cytology and absence of prominent nucleoli differentiates small acinar adenocarcinoma. Double layering of cells is mostly seen but in some conditions because of the marked secretory cell atrophy it may be difficult to appreciate. In such cases, stains highlighting the basal cell compartment may be employed.[34]

Post-atrophic hyperplasia

2–3% of prostatic needle biopsy cases show post-atrophic hyperplasia, an uncommon histological process. Also referred to as partial atrophy or hyperplastic atrophy and is usually found in the peripheral zone. But it is difficult to know if post-atrophic hyperplasia represents a normal or hyperplastic focus undergoing atrophy (partial atrophy) or secondary hyperplasia occurring in atrophic areas. Post-atrophic hyperplasia consists of a combination of atrophic acini and contain more abundant clear or amphophilic cytoplasm and appear hyperplastic. This architecture of post-atrophic hyperplasia may cause diagnostic confusion with adenocarcinoma. However some degree of lobular architecture and basal cells are generally maintained and are usually recognized even at the H&E level.

The prudent use of immunostains is important. There is a discontinuous layer of basal cells in post-atrophic hyperplasia whereas the basal cell layer is completely absent in small acinar carcinoma. Luminal crystalloids and even small amounts of basophilic luminal mucus may be present in post-atrophic hyperplasia in rare instances.[35,36]

Reactive atypia

Glands in acute or chronic prostatitis can exhibit epithelial atypia and may sometimes be present in association with prostatic ischemia (infarction). Reactive atypia can be confused with adenocarcinoma. The glands are atrophic in most cases of reactive atypia and there may be some associated basal cell or transitional cell hyperplasia.[37]

Nephrogenic metaplasia

Nephrogenic metaplasia (adenoma) is uncommonly encountered in the subjacent prostatic tissue and prostatic urethra. This may be present as flat or nodular

abnormality or an exophytic (papillary) lesion. Nephrogenic metaplasia displays exophytic papillary and tubulocystic elements. Nephrogenic metaplasia shows small acini with scanty cytoplasm. Small size of the acini, cystic dilatation and inflamed stroma separates nephrogenic metaplasia from small acinar carcinoma. Immunostains may be helpful sometimes but not all cases of nephrogenic metaplasia.[38]

Squamous metaplasia

Squamous metaplasia is usually seen at the periphery of infarcts, after transurethral resection, hormonal manipulation, or sometimes without any predisposing cause.[39] (Fig. 20)

Basal cell hyperplasia

Basal cell hyperplasia is typically seen as a part of the continuum of nodular hyperplasia in samples from the transition zone of prostate. In recent times researches say that basal cell hyperplasia may also affect the peripheral zone. It is identified in transurethral resection specimens but may be encountered in needle biopsies as well. Basal cell hyperplasia may also occur in association with atrophy and frequently in the setting of antiandrogen therapy. Microscopically characterized by uniform round glands with nodular expansion and associated cellular stroma. (Fig.21) It may be complete or incomplete.

Basal cell hyperplasia may be confused with adenocarcinoma. The nodular arrangement, association with ordinary nodular hyperplasia, cellular uniformity and lack of prominent nucleoli serves to separate this condition from cancer. The distinction relies on the recognition of uniform cytological and nuclear features and in some instances may require immunohistochemical staining.[40]

Sclerosing adenosis

Young and Clement in 1987 first used the term sclerosing adenosis of prostate to describe an infrequent prostatic proliferative lesion which look like sclerosing adenosis of breast.[41]

Sclerosing adenosis though uncommon, it is largely restricted to the transition zone but generally found in transurethral resectates or radical prostatectomy specimens. This condition is described as a vaguely circumscribed proliferation of variably sized small glands surrounded by a cellular and often edematous stroma. Recognized sometimes on low power as a lightly basophilic stroma. Tiny microacini, cords, solid clusters and single cells are seen. A double layer is present but may be difficult to appreciate with the H&E stain.[42]

Verumontanum mucosal gland hyperplasia

Verumontanum mucosal gland hyperplasia is identified as an incidental finding with one or more foci of hyperplastic verumontanum mucosal glands. This feature is rarely encountered in needle biopsy specimens. Microscopy reveals closely packed relatively uniform round glands containing numerous corpora amylacea. Basal cells are usually identified and there is a lack of nuclear features of malignancy. Acinar pattern, cellular uniformity, basal cells and prominent corpora amylacea are features suggesting hyperplasia over low grade adenocarcinoma.[43]

Hyperplasia of mesonephric glands

Mesonephric gland remnants are not often identified in prostatic specimens. 0.6% of mesonephric remnants were identified in a series of close to 700 transurethral resectates. Hyperplasia in mesonephric gland remnants may be

mistaken for adenocarcinoma. The hyperplastic mesonephric glands have an infiltrative appearance and are small. Epithelial tufting, tubular dilatation and micropapillary formations are also seen. They may demonstrate extra prostatic extension and perineural spread, both features are seen in carcinoma. Dense eosinophilic luminal substance in small glands contrast with the loose granular eosinophilic material typical of small acinar carcinoma. Immunohistochemistry for identifying basal cells may be helpful in difficult cases.[44]

Clear cell cribriform hyperplasia

Areas of prominent cribriform glands are occasionally displayed in benign nodular hyperplasia and rarely the histologic picture is dominated by this cribriform process. Crowded proliferation of complex glands and lack of cytological atypia is characteristic of cribriform hyperplasia. The cells in the central area are cuboidal to low columnar secretory-type cells with clear cytoplasm, uniform round nuclei without nuclear atypia and nucleolar enlargement. Periphery shows prominent basal cell layer. Due to its peculiar architecture, both prostatic intraepithelial neoplasia and cribriform adenocarcinoma enters the differential diagnosis of cribriform hyperplasia. Low power nodularity, presence of basal cells, cellular stroma and lack of significant cytologic atypia separates cribriform hyperplasia from cribriform carcinoma.[45]

Paraganglion

Prostatic and periprostatic tissue show paraganglionic tissue may be encountered within, usually in the latter. Paraganglia are small, solid nests of cells often with a 'zellballen' arrangement and cytoplasm is amphophilic or clear. Background shows delicate network of capillaries. The nuclei appears hyperchromatic but nucleoli and other features of adenocarcinoma are not seen.

Fibrous stroma separates the islands of paraganglionic tissue. Paraganglionic tissue can mimic the fused gland pattern of adenocarcinoma Gleason score-4.[46]

Degenerative changes in lymphocytes and stromal cells

Signet ring-like morphology can occur when lymphocytes and sometimes stromal cells undergo degenerative changes. When such change is prominent, it can resemble high-grade adenocarcinoma composed of individual signet ring cells. The artifactual signet ring-like pattern initially described in transurethral resectates, may be found in needle biopsies.[47]

PREMALIGNANT LESIONS OF PROSTATE

Premalignant lesions of the prostate include Prostatic intraepithelial neoplasia (PIN), particularly high-grade PIN (HGPIN) and atypical small acinar proliferation (ASAP). PIN refers to the precancerous end of a morphologic spectrum involving cellular proliferation within prostatic acini, ductules and ducts. ASAP and HGPIN should not be used interchangeably, these two categories differ from each other. Neither ASAP nor HGPIN can metastasize. The enlargement of prostate occurs due to nodular prostatic hyperplasia but this is unrelated to HGPIN. Areas in the prostate may have palpable nodules or other areas may indicate cancer. None of these physical findings suggests the presence of HGPIN or ASAP.

Prostatic Intraepithelial Neoplasia

Bostwick and Brawer in 1987 introduced the term PIN. Prostatic Intraepithelial Neoplasia, commonly referred to as PIN is an abnormal proliferation within the prostatic ducts, ductules, and large acini of premalignant foci of cellular dysplasia and carcinoma in situ without stromal invasion.[48,49]

An autopsy study from older men of step-sectioned whole mount prostates showed that the prevalence of PIN in prostates with cancer, increased with age predating the onset of carcinoma by more than five years.[50]

PIN is characterized by cellular proliferation within pre-existing ducts and acini with cytologic changes including nuclear and nucleolar enlargement mimickes cancer. (Fig.22) Depending on the characteristics like cell crowding, pleomorphism, stratification, nuclear enlargement, nucleolar appearance and chromatin pattern initially it was divided into three grades. These are graded into the low grade PIN (corresponding to grade 1 and 2) and high grade PIN (corresponding to grade 3).

Low grade PIN is a common finding in young male patients. Its association with adenocarcinoma has been questioned and was recently removed from World Health Organization (WHO) classification. Four main patterns of high-grade PIN are tufting, cribriform, micropapillary and flat. Although most cases have multiple patterns, tufting pattern is the most common and is present in 97% of cases.[39] In an analysis of 17 studies, in which a total of 87,713 patients underwent biopsy, 3735 (4.26%) patients had HGPIN. The largest contributors to this study were Orozco et al, with 62,537 patients (of whom 4.1% had HGPIN), and Novis et al, with 15,753 patients (of whom 3.9% had HGPIN).[51, 52]

Basal cell–specific monoclonal antibodies targeted against high–molecular weight keratin is used to identify HGPIN cells. Normal prostatic epithelial cells stained consistently with these antibodies, exhibiting an intact, continuous, circumferential basal cell layer. Receptors for these antibodies are lost in cancer cells. Basal cell disruption affects 56% of patients with HGPIN and is usually found in glands adjacent to invasive cancer. HGPIN correlates with the degree of

disruption. More than one third of the basal cell layer is lost in 52% of foci that contain HGPIN. Similar genetic alterations include loss of heterozygosity (LOH) at 8p22, 10q11.2 and gain of chromosomes 7, 8, 10, and 12 are seen in both High-grade PIN and prostate cancer. Modifications in oncogene Bcl2 expression and RER+ phenotype were similar for PIN and prostate cancer.[53]

Atypical small acinar proliferation (ASAP)

The category ASAP includes a group of lesions (Adenosis, intraductal hyperplasia, atypical adenomatous hyperplasia, and acinar atypical hyperplasia) that have unpredictable clinical significance. Some ASAP lesions mimic cancer, and in many occasions, focal carcinoma may be present, but architectural atypia, cytological and histochemical features are insufficient to establish a definitive diagnosis of cancer.

Borboroglu et al., in their study reported detection rates of cancer on repeat biopsy was ranging from 25-79% for HGPIN and 21-51% for ASAP.[54]

Moore et al., evaluated the biopsy results of 105 men to further evaluate a finding of HGPIN or ASAP, repeat extended biopsies were performed and found that repeat biopsy revealed cancer more often in patients with ASAP than in those with HGPIN. In the HGPIN group, based on first repeat biopsy results, cancer was diagnosed in 1 (4.5%) of 22 men and in 0 of 11 based on a second repeat biopsy result. The results in the ASAP group were much different. 19 (36%) of 53 men in their first repeat biopsy revealed cancer and 13 (16%) of 19 on a second repeat biopsy.[55]

When HGPIN and ASAP is identified, as these entities are often found in prostates in which prostate cancer cells are present, Pathologist should carefully hunt tissue specimens for evidence of cancer. Indeed, it was because of this association that these lesions came to be considered noncancerous precursors to the development of actual prostate cancer. A follow-up protocol is usually initiated if no cancer cells are identified in the patient, since there is a higher risk of prostate cancer in these individuals. The follow-up protocol consists of physical examination, serum PSA estimation and possibly repeat biopsies. However, the presence of a small amount of HGPIN in only 1-2 cores is considered to be insignificant and these patients require no special follow-up. ASAP represents a potentially more serious situation. The finding of ASAP in even a single biopsy sample requires a follow-up biopsy at 6 months. Urologist has to initiate therapy with repeated findings of ASAP.[56]

WHO CLASSIFICATION OF TUMORS OF THE PROSTATE- 2016.[57]

Epithelial tumors

Glandular neoplasms

- Acinar adenocarcinoma
    - Atrophic
    - Micro cystic
    - Foamy gland
    - Signet ring-like cell
    - Clear cell adenocarcinoma
    - Pleomorphic giant cell
    - Sarcomatoid
- Prostatic intraepithelial neoplasia, high-grade

- lntraductal carcinoma
- Ductal adenocarcinoma
    - Cribriform
    - Papillary
    - Solid
- Urothelial carcinoma

Squamous neoplasms

- Adenosquamous carcinoma
- Squamous cell carcinoma

Basal cell carcinoma

Neuroendocrine Tumors

- Adenocarcinoma with neuroendocrine differentiation
- Well differentiated neuroendocrine tumor
- Small cell neuroendocrine carcinoma
- Large cell neuroendocrine carcinoma

Mesenchymal Tumors

- Stromal tumor of uncertain malignant potential
- Stromal sarcoma
- Leiomyosarcoma
- Rhabdomyosrcomaa
- Leiomyoma
- Angiosarcoma
- Synovial sarcoma

- Inflammatory myofibroblastic tumor
    - Osteosarcoma
    - Undifferentiated pleomorphic sarcoma
    - Solitary fibrous tumor
    - Solitary fibrous tumor, malignant
    - Haemangioma
    - Granular cell tumor

Haematolymphoid tumors

- Diffuse large B-cell lymphoma
- Chronic lymphocytic lymphoma/ small lymphocytic lymphoma
- Follicular lymphoma
- Mantle cell lymphoma
- Acute myeloid leukaemia
- B lymphoblastic leukaemia/ lymphoma

Miscellaneous tumors

- Cystadenoma
- Nephroblastoma
- Rhabdoid tumor
- Germ cell tumors
- Clear cell adeno carcinoma
- Melanoma
- Paraganglioma
- Neuroblastoma

Metastatic tumors

CARCINOMA PROSTATE

Among men in the United States, Carcinoma of prostate is the most common internal malignancy and is responsible for 10% of cancer related death in men.[39] It constitutes about 5% of all male cancers in India. The prevalence of pathologic prostate cancer is extremely high and increases with age. In autopsy series pathologic prostate cancer is seen in men in their 20s and 30s and increases to more than 80% over the age of 70.[58]

The vast majority of prostatic cancers was acinar adenocarcinomas. Histological variants of PCa have been variably defined. One approach is to consider two groups of variants. Histological variants of acinar adenocarcinoma were included in the first group and the second group comprises the non-acinar carcinoma variants or types. Variants of usual acinar adenocarcinoma defined in 2016 by the WHO include pseudo hyperplastic, atrophic, foamy, signet ring (Fig.23), oncocytic, colloid and lymphoepithelioma-like carcinomas. Other types of Pca that originate in the prostate includes ductal adenocarcinoma, sarcomatoid carcinoma, squamous and adenosquamous carcinoma, basal cell carcinoma, urothelial carcinoma and neuroendocrine tumors, particularly small-cell carcinoma accounts for about 5–10% of carcinomas.[57,59,60]

Morphology

Diagnosis of PCa requires a synthesis of a group of histological attributes that allows for a definitive diagnosis. Epstien J I[61] in his review has observed that infiltrative small and crowded glands favors malignancy. Scattered neoplastic glands infiltrate widely between larger benign glands at the edge of most adenocarcinomas. One first has to identify the normal non-neoplastic prostate and then search for

glands that do not fit morphologically in order to identify limited amounts of cancer on needle biopsy material. At low power magnification presence of a focus of crowded glands raises a suspicion of carcinoma.

The second architectural pattern that is suspicious for adenocarcinoma of the prostate is the presence of small glands situated between larger benign glands. Benign glands are identified by their large size, branching and papillary in folding. The presence of small neoplastic glands situated in between benign glands is a manifestation of their infiltrative nature. When small atypical glands are seen on both sides of a benign gland it is even more diagnostic of malignancy. Cytologically hyperchromasia, prominent nucleoli with enlargement and mitotic figures are in favour of malignancy(Fig.24). Sharp luminal borders with amphophilic cytoplasm are seen in malignancy whereas normal acini have pale to clear cytoplasm.

Prostatic intraluminal crystalloids (Fig.25) that appear in various geometric shapes such as hexagonal, triangular, rectangular and rod-like structures are dense eosinophilic crystal-like structures and seen in malignant acini in 25% of cases.

Acini of prostatic adenocarcinoma have acidic sulfated and non-sulfated mucin in up to 52% of cases, that appears as amorphous or delicate thread-like faintly basophilic secretions in routine H&E sections.[61, 62]

Bostwicket et al., describes collagenous micronodules (mucinous fibroplasia) as specific but incidental and infrequent finding in prostatic adenocarcinoma. These are of microscopic nodular masses of paucicellular eosinophilic fibrillar stroma that impinge on lumen of the acini seen in mucin-producing adenocarcinoma and is due

to extravasation of acidic mucin into the stroma.[63] Glomerulations a sign of malignancy, consist of glands with a cribriform proliferation which is not transluminal.[60]

Criteria for diagnosis of prostatic adenocarcinoma[64]

Major criteria

- Architectural: infiltrative small glands or cribriform glands too large or irregular to represent HGPIN
- Single cell layer (absence of basal cells)
- Nuclear atypia: nuclear and nucleolar enlargement

Minor criteria

- Intraluminal wispy blue mucin (blue-tinged mucinous secretions)
- Pink amorphous secretions
- Mitotic figures
- Intraluminal crystalloids
- Adjacent high-grade PIN
- Amphophilic cytoplasm
- Nuclear hyperchromasia

Collagenous micronodules and glomerulaions along with perineural invasion are features of prostate cancer, other than metastasis.

Perineural invasion (PNI)

Perineural invasion by malignant cells can be found in 11–37% of needle biopsy specimens with carcinoma, but with minimal or limited prostate cancer occurs only in 0–3% of tissue. In the absence of other features in support of

carcinoma, the glands in question should circumferentially (i.e. 100%) surround the nerve in order for perineural invasion to be diagnostic of adenocarcinoma. (Fig.26) In cases where there are other histological features supporting the diagnosis of prostate cancer, perineural invasion that is less than circumferential can help to form a diagnosis of malignancy. Benign glands abutting the prostatic peripheral nerve needs to be distinguished from true PNI which is representative of adenocarcinoma of the prostate.[65, 66]

David Merrilees et al.,[67] in his study on PNI in radical prostatectomy observed 90% of cases with this feature. Diameter of nerves exhibiting perineural invasion ranged from 11 to 680 μm. Perineural invasion density ranged from 6 to 96%.

Lymphovascular Invasion

Lymphovascular invasion (LVI) indicates whether microscopic lymph-vascular invasion is identified. LVI includes vascular invasion, lymphatic invasion, or lymphovascular invasion. (Fig.27) By AJCC convention, LVI does not affect the Tumor(T) category indicating local extent of tumor unless specifically included in the definition of a T category.[15]

Extra prostatic Extension:

Extra prostatic extension (EPE) is the presence of tumor beyond the confines of the prostate gland. Tumor involves the loose connective tissue beyond or in the plane of fat, even in the absence of direct contact between the tumor and the adipocytes, indicates EPE. When perineural spaces in the neurovascular bundles are involved by the tumor, even in the absence of periprostatic fat involvement may also be reported as EPE. EPE is determined when the tumor extends beyond the confines

of the normal glandular prostate, in anterior and apical prostate and bladder neck regions areas where there is paucity of fat. The specific location(s) and the number of sites (blocks) of EPE are useful to report. Labelled as focal and nonfocal EPE. Focal EPE is involvement of a few neoplastic glands, outside the prostate or a tumor involving less than one HPF in 1 or 2 sections and more extensive spread beyond the prostatic edge is nonfocal EPE.[68]

Grading of Prostatic cancer:

Of the many proposed systems, currently the most widely accepted and used is the Gleason system for the grading of prostate cancer. Most recently, the system has been endorsed by the World Health Organization. Gleason's system is based entirely on the architectural pattern of the tumor, without taking cytologic features into account. It was (and remains) relatively unique among pathologic grading systems.

In 1966, Donald F. Gleason, created the Gleason grading system based architectural features of prostate cancer on low-power examination. The initial description of this system was based on a study of 270 patients from the Minneapolis Veterans Administration Hospital (Minneapolis, Minnesota). Later the study was expanded to include 1032 men in 1974. With more and more experience, Gleason made further refinements to the system in 1974 and 1977.[69,70]

Veteran's Administration Cooperative Urological Research Group (VACURG) was established in the 1960s to study the treatment modalities and prognostic parameters in carcinoma prostate. All PCa's were graded by Dr. Gleason as part of the study, which is still being used with modifications.[71]

Five grading categories, designated patterns, were defined as follows:

Pattern 1: Very well differentiated small and closely packed glands forming a circumscribed tumor mass.

Pattern 2: Similar to pattern 1 but with less well circumscribed glands showing greater variation in both size and shape.

Pattern 3: Well-formed discrete units of variable sized individual glands. (Fig.28)

Pattern 4: Closely packed, poorly formed/ fused (Fig.29) / glomeruloid / cribriform glands. (Fig.30)

Pattern 5: Lack of gland formation with sheets of tumor/ individual cells/ cords/ solid nests of cells and linear arrays. (Fig.31)

In this architectural system, all tumors fall into a 5-grade system representing a continuum of progressively complex morphologies. More than one histological pattern was present in most cases and he designated the predominant pattern as the primary pattern, while the subordinate pattern was designated the secondary pattern.

The Gleason score is obtained by adding primary and secondary patterns in a total out of ten. The pattern is considered as both the primary and secondary, if only one pattern is present then this.

Grading system[15, 57]

Current system differs from original and scores 2 to 5 are no longer assigned on biopsy.

Grade group 1	Gleason score 6 - Only individual discrete well-formed glands
Grade group 2	Gleason score 3+4 = 7 - Predominantly well-formed glands with lesser component of poorly formed/fused/cribriform glands.
Grade group 3	Gleason score 4+3 = 7 - Predominantly poorly formed/ fused/ cribriform glands with lesser component of well-formed glands.
Grade group 4	Gleason score 4+4 = 8; 3+5 = 8; 5+3 = 8 - Only poorly formed/fused/cribriform glands (or) predominantly well-formed glands and lesser component lacking glands (or) predominantly lacking glands and lesser component of well-formed glands
Grade group 5	Gleason scores 9-10 - Lack gland formation (or with necrosis) with or without poorly formed / fused / cribriform glands

Description of the amount of pattern 4 in the setting of Gleason Score 7 (3+4 versus 4+3) is prognostically important. Presence of any Gleason pattern 4 is typically considered clinically significant prostate cancer and is often important in the clinical decision-making process. In the most recent Partin tables for prognostic purposes, Gleason score 7 (3+4) and Gleason score 7 (4+3) are considered separately.[72]

Lower-grade secondary patterns comprising <5% of the tumor area should be ignored in a high grade cancer. For example, a needle biopsy core that is 100% involved by cancer, with 98% Gleason pattern 4 and 2% Gleason pattern 3, would be diagnosed as Gleason score 4+4=8. While any proportion of higher secondary

pattern, even if <5% of tumor area, should contribute to the score. For example a needle biopsy that is entirely involved by cancer with 98% Gleason pattern 3 and 2% Gleason pattern 4 would be diagnosed as Gleason score 3+4=7.

If a tertiary pattern was present then the final score should be derived from the primary pattern, with the (higher) tertiary pattern being reassigned as the secondary pattern. Consequently, tumors with Gleason score 3+4 and a tertiary pattern 5 would be recorded as Gleason score 3+5=8. In cases where there are three patterns consisting of patterns 2, 3, and 4, one would ignore the pattern 2 and the biopsy would be called Gleason score 3+4=7 or Gleason score 4+3=7, depending on whether pattern 3 or pattern 4 was more prevalent.

Needle biopsy with different cores showing different grades: To assign individual Gleason scores to separate cores as long as the cores were submitted in separate containers or the cores were in the same container yet specified by the urologist as to their location (ie, by different color inks). In addition to giving separate cores individual Gleason scores, one has the option to also give an overall score at the end of the case.[15, 57]

Staging

The 2010 revision of the American Joint Committee on Cancer/ Union International Centre on Cancer (AJCC/ UICC) tumor, node and metastasis (TNM) system is the most widely used staging system at this time. Pathologic staging is usually performed after surgical resection of the primary tumor.

Pathologic staging depends on pathologic documentation of the anatomic extent of disease, whether or not the primary tumor has been completely removed.

pT entails a resection of the primary tumor or biopsy adequate to evaluate the highest pT category, pN entails removal of nodes adequate to validate lymph node metastasis, and pM implies microscopic examination of distant lesions. [15]

Intraprostatic adipose tissue does not exist, so identification of fat invasion in needle biopsies constitutes T3a cancer. Similarly, T3b cancer can be identified by needle biopsy of seminal vesicles. Because of the availability of various imaging tools for the detection histopathological assessment of bone biopsy is rarely needed for the diagnosis of bone metastasis.[73]

Molecular genetics

Great progress in understanding the molecular basis of PCa and the genomic alterations of the underlying the disease has occurred over the past decade. Next-generation sequencing has allowed the classification of PCa at multiple strata of molecular information, incorporating data at genomic, proteomic, transcriptomic, and epigenetic levels.

In early genomic and transcriptomic analyses, prostate tumors were able to be stratified based on patterns of somatic copy number alterations (SCNAs) and mRNA expression signatures. Considerable evidence exists that PCa is driven by cardinal genetic alterations that activate oncogenes and inactivate tumor suppressors, these result most commonly from structural genomic changes including deletions involving the NKX3.1 and phosphatase and tensin homologue tumor suppressor genes (PTEN), and amplifications of the androgen receptor (AR) and MYC genes.

Recently published data from The Cancer Genome Atlas (TCGA) supports the major molecular subclasses of localized PCa can be divided into E26

transformation specific (ETS) - rearrangement PCa (PCa with rearrangements and overexpression of ERG, ETS Variant 1 (ETV1), ETV4, or other ETS family transcription factors), Speckle-type POZ protein (SPOP)/Chromodomain Helicase DNA Binding Protein 1(CHD1) altered cancers and several smaller categories.[74]

ETS-rearranged tumors are generally enriched in genomic alterations in the Phosphatidyl Inositide 3-Kinase (PI3K) and p53 signaling pathways, whereas other specific SCNAs predominate in SPOP-mutant cancers. A subset of ETS-negative cancers (including SPOP mutant cancers) show overexpression of Kazal type 1 (SPINK1), a secreted serine peptidase inhibitor, associated with poor prognostic features, is another marker commonly used for disease classification. Those overexpressing SPINK1, hold promise as treatment targets.[75] In contrast to well defined genomic lesions, protein altering point mutations are uncommon in PCa, which makes it one of the cancers with lowest rate of such mutations.

Studies have shown fusion of TMPRSS2 and ERG loci at the chromosomal level with subsequent overexpression of the TMPRSS2: ERG transcript and truncated ERG protein product is essentially 100% specific for the presence of PCa.

The ability of a solid tumor to grow without developing its own vascular network is limited by simple oxygen and or nutrient diffusion capacity. The development of a pro-angiogenic capability is necessary for continued growth of tumor. In many tumor types this occurs as an early-stage to mid-stage event in the carcinogenic process as a well-defined transition, termed the angiogenic switch. Vascular endothelial growth factor (VEGF), placental growth factor, angiopoietin 1, fibroblast growth factors, platelet-derived growth factor (PDGF) and epidermal

growth factor are various pro-angiogenic factors that have been shown to play roles in new blood vessel formation during tumor growth.[76]

Although significant biological activity can usually be demonstrated in preclinical models, number of agents that interfere with VEGF / VEGFR signaling have been developed. A non-receptor tyrosine kinase, Src also mediates a diverse array of biological effects in different cell types. Bevacizumab is a humanized monoclonal antibody against VEGF that inhibits VEGFR signaling by binding to and neutralizing the ligand. Other newer therapeutic agents developed include Sunitinib, Aflibercept and Dasatinib.

Isocitrate dehydrogenase-1 (IDH1) metabolic enzyme, is recurrently mutated in several human malignancies including acute myeloid leukemia and gliomas and result in a methylator phenotype. Increased production of the oncometabolite, 2-hydroxyglutarate via neomorphic activity of IDH1 gained through characteristic mutations is thought to result in the inhibition of Tet Methylcytosine Dioxygenase 2, thereby resulting in hypermethylation across the genome.[77]

The integration of multiple genomic platforms in primary PCa allowed for the identification of this rare, novel molecular subclass of PCa which is characterized by IDH1 mutations, most notably at residue R132. These cancers were found to be associated with early age of onset, few SCNAs, and similar to IDH1-mutant gliomas and acute myeloid leukemias, vast, genome-wide hypermethylation, although at disease-specific loci. Although this mutation is uncommon, this mutation may be clinically actionable, as clinical trials with IDH1 inhibitors specific to R132 IDH1-mutants are ongoing in acute myelogenous leukemia and other malignancies.[78]

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) is a method for locating specific antigens in tissues based on antigen antibody recognition. It exploits the specificity of the binding antibody with the antigen at a light microscopic level.[79]

The enzymatic label (Horseradish peroxidase) developed by Avaremeas and by Nakane and colleagues, allowed visualization of the labelled antibody by light microscopy in the presence of a suitable colourogenic substrate system.

Taylor in 1974 successfully demonstrated antigens in formalin fixed paraffin embedded (FFPE) tissues. Critical issue in the development of immunoperoxidase technique was related to the need to achieve greater sensitivity that would facilitate staining of FFPE tissues from a simple one step direct conjugate method to multiple step detection techniques such as peroxidase antiperoxidase (PAP), avidin-biotin conjugate (ABC), and biotin streptavidin (B-SA) methods and would eventually lead to amplification methods (such as tyramide) and highly sensitive polymer-based labelling system.[80]

The use of IHC as an auxiliary in the diagnosis of adenocarcinoma is a common practice in Uropathology. Application of Immunohistochemistry in PCa includes confirmation or exclusion of prostate cancer, identification of atypical cells within benign prostatic appearences, identification of genotypic subtypes and finally prediction of behaviour of prostate cancer. It also helps to distinguish benign mimics from malignancy. Markers fall into second category are those employed as diagnostic adjuncts (CK34βE12) and those identify cellular phenotypic change. Markers used to identify prostatic origin of neoplasm are PSA and PSMA. To avoid rebiopsies and to enhance their diagnostic capabilities, Pathologists frequently use

immunohistochemistry. Still there are reports of false-positives and false-negatives for use of the combined CK34βE12 and p63 cocktail.

Recognition of mimickers of adenocarcinoma by careful routine microscopy coupled with Immunhistochemistry will lead to a correct diagnosis. Immunohistochemical studies aimed at identifying prostatic basal cells (CK34βE12, CK5/6, p63), prostatic secretory cells (PSA, PAP, CD57), neuroendocrine cells (chromogranin, synaptophysin) and inflammatory cells (LCA, CD68) may be required to resolve this diagnostic dilemma. Another marker α-methylacyl-CoA racemase (P504S) appears to be of value in supporting a diagnosis of adenocarcinoma, especially when one is dealing with small foci.[81]

PSA is a 33-kD serine protease that is used widely to confirm immuno histochemically the prostatic origin of metastatic carcinoma. It is not entirely prostate-specific, as PSA has also been detected in carcinomas of the ovary and the breast, including male breast cancer and other tissues.

Prostate-specific membrane antigen (PSMA) is a transmembrane protein that is expressed strongly by most PCa and their metastases and has therefore been recommend as a diagnostic marker.[82] PSA and PSMA are both targets of androgen signalling and is also regulated in prostate cancer. It is non-specific as it is expressed by other human tissues.

Novel markers available are Prostein, coded by the gene SLC45a3, first identified as a prostate-specific transcript by Xu et al.[83] in 2001 and ERG expression although not sensitive is highly specific for carcinoma prostate in a metastatic site.

p63:

p63, a homologue is expressed in the basal cells of prostate, located in 3q27. This encodes six isoforms, distinction between these isoforms by mRNA expression is important when interpreting the biological phenotype of cells, that contain identified protein. Shift in balance between different isoforms inhibits or even promotes normal or malignant growth. This depends upon expression of other co-acting genes simultaneously. [84]

The use of antibodies against p63 and high molecular weight cytokeratin has been recommended as adjuncts in confirming PCa.

High Molecular Weight Cytokeratin (HMWCK):

Originally reported in 1983, monoclonal antibody clone 34βE12 (earlier known as CK 903, EAB 903, hP 34) to HMWCK's. This immunostain is used to demonstrate basal cells which in turn discriminate between early prostatic malignancy and atypia from non-neoplastic mimickers of carcinoma. In a comparison study of basal cell detection, have found out that it should be employed only after use of microwave retrival protocol. HMWCK assists in differentiating basal call hyperplasia from carcinoma or high grade PIN.[85]

Qualitative differences are identified between CK34βE12 and p63 in the staining pattern and have been found significant where p63 staining is offered in diagnostic utility than 34βE12. In such condition p63 appeared as strong but discontinuous in atypical glands and adjacent benign glands. But CK34βE12 failed to stain the above mentioned structures. This emphasises the necessity for use of defined protocol. To avoid such difficulties, various cocktails of antibodies are suggested (eg. AMCAR).

According to Epstein et al., basal cell cytokeratin is expressed in up to 0.3% of cases, the negativity of basal cell markers is complemented by a positive indicator of malignancy. Basal cells in the prostate can be labelled by a wide range of other markers that can be employed diagnostically. Among others, P-cadherin, D2-40, CD109 or BCL-2 has been suggested.

Gown and Vogel[86] in 1984, reported the use of a monoclonal antibody anti-high molecular weight cytokeratin (CK34βE12) to mark basal cells of the prostate that was later demonstrated as a characteristic of benign glands that retain the basal cell layer.

In another series, Wojno and Epstein[87] used CK34βE12 to diagnose adenocarcinoma in suspicious glands identified in needle prostate biopsies.

Shah et al.[88] later proposed the combined use of p63, a homolog of the p53 tumor suppressor protein, as an auxiliary marker for the determination of cancer since it is also a protein expressed selectively by the basal cells of epithelial organs, including the prostate gland.

MATERIALS & METHODS

Study Design: Cross sectional Study of retrospective data and present data. Retrospective data from January 2016 to December 2016 was used.

Study Setting: Department of Pathology, Sree Mookambika Institute of Medical Sciences.

Duration of study: Two years

Number of groups to be studied: 1

Detailed description of group: All prostatic specimens received in histopathological lab

Sample size of each group: One group with 50 samples

Total sample size of study: 50

Scientific basis of sample size used in study:

Sample size was calculated using the formula

$N = 4pq/d^2$

P = Prevalence

q = 100 – p

d = Allowable error (5-20%p)

Prevalence: 68%

4 x 68 x 32= 8704

20% of 68 = 13.6

$8704/ (13.6)^2$= 47 Samples

Sampling technique: Convenient Sampling.

Inclusion criteria:

- All prostatic biopsies (received from Department of Urology for histopathological examination).

Exclusion criteria:

- Those Cases where specimen sample seems inadequate for histopathological reporting.
- Histopathologically proved metastatic carcinomas to prostate.
- Men below 40 years of age.

Procedure in detail:

After getting approval from Institutional Human Ethical Committee (IHEC), clinical history and results of relevant investigations done were collected from the patient case files. Prostatic needle biopsies and Transurethral resection of prostate (TURP) specimens were the usual specimens received in the Pathology department in adequate 10% formalin.

After a detailed specimen description, sampling was done. Entire tissue was processed in needle biopsies. In cases of TURP approximately 2 grams of tissue was processed for first 12 grams in 6 cassettes and then for every 5 grams of tissue bit one cassette of 2 grams is added and embedded. The tissue bits were processed manually and paraffin blocks were prepared.

Tissue sections of 4- 6µm thickness were cut and stained by haematoxylin and eosin (H & E) for histopathological study. The sections were studied extensively and the results were noted in the proforma.

For retrospective cases, the histopathology reports, slides and paraffin blocks were retrieved from the archives from January 2016 to December 2016. Sections were cut from the paraffin blocks in a similar manner.

Then 3-4 μm sections were cut from a paraffin block of premalignant & malignant tissues and taken on glass slide coated with adhesive (Poly- Lysine) for immunohistochemistry (IHC) to detect for High molecular weight Cytokeratin (Cytokeratin 34BetaE12 /keratin 903) and p63. Procedure in brief as follows,

Air dry the slide for 2 hours at 58ºC. Deparaffinise, dehydrate and rehydrate tissues. Subject tissues to heat epitope retrieval using immune DNA retriever citrate. Wash IHC wash buffer. Place slide in polydetector peroxidase blocker for 5 minutes. Wash IHC wash buffer. Incubate sections with the primary antibody. Wash IHC wash buffer. Cover tissues with polydetector HRP label. Incubate for 45 minutes. Rinse with IHC wash buffer. Prepare DAB (deaminobenzidine) by adding one drop of polydetector DAB chromogen per ml of polydetector DAB buffer and mix. Cover tissue with prepared DAB substrate chromogen solution. Incubate for 5 minutes. Rinse with Deionised water. Counterstain with hematoxylin, then dehydrate, clear and mount the slide with DPX. Positive and negative controls will be run with each batch of slides.

H &E stained slides were studied for the tumor histology - benign or premalignant or malignant, benign mimickers of malignancy, inflammation and Gleason scoring system in case of malignancies. The immunostained slides were examined for basal cell cytoplasmic staining of High molecular weight cytokeratin (CK 34BetaE12) and nuclear staining in P63.

Basal cell staining intensity was scored as follows: 1 (negative), 2 (weak), 3 (moderate) or 4 (strong positive). Percentage basal cell staining was scored from 0-100%. Basal cell staining was considered positive if the density was 2+ or more and >10% glands were stained and negative if the score was 1+ or less and ≤ 10% of the glands were stained.

Statistical methods of analysis

1) Significance level decided before starting of study: 5%
2) Statistical test used in data analysis: Logistic Regression Analysis & Chi-Square test
3) Software used for statistical analysis:
    - Data was entered in Microsoft Excel
    - Analysis was by SPSS Software Trial Version 20.0.

RESULTS

The total number of prostatic biopsies studied during the period of two years was 50.

AGE DISTRIBUTION:

Table 1 : Age Distribution

Age Range	No. of patients	Percentage %
50-59	3	6
60-69	22	44
70-79	19	38
80-89	5	10
90 – 99	1	2
Total	50	100

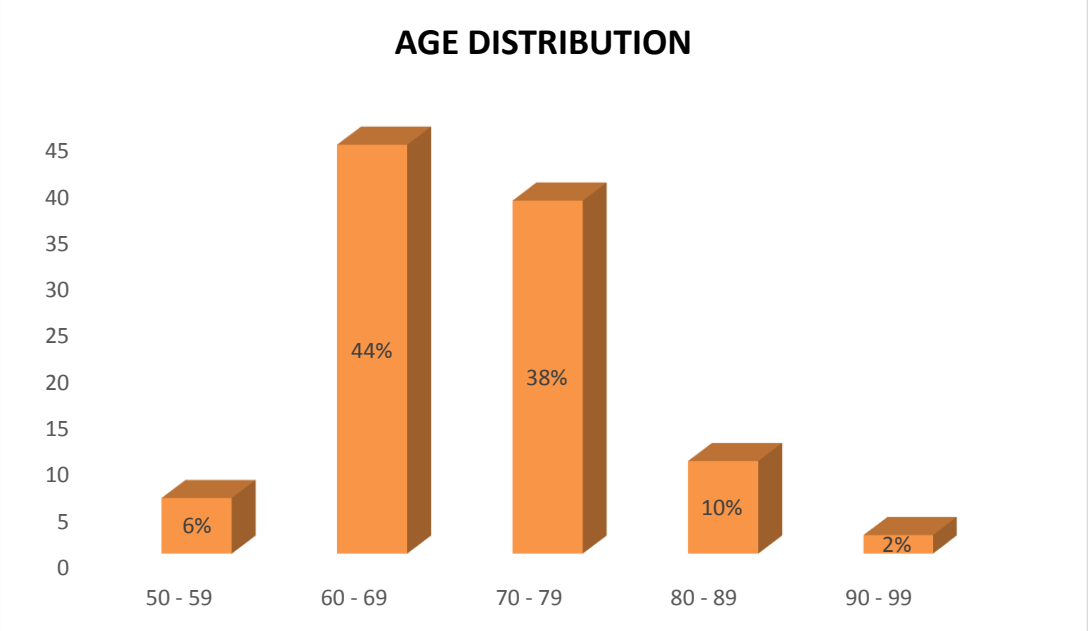

Fig. 1: Age Distribution

Most common age group sampled were between 60 to 69 years.

CLINICAL PRESENTATION

Table 2 : Clinical Presentation

Clinical Presentation	Frequency	Percentage %
Urinary symptoms	24	48
Hard prostate alone on DRE	2	4
Both features	24	48
Total	50	100

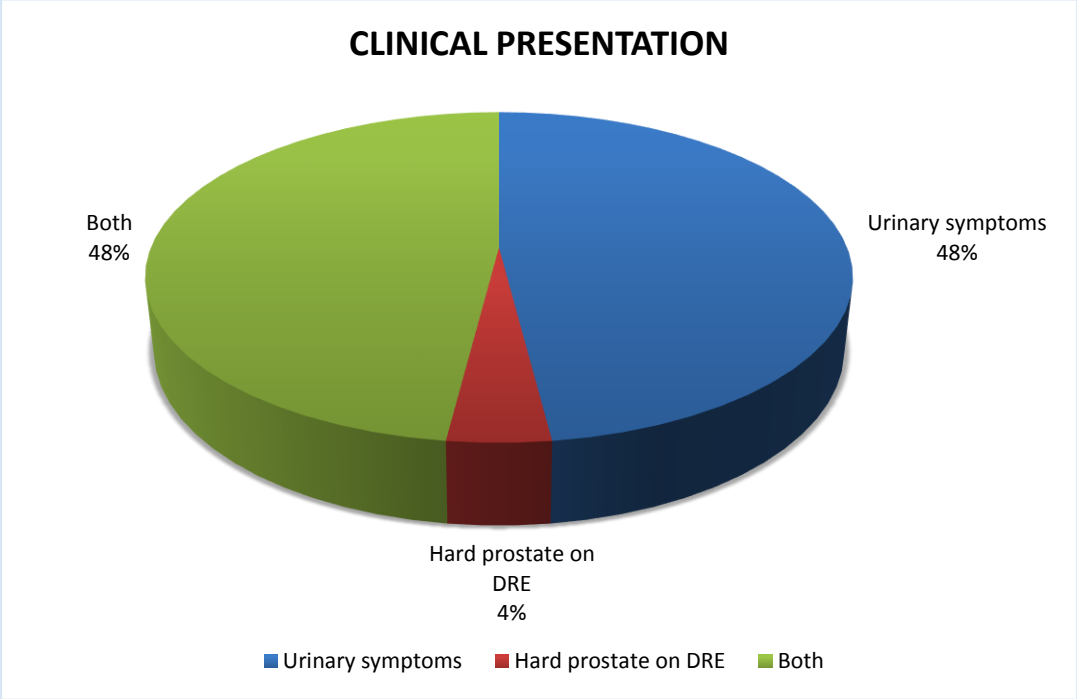

Fig. 2: Clinical Presentation

Majority of the patients presents with urinary symptoms like urgency, increased frequency and dribbling and hard prostate on digital rectal examination. Hard prostate alone was seen in 4% of the cases detected incidentally.

NATURE OF SPECIMEN

Table 3: Nature of Specimen

Nature of specimen	Frequency	Percentage %
TURP	39	78
Trucut biopsy	11	22
Total	50	100

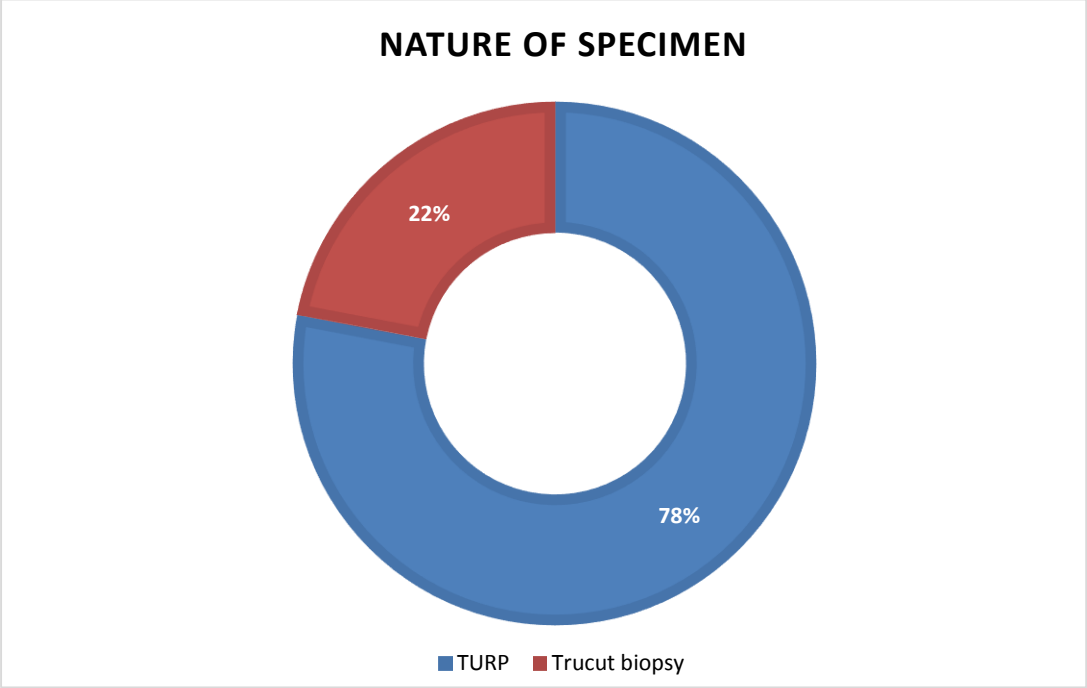

Fig. 3: Nature of Specimen

TURP specimens accounts for 78% during this study period.

H & E DIAGNOSIS

Table 4: H & E Diagnosis

DIAGNOSIS	Frequency	Percentage %
Benign (hyperplasia)	32	64
High grade PIN	1	2
Carcinoma	17	34
Total	50	100

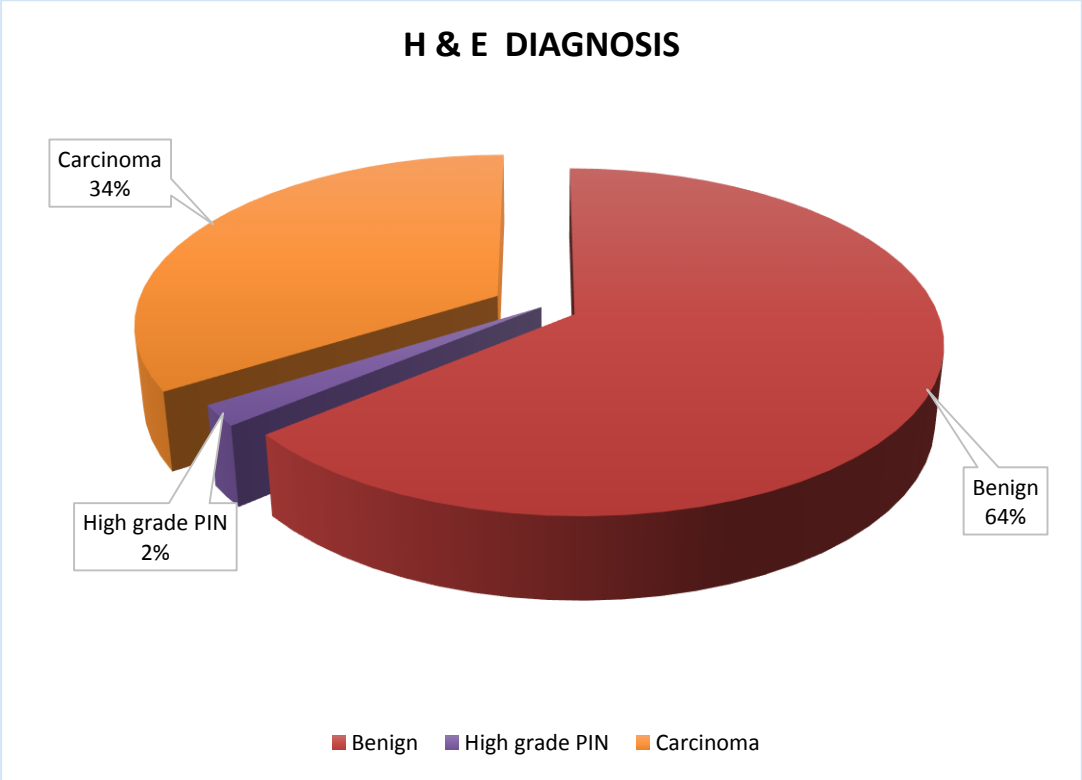

Fig. 4: H & E Diagnosis

A histopathological diagnosis was made by H and E; most were benign, amounting to 64%. 34 % showed carcinoma and 2% was diagnosed as PIN.

INFLAMMATION AND INFARCTION

Table 5: Inflammation and Infarction

Inflammation	Frequency	Percentage %
Prostatitis	14	28
Granulomatous prostatitis	1	2
Abscess	4	8
Infarction	4	8
Absent	27	54
Total	50	100

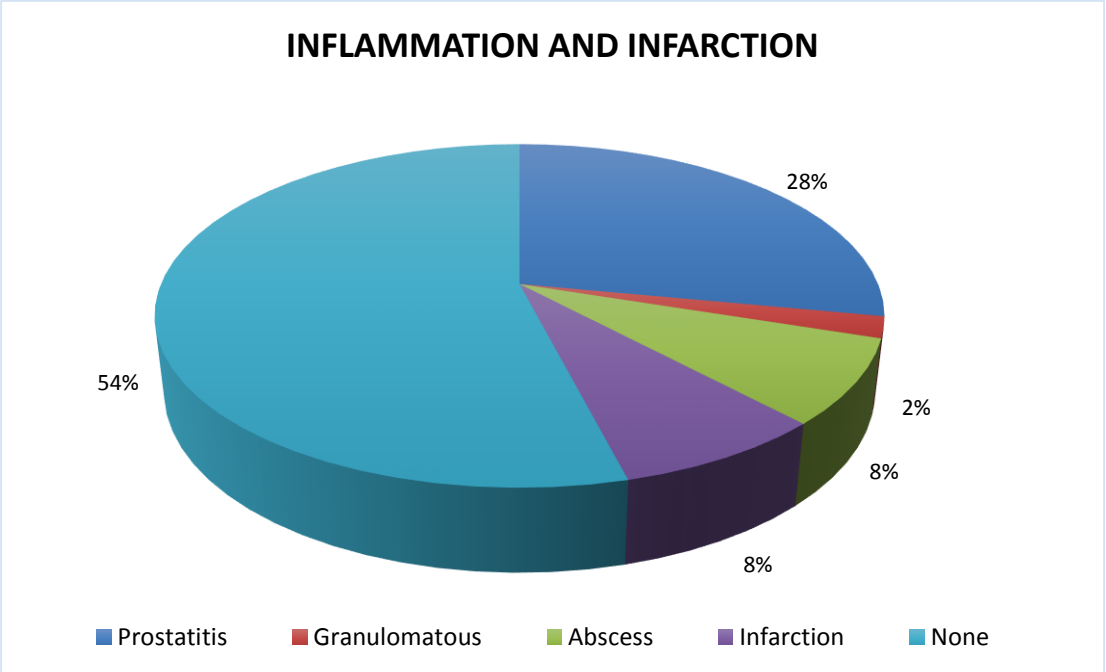

Fig. 5: Inflammation and Infarction

Prostatitis was seen in association with Nodular hyperplasia of prostate. Diagnosed only in the absence of malignancy, High grade PIN and atypical hyperplasia. Prostatitis being the most common type 38%. Granulomatous prostatitis was rare and accounts for 2%. Abscess was seen in 8% of the cases.

PSEUDO NEOPLASTIC LESIONS

Table 6: Pseudo Neoplastic Lesions

Pseudo Neoplastic Lesions	Frequency	Percentage %
Basal cell hyperplasia	16	32
Squamous metaplasia	3	6
Atrophy	0	0
Absent	31	62
Total	50	100

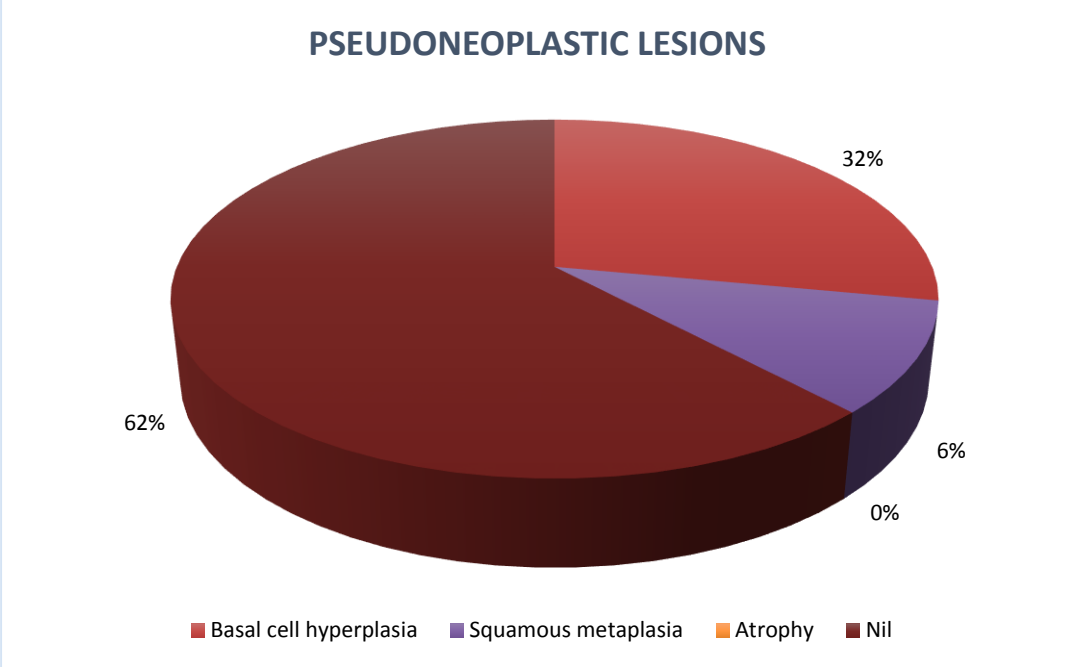

Fig. 6: Pseudo Neoplastic Lesions

Among the various pseudo neoplastic lesions basal cell hyperplasia and squamous metaplasia were noted. Basal cell hyperplasia was seen in 32% cases and in association with benign prostatic hyperplasia. Squamous metaplasia notes in 6% of the cases.

HISTOPATHOLOGICAL EXAMINATION: ARCHITECTURE

The following architectural features were studied. More than one finding was allowed in the same biopsy.

Table 7: Histopathological Examination: Architecture

Architectural Feature	Frequency	Percentage
Benign glands	32	64
Small neoplastic glands	4	8
Small and cribriform glands	10	20
Cribriform and sheets	3	6
Sheets	1	2
Total	50	100

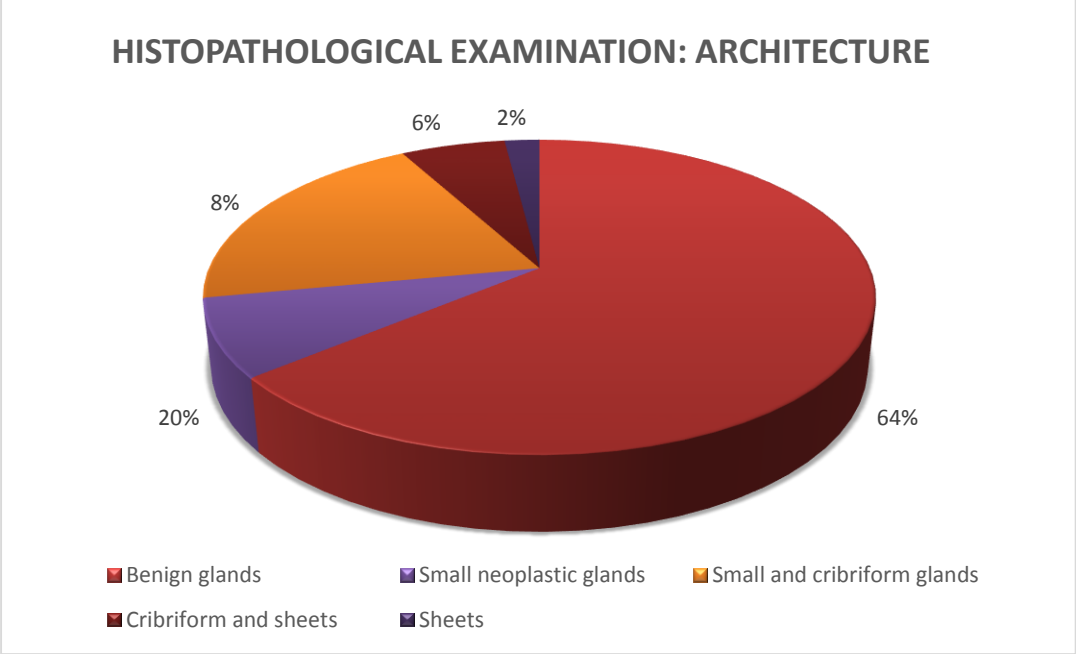

Fig. 7: Histopathological Examination: Architecture

Benign glands predominate and accounts for 64% followed by small and cribriform glands 20% with the least one being sheets 2%

HISTOPATHOLOGY: CYTOLOGICAL FEATURE

Table 8: Histopathology: Cytological Feature

Cytological Feature	Frequency	Percentage %
Nucleomegaly only	5	10
Nuclear Pleomorphism and nucleoli	12	24
Signet ring cell	1	2
None	32	64
Total	50	100

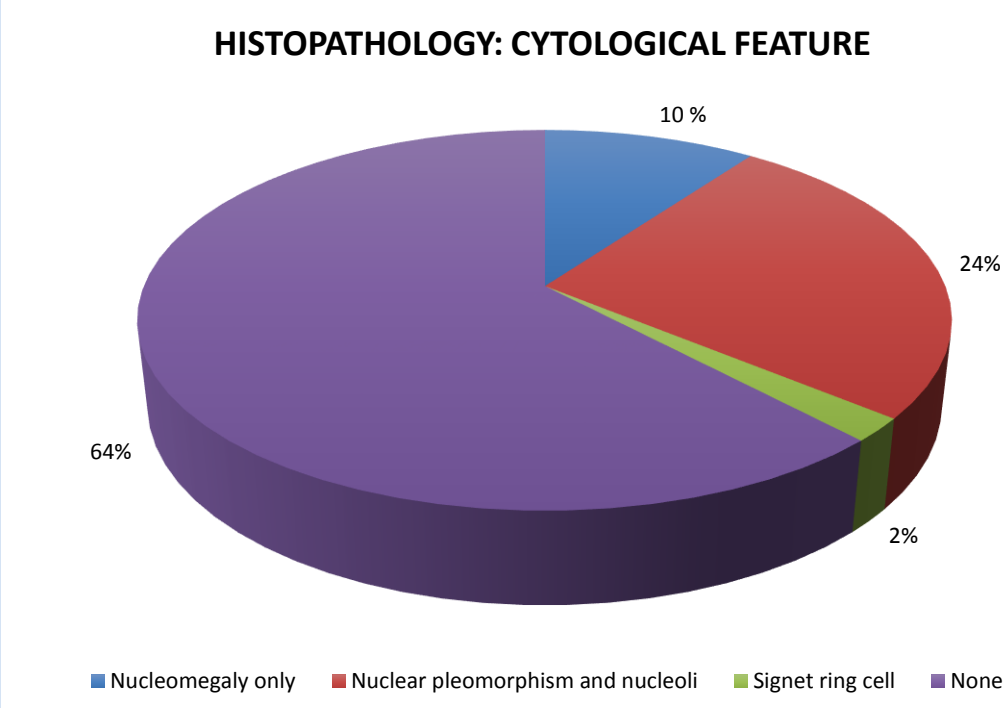

Fig. 8: Histopathology: Cytological Feature

Cytological features suggest presence of malignant glands – nuclear pleomorphism and nucleoli being the most common finding seen in carcinoma, followed by nucleomegaly in High Grade PIN and in atypical hyperplasia.

EVALUATION OF GLEASON SCORE:

Table 9: Evaluation of Gleason Score

Gleason Score	Number of cases	Percentage %
3 + 3	3	17.5
3 + 4	7	41
4 + 3	3	17.5
4 + 4,3+5,5+3	0	0
4 + 5	1	6
5 + 4	2	12
5 + 5	1	6
Total	17	100

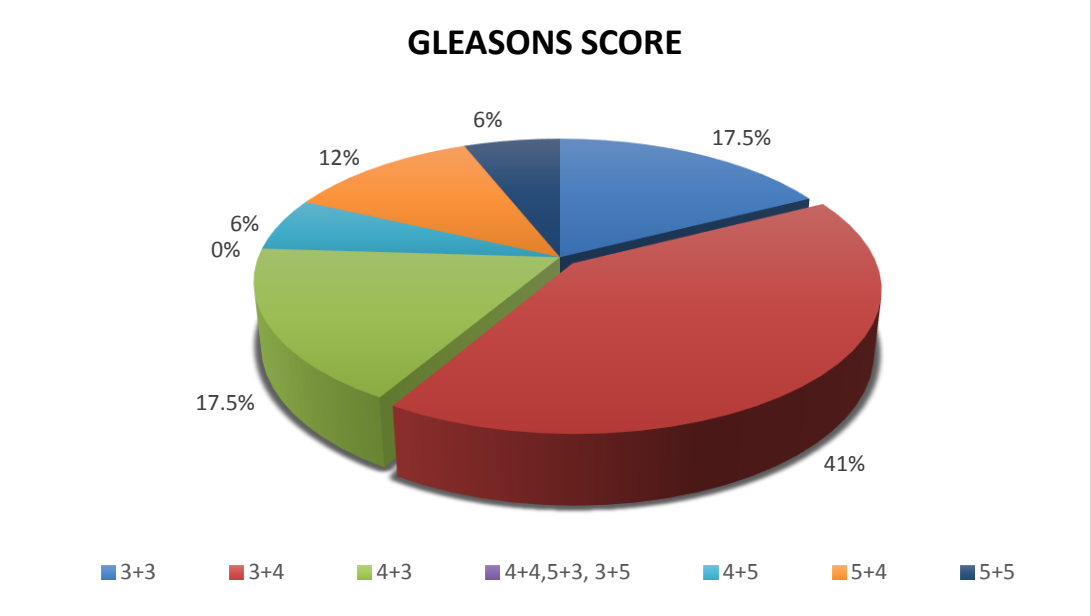

Fig. 9: Evaluation of Gleason Score

The predominant pattern was 3, 4 amounting to 41% and followed by 3,3 and 4,3 each of which showed 17.5%. None of the cases shows tertiary component.

DIAGNOSIS OF CARCINOMA: GLEASON SCORE

Table 10: Diagnosis of Carcinoma: Gleason Grade Group

Gleason Group	Number of cases	Percentage %
Group 1	3	18
Group 2	7	41
Group 3	3	18
Group 4	0	0
Group 5	4	23
Total	17	100

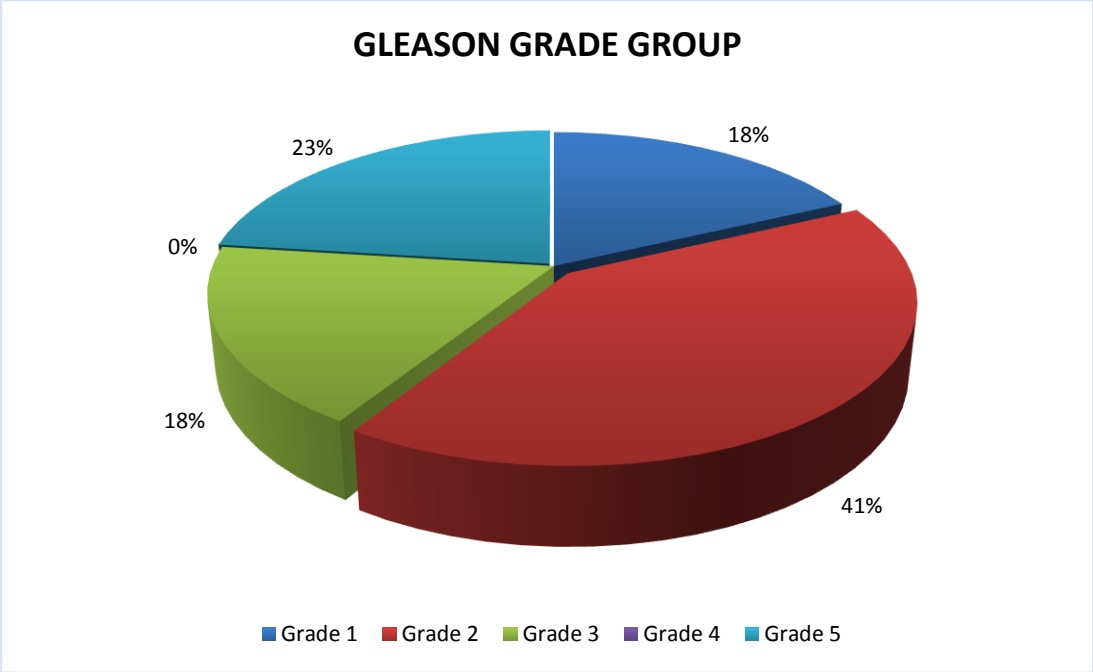

Fig. 10: Gleason grade

The most common Gleason score encountered were grade 2(41%) followed by Score 1 and 3(18%)

OTHER SPECIFIC FEATURES OF MALIGNANCY

Table 11: Other Specific features of malignancy

Feature	Frequency	Percentage %
Perineural invasion	8	46
Lymphovascular invasion	2	12
Glomerulations	2	12
Extraprostatic extension	0	0
Both perineural invasion and LV emboli	4	24
Absent	1	6
Total	17	100

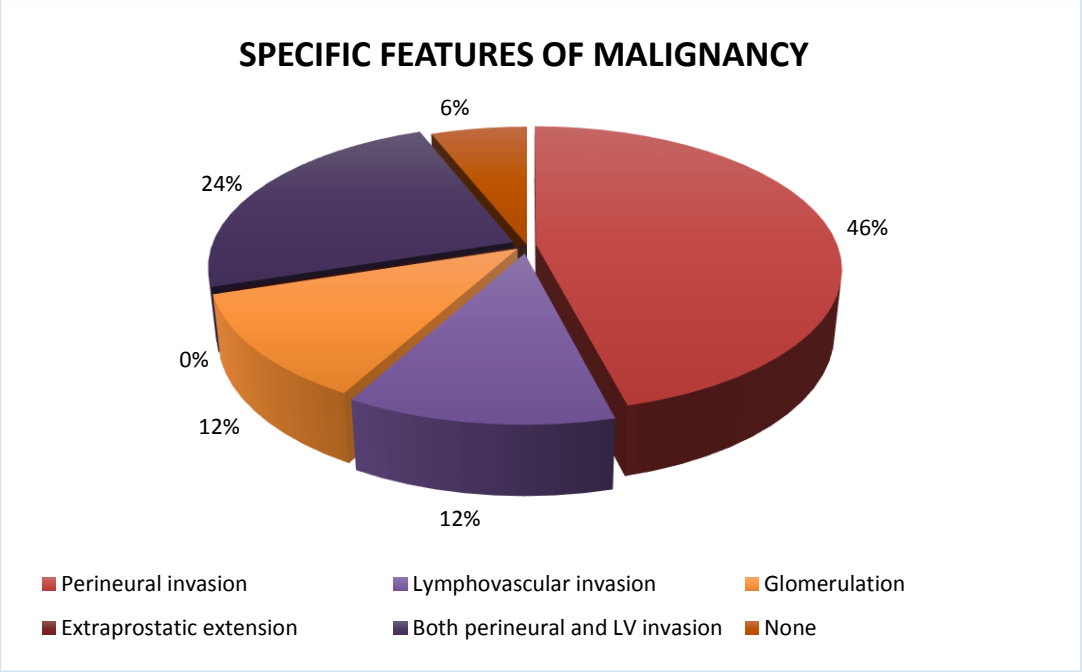

Fig. 11: Other specific features of malignancy

Of the absolute features of malignancy studied, PNI was most common, seen in 46% of all carcinoma followed by lymphovascular emboli 12%. Both are seen in 24% of cases.

PERINEURAL INVASION

Table 12: Perineural invasion and relation with Gleason score

Gleason score	Frequency	Percentage %
Score 6	1	8
Score 7	7	58
Score 8	0	0
Score 9	3	26
Score 10	1	8
Total	12	100

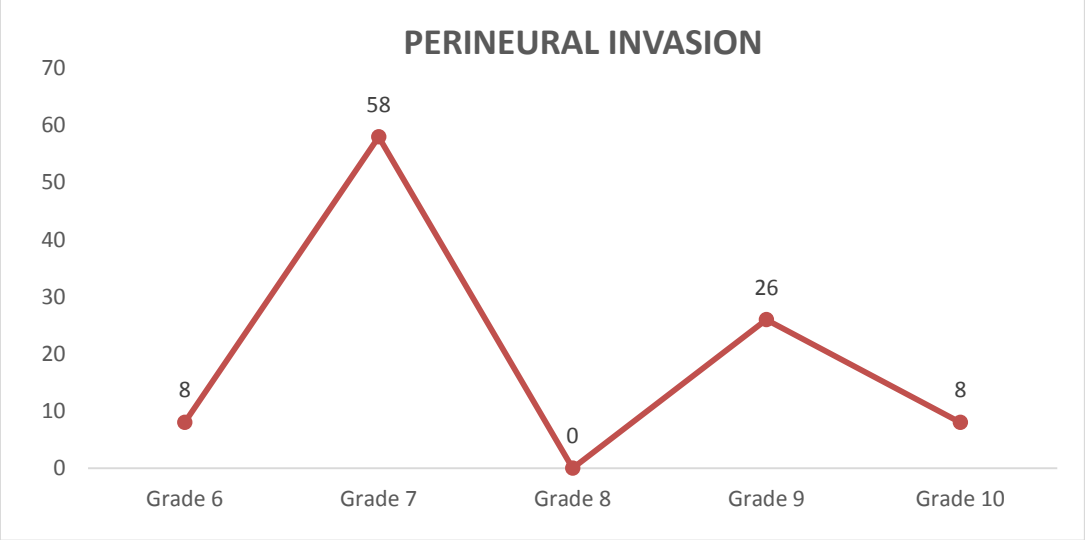

Fig. 12: Perineural Invasion

Perineural invasion is associated with Gleason score 7 is 58% and score 9 is 26%.

HISTOPATHOLOGY: INTRAGLANDULAR CONTENTS

Table 13: Histopathology: Intraglandular Contents

Intraglandular Contents	Frequency	Percentage %
Corpora amylacea	27	54
Crystalloids	2	4
Calcification	3	6
Wispy blue mucin	0	0
Crystalloid and eosinophilic debri	1	2
None	17	34
Total	50	100

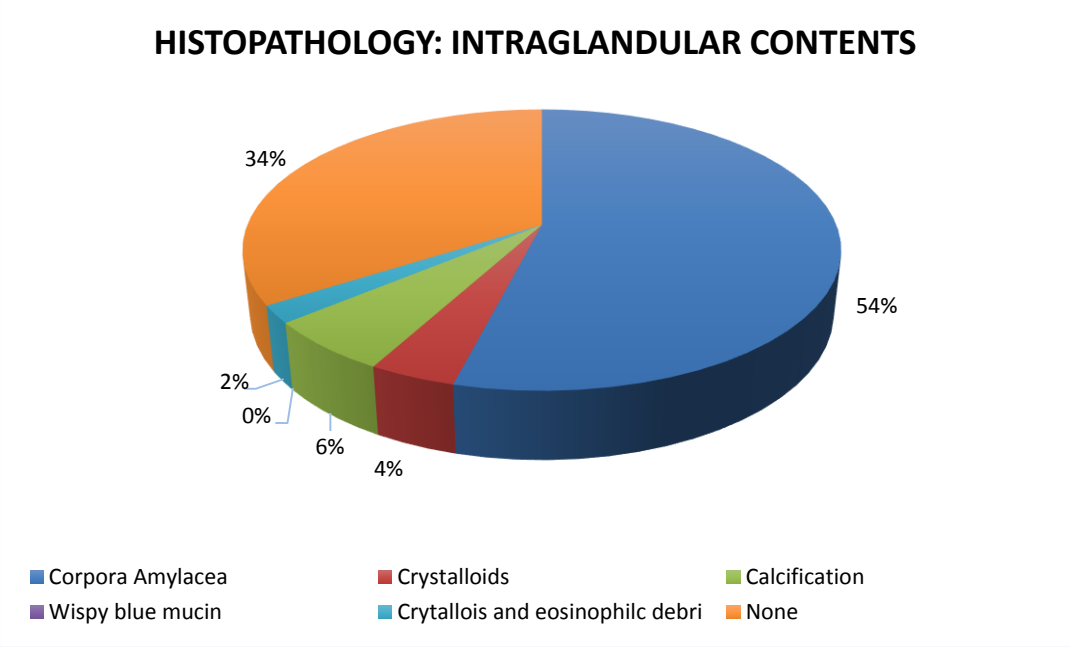

Fig. 13: Histopathology: Intraglandular Contents

The most common intraglandular finding was corpora amylacea, seen only in benign glands. Crystalloids and eosinophilic material, that suggest malignancy was seen in 4 and 1 % of cases respectively.

IMMUNOHISTOLOGICAL EVALUATION OF PREMALIGNANT AND MALIGNANT LESIONS

A total of 18 cases were diagnosed as premalignant and malignant. Presence or absence of basal cells are important in differentiating malignant cases from benign and pseudo neoplastic lesions. These basal cells are present in benign glands but are completely absent in malignant glands. To identify the basal cells, basal cell markers like p63 and CK 34BetaE12 are useful. P63 stains nuclear positivity while CK 34BetaE12 is cytoplasmic.

Basal cell staining density was scored as

3+ - strong

2+ - moderate

1+ - weak

0 - negative

Percentage of basal cell staining was scored from 0% to 100%.

Basal cell staining was considered positive only if the staining density was 2+ or more and >10% of the glands were stained

Basal cell staining was considered negative if the score was 1+ or less and ≤10% of the glands were stained.

For both stains when staining is positive it is considered benign, (Fig.32, 34) and is considered malignant if basal staining is negative. (Fig.33, 35). Intermediate between these is the PIN which shows intermittent or patchy staining.

Table 14: IHC Evaluation

Sl. No	HP. NO	p63		CK 34BetaE12		IHC Diagnosis
		Staining	**Other findings**	**Staining**	**Other findings**	
1	713/16	0	-	0	-	Malignant
2	839/16	3+ (intermittent)	-	2+ (intermittent)	-	PIN
3	2308/16	0	Entrapped benign glands	0	Entrapped benign glands	Malignant
4	551/16	0		0		Malignant
5	906/16	0		1+(<10%)		Malignant
6	1061/17	0		0		Malignant
7	864/17	1+		1+(<10%)		Malignant
8	1263/17	0		0		Malignant
9	1555/17	0		0		Malignant
10	1178/17	0		1+(<10%)		Malignant
11	798/17	0	Entrapped benign glands	0	Entrapped benign glands	Malignant
12	1558/17	0		0		Malignant
13	347/17	0		0		Malignant
14	3631/17	0	Entrapped benign glands	0	Entrapped benign glands	Malignant
15	13/17	0		0		Malignant
16	2299/17	0		1+ (<10%)		Malignant
17	6678/17	0	Entrapped benign glands	0	Entrapped benign glands	Malignant
18	2302/17	0		0		Malignant

Out of the 18 cases, 17 cases were diagnosed as malignancy. Among the malignancies one case stained with p63 showed staining (1+) but it was involving <10% of atypical glands. So this was considered malignant.

Four cases stained with CK 34BetaE12 showed staining (1+) (Fig.36) and was involving <10% of glands and hence considered as malignant.

Four of the cases showed few glands with continuous basal cell staining pattern. These are considered as entrapped benign glands (Fig.37) and correlated with H and E stained sections. This finding was noted only in TURP specimens.

Remaining one case shows intermittent staining pattern, which was diagnosed as PIN.

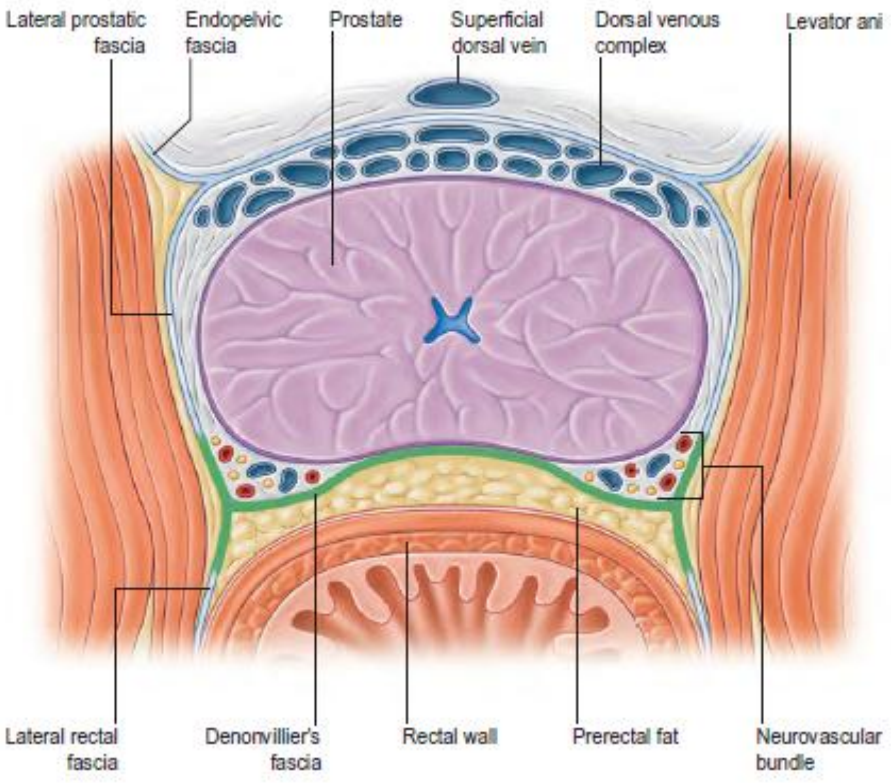

Fig. 14: Anatomy of Prostate

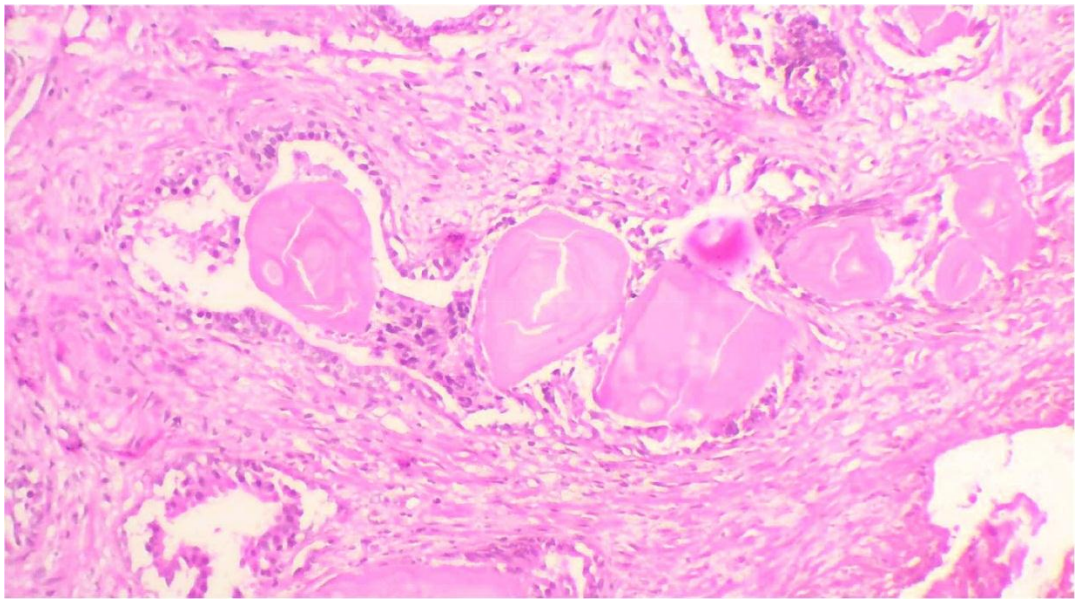

Fig. 15: Corpora Amylacea (10x)

Fig. 16: TURP – Gross

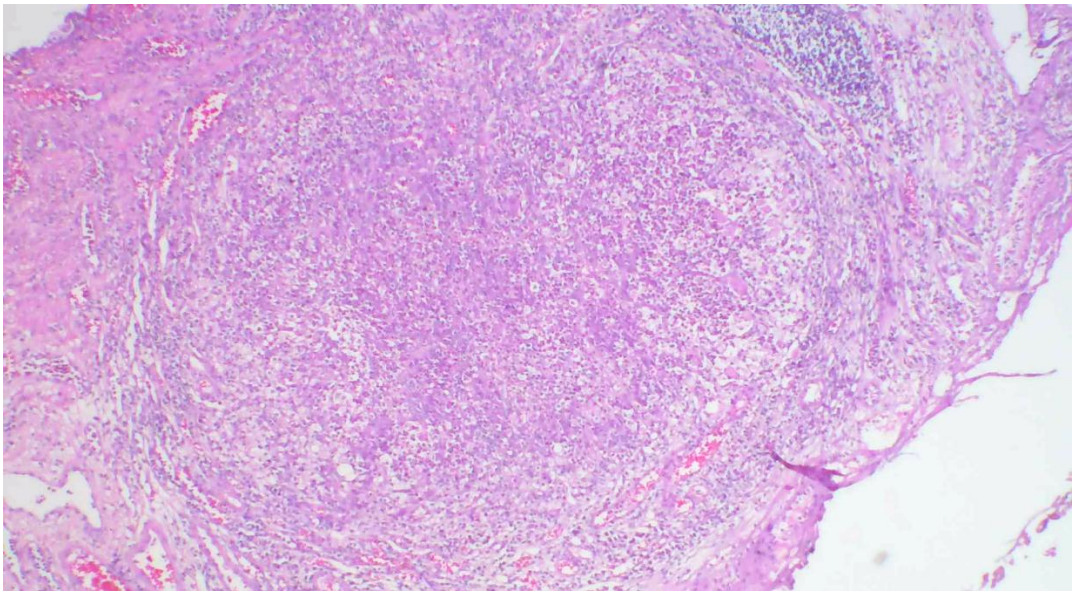

Fig. 17: Prostatic Abscess (4x)

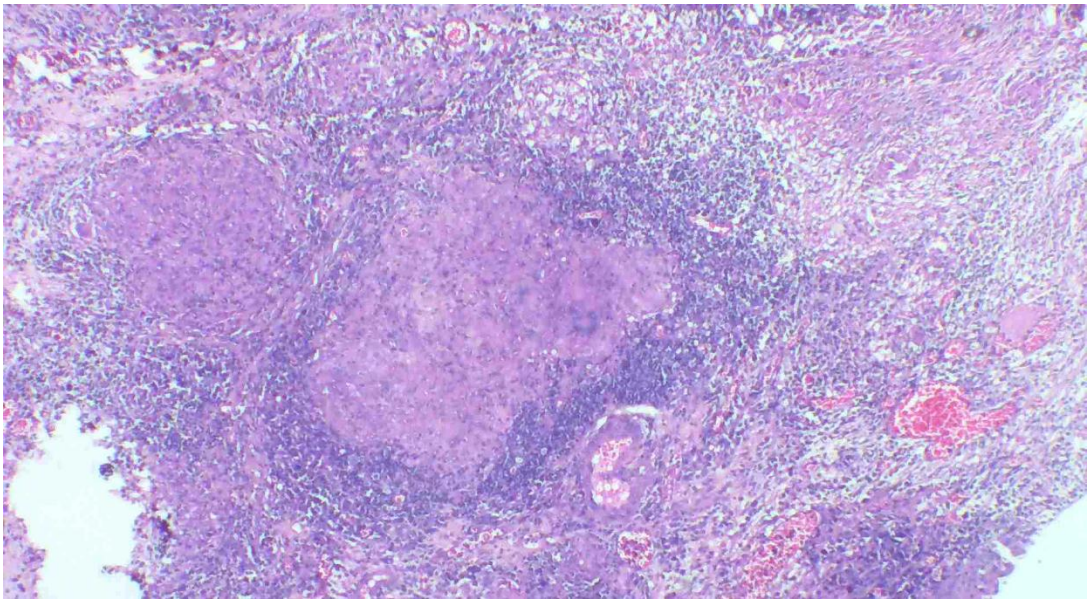

Fig.18: Granulomatous Prostatitis (4x)

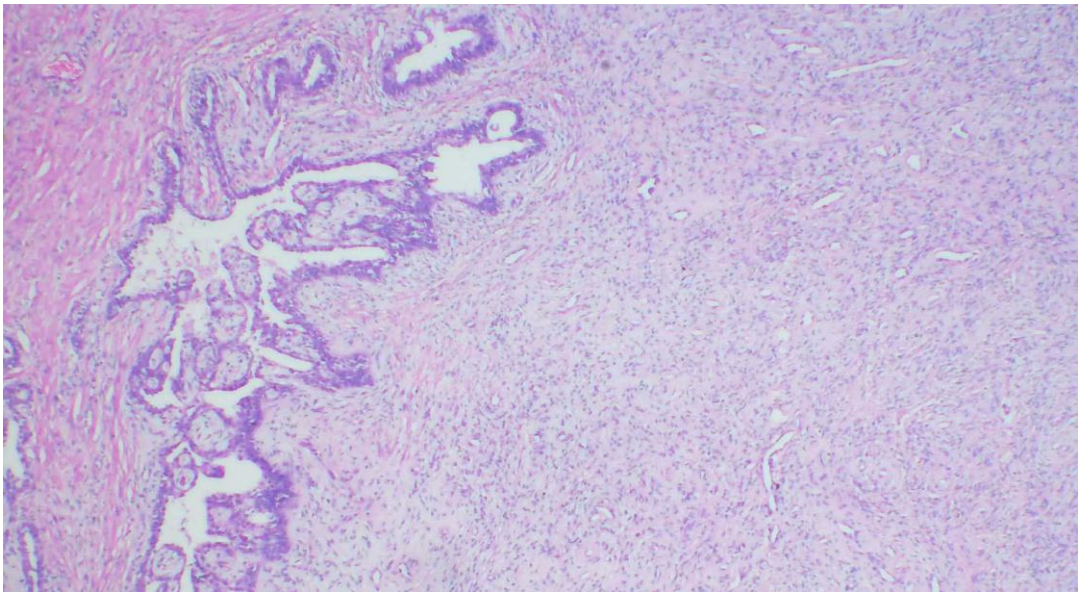

Fig.19: Benign Nodular Adenomyomatous Hyperplasia (4x)

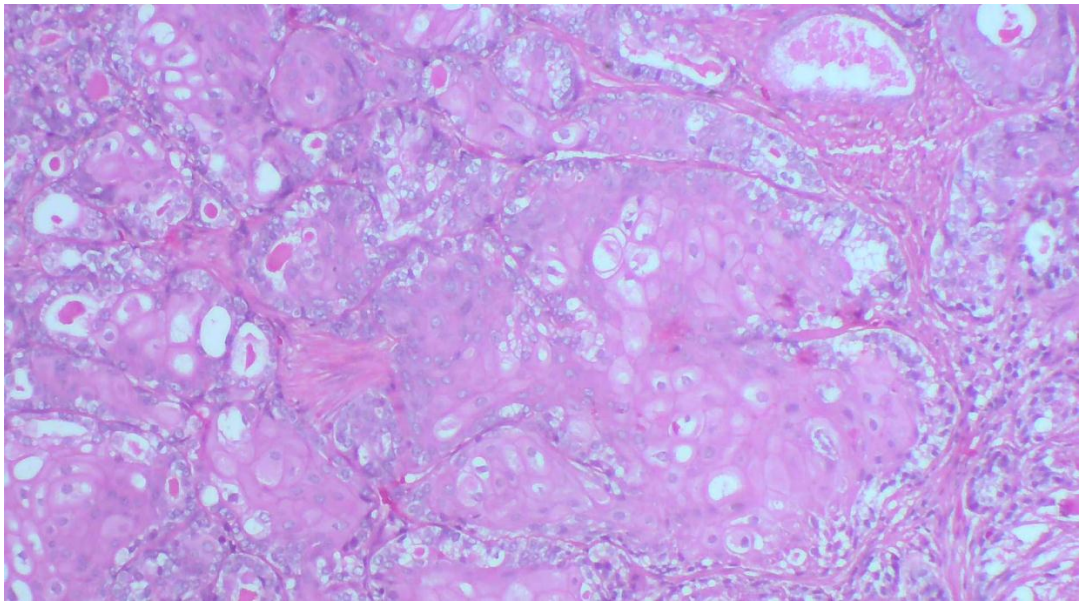

Fig. 20: Squamous Metaplasia (10x)

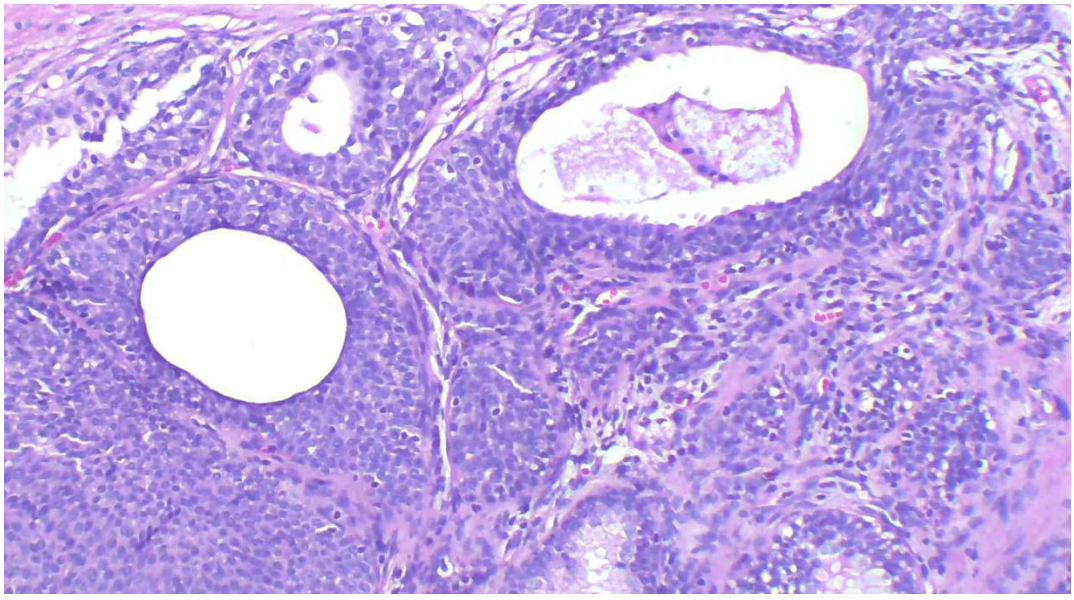

Fig. 21: Basal cell Hyperplasia (10x)

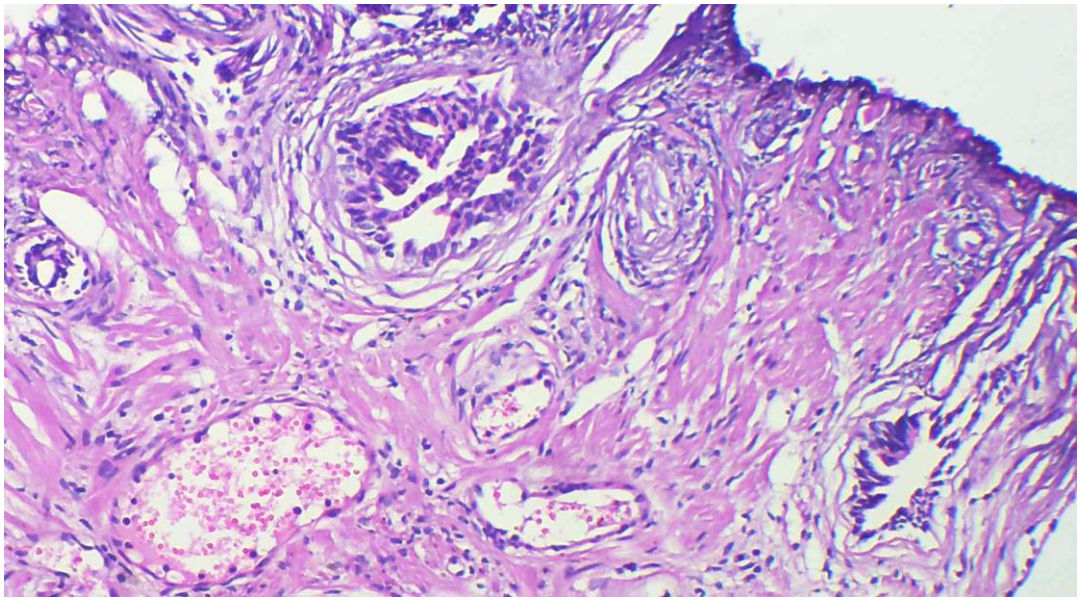

Fig. 22: High Grade PIN (4x)

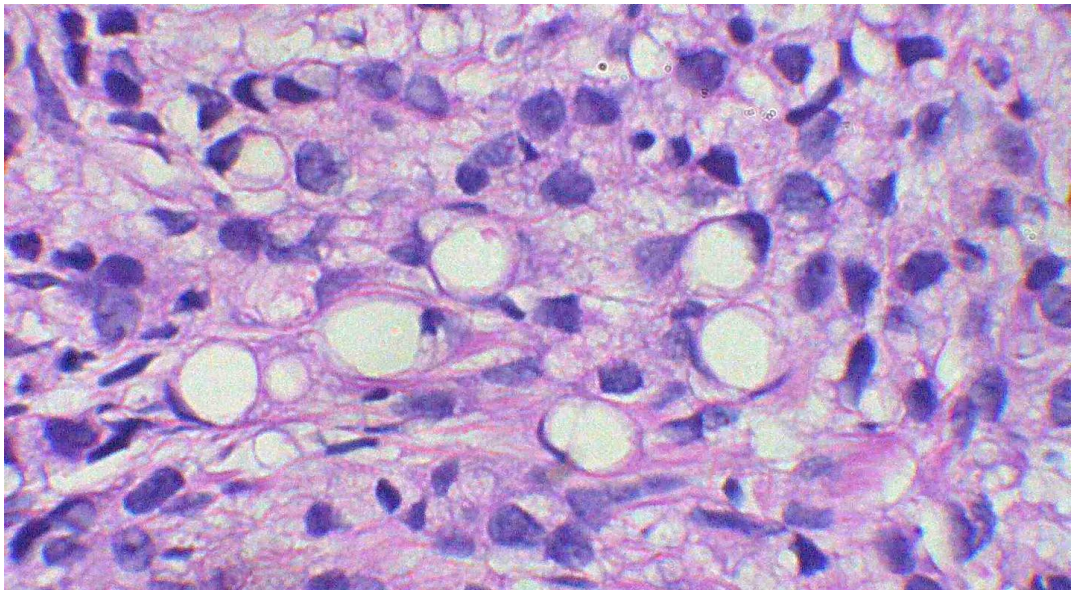

Fig. 23: Signet ring cells (40x)

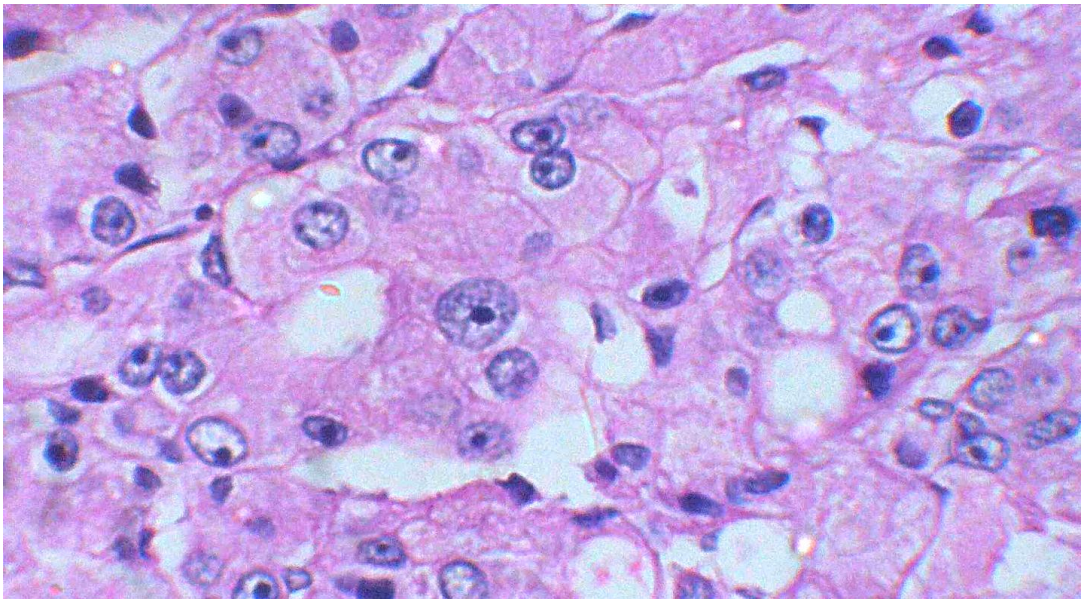

Fig. 24: Atypical cells having eosinophilic cytoplasm vesicular nucleus, prominent nucleoli (40x)

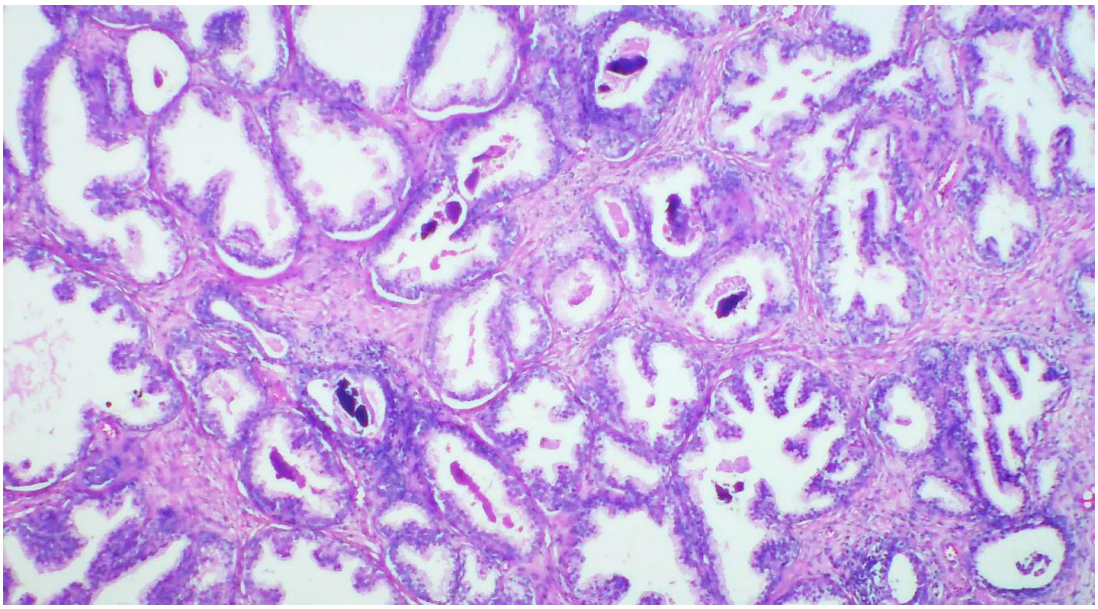

Fig. 25: Intraluminal Crystalloids (10x)

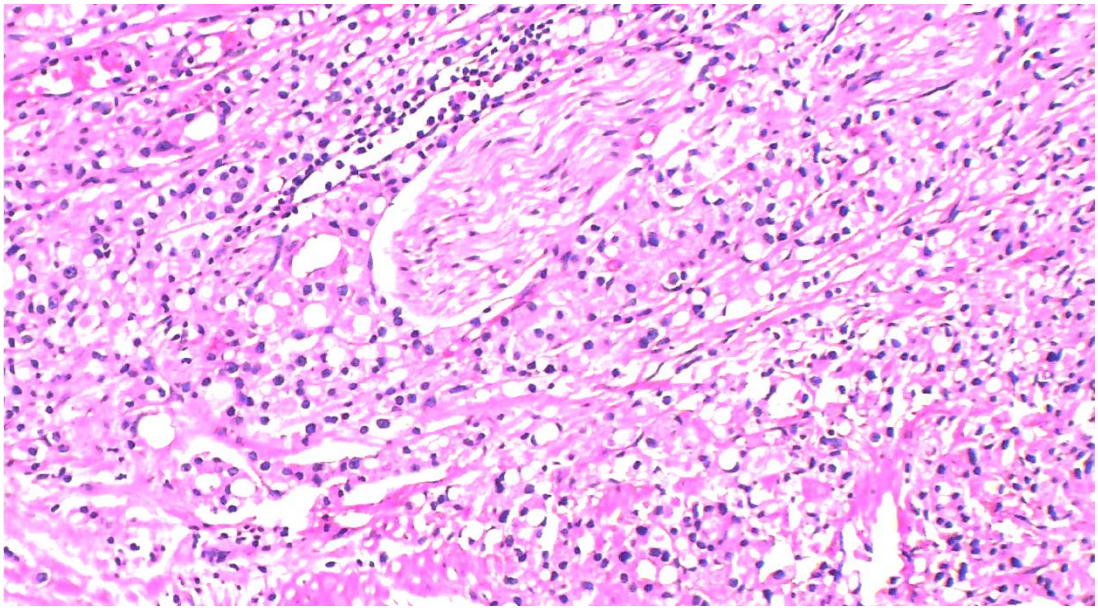

Fig. 26: Perineural invasion (10x)

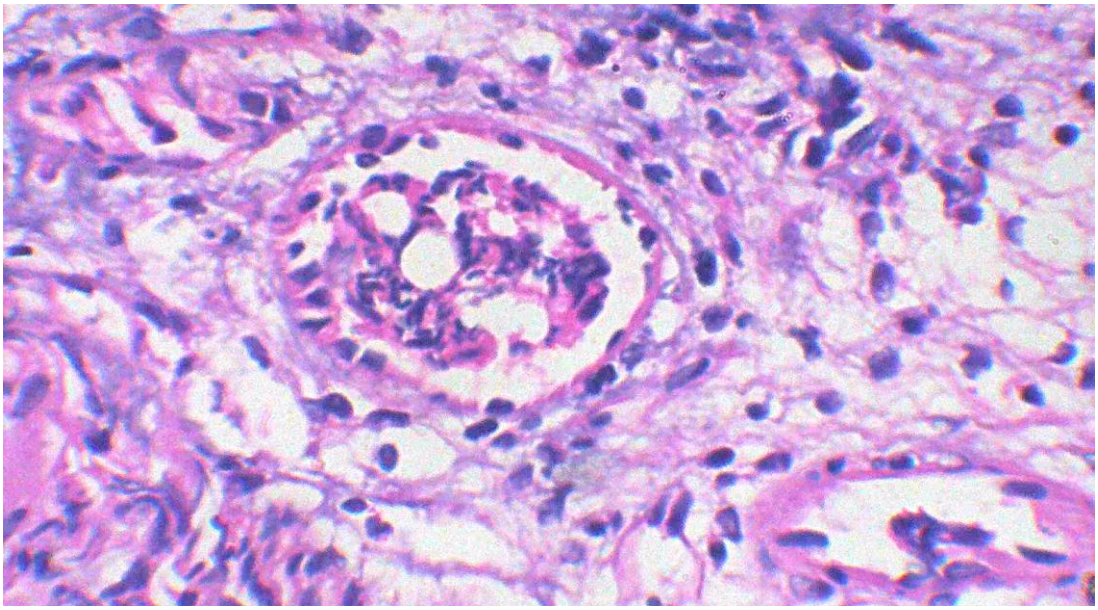

Fig. 27: Lymphovascular emboli (10x)

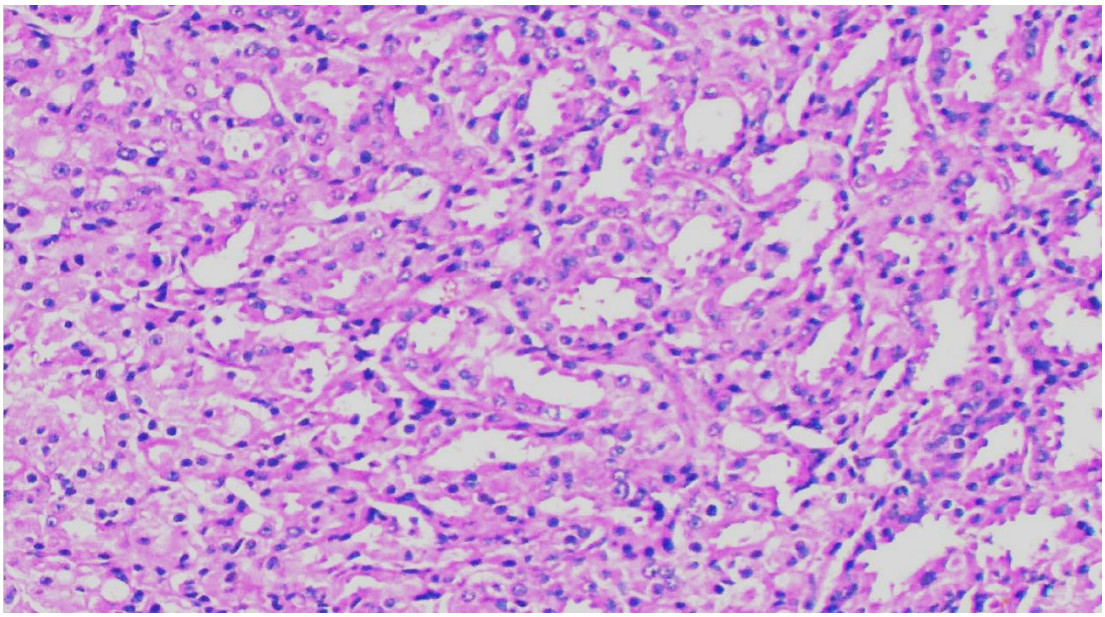

Fig. 28: Small well-formed glands – Gleason score 3 (4x)

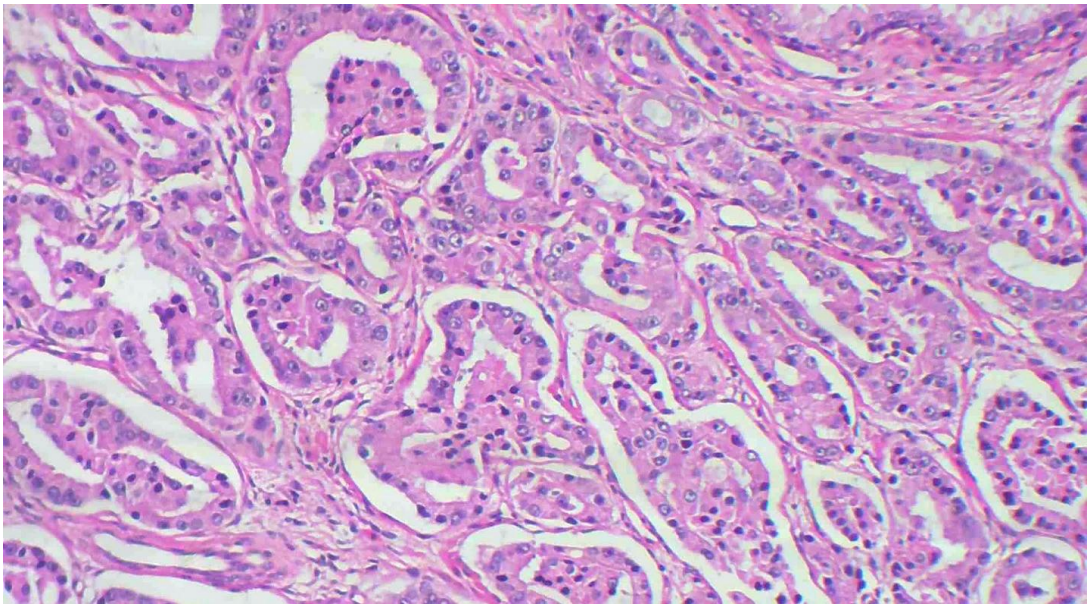

Fig.29: Fused glands – Gleason score 4 (4x)

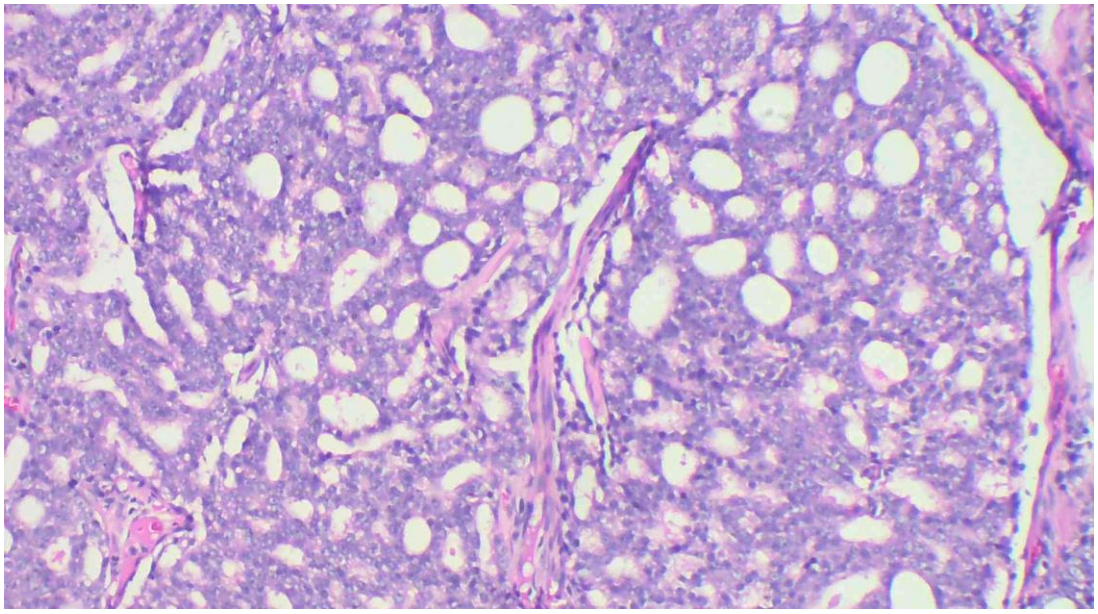

Fig. 30: Cribriform pattern – Gleason score 4 (4x)

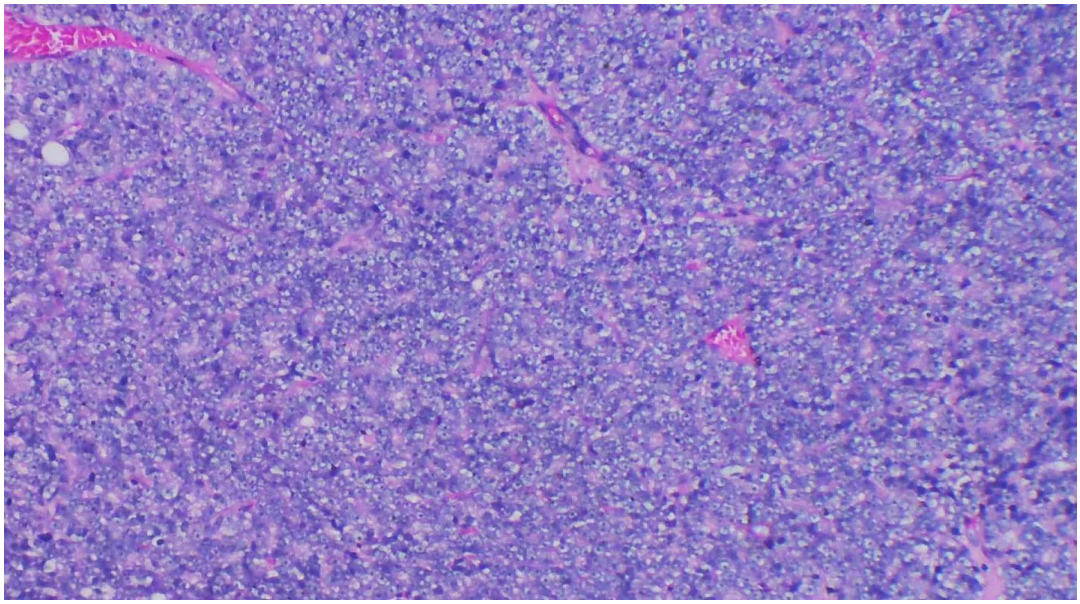

Fig. 31: Sheets of tumor cells - Gleason score 5 (4x)

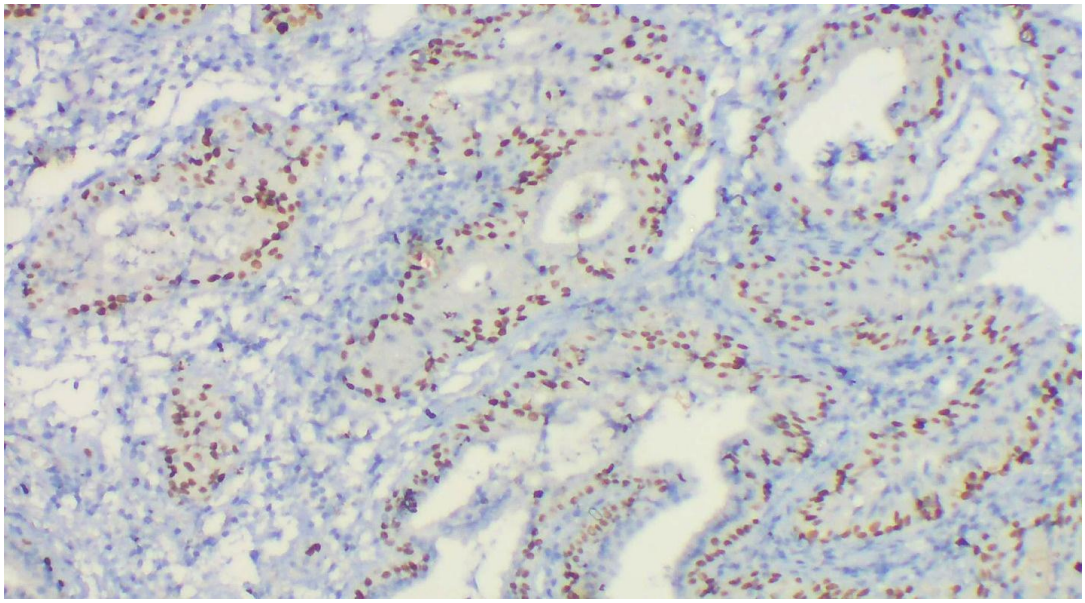

Fig. 32: Benign glands - p63 Nuclear positivity for basal cells (10x)

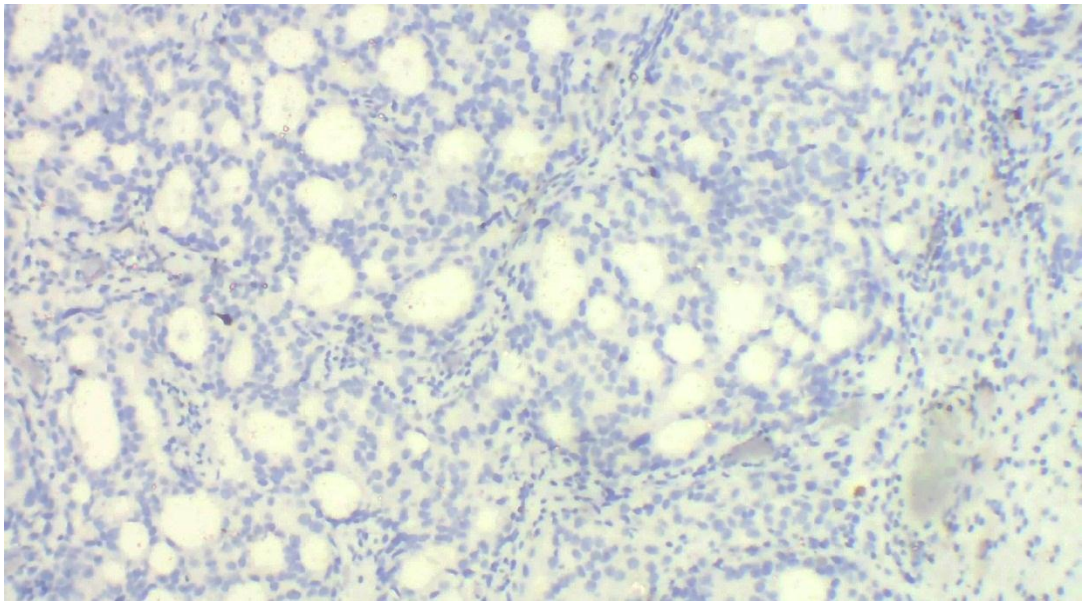

Fig. 33: Malignant glands - p63 Absence of basal cells (10x)

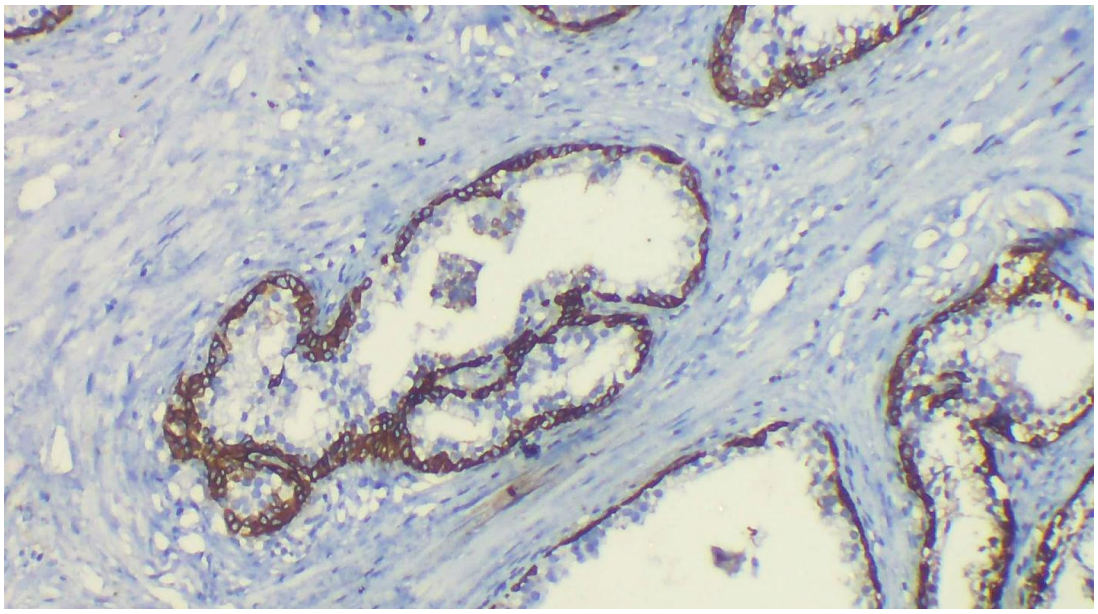

Fig. 34: Benign glands – CK34betaE12 Cytoplasmic positivity for basal cells (10x)

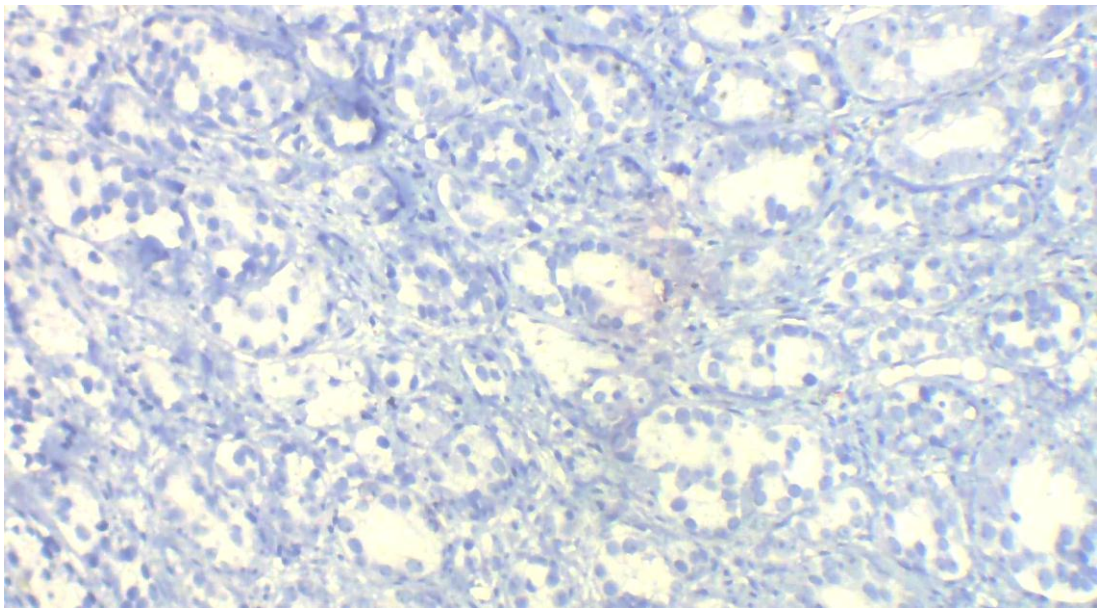

Fig. 35: Malignant glands – CK34BetaE12 Absence of basal cells (10x)

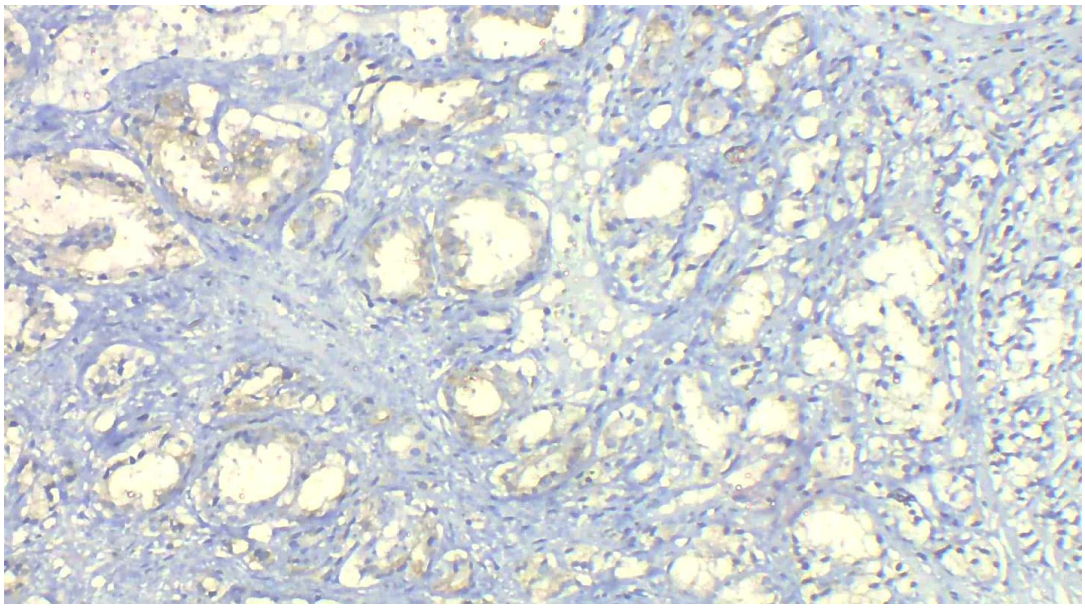

Fig. 36: CK34BetaE12 – 1+ staining pattern (10x)

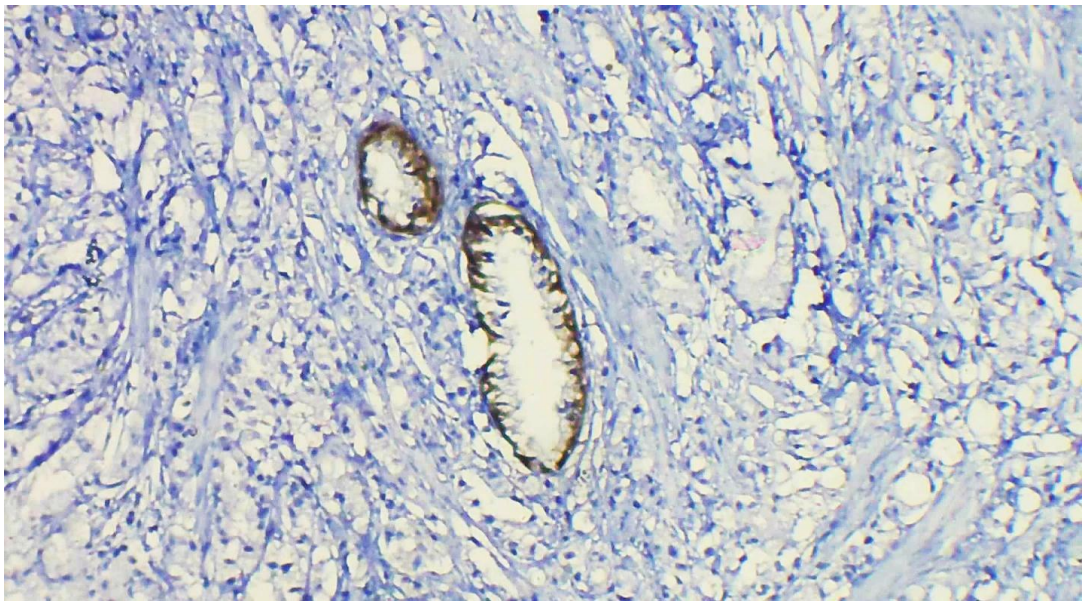

Fig. 37: CK34BetaE12 - Entrapped benign glands with presence of basal cells (10x)

DISCUSSION

Prostatic specimens constitute a good percentage of the surgical pathology workload. This study was undertaken to evaluate the various histological lesions in prostatic specimens.

CLINICAL FEATURES:

The age of the patients in our study ranged from 50 years to 93 years; however, the predominant population was in the 6th to 7th decade with a mean age of 69 years. Mean age for non neoplastic and neoplastic cases was 71 and 76 respectively. No significant difference was noted in the mean age of the both groups.

The results of the present study agree with the studies by George and Thomas,[89] in which the mean age was 66.81 years, and by Barakzai et al.,[90] in which the mean age was 66.9 years. This is also similar to the screening procedures in the studies by Physicians Health Study (PHS) and the Health Professionals Follow-Up Study (HPFS).[91.] The decline in the number of cases beyond the age of 80 years reflects the average life span of people in our country.

Most of the patients had nonspecific symptoms related to urinary tract; like urinary hesitancy, or repeated urinary tract infections along with hard prostate on digital rectal examination.

SPECIMEN:

Transurethral resection of prostate specimens were more compared to core needle biopsies accounting for 78% and 32% respectively. Most of the core biopsies are done for suspected malignancies and these were done from hard nodules under digital guidance.

PROSTATITIS:

Prostatitis accounts for 42% of which chronic prostatitis is noted in 38% and abscess in 8% of the cases studied. Mittal et al.[92] in a study of 185 prostatic specimens reported that prostatitis constituted 38.4% of the cases, of which 26.3% of cases were chronic prostatitis, and acute prostatitis with or without abscess formation was noted in 12.1% of cases.

Granulomatous prostatitis was seen in 1 out of 50 cases (2%) similar to studies done by Anjorin et al.[93]

Nickel et al., in their review, quotes the REDUCE[94] (REduction by DUtasteride of prostate Cancer Events) trial, where 80% of patient biopsies were found to have some degree of inflammation.

PSEUDONEOPLASTIC LESIONS:

Pseudoneoplastic lesions includes basal cell hyperplasia, seminal vesicle, cowper's gland, atrophy, cribriform hyperplasia, nephrogenic metaplasia and squamous metaplasia. These lesions can cause confusion with malignancy. In our study BCH was the common entity in the pseudoneoplastic group and accounted for 28% (14 cases) of the total cases. All cases had associated nodular hyperplasia of prostate. Thorson et al.[95] studied 500 needle core biopsy samples and found the incidence of BCH in the setting of usual nodular hyperplasia to be in the range of 3.1% to 8.9%.

The present study also showed squamous metaplasia in 3 cases, accounting for 6% of the cases. Study done by Abdollahi et al., who found squamous cell metaplasia in 4 cases (0.3%) in their study.[96]

INTRALUMINAL CONTENTS:

Intraluminal contents studied were corpora amylacea, eosinophilic dense material, crystalloids and wispy blue mucin. The most common finding was corpora amylacea, seen in 54% of the total biopsies received, but few of these were seen in cases with malignant diagnosis which are associated with Nodular hyperplasia of adjacent glands in TURP specimens. James D Christian et al[97] observed that corpora amylacea were located in malignant acini in 0.4 % of needle biopsies.

Of the other three intraglandular materials seen in malignancy, crystalloid was the most common accounting to 4 % of all biopsies. This is far less than that described by Del Rosario AD et al., [98] who observed such crystals in 14 to 36% of cancers.

Varma et al., [99] has described presence of faintly basophilic intraluminal mucin in up to 52% of cases, but no such material was demonstrated in the present study probably because of different staining characteristics.

HISTOPATHOLOGY

The architectural features studied included normal prostatic glands, neoplastic small glands separated by stroma, fused glands and neoplastic cells forming sheets. Benign glands show double lining epithelium. Malignant glands show absence of basal layer along with cellular atypia.

Benign prostatic glands were seen in 62% of cases and was the most common finding observed; but it is not exclusively seen in benign conditions. Sarah Karre et al[100] observed that the intervening benign glands diagnosed as malignant should be included in assessing the tumor mass.

Small infiltrating glands, back to back arrangement of these glands and sheets of neoplastic cells, which are signs of malignancy, were seen in 38 % of cases.

CELLULAR FEATURES

The cellular feature studied included cytoplasmic amphophilia, nuclear pleomorphism, nucleomegaly and nucleoli. Nuclear pleomorphism was the most common finding, observed in upto 26% of cases which are malignant lesions. Nucleolar enlargement alone was seen in 10% of total cases. Nucleolar enlargement, as reviewed by Epstein constitutes one of the most important diagnostic features, but may be seen in benign mimickers as well.

HIGH GRADE PIN:

The presence of an isolated PIN (PIN in the absence of carcinoma) should be reported in biopsy specimens, especially if more than 1 site is involved. High-grade PIN in a biopsy without evidence of carcinoma has in the past been a risk factor for the presence of carcinoma on subsequent biopsies. But reporting of PIN in biopsies with carcinoma is considered optional.

In our study High grade PIN accounts for 2% of the cases studies which is similar to studies done by Oliai et al,[101] mean number of HGPIN glands was 1.36%.

H & E DIAGNOSIS:

In our study, of 50 cases, 32 (64%) were nonneoplastic lesions, premalignant lesions 1 (2%) whereas neoplastic lesions constituted 17 of the total cases (34 %). This agrees with the studies conducted by Mittal et al,[93] Anjorin et al,[94] and George et al,[89] in which nonneoplastic lesions formed the bulk of the cases.

The prevalence of PCa in the present study was 34%, i.e., 17 of 50 cases. Similar results were obtained by Anjorin et al.[94] and Rekhi et al[102]. Most cases of prostate cancer are diagnosed after 50 years of age, but prostate cancer can be seen in younger adults. The frequency increases with age. Majority of cases were seen in the age group of 71 to 80 years, closely followed by the age group of 61 to 70 years. The time trends in detection of carcinoma at an early age have been greatly affected because more and more latent cases are diagnosed with the increasing use of PSA.

VOLUME OF TISSUE INVOLVES IN MALIGNANT CASES

The designation of the percentage of cancer tissue in transurethral samples is important. When prostate cancer is discovered incidentally (i.e., discovered in specimens submitted for clinically benign disease, usually Nodular hyperplasia of prostate), the percentage involvement is used to determine the clinical T_1 substage, with ≤5% involvement being T_1a and >5% being T_1b.

For needle core biopsy specimens, the number of positive cores out of the total number of cores should always be reported, except in situations where fragmentation precludes accurate counting. The estimated percentage of prostatic tissue involved by tumor and/or the linear millimeters of the tumor should also be reported.

In our study malignant cases was seen in 6 of the TURP specimens and 11 of the needle biopsy cases. All the specimens showed tumor volume more than 5% with a mean value of 66%.

GLEASON'S SCORE (SUM):

Gleason grading system is recommended for use in all prostatic specimens containing adenocarcinoma, with the exception of those showing treatment effects.

Gleason score is the sum of the primary (most predominant in terms of surface area of involvement) Gleason grade and the secondary (second most predominant) Gleason grade. In needle biopsy specimens, Gleason score is the sum of the primary (most predominant) Gleason grade and highest Gleason grade and is recommended that Gleason scores be assigned for each separately identified core. However, some pathologists may choose to report an overall Gleason score for that specimen. The highest Gleason score should be provided in the summary.

In needle biopsy specimens where there is a minor secondary component (<5% of tumor) and where the secondary component is of higher grade, the latter was reported. Conversely, if a minor secondary pattern is of lower grade, it was not be reported. When more than 2 patterns are present, and the worst grade is neither the predominant nor the secondary grade, the predominant and highest grade was chosen to arrive at a score. For transurethral resection specimens, the above grading principles also apply.

A score of 7(3+4 and 4+3) was seen predominantly, constituting 41% and 17.5% respectively, a score of 6 and 9 showed equal proportion given in 17.5% and score of 10 is seen in 6% of the cases. Various other studies including ours highlight a score of 7 as a predominant score.[103]

Based on the grade grouping endorsed by ISUP, our study showed group grade 2 as the predominant group amounting for 41% followed by group grade 1 and 3 both of which showed 18%.

Gleason score ≤6 were reported to have negligible risk of prostate cancer-specific mortality at 15 to 20 years; hence the importance for scoring 7 and above.

Kiril et al[104] states that grade 5 is a rare diagnosis seen in 4.1 % of cancer biopsies, he reviews ISUP guidelines which recommends even tertiary grade 5 be reported as secondary.

OTHER FEATURES SPECIFIC FOR CARCINOMA:

Three histopathological features diagnostic of carcinoma are glomerulations, lymphovascular invasion and perineural invasion (PNI). Perineural invasion is regarded as pathognomonic of prostate cancer if there is circumferential or intraneural invasion by the tumor cells.

A study conducted on 302 needle biopsies by Bastacky et al.[105] showed perineural invasion in 20% of biopsies with cancer which was less compared to our present study in which perineural invasion alone was found in 8 of the malignant cases (46%) and was not observed in any of the benign cases studied.

Lymphovascular invasion alone was noted in 12% cases and both perineural and lymphovascular invasion noted in 24% in our present study.

Glomerulation, which are cribriform proliferation that are not transluminal were considered as grade 4 and was noted in 12 % of cancer diagnosis.

Extraprostatic extension is tumor involving loose connective tissue, even in the absence of direct contact between the tumor and the adipocytes and also when the tumor involves perineural spaces, even in the absence of periprostatic fat involvement. None of the cases in our study showed extra prostatic extension.

IMMUNOHISTOCHEMISTRY:

The IHC panel for PCa usually includes basal cell–specific marker p63 and CK34βE12. p63 is expressed in the nuclei of basal cells, whereas CK34βE12 stains

their cytoplasm. Negative staining must be interpreted with caution because cytokeratin is formalin sensitive and a progressive loss of immunoreactivity can be seen in prolonged formalin fixation.

IHC was done 18 cases that showed atypical glands. The usefulness of CK34βE12 and p63 relies on the fact that PCa lacks basal cells; therefore, lack of basal cell staining in an atypical lesion lends support to the diagnosis of PCa, whereas the presence of basal cell staining in general rules out cancer, with the exception of rare cases of basal cell staining in typical PCa by reactivity to CK34βE12. Few benign glands showed lack of basal staining and some entrapped benign glands were noted in TURP specimens.

Basal cell immunohistochemical analysis, however, has some limitations. A patchy staining pattern seen in certain atypical lesions might create diagnostic confusion. Oliai et all in their study have shown that rare lesions with the appearance of PCa show CK34βE12 staining in a basal cell distribution either from retention of basal cells by early invasive cancer or from HGPIN outpouching[101]

p63 is slightly more sensitive in identifying basal cells than CK34βE12 according to our study. Because compared to p63 four cases stained by the latter showed 1+ staining intensity. Shah et al[88] similarly found that p63 is more sensitive than CK34βE12 in identifying the basal cells, particularly in TURP specimens, offering slight advantage over CK34βE12 in diagnostically challenging cases and thus p63 may be used as an alternative to CK34βE12 stain for difficult prostatic lesion.

Varma et al[99] studied the deleterious effects of prolonged formalin fixation on the CK34βE12 staining pattern of benign prostate glands and its effects on

different antigen pretreatments. They concluded that CK34βE12 immunoreactivity is dependent on optimal fixation and the immunohistochemical protocol.

Multhaupt et al[106] found the critical role of antigen retrieval in CK34βE12 immunoreactivity. 88% of benign glands in the transition zone obtained by TURP lost their antigenicity if antigen retrieval was not used.

Parson et al[107] did report a small percentage of PCa cases staining positive for both p63 and 34βE12. Similar positivity was observed by Varma et al.[99] The staining intensity was very weak, occurred in <1% of cells, that was not significant to create any problem in interpretation. Possible explanations are false positive reactions because of formalin fixation, entrapped benign glands, PIN glands entrapped within malignant glands or heterogeneity within tumor.

CONCLUSION

Prostatic disease is responsible for significant mortality and morbidity in elderly men throughout the world. It is uncommon to under diagnose small focus of prostatic adenocarcinoma or over diagnose benign lesions mimicking cancer.

Our study focused on all prostatic lesions – benign, premalignant and malignant. Most of the patients presented with urinary symptoms along with hard prostate on digital rectal examination. Nodular prostatic hyperplasia forms the major part of the study (64%). 36% cases showed histological diagnosis of cancer and atypical / suspicious glands. Though the diagnosis of prostatic carcinoma can be made on morphological features and graded according to Gleason's grading, sometimes it can be challenging when pathologists are faced with certain problems like crushing artifact, limited tissue sample and benign mimickers. In such case, Immunohistochemistry using basal cell markers like p63, CK34βE12 can be useful.

For both types of stains positive staining was taken as an evidence of benignity whereas negative staining was taken as malignancy and discontinuous or intermittent staining was taken as premalignant.

Immunostains were done in 18 cases having suspicious and atypical glands. Out of these 18 cases 17(34%) were diagnosed as malignancy and one (2%) was diagnosed as PIN. It was found that immunohistochemical p63 staining is diagnostically reliable in identifying basal cells and compares favorably with CK34βE12 staining. In addition, p63 staining which shows a nuclear reaction is easy to interpret than CK34βE12 which shows cytoplasmic reaction.

Absence of basal staining may be attributed to the true absence of basal cells or diminished or absent gene expression of basal cell markers. 1+ staining pattern with CK34βE12 was noted in 3 cases with atypical glands but this was less than

10%, hence diagnosed as malignancy. Technical variabilities including surgical procedures, formalin fixation and antigen retrieval methods could be another cause of negative basal cell reaction.

This study has demonstrated that p63 has the same specificity and almost the same sensitivity as CK34BetaE12, and may also be utilized in distinguishing PCa from benign prostatic lesions and pseudo neoplastic lesions of prostate. Ultimately, understanding the potential pitfalls of IHC stains and paying careful attention to morphologic details are crucial to prevent the false-positive and false-negative diagnosis.

SUMMARY

- During the study period, 50 cases of prostatic biopsies were obtained and histomorphology studied.
- Age distribution was between 50 – 93 years, with 44% falling between 6^{th} – 7^{th} decades.
- Common manifestations were urinary symptoms like reduced flow, urinary incontinence and hard prostate on digital rectal examination.
- Out of 50 cases 78% of the cases are Transurethral resection specimens.
- 64% cases were diagnosed as Nodular hyperplasia, 2% as PIN and the remaining 34% was diagnosed as malignancy.
- Prostatitis is more commonly noted along with nodular prostatic hyperplasia 28%. Granulomatous was seen in 2% of the cases.
- Pseudo neoplastic lesions like basal cell hyperplasia and squamous metaplasia were noted accounts for 32% and 6% respectively.
- 62% of the patients showed benign glands rest being atypical or suspicious glands. Atypical glands showed predominance of small glands and cribriform pattern (24%), cribriform and sheets in 6% and sheets of tumor cells alone was noted in 2% cases.
- Corpora amylacea (54%) is the most common intraluminal contents and that is noted in benign glands. Luminal crystalloids seen in 4% of the cases and all were seen association with malignancy.
- Adenocarcinoma diagnosed were graded according to Modified Gleason grading, IUSP 2015 guidelines. Gleason grade 2 was seen in 41% cases with score 3+4 = 7.

- One case with signet ring component noted, no other variants were detected.
- Associated factors in malignancy like perineural invasion 46% lympho vascular emboli 12% were noted. Both findings were seen in 24% of the cases studied. Extra prostatic extension not detected.
- Immunohistochemistry (p63 and CK34βE12) was done in 18 cases of malignancy and suspicious of malignancy. One case was diagnosed as High Grade PIN and remaining cases were confirmed as malignancy.
- p63 is expressed in the nuclei of basal cells, whereas 34βE12 stains their cytoplasm. 4 cases showed entrapped benign glands. p63 is slightly more sensitive in identifying basal cells than CK34βE12 according to our study. Because compared to p63, four cases stained by CK34βE12 showed 1+ staining intensity. This false positive reaction may be because of formalin fixation and antigen retrieval.

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APPENDICES

APPENDIX - 1

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

KULASEKHARAM

RESEARCH COMMITTEE

CERTIFICATE

This is to certify that The Research Protocol Submitted by DR. S.S. MEGA SAMLY

~~*Faculty*~~ */ Post Graduate from Department of* PATHOLOGY

Titled HISTOPATHOLOGICAL STUDY OF PROSTATIC BIOPSIES WITH REFERENCE TO IMMUNOHISTOCHEMISTRY ON PREMALIGNANT AND MALIGNANT LESIONS

is approved by the Research Committee.

Chair Person

Prof. & H.O.D.
Dept. of Bio-Chemistry
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

Convenor

Prof. & H.O.D.
Dept. of Physiology
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

Date : 15.11.2016

APPENDIX - 2

INSTITUTIONAL HUMAN ETHICS COMMITTEE

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES,
KULASEKHARAM, TAMILNADU

Communication of Decision of the Institutional Human Ethics Committee(IHEC)

SMIMS/IHEC No: 1 /Protocol no: 23 / 2016

Protocol title: HISTOPATHOLOGICAL STUDY OF PROSTATIC BIOPSIES WITH REFERENCE TO IMMUNOHISTOCHEMISTRY ON PREMALIGNANT AND MALIGNANT LESIONS
Principal Investigator: Dr. S.S.Mega Samly
Name& Address of Institution: Department of Pathology Sree Mookambika Institute of Medical Sciences, Kulasekharam ☑ New review ☐ Revised review ☐ Expedited review
Date of review (D/M/Y): 15.12.2016 Date of previous review , if revised application:
Decision of the IHEC: ☑ Recommended ☐ Recommended with suggestions ☐ Revision ☐ Rejected
Suggestions/ Reasons/ Remarks:
Recommended for a period of : one year

Please note*

- Inform IHEC immediately in case of any Adverse events and Serious adverse events.
- Inform IHEC in case of any change of study procedure, site and investigator
- This permission is only for period mentioned above. Annual report to be submitted to IHEC.
- Members of IHEC have right to monitor the trial with prior intimation.

Signature of Member Secretary IHEC

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES ★ KULASEKHARAM ★
IHEC
Date 21/12/16

APPENDIX - 3

H & E STAINING:

- Sections - 3-4 micron thickness
- Deparaffinize in xylene – 2 changes 5 minutes each.
- Rehydrate in absolute alcohol and 50% alcohol 1 min each.
- Wash in running tap water for 5 minutes.
- Stain with Harris hematoxylin for 10 minutes.
- Running tap water wash for 5 minutes.
- Differentiation with Acid alcohol 1 dip.
- Running tap water wash for 5 minutes.
- Stain with eosin for 30 seconds
- 75%, 80% and 95% alcohol 1 minute each
- Absolute alcohol 1 minutes
- Clear in xylene – 3 changes 1 minute each.
- Mount with DPX.

APPENDIX - 4

IMMUNOHISTOCHEMICAL STAINING

Thinner sections (3 microns) of formalin fixed paraffin embedded tissue blocks are taken on Poly L lysine coated slides, and processed through following steps:

- Xylene I 5 min
- Xylene II 5 min
- Alcohol (100%) 1 min
- Alcohol (50%) 5 min
- Tap water 3 min
- Buffer wash (Citrate buffer at pH 6.2)
- Antigen retrieval by pressure cooker method
- Wash in running tap water 5 minutes
- Rinse in distilled water
- Buffer wash (tris buffer) 2 x 5 minutes
- Peroxidase block 5 minutes
- Buffer wash 2 x 5 minutes
- Power(protein) block 5 minutes
- Incubate in Primary Antibody 30 minutes
- Buffer wash 2 x 5 minutes
- Super enhancer (incubation with Post primary) 30min
- Buffer wash 2 x 5 minutes

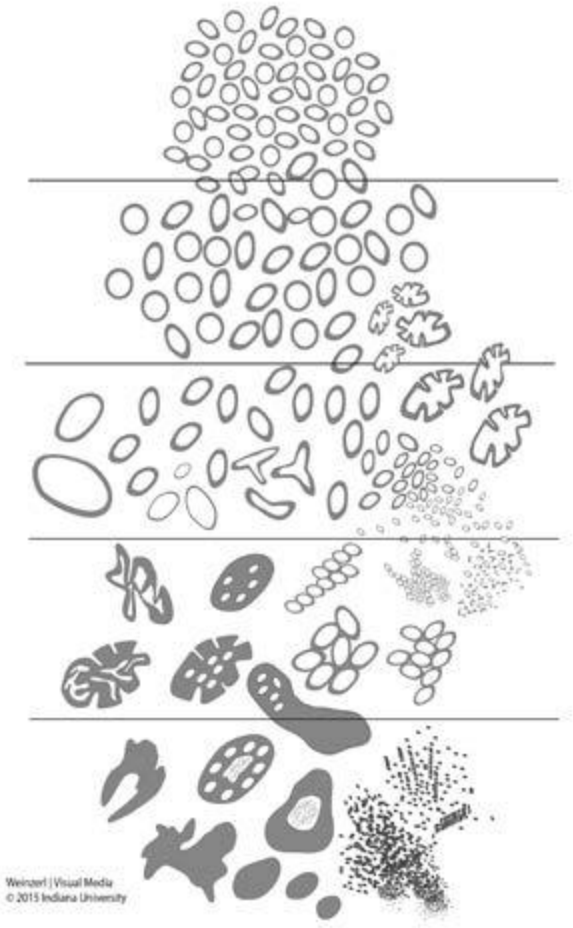
- Incubate with Polymer HPR 30 minutes
- Buffer wash 2 x 5 minutes with gentle rocking
- DAB (Freshly prepared) for 5 minutes
- Running tap water 5 minutes
- Hematoxylin counterstain for 15 seconds
- Rinse in running tap water for 5 minutes
- Dehydrate
- Clearing
- Mount in DPX

APPENDIX - 5

GLEASON GRADE GROUPS:

Grade Group	Gleason Score	Definition
1	≤6	Only individual discrete well-formed glands
2	3+4=7	Predominantly well-formed glands with lesser component of poorly formed/fused/cribriform glands
3	4+3=7	Predominantly poorly formed/fused/cribriform glands with lesser component of well-formed glands
4	4+4=8	Only poorly formed/fused/cribriform glands
	3+5=8	Predominantly well-formed glands and lesser component lacking glands (or with necrosis)
	5+3=8	Predominantly lacking glands (or with necrosis) and lesser component of well-formed glands
5	9-10	Lack gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands

Illustration of various grades by Gleason. ISUP 2015



APPENDIX - 6

CASE PROFORMA

Identity Number : Date :

AGE/SEX : Phone No :

ADDRESS :

PRESENTING COMPLAINTS :

(Dysuria, Hesitancy, Frequency,

Dribbling, Retention, Urgency,

Hematuria, Fever)

INVESTIGATIONS :

MACROSCOPY:

- Type of specimen :
- Gross weight & measurement :
- Added description :

FINDING BY MICROSCOPY:

Features	**Finding**	
1.Number of fragments in needle biopsies Number of fragments involved by tumor/ lesion		
2. Volume of tissue involved by tumor/ lesion		
3. Appearance of glands · Nodular adenomatous hyperplasia · stromal hyperplasia · mixed epi and stromal hyperplasia · small glands · cribriform / fused glands . Sheets . Squamous Metaplasia . Basal cell hyperplasia 4. Appearance of stroma · Fibrous hyperplasia · Stromal oedema		
5. Cell morphology . Nuclear pleomorphism . Nucleoli . Mitosis		

6. Inflammation and infarction . Prostatitis . Granulomatous inflammation . Abscess . Infarction . None			
7. Luminal contents · Corpora amylacea · Crystalloids · Mucinous secretion . Eosinophilic debri . Calcification			
8. PIN			
9. Malignancy +/- If yes, volume of infiltrate			
10. Gleason scoring (malignancy) • 3+3 • 3+4 • 4+3 • 4+4 • 5+3 • 3+5 • 5+5	Gleaon's grade: • Grade 1 • Grade 2 • Grade 3 • Grade 4 • Grade 5		
11. Associate prognostic factors: • Perineural invasion • LV emboli • Whispy mucinous material			
12. H & E Diagnosis • Benign • PIN • Carcinoma			
13. Immunostaining • Indication - atypical/suspicious glands • cytokeratin 34βE12 • p63	Positive %	Negative %	Others

INFERENCE:

APPENDIX - 7

ABBREVIATIONS

AAH	-	Atypical Adenomatous Hyperplasia
ABC	-	Avidin-Biotin Conjugate
AJCC	-	American Joint Committee on Cancer
ASAP	-	Atypical Small Acinar Proliferation
CAP	-	College of American Pathologist
DRE	-	Digital Rectal Examination
ELISA	-	Enzyme Linked Immuno Sorbant Assay
EPE	-	Extra Prostatic Extension
FFPE	-	Formalin Fixed Paraffin Embedded
H&E	-	Hematoxylin and Eosin
IDH 1	-	Isocitrate Dehydrogenase 1
ISUP	-	International Society of Urologic Pathology
KLK3	-	Kallikrein-3
LOH	-	Loss of Heterozygosity
LVI	-	Lympho Vascular Invasion
NIH	-	National Institute of Health

PAP	-	Peroxidase Anti Peroxidase
PCa	-	Prostatic Carcinoma
PIA	-	Proliferating Inflammatory Atrophy
PIN	-	Prostatic Intraepithelial Neoplasia
PNI	-	Perineural Invasion
PSA	-	Prostate Specific Antigen
TURP	-	Trans Urethral Resection of Prostate
UICC	-	Union International Centre on Cancer
VACURG	-	Veteran’s Administration Cooperative Urological Research Group
WHO	-	World Health Organization

APPENDIX - 7

MASTER CHART

Sl. No	HP. No	Age	Type of bx	Clinical presentation	Volume of tissue infiltrated	Gland morphology	Hyperplasia	Inflammation/ Infarction	Mimikers	Luminal contents	Cell morphology	Gleasons Score	Gleason grade group	Prognostic factors	Diagnosis	P63	Staining pattern	CK 34 beta E12	staining pattern	Entrapped benign glands
1	1358/16	71	1	1	0	1	1	3	2	1	0	0	0	0	1	2	4	2	4	0
2	2031/16	64	1	1	0	1	3	1	0	1	0	0	0	0	1	2	4	2	4	0
3	1984/16	65	1	1	0	1	1	2	0	1	0	0	0	0	1	2	4	2	4	0
4	713/16	77	2	3	60	3	0	0	0	2	1	2	2	3	3	0	0	0	0	0
5	2097/16	65	1	3	0	1	3	1	1	0	0	0	0	0	1	2	4	2	4	0
6	2742/16	62	1	1	0	1	1	0	0	1	0	0	0	0	1	2	4	2	4	0
7	839/16	62	1	2	0	2	0	3	2	1	1	0	0	3	2	1	3	1	2	0
8	3027/16	70	1	1	0	1	1	3	1	1	0	0	0	0	1	2	4	2	4	0
9	4191/16	64	1	3	0	1	1	1	0	1	0	0	0	0	1	2	4	2	4	0
10	4478/16	78	1	1	0	1	0	0	0	0	0	0	0	0	1	2	4	2	4	0
11	2308/16	79	1	3	20	2	1	1	0	0	1	1	1	6	3	0	0	0	0	1

12	7040/16	67	1	1	0	1	1	0	1	1	0	0	0	0	1	2	4	2	4	0
13	7100/16	70	1	1	0	1	2	1	1	0	0	0	0	0	1	2	4	2	4	0
14	7310/16	65	1	3	0	1	3	0	0	1	0	0	0	0	1	2	4	2	4	0
15	420/17	70	1	3	0	1	1	4	1	1	0	0	0	0	1	2	4	2	4	0
16	551/16	83	1	1	80	3	0	0	0	1	2	3	3	2	3	0	0	0	0	0
17	906/16	74	2	3	95	3	0	0	0	0	2	3	3	5	3	0	0	0	1	0
18	1264/17	60	1	1	0	1	1	3	0	1	0	0	0	0	1	2	4	2	4	0
19	4247/16	50	1	1	0	1	1	0	0	3	0	0	0	0	1	2	4	2	4	0
20	2060/17	66	1	3	0	1	1	1	1	1	0	0	0	0	1	2	4	2	4	0
21	1061/15	65	2	3	70	2	0	0	0	0	1	1	1	1	3	0	0	0	0	0
22	864/16	72	2	3	50	2	0	0	0	1	1	1	1	2	3	0	1	0	1	0
23	3785/17	86	1	1	0	1	1	1	0	0	0	0	0	0	1	2	4	2	4	0
24	3292/17	68	1	1	0	1	3	1	0	1	0	0	0	0	1	2	4	2	4	0
25	1263/17	94	2	3	90	3	0	0	0	0	2	2	2	1	3	0	0	0	0	0
26	2163/17	67	1	1	0	1	1	0	1	1	0	0	0	0	1	2	4	2	4	0
27	3554/17	60	1	3	0	1	1	2	0	0	0	0	0	0	1	2	4	2	4	0
28	4379/17	60	1	1	0	1	1	1	0	1	0	0	0	0	1	2	4	2	4	0
29	1555/17	78	2	3	80	4	0	0	0	0	2	5	5	1	3	0	0	0	0	0
30	4529/17	73	1	1	0	1	1	0	1	1	0	0	0	0	1	2	4	2	4	0
31	4668/17	57	1	1	0	1	1	0	1	1	0	0	0	0	1	2	4	2	4	0
32	1178/17	77	2	3	70	3	0	0	0	0	2	2	2	5	3	0	0	0	1	0
33	5405/17	73	1	1	0	1	3	1	1	1	0	0	0	0	1	2	4	2	4	0
34	6028/17	67	1	3	0	1	1	1	2	1	0	0	0	0	1	2	4	2	4	0

35	6458/17	70	1	1	0	1	1	0	1	1	0	0	0	0	1	2	4	2	4	0
36	6976/17	70	1	1	0	1	3	0	0	1	0	0	0	0	1	2	4	2	4	0
37	7193/17	55	1	3	0	1	1	1	1	3	0	0	0	0	1	2	4	2	4	0
38	798/17	67	1	1	90	3	0	0	0	0	2	2	2	1	3	0	0	0	0	1
39	1555/17	86	2	3	40	3	0	0	0	2	2	2	2	0	3	0	0	0	0	0
40	7268/17	68	1	1	0	1	1	0	0	1	0	0	0	0	1	2	4	2	4	0
41	347/17	83	2	3	80	4	0	0	0	0	2	4	5	1	3	0	0	0	0	0
42	7481/17	72	1	3	0	1	3	2	1	3	0	0	0	0	1	2	4	2	4	0
43	3631/17	72	1	3	50	4	0	2	0	0	2	5	5	1	3	0	0	0	0	1
44	2693/17	65	1	2	0	1	1	1	1	1	0	0	0	0	1	2	4	2	4	0
45	2700/17	64	1	1	0	1	1	1	0	1	0	0	0	0	1	2	4	2	4	0
46	13/17	82	2	3	70	3	0	0	0	0	2	2	2	1	3	0	0	0	0	0
47	2299/17	68	2	3	60	3	0	0	1	0	2	2	2	1	3	0	0	0	1	0
48	3245/17	65	1	1	0	1	1	0	1	1	0	0	0	0	1	2	4	2	4	0
49	6678/17	75	1	3	95	3	0	0	0	0	2	3	3	5	3	0	0	0	0	1
50	2302/17	71	1	3	30	5	0	0	0	2,4	3	6	5	5	3	0	0	0	0	0