A STUDY ON MUCIN HISTOCHEMISTRY AND p63 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS

Dissertation submitted in

Partial fulfillment of the requirements for the degree of

M.D. PATHOLOGY

BRANCH- III

INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI- 600003

THE TAMILNADU DR M.G.R. MEDICAL UNIVERSITY

CHENNAI

MAY - 2019

CERTIFICATE

This is to certify that this Dissertation entitled **"A STUDY ON MUCIN HISTOCHEMISTRY AND p63 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS"** is the bonafide original work of **DR.N.KIRUTHIKA**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in May 2019.

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DECLARATION

I, **Dr.N.KIRUTHIKA,** solemnly declare that the dissertation entitled **"A STUDY ON MUCIN HISTOCHEMISTRY AND P63 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS"** is the bonafide work done by me at the Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.Pappathi.S, M.D., DCH,** Professor of Pathology and **Dr.K.Indumathi,** M.D., DCP, Assistant professor of Pathology, Institute Of Pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place : Chennai

Date : **DR.N.KIRUTHIKA**

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CERTIFICATE OF APPROVAL

To
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Madras Medical College, Chennai

Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON MUCIN HISTOCHEMISTRY AND P63 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS " NO.26092016** .

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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ABBREVIATIONS

AAH	**-**	**atypical adenomatous hyperplasia**
PIN	**-**	**prostatic intraepithelial neoplasia**
PAS	**-**	**periodic acid schiff**
BPH	**-**	**benign prostatic hyperplasia**
LUTS	**-**	**lower urinary tract symptoms**
PBCR	**-**	**population based cancer registries**
AAR	**-**	**age adjusted incidence rates**
MAPC	**-**	**mean annual percentage change**
EAPC	**-**	**estimated annual percentage change**
OCP	**-**	**organochlorine pesticides**
HCH	**-**	**hexachlorocyclohexane**
AR	**-**	**androgen receptor**
HSP	**-**	**heat shock protein**
DHT	**-**	**dihydroxytestosterone**
HRPC	**-**	**hormone resistance in prostate cancer**
BCH	**-**	**basal cell hyperplasia**
CCCH	**-**	**clear cell cribriform hyperplasia**
DRE	**-**	**digital rectal examination**
PSMA	**-**	**prostate specific membrane antigen**
BLSA	**-**	**Baltimore Longitudinal study of aging**

CONTENTS

INTRODUCTION

INTRODUCTION

Benign prostatic hyperplasia and prostatic carcinoma are the two most common diseases involving men in older age. The prostatic cancer is the second most frequently diagnosed cancer in men , sixth most common cause of cancer death in males worldwide and fifth most common cancer overall[1]. As prostatic cancer is a disease of older age,by 2030,the proportion of people above 65 years will increase from 12.4% to 19.6% , the number of prostate cancer cases will quadruple.

According to world cancer stat facts, the estimated new cases in 2018 is about 164,690 with percentage of all new cancer being 9.5%. Estimated deaths in 2018 is about 29,430 and percentage of all cancer deaths being 4.8%.The percentage of overall survival rate is about 98.2% from 2008-2014. Prostatic cancer is rare below 40 years and about 70% of cases occur after 65 years of age. The lifetime risk of being affected by prostate cancer is 1. There is a significant variation in geographic incidence with Asians having the lowest incidence rates of prostate cancer at about 107.2 per 100,000.Incidence of prostatic cancer in persons with family history increases about two to four times higher than in control populations. Those with a family history of prostate cancer tend to have earlier onset of disease about six or seven years earlier than controls about 40% of those cancers diagnosed below the age of 55.

Charles C Huggins recieved a Nobel prize in 1941 for his noble work in identifying that androgens play an importsnt role in development, growth and

treatment of prostate cancer.

The diagnosis of limited well differentiated adenocarcinomas of prostate is one of most difficult areas of surgical pathology. Benign hyperplasia can sometimes mimic adenocarcinomas and differentiation between the two and early diagnosis of prostatic carcinoma is crucial. It should also be differentiated from benign lesions, mimickers and premalignant lesions such as prostatic intraepithelial neoplasia(PIN) and atypical adenomatous hyperplasia (AAH).

Mucins are present in the tissues or are secreted by the glands. The normal prostatic glands secrete neutral mucosubstance. Numerous reports have claimed that acidic mucin is absent in benign prostatic glands and is present in prostatic adenocarcinomas. Whereas PAS is positive in both benign prostatic hyperplasia(BPH) and prostatic adenocarcinoma(Pca).

Prostatic adenocarcinomas are differentiated from benign hyperplasia of prostate by absence of basal cell layer. Therefore basal cell marker (p63) is useful in differentiating benign hyperplasia of prostate from prostatic adenocarcinoma.

As special stains will be cost effective and simplicity of its procedure,it can be used even in lower centres for diagnosis of prostatic adenocarcinoma and its differentiation from benign prostatic hyperplasia.

This study is undertaken to demonstrate that acidic mucin maybe an adjunctive aid in the diagnosis of prostatic adenocarcinomas from benign prostatic hyperplasia and to correlate and confirm with P63 expression.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

1. To study the mucin histochemistry in benign prostatic hyperplasia and prostatic adenocarcinoma.

2. To study alcian blue staining among different grades of prostatic adenocarcinoma.

3. To correlate and confirm with p63 expression in benign prostatichy perplasia and prostatic adenocarcinoma.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY:

Prostate is a pear shaped encapsulated accessory sex gland situated at the apex of urinary bladder in males. A healthy prostate in a normal adult weighs about 20 grams and measures about 4x3x2 cm. It has a true internal connective tissue capsule and a false external capsule, which derived from the pelvic fascia. It can be broadly divided into an inner periurethral zone and an outer cortical zone. This classification is important because, the outer zone is the most common site for adenocarcinoma whereas the inner zone is the most common site for benign prostatic hyperplasia.[55,56] It can be further classified based on embryology and pathological features into peripheral, central,transitional and periurethral regions.[57]

Transition zone

It is the portion of prostate surrounding the pre prostatic urethra and constitutes about 5-10% of the prostate volume. A dramatic increase of the transition zone volume is mainly due to benign prostatic hyperplasia and causes lower urinary tract symptoms (LUTS)

Central zone:

The transition zone is surrounded by the conical central zone. It extends from the base of the prostate gland to the verumontanum. This region forms a funnel or a ring like zone which comprises the ejaculatory ducts which are

present posterior to the pre prostatic urethra. It constitutes about 25% of the prostate volume.

Peripheral zone

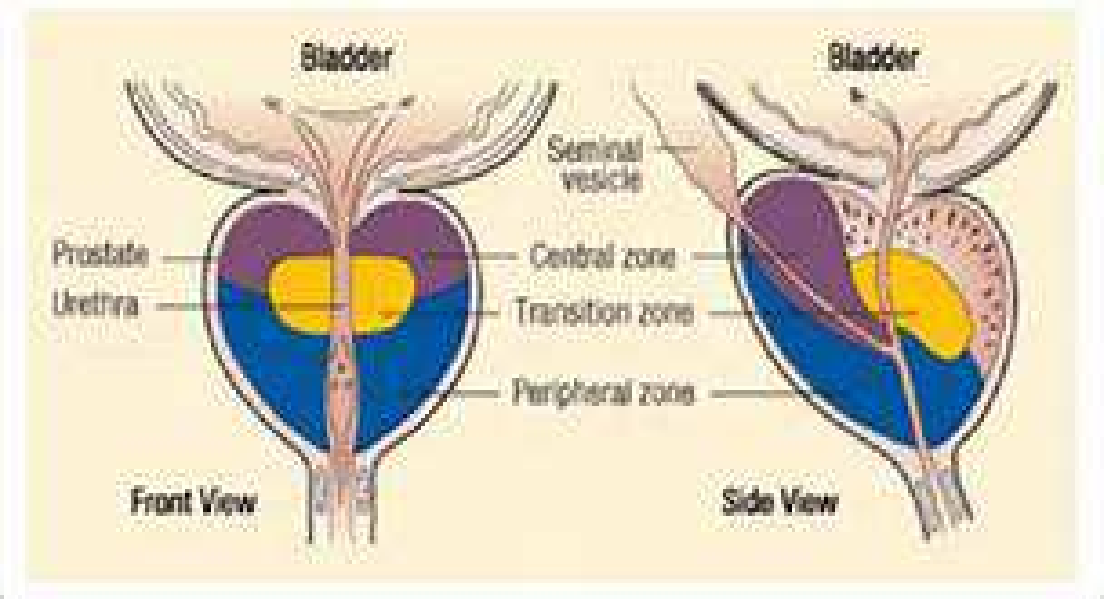
It is the outermost portion of the prostate gland surrounding the central zone posteroanteriorly and most of the transition zone.

It constitutes about 75% of the total prostatic volume.

Anterior fibromuscular stroma:

It is present anterior to the urethra and extends into the transition zone.It constitutes about 5% of the total prostatic volume.

Figure 1 Anatomy of Prostate



HISTOLOGY:[74]

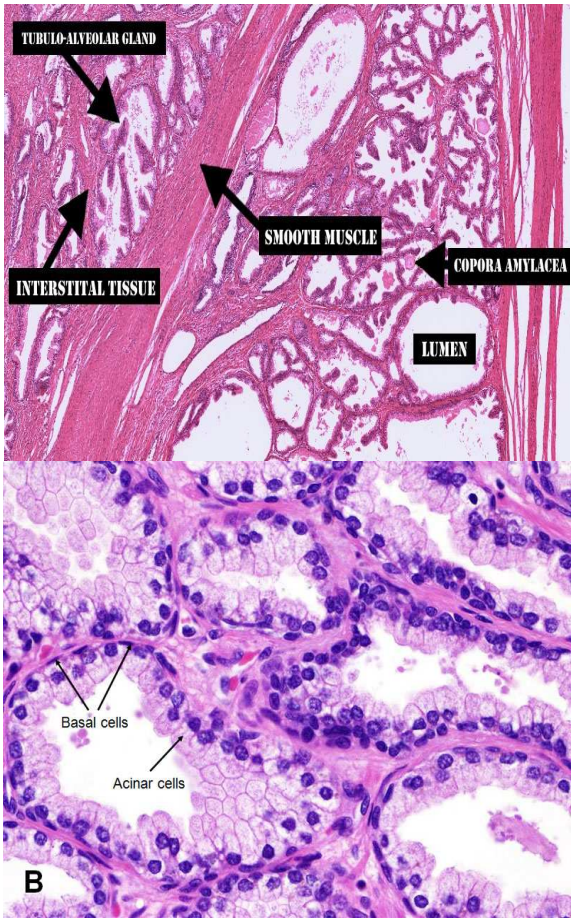
It is a partially encapsulated organ with capsule covering posterior and lateral aspects. The anterior and apical surfaces are covered by anterior fibromuscular stroma which is part of the gland itself.

The prosate gland is composed of glands and stroma.The glands are of branched tubule-acinar type embedded in a fibromuscular stroma.

The epithelium has a convoluted pattern and is thrown into folds, sometimes into papillary pattern. The glands are lined by two layers of epithelium. Luminal epithelium is tall columnar with prominent round basal nuclei and pale staining eosinophilic cytoplasm. Other type is basal cells which are stem cells and becomes prominent in prostatic hyperplasia.Some glands may show inspissated secretions forming spherical concretions-corpora amylacea. Corpora amylacea increase in number with increase in age and it may become calcified.

The supporting stroma is composed of collagenous fibrous tissue and smooth muscle fibres.

FIGURE 2: NORMAL HISTOLOGY OF PROSTATE.



EPIDEMIOLOGY:

Prostate is the second leading site of cancer among males in large Indian cities like Delhi, Kolkatta, Pune and Thiruvananthapuram and third leading site of cancer in cities like Bangalore and Mumbai and it is among the top ten leading sites of cancers in the rest of the population based cancer registries (PBCRs) of India.[18] In Chennai, prostate cancer is 4th most common cancer.

According to GLOBOCON 2018, prostate cancer incidence is 7.1% worldwide. Lathika *et al* [2]analyzed the time trends in the incidence of prostate cancer for different age groups of the Indian population reported using relative difference and regression approaches covering major cities like Ahmedabad,

Bangalore, Chennai, Delhi, Mumbai, Karunagappalli, Nagpur, Pune, and Thiruvananthapuram.

The estimated age-adjusted incidence rates (AARs) of prostate cancer in India as a whole was 3.7/10 ^{5} persons during the year 2008.

The mean annual percentage change (MAPC) in the crude incidence rates ranged from 0.14 in Ahmedabad to 8.6 in Chennai. Peak incidence was observed in the age group above 65 years, indicating that prostate cancer was a cancer of the elderly. Chennai also recorded the highest MAPC of 5.66 in the age group of patients above 65 years.

The estimated annual percentage change (EAPC) in the AAR ranged 0.8-5.8 in the various registries. Increase in the trend was seen in men aged 55-64 years in Bangalore, Chennai, and Mumbai during 1983-2002.

In other study by yoele et al, maximum increase in AAR was noted for Chennai registry (4.95%) and the least for Mumbai registry (0.89%)[3]

Swaminathan *et al*. study showed that the average annual age-standardized rate for prostate cancer had a significant increase by 47% during the period of 2002-2006 in Chennai compared to the previous years. Their study also showed that prostate cancer had become the ninth most common cancer in Tamil Nadu.[4]

Herbert *et al*. compiled data available from various cancer registries and observed that the average annual cancer incidence rate for prostate cancer in

India ranged 5.0-9.1 per 100,000/year. In India ,of all prostate cancers, 85% were detected late (stages III and IV).A significant notable difference was also observed between the rural and urban areas in India. [5]

RISK FACTORS:

1) AGE; In 2013,Singh *et al.* [9] studied the relationship of lifestyle, age, and BMI with PSA levels in benign prostatic hyperplasia (BPH) and prostate cancer in the North Indian population. They observed that the mean age of prostate cancer patients (67.56 ± 5.72 years) was significantly higher than that of BPH patients (63.56 ± 7.92 years).
2) HORMONAL STATUS: Prostatic cancer is hormone dependent that is it develops in older men with circulating androgens.

Castration done before puberty protects against prostatic cancer.

Patients with hyperestrogenism due to liver cirrhosis have lower incidence.

Therapeutic castration and antiandrogen treatment causes tumour regression.

3) DIET: Increased consumption of fat and carcinogens in charred red meat, lycophenes in tomatoes,soy products and vitamin D are suspected to play a role.

In a study conducted by Terry *et al.* [7] had observed a reduced risk of prostate cancer for fish eaters.

Heterocyclic amines produced during cooking of red meat and pyrolysates produced during cooking of meat over charcoal/smoke had been observed as a reason for increased prostate carcinogenesis in the non vegetarians. [8]

4) SMOKING: In 2010, Huncharek *et al*., showed an increased risk of prostate cancer in chronic smokers[6]
5) OBESITY: Amling *et al*. [10] and Freedland *et al*. [11] showed positive correlation of obesity and BMI to prostate cancer.
6) ENVIRONMENTAL FACTORS: As India is an agricultural country, exposure to pesticides and other agricultural chemicals is inevitable. Banerjee *et al*[12] study reported that pesticides, mainly organochlorine pesticides (OCPs), could be called as xenoestrogenic pesticides as they possessed estrogenic properties. OCPs such as 1, 1, 1-hexachlorocyclohexane (HCH), dieldrin, and endosulfan are the most commonly used xenoestrogenic OCPs in India. As prostate cancer is an estrogen-dependent cancer, these pesticides might increase the risk of prostate cancer incidence in the population exposed to these carcinogenic agents.
7) PREMALIGNANT LESIONS: Nodular hyperplasia is not a predisposing factor but both the conditions may occur simultaneously.

High grade prostatic intraepithelial neoplasm is a premalignant condition for prostatic adenocarcinoma.

GENETICS:

Genetic association is seen in about 5-10% of prostatic cancers. The risk is twice with single first degree relative with prostatic cancer and the risk increases to five fold with two first degree relative with prostatic cancer.

It also occurs at an earlier age in patients with strong family history. A_2 allele of the CYP17 polymorphism has also been reported to be associated with an increased risk of prostate cancer in smokers and nonvegetarians. [13]

BRCA2 germline mutation is associated with 20 fold increased risk of developing prostatic cancer.

Increased risk is associated with germline mutation of HOXB13, chromosomal rearrangements in coding sequence of ETS family transcription factor next to androgen regulated TMPRSS2 promoter.

Other genetic alterations include amplication of 8q24 locus(MYC oncogene), deletions of PTEN tumour .

The commonest genetic alteration found in about half of all cases is fusion of androgen responsive serine protease gene TMPRSS2 (21q22.2) with one of the ETS transcription factor gene family members[30].

ETS transcription factor gene family includes ERG(21q22.2), ETV1 (7p21.2), ETV4 (17q21) , ETV5(3q27) with ERG accounting for about more than 90% of cases. this molecular alteration seems to be an earlier event in pathogenesis as it is seen in high grade prostatic intraepithelial neoplasia.

Expression of fusion transcript is downregulated as the tumour progresses to become androgen resistant[31].

Advanced stage shows TP53 loss, deletion of RB gene and amplification of androgen receptor gene locus.

Hypermethylation of glutathione s transferase (GSTP1) gene is the most common epigenetic alteration which downregulates GSTP1 expression.

RB,CDKN2A,MLH1,MSH2 and suppression of Wnt pathway signaling (APC) are other epigenetic modifications seen in prostatic cancers.

HER 2 gene amplication is seen in about one third of prostatic adenocarcinomas and it correlates with tumour grade,stage and non diploid DNA content[32].

PATHOGENESIS:

Androgen receptor (AR) signaling is important for prostate differentiation, function as well as for prostate cancer growth and progression. The human AR is encoded by a single copy gene on the X-chromosome (Xq11.2-q12).

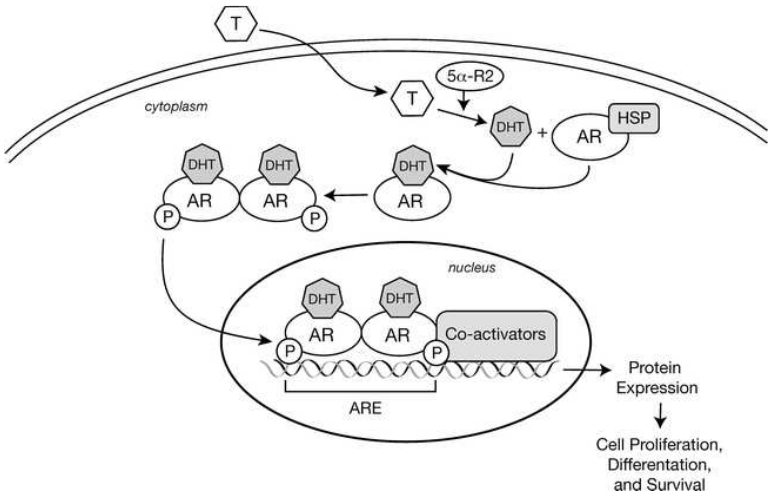
Although there is some evidence that the length of the poly-glutamine repeat correlates with prostate cancer risk, there is no strong proven relationship.[25]

In the absence of androgens, AR is present in the cytoplasm bound to heat shock proteins (HSP-70 and -90).HSP function is to stabilize the protein

and protect it from degradation. AR activity is regulated by 2 major ligands, testosterone and dihydrotestosterone (DHT). by in the prostate. Prostate converts testosterone to DHT by 5α-reductase. DHT is more potent than testosterone and has 10 times higher binding affinity for AR than testosterone and is the primary androgen bound by AR. DHT binding to the AR results in the recruitment of protein kinases, leading to phosphorylation of many serine residues. Phosphorylation of the AR leads to many functions such as protection from proteolytic degradation, stabilization, and transcriptional activation.[26] The transactivation of AR involves several coregulatory proteins that are able to differentially respond to a changing microenvironment to regulate specific gene targets involved in cell growth and survival.[27]

In the normal prostate epithelium, there is a balance between the rate of cell proliferation and the rate of apoptosis; which is lost in prostate cancer leading to tumor growth.[28]

FIGURE 3: MECHANISM OF PATHOGENESIS OF PROSTATIC ADENOCARCINOMA:



Mechanism of ligand-dependent gene transactivation by the androgen receptor. Testosterone (T) inside the prostate epithelial cell is converted to dihydrotestosterone (DHT) by 5α-reductase. DHT binds to AR causes dissociation of the AR-heat shock protein (HSP) complex, dimerization, and translocation to the nucleus. AR binds to androgen response elements (ARE) and recruits multiple co-activators to enhance transcription.

Mechanisms of Hormone Resistance in Prostate Cancer

The mechanisms involved in the emergence of HRPC despite sustained androgen ablation and/or the use of AR antagonists can be classified into 3 general categories:

DNA-based alterations in the AR gene, such as amplification or point mutations, AR- growth factors crosstalk, and activation of alternative pathways of survival and proliferation.[29]

CLINICAL EXAMINATION:

Early localized cancer is asymptomatic.

Urinary symptoms such as hematuria , dysuria,increased frequency occur in later stages because most of the tumour occurs in peripheral prostate.

Very advanced stage cancer may present as vertebral metastasis with back pain. Digital rectal examination and PSA levels help in detection of early prostatic cancers.

Skeletal surveys and radionucleotide bone scanning confirms osteoblastic metastasis.

Diagnostic triad include serum PSA ,digital rectal examination and transrectal ultrasonography for early prostatic carcinoma detection.[20]

DIGITAL RECTAL EXAMINATION AND ULTRASONOGRAPHY:

Early carcinomas cannot be distinguished from nodular hyperplasia, granulomatous prostatitis, tuberculosis, infarct or lithiasis by rectal examination alone. Pathological examination of prostatic tissue is confirmatory in such cases.

As most of the prostatic carcinomas are located in the peripheral zone, it may be detected by DRE when the volume is > 0.2 mL. In about approximately 18% of cases, carcinoma is detected by DRE alone, irrespective of PSA level. DRE in patients with PSA level < 2 ng/mL has a positive predictive value of about 5-30%. Abnormal DRE is associated with an increased risk of higher Gleason score and is an indication for biopsy.

Transrectal ultrasound can detect tumours as small as 5 mm in diameter.[19] However, 30% of prostatic tumours are missed on transrectal ultrasound and hence it is not a valuable screening tool.

SERUM PSA LEVELS;

PSA has been widely used as a screening test for prostate cancer. Prostatic epithelium synthesizes PSA which is a serine protease regulated by androgen. It cleaves and liquefies coagulum formed after ejaculation.Though elevated serum PSA is specific to prostate, it is not specific to prostatic tumour.

Although it is used as a screening test it lacks both specificity and sensitivity.

It serum levels is also elevated in benign prostatic hyperplasia,infarction of nodular hyperplasias, prostatitis, instrumentation of prostate and even after ejaculation. A serum value of 4ng/mL is taken as normal cutoff in most laboratories but in about 20-40% of early localized prostatic tumours the serum value maybe 4ng/mL or even lower than that.

So some guidelines take 2.5 ng/mL as cutoff value.

Serial assessment of serum PSA values is used in assessment of response to therapy. Increase in serum PSA level following therapy for localized tumour indicates recurrent or disseminated disease.

Immunohistochemical localization of PSA on tissue sections can be used to find whether a metastatic tumour originated in prostate.

Modifications in PSA include PSA density,PSA velocity, ratio of free and bound PSA in serum and age specific reference values.

AGE SPECIFIC SERUM PSA LEVELS:

In 2007,Ganpule *et al*. [14] study observations on age-specific PSA and PSA density values in a community-based Indian population in Gujarat showed that the mean PSA values increased from 2.1 ng/mL in the age group of 40-49 years to 5.0 ng/mL in the ag group of >70 years. Similarly, the mean PSA density also increased from 0.15 to 0.2 ng/mL in the same age group of patients.

Men with hyperplastic prostate produce more PSA than men with smaller glands.

Older men have increased incidence of BPH and hence have more serum PSA levels.

The upper age specific serum PSA values:

2.5ng/mL for men aged 40-49years.

3.5ng/mL for men aged 50-59 years.

4.5ng/mL for men aged 60-69 years.

6.5ng/mL for men aged 70-79 years.

PSA DENSITY:

Serum PSA density identifies the contribution of benign prostatic tissue to serum PSA level.

It is usually calculated by dividing the total serum PSA level by estimated gland volume to calculate PSA produced per gram of prostate tissue. Gland volume is estimated using transrectal ultrasound.

PSA VELOCITY:

It is the rate of change of PSA. More rapid increase in PSA level is seen in men with prostatic cancer than in men without prostatic cancer. The rate of change in PSA that distinguishes between prostatic cancer and without prostatic cancer is 0.75 ng/mL per year.

For PSA velocity measurement, atleast three PSA measurements in about 1.5 to 2 years should be done as there is about 20% variability between repeat PSA measurements.

RATIO OF FREE AND BOUND PSA:

Immunoreactive PSA exists in two forms-a major fraction bound to alpha 1 antichymotrypsin and a minor free fraction. The percentage of free PSA is lower in men with prostatic tumour than in men with benign prostatic diseases.

In 2011,Shah *et al*. [15] in a hospital-based study found than the free PSA (f PSA) levels correlated with the age of the patient. The mean f PSA levels (ng/mL) among the four age categories (<45 years, 45-60 years, 60-75 years, and >75 years) were 0.49 ± 0.13 ng/mL, 0.69 ± 0.10 ng/mL, 1.94 ± 0.04 ng/mL, and 2.33 ± 0.43 ng/mL, respectively.

In Chennai, a study conducted by Atish *et al*. [16] evaluated the free-to-total PSA (f/t PSA) ratio to distinguish BPH and prostate cancer in the age group of 40-75 years. They observed that f/t PSA ratio was decreased significantly in prostate cancer compared to BPH.

One study has shown that a cutoff for biopsy in symptomatic men with negative digital rectal examination (DRE) in India could safely be raised to 5.5 ng/mL, which could avoid about 10% of men unnecessarily subjected to biopsy. [17]

ROLE OF OTHER GENES IN DIAGNOSIS OF PROSTATIC TUMOUR:

PCA3 is overexpressed in about 95% of prostatic tumours.it is a non coding RNA.

Urine PCA3 is used as an additional marker in patients with elevated serum PSA levels but negative prostate biopsy. Elevated urine PCA3 levels is associated with increased risk of positive repeat prostate biopsy.

Combination of urinary PCA3 and urinary TMPRSS2-ERG fusion DNA have increased sensitivity and specificity than PSA screening alone.

CYTOLOGY:

According to Epstein et al,.[21]the accuracy of needle biopsy is 85.6% and that of aspirates was 86.6%,together the accuracy is 95.8%. Inspite of this, aspiration cytology has fallen into disuse because of large number of false negative reports.

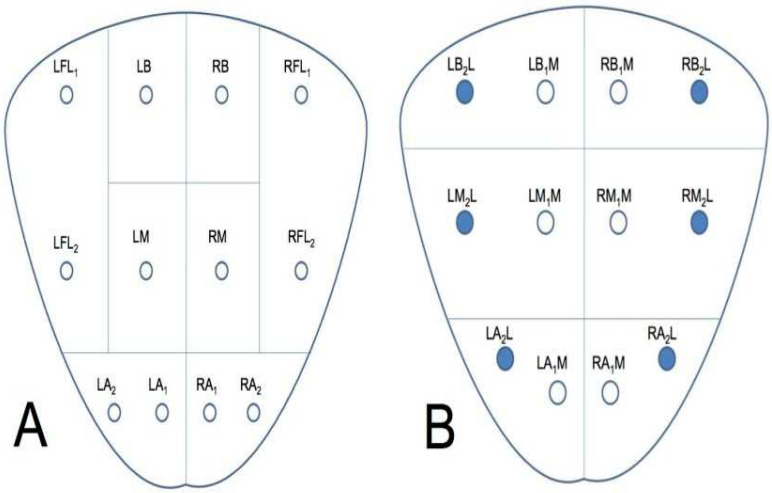
Poorly differentiated and moderately differentiated carcinomas are easy to diagnose whereas well differentiated tumour diagnosis is difficult.

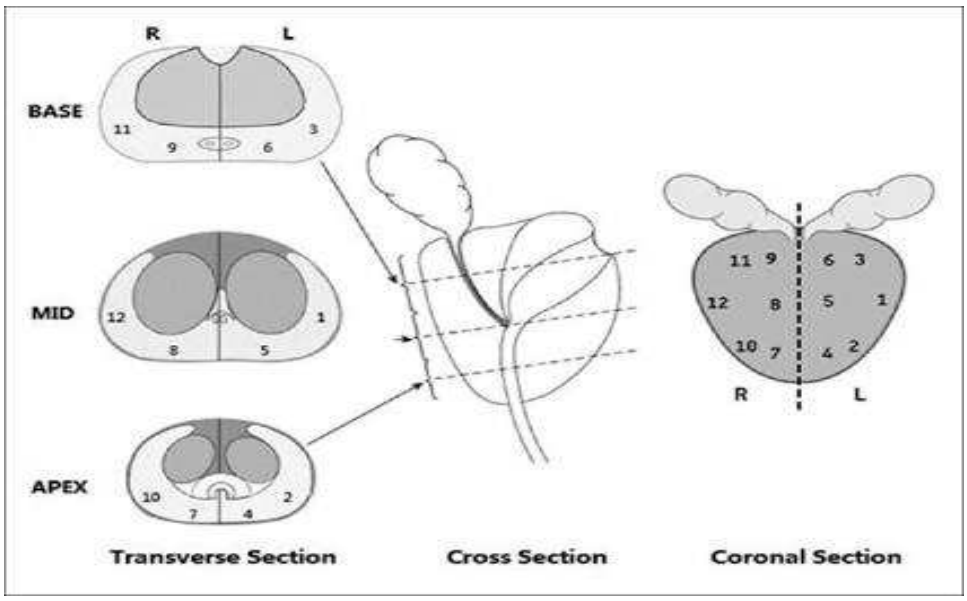
BIOPSIES:

Needle biopsy can be done either perineal or transrectal. Transrectal route is more preferred.

Automated spring loaded 18 gauge biopsy gun is recently being used. Six core technique (sextant biopsies) is used routinely[22]. However, 12 core shows higher yield by 31% especially when the cores are taken from lateral lobes.

FIGURE 4 & 5: SITES OF PROSTATIC BIOPSY:





It has been found that if five blocks or 12 g of randomly selected tissue submitted, the probability of detection of carcinoma is approximately 90% and it rises to 98% with examination of eight blocks.[23]

HANDLING OF PROSTATE SPECIMENS:[24]

RADICAL PROSTATECTOMY:

Specimen should be oriented, margins inked and fixed overnight.

Vas deferens and proximal bladder neck margins should be shaved.distal apical margin is obtained by amputating the distal 1 cm of apex and sectioning the so obtained cone perpendicularly to the cut edge.

Cut serial sections at 2-3 mm from apex to base.

Description:

Weight, dimensions and organs received should be noted. Location in prostate, size,colour,borders,capsular and periprostatic involvement of tumour should be noted. Whether urethra and seminal vescicle is involved by tumour should be noted.

Any adjacent nodular hyperplasia should be noted.

Sections for microscopic examination;

Vas deferens margin.

Proximal bladder neck margin.

Right and left distal apical margin.

Proximal, mid and distal portions from each seminal vescicle.

Adequate sections from the tumour.

TRANSURETHRAL RESECTION (TURP);

Specimen should be weighed and examined carefully for hard yellow areas which represents carcinomatous areas. Size ,shape and colour of fragments should be noted.

Sections:

If all fragments received in single container: all of specimen until four cassettes. If excess, one additional cassette for each additional 10 g of tissue.

If received fragments are identified as from which lobe they were taken then, all of specimen upto 4 cassettes. If excess, one cassette for each additional 10 g of tissue.

If carcinoma is identified microscopically in a lobe that was not entirely submitted then the remainder of the tissue should be processed entirely regardless of the amount.

SUPRAPUBIC PROSTATECTOMY FOR NODULAR HYPERPLASIA:

Specimens should be sliced every 3mm after fixation and examined for carcinomatous areas.

Sections:

Three sections from each left and right lobe.

One section from middle lobe.

PROSTATIC INTRAEPITHELIAL NEOPLASIA:

In 1926, Orteil[33] first described premalignant changes in prostate. In 1989, the term prostatic intraepithelial neoplasia (PIN) was coined and is defined as a cytologic alteration in architecturally normal glands.[35] Prostatic intraepithelial neoplasia is more common in the peripheral zone of the prostate (75%–80%), as in prostatic adenocarcinoma, and is extremely rarely in the

central zone (<5%).[76-78] The frequency of HGPIN in needle biopsy specimen is about 5% to 16% and in transurethral resection of the prostate specimens it is about 2.3% and 4.2%.[75]It comprises an intraluminal proliferation of the secretory epithelium revealing a spectrum of atypical cytological changes ranging from minimal changes to those that are indistinguishable from carcinoma.[34]

According to McNeal Bostwick criteria,three grades of PIN were identified.[36] Recently, PIN isdivided into two grades (low-grade and high-grade) instead of the previous three-grade system. Low-grade includes PIN 1 and PIN 2; high-grade includes PIN 3.

McNeal,[37]also described atypical adenomatous hyperplasia (AAH). It is characterized by an architectural alteration in cytologically unremarkable glands[38] .High grade PIN is the most likely precursor of carcinoma prostate because of its greater association with prostatic carcinoma. The other premalignant lesion AAH and its more common association with nodular hyperplasia than adenocarcinoma makes it a possible premalignant lesion to transition zone adenocarcinoma.

Histological features of PIN:[83]

At low power:

- Ducts are lined by darker cells.
- The ducts are thicker than normal ducts.
- It has a complex intraluminal patern of growth.

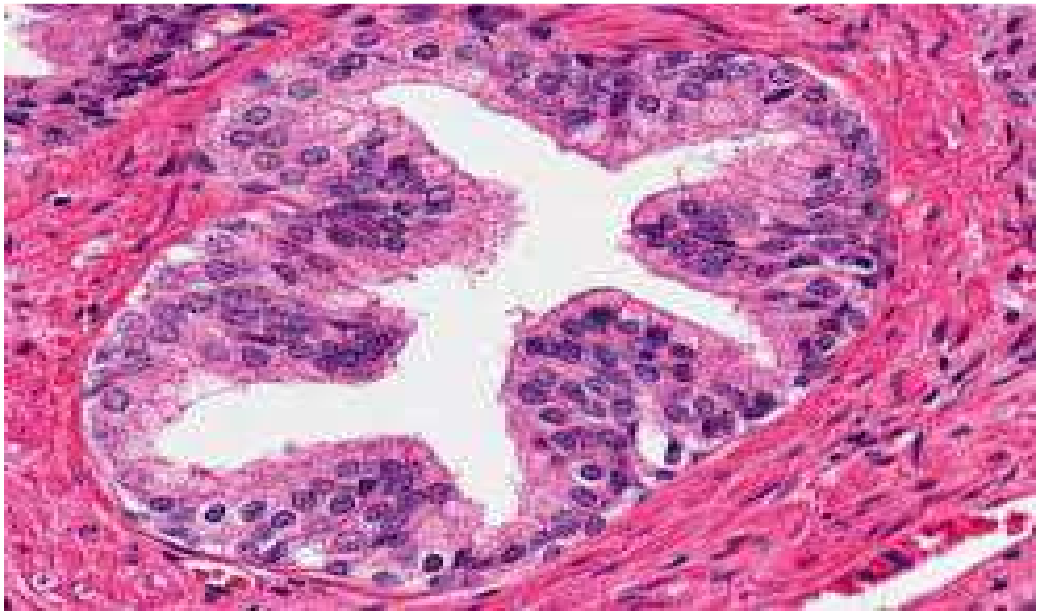
At high power:

- nuclear enlargement and nuclear stratification.
- Hyperchromasia
- Prominent nucleoli.

Histological features of Low grade PIN:

- Epithelial proliferation with cellular crowding.
- Nuclear stratification and nuclear enlargement.
- Nucleoli rare, if seen will be small.

FIGURE 6: HISTOLOGY OF LOW GRADE PIN

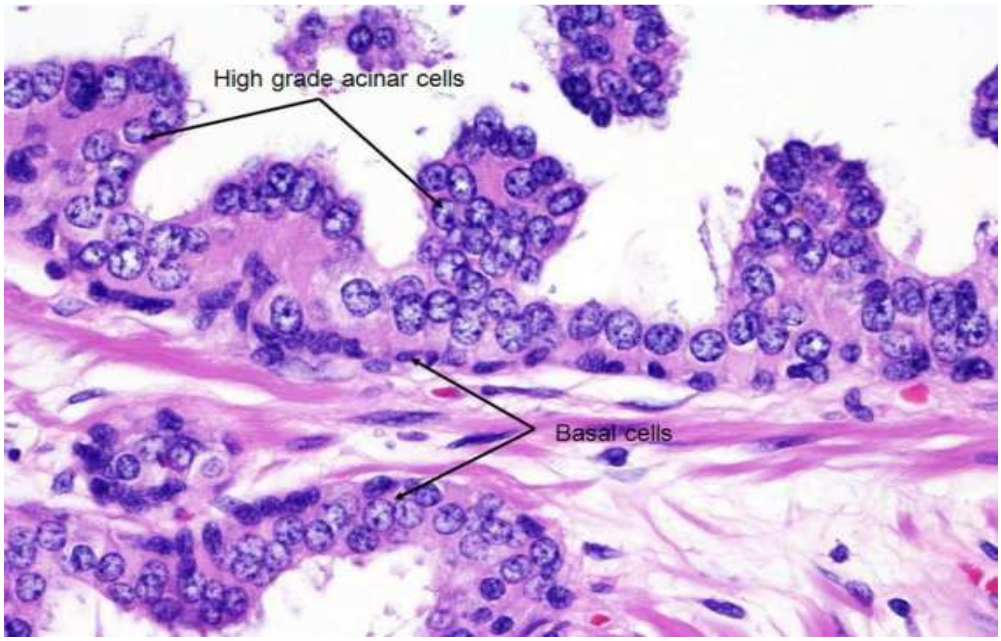


Histological features of High grade PIN:

- Nuclear enlargement and hyperchromasia
- One or more large nucleoli with clear halos.
- Mitotic figures are rare.

- Common patterns of HGPIN: tufting pattern(87%),micropapillary pattern(85%),cribriform pattern(32%),flat pattern(28%).[79]

FIGURE 7: HISTOLOGY OF HIGH GRADE PIN:



other histologic variants of PIN:[80-82]

- signet ring variant.
- Mucinous variant.
- Small cell neuroendocrine variant.
- Foamy variant.
- Inverted variant.
- With squamous differentiation.

BENIGN MIMICKERS OF PROSTATE CARCINOMA;

- **Prostatic atrophy:**

It is also known as simple or cystic atrophy and usually involves an entire lobe. It is characterized by presence of both atrophic and hyperplastic glands arranged in multiple lobules separated by fibrotic stroma.It is differentiated from prostatic adenocarcinoma by presence of inflammatory cells,corpora amylacea and discontinuous basal layer.

PROSTATIC HYPERPLASTIC LESIONS:

- **Benign prostatic hyperplasia:**

It is composed of glands varying from small and crowded glands to large glands with cystic dilatation exhibiting complicated growth pattern, such as papillary and branching. It is differentiated from adenocarcinoma by lacking malignant nuclear features and presence of basal cell layer. Adenocarcinoma shows the presence of luminal crystalloids or blue mucin at low power.

- **Basal cell hyperplasia:** BCH occurs in about 10% of peripheral zone by needle core biopsy. It is associated with the androgen therapy-related atrophy. It may present as glands with multilayered basal cells to solid basaloid nests composed of hyperplastic basal cells having basophilic cytoplasm and bland nuclear features without nucleoli. Morphologically, it can be florid, pleomorphic or atypical. Atypical BCH is chararcterised by proliferation of basal cells with prominent nucleoli and exhibiting nuclear atypia, intraluminal

secretions, intracytoplasmic hyaline globules, and very few mitoses. IHC for basal cells will help in distinguishing from prostatic adenocarcinoma.

- **Clear cells cribriform hyperplasia (CCCH):**

CCCH has been considered as a morphological variant of BPH. It consists of enlarged glands composed of anastomosing clear cells forming a cribriform growth pattern. It should be differentiated from high-grade PAA with cribriform pattern and can be differentiated by the presence of the nodular proliferation of cells with bland cytology in a cellular fibrous stroma, and the presence of basal cell layer.

- **Atypical adenomatous hyperplasia.:**

It is also known as adenosis.It is a well circumscribed lesion with a lobular appearance composed of small,round densely packed disorderly glands with medium sized nucleoli and luminal crystalloids.Basal can be either present or discontinuous.

- **Sclerosing adenosis:** It is similar to the lesion in breast. It is composed of variable sized glands lined by clear secretory cells and dark staining basal cells in a prominent sclerotic stroma. It may contain intraluminal mucin and prominent nucleoli.

METAPLASTIC LESIONS:

- **Mucinous metaplasia:**

It occurs in peripheral zone and composed of glands lined by tall columnar cells with rich mucin and basal nuclei. It resembles Cowper glands and positive for PAS, Alcian blue and Mucicarmine.

- **Paneth cell metaplasia:**

It can be neuroendocrine differentiation,exocrine differentiation or intestinal Paneth cell differentiation. It is usually associated with HGPIN and prostatic adenocarcinoma.

NON PROSTATIC LESIONS:

- **Nephrogenic adenoma:**

It is also known as adenomatoid or nephrogenic metaplasia.It is metaplastic response of urothelium to injury and is composed of following structural patterns- tubular, microcystic or papillary pattern exhibiting hobnail like or flat cells.

Wolffian duct:

Wolffian duct remnants are very rare in adults and has a lobular architecture composed of different sized glands without basal cells.It may also show papillary hyperplasia, intraluminal eosinophilic secretion ,fibrocystic change and sometimes infiltrative pattern. It can be differentiated from prostatic adenocarcinoma by negativity for PSA and PSAP.

- **Seminal colliculus:**

It is present near the entrance of seminal vesicles known as posterior urethral valves. They show nuclear pleomorphism and complex architecture and can be differentiated from adenocarcinoma by presence of basal cells.They contain cytoplasmic golden brown lipofuschin and luminal corpora amylacea.

- **Rectal glands:**

They are most likely to be found in TRUS guided prostatic biopsy. It is chararcterised by presence of distorted,hyperplastic glands containing intraluminal and extracellular mucin with prominent nucleoli and absence of basal cells. It can be differentiated by absence of PSA and PSAP staining.

TYPES OF PROSTATIC CARCINOMA:

Prostatic carcinoma is mainly divided into two categories

1) adenocarcinoma of secondary ducts and acini.
2) Adenocarcinoma of primary ducts.

Histologic features favouring Adenocarcinoma:[84]

CRITERIA FOR ADENO CARCINOMA

- Enlarged nuclei
- Nuclear hyperchromasia.
- Prominent nucleoli.
- Amphophilic cytoplasm.
- Sharp luminal border.
- Apoptotic bodies.
- Mitotic figures.
- Blue tinged mucinous material.
- Pink amorphous material.
- Crystalloids.
- Mucinous fibroplasias.
- Perineural invasion.
- Glomerulations.

ADENOCARCINOMA OF SECONDARY DUCTS AND ACINI:

SITE OF ORIGIN:

Most adenocarcinomas arise in the peripheral region of prostate[85]. Periurethral region is involved in later stages of the disease[86].Rarely carcinoma can occur in transitional zone.

HISTOLOGIC FEATURES:

- There are four major cytological patterns[87]-medium sized glands, small glands, diffuse individual cell infiltration and cribriform pattern.Gland forming types are usually lined by single layer of epithelium but occasional stratified epithelium may mimic late prostatic intraepithelial neoplasia. Most of the carcinomas exhibit a combination of the above four cytological patterns either synchronously or metachronously.
- The neoplastic cells show nuclear enlargement, irregular nuclear contour, hyperchromasia and macronucleoli[88] measuring more than 1 micron in diameter.
- Malignant glands contain intraluminal wispy blue mucin. Carcinomas with medium sized glands appear as closely placed glands with irregular outline and scanty intervening stroma under low power.

Carcinomas with small glands appear as expansive nodules with regular round small sized individual glands.

Diffuse individual cell infiltration pattern resembles lobular carcinoma of breast. Cribriform pattern is considered as intraductal carcinoma as the basal layer is preserved[89].

Glomeruloid pattern is recently discovered which is characterized by intraluminal ball like clusters of tumour cells[90].

Squamous metaplasia is uncommon and presents as high grade carcinomas and has poor prognosis[91].

Perinuerial involvement by the tumour cells is common. It is due to spread of neoplastic glands along the planes of least resistance and its presence in needle biopsy indicates Mucinous fibroplasias or collagenous micronodules is deposition of basophilic ground substance in the surrounding stroma.[92]

PROTEIN CRYSTALLOIDS:[93]

10-23% of prostatic carcinomas especially the ones having medium sized glands show protein crystalloids in their lumen.

Although it is seen in benign glands, its presence is an indicator of malignancy. They predominantly contain inorganic sulfur.

VARIATIONS IN PROSTATIC ADENOCARCINOMA:

- Prostatic adenocarcinoma with atrophic features:

It is composed of tumour cells with scanty cytoplasm and the nuclei occupies the entire cell. It mimics benign hyperplasia and is differentiated by presence of infiltrative growth and cytological features of malignancy.

- Pseudohyperplastic variant:

It shows papillary folding, branching, corpora amylacea and microcystic pattern, all resembling hyperplasia. It is differentiated by cytological features of malignancy and adjacent PIN[95].

- Foamy gland variant:

Grossly the tumour has soft consistency and a bright yellow colour and hence difficult to identify on digital examination.

The tumour cell cytoplasm has finely granular appearance or clear foamy appearance due to accumulation of lipids.

It has a low gleason score but is an aggressive tumour.[94]

CARCINOMAS OF PRIMARY DUCTS:

It occurs in large ducts in periurethral region[96].

Grossly it is either polypoid villus or an infiltrative growth involving urethra.

TYPES OF PRIMARY DUCT CARCINOMA:

Large duct adenocarcinoma;

Microscopically it shows large dilated ducts arranged in cribriform or papillary pattern lined by columnar pseudostratified epithelium [97]with occasional clear cell (mesonephroid).

Occasionally it shows pagetoid spread in the prostatic urethra.

It presents at more advanced stage and has higher short term survival rate. Endometrioid(endometrial)type adenocarcinoma[98]:

It is a variant of large duct adenocarcinoma.

It arises from prostatic utricle which is a mullerian remnant.

Primary urothelial carcinoma:[99]

It constitutes less than 2% of prostatic carcinomas.

It arises from the outer portion of periurethral ducts draining into urethra which is lined by urothelium.

Primary urothelial carcinoma from urethra or bladder should be excluded before making a diagnosis of primary urothelial prostatic carcinoma.

Mixed adenocacinoma and urothelial carcinoma of prostate:

It contains both glands and urothelial component in varying proportions.

OTHER MICROSCOPIC VARIANTS:

CARCINOMA WITH NEUROENDOCRINE FEATURES[100]:

About 80% of normal or hyperplastic prostate have endocrine cells with argentaffin-argyrophil properties, serotonin, calcitonin,bombesin,somatostatin and dense core granules on ultastructural examination.

About 10% to half of typical adenocarcinomas show endocrine features. Prostatic carcinoma with neuroendocrine differentiation typically express estrogen inducible pS2 protein.

Some prostatic carcinoma resemble typical or atypical carcinoid tumour. Strong positivity for PSA and PAP indicates prostatic origin. The endocrine

component shows positivity for adrenocorticotropic hormone, beta endorphin and calcitonin.

Small cell carcinoma: It presents as pure formor associated with adenocarcinoma either synchronously or metachronously.

It causes cushing syndrome or inappropriate anti diuretic hormone secretion.

It is an aggressive tumour and should be differentiated from high grade prostatic adenocarcinoma.

Large cell neuroendocrine carcinoma: In most cases it arises after long term hormonal therapy for prostatic adenocarcinoma.

MUCIN SECRETING ADENOCARCINOMA[101]:

This tumour contains large amounts of intracellular and extracellular mucin comprising about 25% of tumour.

Microglandular, comedo,cribriform,hypernephroid and solid variants can be seen.

The mucin secreted by well differentiated adenocarcinomas is non-o-acylated sialomucins whereas poorly differentiated adenocarcinomas secrete mono-o-acylated sialomucins but mucinous adenocarcinomas secrete mono- , di-, and tri-o-acylated sialomucins.

MUC2 expression is seen.PSA and PAP are positive.

In contrast to usual adenocarcinomas, this type shows rare bone metastasis, lesser response to radiation therapy and are not hormone dependent.

It should be differentiated from large bowel mucinous carcinoma extension and Cowper's gland carcinoma.

SQUAMOUS CELL CARCINOMA;

It is a rare carcinoma and it can occur either de nova or following hormonal therapy. Grossly presents as a circumscribed nodule in transition zone[102]. Closely related to adenosquamous carcinoma.

ADENOSQUAMOUS CARCINOMA:

It occurs either de nova or following radiation or hormonal therapy.

SIGNET RING CARCINOMA:

It is a highly malignant neoplasm composed predominantly of signet ring cells.

It can have solid, acinar or Indian file pattern.

The cells contain microvilli lined intracytoplasmic lumen[103].

ADENOID BASAL CELL TUMOUR[104]:

Also known as basal cell carcinoma and adenoid cystic like tumour. It resembles adenoid cystic carcinoma of salivary gland but has a more indolent course. Microscopically,it presents as an expansile growth,multinodularity with cribriform pattern and with luminal- basal lumina like material with

surrounding fibromyxoid stroma. Squamous differentiation and basal cell hyperplasia can be seen.

PSA and PAP is usually negative or focally positive.

This variant should be differentiated from adenocarcinoma with cribriform form of glands ,basal cell hyperplasia, basaloid carcinoma and true adenoid cystic carcinoma.

BASALOID CARCINOMA:

It is a highly aggressive neoplasm and should be differentiated from aenoid basal cell carcinoma. This carcinoma shows elevated expression of BCL2 and high Ki 67 index.

SARCOMATOID CARCINOMA:

The epithelial component is mostly commonly adenocarcinoma but squamous features can also be seen. Sarcomatoid element is either non specific spindle cells or giant cell features showing differentiation toward cartilage , skeletal muscle or bone[105]. Pleomorphic giant cell adenocarcinoma is a subtype of sarcomatoid carcinoma.

LYMPHOEPITHELIOMA LIKE CARCINOMA;

TUBULOCYSTICNCLEAR CELL ADENOCARCINOMA:

Rare cases similar to mullerian type clear cell adenocarcinoma and clear cell carcinoma of renal cell carcinoma have been reported.

TUMOUR METASTASIS:

Prostatic carcinoma initially spreads within the prostate,ducts and acini, fibromuscular stroma , perineural spaces and blood vessels.[40] Invasion of fibromuscular layer of prostate (capsule) is more common. It may also spread to seminal vescicles, distal aspect of the gland, bladder and very rarely prostatic urethra and rectum.

Seminal vescicle invasion should be diagnosed only when the muscular wall of the organ is infiltrated by the tumour.[41]

Rectal invasion is rare because of Denonvillier's fascia which covers the posterior aspect of the prostate.[42] It can present as anterior rectal mass , subserosal implants or as circumferential infiltration causing annular rectal stricture. Prostatic carcinoma most commonly metastasize to the skeletal system and lymph nodes.

SKELETAL METASTASIS:

Skeletal metastasis can be either multiple or solitary. Most commonly multiple. They are chararcteristically osteoblastic but can also be mixed or even osteolytic. Lumbar spine, sacrum and pelvis are the more common sites. Spread to these sites is via Batson's vertebral plexus[43] .Metastasis to other bones is through systemic circulation. Spinal metastasis may present as cord compression due to epidural mass and base of skull metastasis may present as cranial nerve defects.

Radiographically, osteoblastic metastasis should be differentiated from Paget disease and osteosarcoma. Microscopic examination shows clusters of malignant glands surrounded by abundant new bone formation. Patient may present with hypocalcemia, hypophosphatemia and serum alkaline phosphatase.

NODAL METASTASIS:

The tumour first spreads to pelvic group and later to retroperitoneal nodes. If retroperitoneal nodal metastasis occurs in absence of pelvic nodal group, then the patients are likely to have liver and lung metastasis. Periprostatic, periseminal vescicle and perirectal nodes can also be involved[44].

Sometimes, left supraclavicular and mediastinal nodes can also be involved. When these nodes are involved, the tumour is usually poorly differentiated and immunohistochemical staining for PAP and PSA is used for diagnosis.

OTHER METASTATIC SITES:

Lung metastasis present with massive pleural effusion and mostly exhibit lymphangitic pattern of spread. Microacinar, tubulopapillary and carcinoid like growth patterns can be seen. when large ducts are involved they mimic metatatic colonic carcinoma. Other sites include testis, breast in patients taking estrogens, liver, adrenal gland, dura, eye, skin, umbilicus, penis and salivary gland.

GLEASON MICROSCOPIC SCORING SYSTEM;

The Gleason scoring system is named after Donald Gleason, a pathologist who developed it with his colleagues at that facility in the 1966s.[50]

The (2005 ISUP modified) [51,52]Gleason score of prostatic biopsy includes the Gleason grade or the most extensive primary pattern plus the second most common pattern that is the secondary pattern, if two are present. If only one pattern is present then it should be doubled to yield the Gleason score.

Gleason grading of prostatic adenocarcinoma should be typically performed using the 4x objective, although in certain instances such as in back-to-back glands arrangement and in fused glands require higher magnification at 10x objective[54].

For three grades, the Gleason score is calculated by adding the most common grade plus the highest grade, irrespective of its extent. When a carcinoma is largely grade 4/5, identification of < 5% of Gleason grade 2 or 3 glands should not be included in the Gleason score.

In addition to reporting of the carcinoma features for each biopsy, an overall Gleason score based on the carcinoma-positive biopsies shoud be given.

The 2014 ISUP Gleason grading conference of prostatic carcinoma has introduced the concept of the grade groups of PCa, in order to:

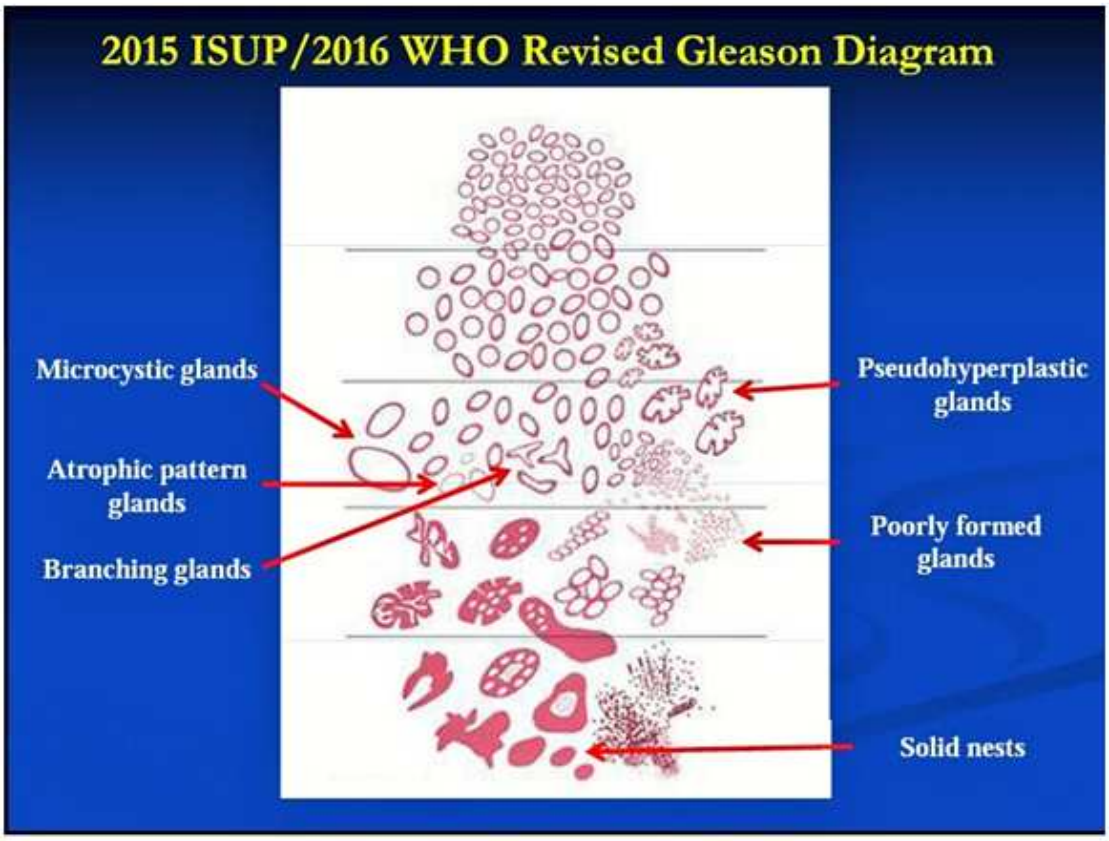
1. align the PCa grading with the grading of other carcinomas;

2. eliminate the anomaly that the most highly differentiated PCas have a Gleason score 6;

3. to further codify the clinically highly significant distinction between Gleason score 7 (3 + 4) and 7 (4 + 3) PCa.

The ISUP 2015/2016 WHO prostate cancer grade groups therefore range from 1-5.

FIGURE 8: ISUP 2015/ WHO 2016 REVISED GLEASON SCORE:



Problems with the Current Gleason System[53]:

1) Scores 2-5 are currently no longer assigned and certain patterns that Gleason defined as a score of 6 are now graded as 7, thus leading to

contemporary Gleason score 6 cancers having a better prognosis than historic score 6 cancers.

2) The combination of Gleason scores into a 3-tier grouping (6,7,8-10) is used most frequently for prognostic and therapeutic purposes, despite 3+4=7 vs. 4+3=7 and 8 vs. 9-10 having very different prognoses.

3) In practice the lowest score is now assigned a 6, although it is on a scale of 2-10. This leads to a logical yet incorrect assumption on the part of patients that their cancer is in the middle of the scale, compounding the fear of their cancer diagnosis with the belief that the cancer is serious, thus leading to an expectation that treatment is necessary.

New Grading System[53]

The new 5 Grade Group system has been developed based on a study of >20,000 prostate cancer cases treated with radical prostatectomy and >5,000 cases treated by radiation therapy.

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 a lesser component of poorly-formed/fused/cribriform glands

Grade Group 3 (Gleason score 4+3=7) – Predominantly poorly formed/fused/cribriform glands with a lesser component of well-formed glands

Grade Group 4 (Gleason score 8)

- Only poorly-formed/fused/cribriform glands or

- Predominantly well-formed glands with a lesser component lacking glands or

- Predominantly lacking glands with a lesser component of well-formed glands

Grade Group 5 (Gleason scores 9-10) – Lacks gland formation (or with necrosis) with or w/o poorly-formed/fused/cribriform glands

For cases with >95% poorly-formed/fused/cribriform glands or lack of glands on a core or at Radical prostatectomy, the component of <5% well-formed glands is not factored into the grade. Poorly-formed/fused/cribriform glands can also be a more minor component.

PATTERNS IN GLEASON SCORING:

New category	Histological descriptions of new grading categories
Grade 1	• Only individual, discrete well-formed glands
Grade 2	• Predominantly well-formed glands with lesser component of poorly formed, fused, and/or cribriform glands
Grade 3	• Predominantly poorly formed poorly formed, fused, and/or cribriform glands with lesser component of well-formed glands
Grade 4	• ***Only*** poorly formed, fused, and/or cribriform glands ***or*** • Predominantly well-formed glands with lesser component lacking gland formation ***or*** • Predominantly lacking gland formation and lesser component of well-formed glands (but poorly formed, fused, and/or cribriform glands can be a minor component)
Grade 5	• Lacks gland formation – or has glands with necrosis -- with or without poorly formed, fused, and/or cribriform glands

FIGURE 9: GLEASON PATTERN I

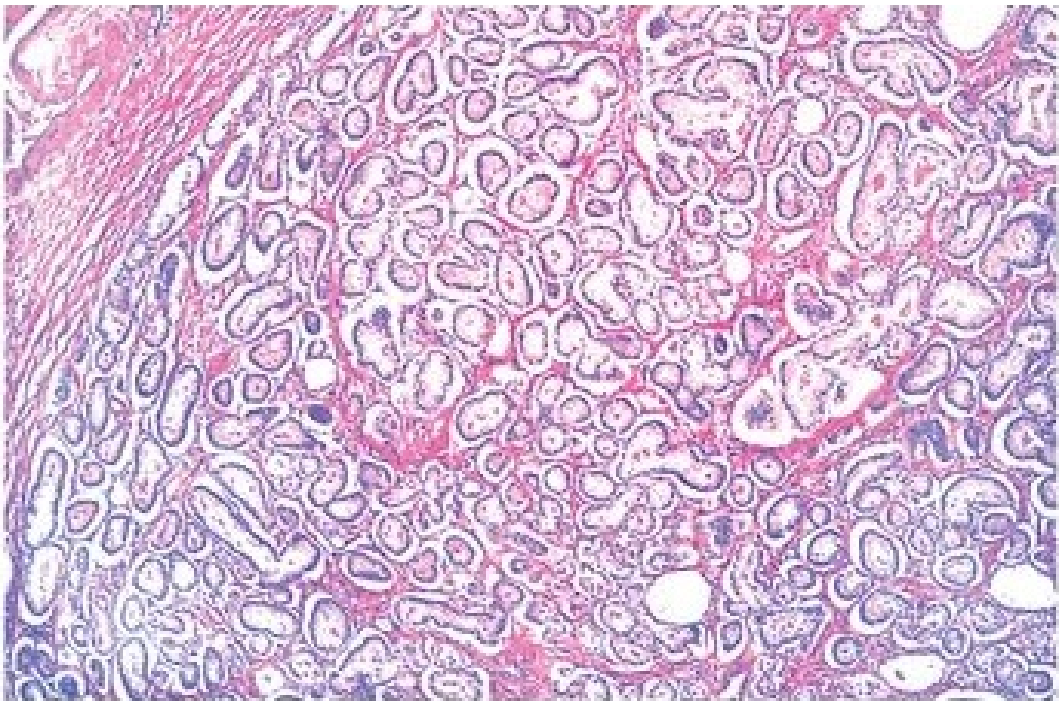


FIGURE 10: GLEASON PATTERN II

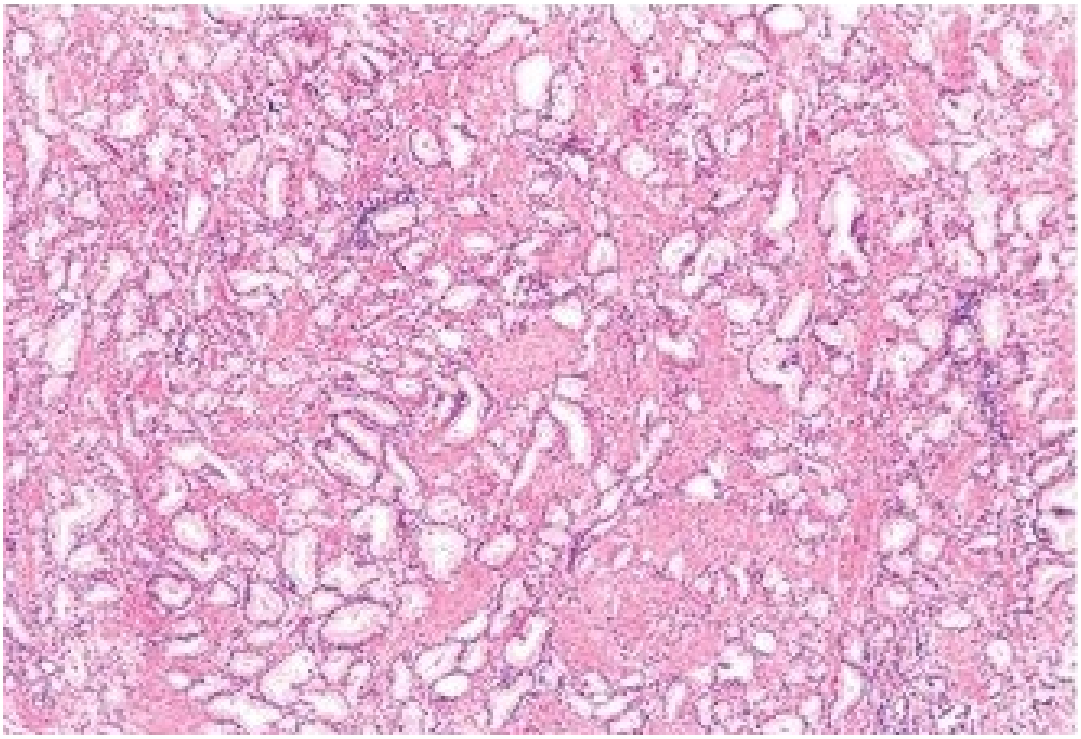


FIGURE 11: GLEASON PATTERN III

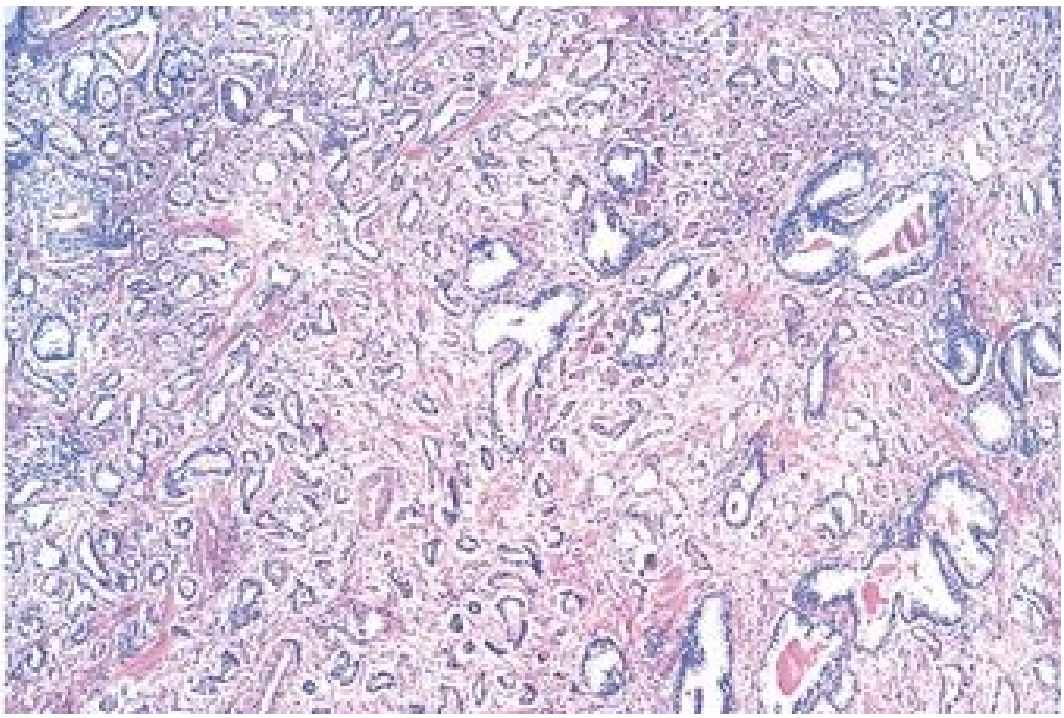


FIGURE 12: GLEASON PATTERN IV

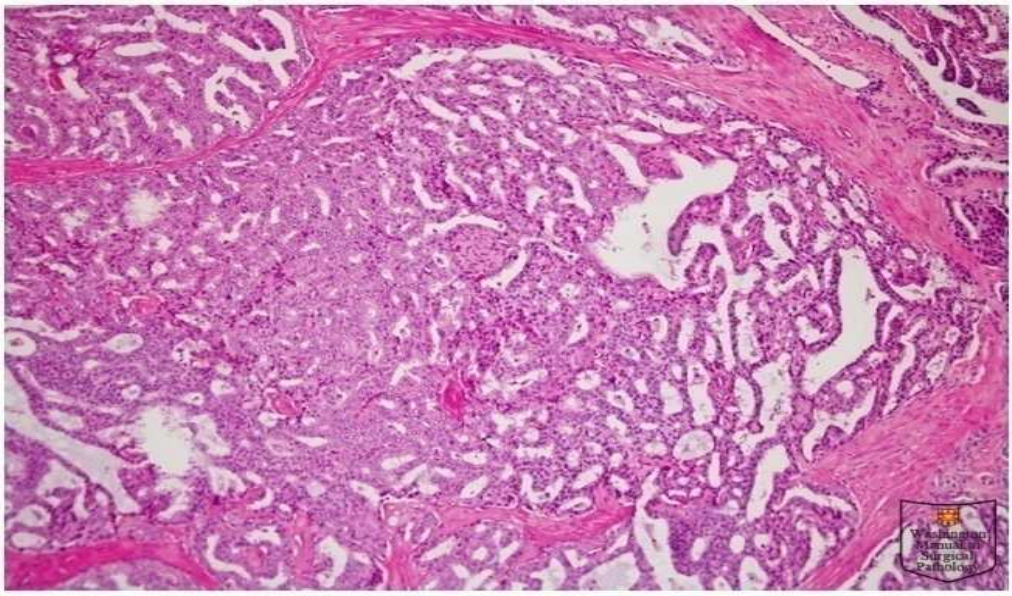
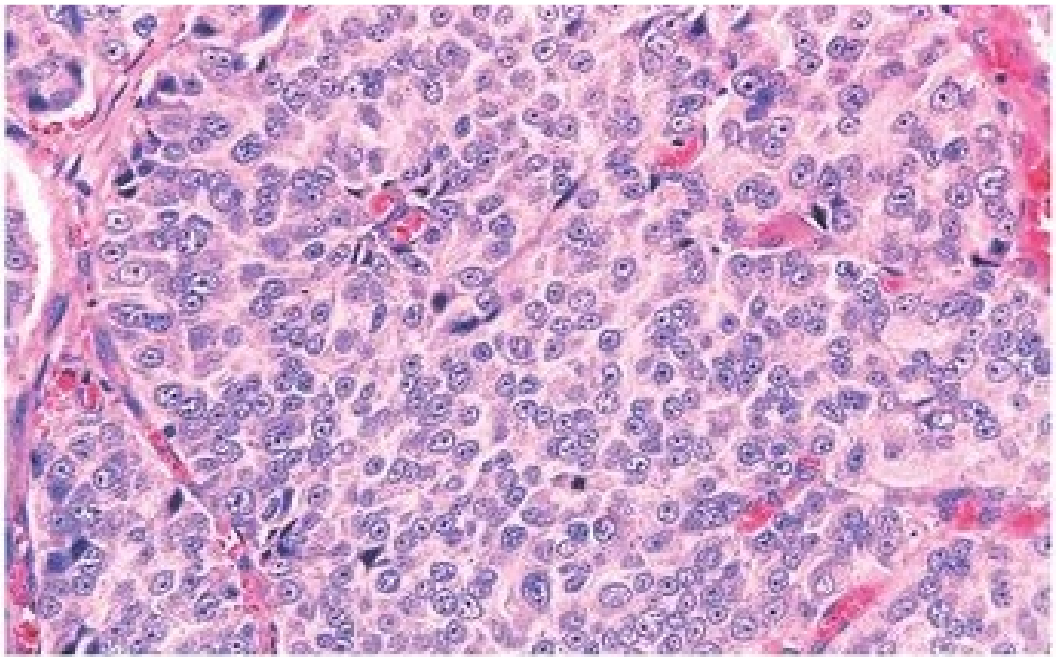


FIGURE 13 :GLEASON PATTERN V



GLEASON GRADE GROUPING:

GRADE GROUP	GLEASON SCORE AND PATTERN
1	Grade 6(3+3)
2	Grade 7(3+4)
3	Grade 7(4+3)
4	Grade 8(4+4, 3+5 , 5+3)
5	Grade 9 or 10(4+5,5+4 , or 5+5)

DIFFERENTIAL DIAGNOSIS:

- Atypical adenomatous hyperplasia.
- Atrophy.
- Crowded benign glands.
- Basal cell hyperplasia.
- Sclerosing adenosis.
- Cribriform hyperplasia.
- Mesonephric hyperplasia.
- Nephrogenic adenoma.
- Squamous metaplasia.
- Transitional cell metaplasia.
- Veromontanum mucosal gland hyperplasia.
- Prostatitis.
- Radiation atypia.
- Malakoplakia.
- Endometriosis.
- Cowper glands.
- Paraganglia in prostate.
- Benign glands adjacent to nerves and skeletal muscle.

HISTOCHEMISTRY:

The normal prostatic secretion is a neutral mucosubstance. In about two third of adenocarcinomas secrete acid mucosubstances.Acidic nature of the mucin should be suspected when the luminal content of the gland is basophilic in routine staining and it is confirmed with Alcian blue or colloidal iron staining.

IMMUNOHISTOCHEMISTRY;

The two important prostatic epithelial markers are PAP and PSA.

PSA has more specificity than PAP.

They do not differentiate between benign and malignant lesions in prostate but very useful in confirming prostatic origin in metastatic tumours.

They are absent in most undifferentiated tumours and in advanced cases following hormonal therapy. They are useful in differentiating poorly differentiated prostatic carcinoma and urothelial carcinomas.

PSA is localized to endoplasmic reticulum,vesicles,vacuoles and glandular lamina whereas PAP is localized to lysosomal granules.

PROSTATE SPECIFIC ANTIGEN:

It is a serine protease member of the family human glandular kallikrein. It is a 34 kD glycoprotein of 237 amino acids.It is exclusively synthesized by prostic acinar and ductal epithelium which is present in normal , hyperplastic and as well as in malignant prostatic tissue[106].

OTHERS IMMUNO MARKERS:

PROSTATE SPECIFIC MEMBRANE ANTIGEN(PSMA):

It is a type II membrane glycoprotein.

It is expressed by both benign and malignant prostatic epithelium with higher extent of staining in malignant epithelium.

It is also positive in high grade prostatic intraepithelial neoplasia (PIN) and in hormone refractory prostatic carcinoma[107].

Its expression correlates with Gleason score and staging.[108]

In addition to prostatic tissue, it is expressed in lesser amount in central and peripheral nervous system, small intestine and salivary gland.It is also positive in endothelial cells of neovasculature of many solid tumours.

PROSTEIN/P501S:

It is localized in golgi complex and hence shows perinuclear cytoplasmic and a speckled pattern. It is also positive in poorly differentiated

and metastatic prostatic carcinoma[109].It is positive even in PSA negative metastatic tumours.

P504S/ALPHA METHYLACYL COENZYME A RACEMASE(AMACR):

It has 97% sensitivity and 92% specificity[110].It is localized to peroxisomes. It I positive in high grade PIN, prostatic carcinoma as well as in both untreated and hormone refractory prostatic carcinoma metastasis. As it is positive in high grade PIN and benign mimics of prostatic carcinoma such as glandular and partial atrophy and in adenosis, it is of limited value as an individual marker. A panel of AMACR, HMWCK and p63 with negative basal cell markers is used for identifying atypical prostatic glands.

HIGH MOLECULAR WEIGHT CYTOKERATINS[111]:

It is used in identifying the presence or absence of basal cells in atypical prostate glands. 34BE12 is the most commonly used HMWCK. CK5/6 can be used alternately.

P63:

It is expressed in basal cells and is absent in secretory cells and neuroendocrine cells of prostate and hence is absent in prostatic carcinoma and is expressed in basal cells of benign glands[45,46,47].

Signoretti *et al* reported that all basal cells express p63 and hence this marker can be useful in distinguishing benign lesions from prostate malignancy [48].

Person *et al* showed that p63 is expressed in normal basal cells and benign prostate hyperplasia (BPH). It can be focally expressed in prostate atrophy and HGPIN, but p63 expression is absent in the majority of prostate adenocarcinomas.[49] Recent studies show that P63 gene is essential for normal stem cell function in prostate.

NKX3-1:

It is a prostate specific androgen regulated homeobox gene involved in tumour differentiation and its loss of function causes carcinogenesis. It is superior to PSA in poorly differentiated prostatic carcinoma[112].

OTHER IHC MARKERS:

HER2/NEU protein is overexpressed in androgen independent prostatic carcinoma.

CDX2 nuclear staining is occasionally present causing difficulty in differentiating from intestinal adenocarcinoma.

They also show positivity for EMA , CEA , Leu7 , cathepsin D, B72.3, parathyroid hormone related protein,gastric acid proteinase gastricism, erythropoietin/erythropoietin receptor and glycoprotein A-80.

Protatic adenocarcinomas show reduced expression of E-cadherin and catenin/E-cadherin complex.

PROGNOSIS:

In 1999,the College of American Pathologists(CAP) involved a group of clinicians, pathologists and statisticians and established the following categories for as prognostic indicators of prostatic carcinoma.[39]

Prognostic factors categorized by CAP:

I- Proven to be of prognostic importance and useful in clinical patient management:

Preoperative serum PSA level

TNM stage grouping

Histologic grade as Gleason score

Surgical margin status.

II- Extensively studied but whose importance remains to be validated:

Tumour volume

Histologic type

DNA ploidy

III- Not sufficiently studied to demonstrate their prognostic value:

Perineurial invasion

Neuroendocrine differentiation

Microvessel density

Nuclear roundness

Chromatin texture

Karyometric factors

Proliferation markers

PSA derivatives

Other factors such as pncogenes , tumour suppressor genes , apoptosis genes.

BENIGN PROSTATIC HYPERPLASIA

INTRODUCTION:

The term nodular hyperplasia as proposed by Moore is a more exact designation. Benign prostatic hyperplasia (BPH) shows nodular enlargement of gland and histologically shows unregulated proliferation of connective tissue, smooth muscle and glandular epithelium within the prostatic transition zone[113]. The weight of the increases above 20 grams which is considered as normal for adult individuals.Prostate tissue is made up of two basic elements: A glandular element composed of secretory ducts and acini and a stromal element composed primarily of collagen and smooth muscle. In BPH, cellular proliferation leads to increased prostate volume and increased stromal smooth muscle tone.

McNeal [114]describes two phases of BPH progression. The first phase shows an increase in BPH nodules in the periurethral zone and the second a significant increase in size of glandular nodules.

CLINICAL FEATURES:

BPH causes physical compression of the urethra resulting in anatomic bladder outlet obstruction (BOO) through two distinct mechanisms[115]: First, the static component which is associated with an increase in prostate volume; second, the dynamic component, associated with an increase in stromal smooth muscle tone. BOO may clinically present as lower urinary tract symptoms (LUTS), urinary tract infections, acute urinary retention (AUR), renal failure hematuria, and bladder calculi[116].

ETIOPATHOGENESIS:

Age

The prevalence of BPH rises markedly with increase in age. Autopsy studies show a histological prevalence of 8%, 50% and 80% in the 4 th , 6 thand 9 th decades of life, respectively[116]. Krimpen and Baltimore Longitudinal Study of Aging (BLSA) cohorts shows that Prostate volume also increases with age, suggesting a prostate growth rate of 2.0% to 2.5% per year in older men[117-119].

Geography

Several international studies have shown geographic heterogeneity in prostate volume and LUTS prevalence. Significantly lower prostate volumes have been observed in men from Southeast Asia when compared to western populations[120].

Genetics

Several studies suggests that there are genetic components to both BPH and LUTS and they show an autosomal dominant pattern of inheritance[121]. Men with inherited forms of BPH tend to have a larger volume prostates and earlier age of onset of clinical symptoms than men with sporadic BPH[122].

Monozygotic twin concordance rates of 63% and 26% have been observed for LUTS and BPH, respectively, with one study estimating that genetic factors may contribute as much as 72% to the risk of high-moderate or severe LUTS among older men[123,124].

Sex steroid hormones: Testosterone, dihydrotestosterone and estrogen

The hormone 5-alpha reductase present in prostatic secretory cells converts testosterone to DHT which is a potent stimulator of prostate growth and in addition to prostate development, it also plays a central role in BPH pathogenesis. Many studies shows that higher serum testosterone concentrations do not promote BPH and even are protective. Several studies have noted a nearly three times increased risk of BPH with increased serum concentrations of DHT and its metabolites than in cases with lowest levels[125].

17b-diol-glucuronide and androstanediol glucuronide are the metabolites of DHT and are surrogate markers for DHT activity, with higher concentrations indicating increased and lower concentrations decreased levels of DHT. Five-alpha reductase inhibitors such as finasteride and dutasteride

decrease serum concentrations of DHT [126] and prevent clinical progression of BPH and LUTS.

Though there is no clear patterns of estrogen, BPH and LUTS, one study has observed increased efficacy for reducing stromal cell proliferation in BPH[127] through the use of selective estrogen receptor modulators in combination with five-alpha reductase inhibitors.

The metabolic syndrome and cardiovascular disease

The metabolic syndrome is a collection of metabolic abnormalities - obesity, glucose intolerance, dyslipidemia and hypertension - that increases the risk of cardiovascular disease and results primarily from dietary and other life-style practices.[128]

One study shows that men diagnosed with at least three components of the metabolic syndrome had an 80% increased prevalence of LUTS compared with those with no components. Other studies have shown that men with heart disease are at significantly increased risk of both BPH and LUTS[129,130]. These observations are important because they suggest novel targets for prevention and treatment.

Obesity

Prior studies observed that greater the amount of adiposity, the greater the prostate volume. Body weight, body mass index (BMI) and waist circumference have all shown to be associated with increased prostate volume[131]. In the BLSA cohort, for example, each 1 kg/m ^{2} increase in BMI corresponded to a 0.41 cc increase in prostate volume. Moreover, obese (BMI $\geq$ 35 kg/m ^{2}) participants had a 3.5-fold increased risk of prostate enlargement compared with non-obese (BMI <25 kg/m ^{2}) participants[132].

Obesity also increases the risks of BPH surgery, initiation of BPH medical therapy and LUTS and decreases the efficacy of finasteride and dutasteride.

Diabetes and disruptions in glucose homeostasis

Higher serum concentrations of IGF-1 and insulin-like growth factor binding protein 3 have been associated with increased risk of clinical BPH[133]. Diabetes, increased serum insulin and elevated fasting plasma glucose have been associated with increased prostate size and increased risks of prostate enlargement. Similarly, diabetic men on medical therapy had decreased risk of moderate/severe LUTS compared with those men not on medications.

Physical activity

Increased physical activity and exercise have consistently shown decreased risks of BPH. Moderate to vigorous physical activity reduced the risk of BPH by 25% relative to a sedentary life-style.[134] The magnitude of the protective effect increases with increasing levels of physical activity.

Diet;[135-137]

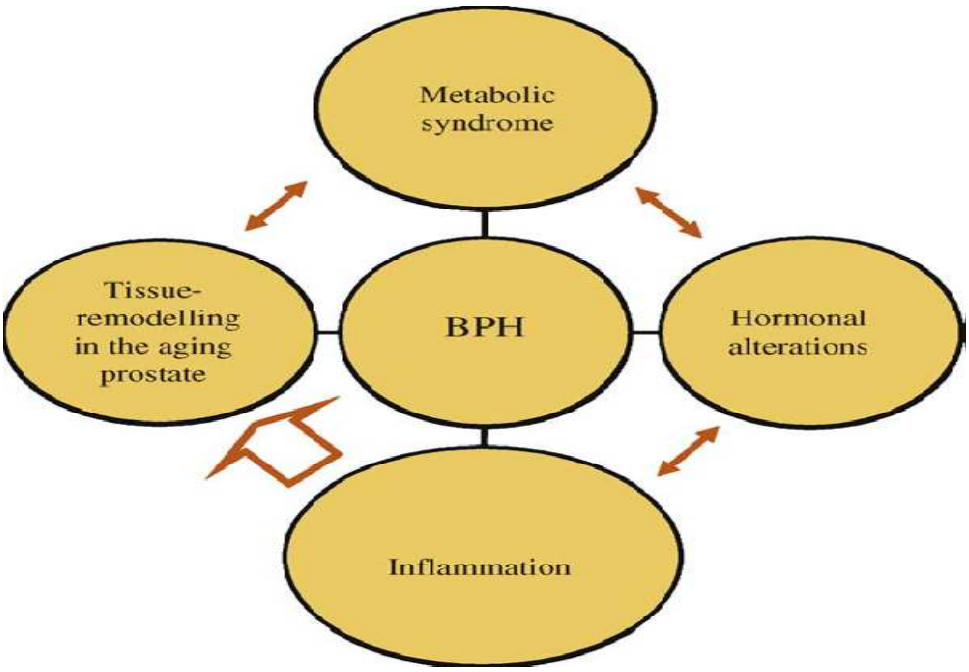
There are some indications that both macronutrients and micronutrients may affect the risk of BPH although the patterns are inconsistent. Foods like red meat, fat, milk and dairy products, cereals, bread, poultry and starch potentially increase the risks of symptomatic BPH whereas vegetables (particularly carotenoids), fruits, polyunsaturated fatty acids, linoleic acid, Vitamin A and Vitamin D potentially decrease the risks of symptomatic BPH. Higher circulating concentrations of micronutrients such as vitamin E, lycopene, selenium and carotene have decresed risks of BPH whereas zinc and vitamin C have been associated with both increased and decreased risk.

Inflammation

The metabolic syndrome which causes systemic inflammation and oxidative stress is the link between inflammation and BPH. Inflammation has been implicated as a cause for prostate carcinogenesis and BPH represents a proliferative pathway promoted by oxidative stress and inflammatory mediators[138]. the extent and severity of the inflammation corresponds to the magnitude of enlargement of prostate. A history of prior infection with

gonorrhea, chlamydia or trichomonosis increases the risk of elevated PSA[139,140]. inhibition of inflammatory pathway by non-steroidal anti-inflammatory (NSAID) use decreases the risk of LUTS, low urinary flow rate, increased prostate volume and elevated PSA[141].

FIGURE 13:PATHOGENESIS AND RISK FACTORS OF BENIGN PROSTATIC HYPERPLASIA.



GROSS FEATURES:

The enlarged gland shows multiple nodules of varying sizes with a gray to yellow colour and a granular appearance are seen projecting above the cut surface. Cross section of the entire gland shows nodular hyperplasia beginning in the periurethral and transitional zones i.e the portions around the urethra and where the ejaculatory ducts enter the urethra.

MICROSCOPIC FEATURES:

Stromal proliferation either concentric or eccentric in the small sinusoidal spaces in the periurethral , periductal and intralobar regions is the earliest change. The stromal component contains more smooth muscle and less elastic tissue than the normal stroma. Hyperplasia of glandular component follows stromal proliferation and so well developed nodules show varying proportions of both components[142].

The glandular component consists of cystically dilated glands containing corpora amylacea which sometimes may be calcified. The glands are lined by flat to columnar epithelium sometimes showing Functional polarization i.e flat to columnar epithelium facing each other in same gland. The epithelial cells have pale cytoplasm, regular centrally located nuclei with inconspicuous nucleoli. Papillary infoldings are commonly seen. The glands also contain continuous basal cell layer above a well developed basement membrane. Small clusters of lymphocytes are seen in the interstitium and around the ducts.

Microscopic variants:

Some variations occur due to overgrowth of one component over the other. Distinctive patterns resembling breast lesions are named accordingly; they include sclerosing adenosis, fibroadenoma like, phylloides tumour like, leiomyoma like, fibromyxoid nodules and bizarre cells in stroma.

IMMUNOHISTOCHEMISTRY:

P27 protein, a negative regulator of the cell cycle is predominantly expressed in epithelial and stromal cells of normal prostatic gland but is negative in nodular hyperplasia[143].

MATERIALS AND METHOD

MATERIALS AND METHODS

It is a prospective and retrospective study conducted on benign prostatic hyperplasia and prostatic carcinoma done in Institute of pathology, the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General hospital, Chennai during the period of two years between September 2015 to August 2017.

SOURCE OF DATA:

INCLUSION CRITERIA:

1.All cases of benign prostatic hyperplasia.

2. All cases of prostatic adenocarcinoma.

EXCLUSION CRITERIA:

1.Prostatitis.

METHOD OF DATA COLLECTION:

Of the total cases of benign prostatic hyperplasia and prostatic adenocarcinoma reported during this study period, p63 expression was studied for 25 cases of benign prostatic hyperplasia and prostatic carcinoma cases. Mucin histochemistry with Alcian blue at pH 2.5 was also done on the same 25 cases of benign prostatic hyperplasia and prostatic adenocarcinoma.Detailed history of the cases regarding age, type of procedure, were obtained for those

50 cases from surgical pathology records. Formalin fixed tissue were cut, processed and paraffin embedded.

4μm thick sections of the paraffin tissue blocks were cut and stained with eosin and hematoxylin.

Slides were collected from slide filing and were reviewed and 25 cases of prostatic adenocarcinoma was graded using the Gleason scoring system.25 cases of benign prostatic hyperplasia were randomly selected from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemical analysis of p63 expression and Alcian blue staining at pH2.5.

25 cases of prostatic adenocarcinoma was selected according to Gleason scoring and subjected to immunohistochemical analysis of p63 expression and Alcian blue staining at pH2.5.Slides were evaluated for expression of p63 and positivity for alcian blue staining. The results were recorded with photographs.

IMMUNOHISTOCHEMICAL ANALYSIS:

Immuno-histochemical analysis of p63 were done in Paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology.

4 μm thick sections from selected formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Heat induced antigen retrieval was done using micro wave method. The p63 antigen is

bound with mouse monoclonal antibody (PathnSitu). Later antigen antibody complex are detected by the addition of secondary antibody conjugated with horse radish peroxidase-polymer and Di-aminobenzidine substrate.

Positive control prostate and squamous cell carcinoma.

DETAILS OF IMMUNOHISTOCHEMICAL MARKER USED IN THE STUDY

ANTIGEN	VENDOR	CLONE	DILUTION	POSITIVE CONTROL
P63	PathnSitu	4A4 Mouse monoclonal	Ready to use.	Squamous cell carcinoma

IMMUNOHISTOCHEMISTRY PROCEDURE

Peroxidase –antiperoxidase immune complex technique which was developed in 1970 by Sternberger is the most commonly used technigue. The biotin-avidin immunoenzymatic technique developed by Heitzman and Richards in 1974 is the latest technique.

- 4 micron thick sections were cut from formalin fixed paraffin embedded tissue
- samples and transferred to positively charged slides.
- The slides were incubated at 58oC for overnight.
- The sections were deparaffinized in xylene for 15 minutes x 2 changes.
- The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.

- The sections were washed in tap water for 10 minutes.
- The slides were then immersed in distilled water for 5 minutes.
- Heat induced antigen retrieval was done with pressure cooker in appropriate temperature with appropriate buffer for 20 to 25 minutes.
- The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
- The slides were then rinsed in distilled water for 5 minutes.
- Wash with appropriate wash buffer for 5 minutes x 2 changes.
- Apply peroxidase block over the sections for 10 minutes.
- Wash the slides in buffer for 5 minutes x 2 changes.
- Cover the sections with protein block for 15 minutes.
- The sections were drained without washing and appropriate antibody was applied
- over the sections and incubated for one hour.
- The slides were washed in wash buffer for 5 minutes x 2changes.
- DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1ml of DAB buffer.
- DAB substrate solution was applied on the sections for 2 minutes.
- The slides were washed in distilled water for 5 minutes.
- The sections were counterstained with Hematoxylin stain.
- The slides were washed in running tap water for 3 minutes.
- The slides were air dried and mounted with DPX.

MUCIN HISTOCHEMISTRY:

4 μm thick sections from selected formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides and Alcian blue staining was done at pH 2.5 and counterstained with nuclear fast.

PROCEDURE OF ALCIAN BLUE STAINING:

Alcian blue stains acid mucosubstances and acetic mucins.Strongly acidic mucosubstances will be stained blue, nuclei will be stained pink to red, and cytoplasm will be stained pale pink.

Solutions and Reagents:

3% Acetic Acid Solution:

Glacial acetic acid ---------- 3 ml

Distilled water ---------- 97 ml

Alcian Blue Solution (pH 2.5):

Alcian blue, 8GX ---------- 1 g

Acetic acid, 3% solution ---------- 100 ml

Mix well and adjust pH to 2.5 using acetic acid.

0.1% Nuclear Fast Red Solution:

Nuclear fast red ---------- 0.1 g

Aluminum sulfate---------- 5 g

Distilled water ----------100 ml

Dissolve aluminum sulfate in water. Add nuclear fast red and slowly heat to boil and cool. Filter and use.

Procedure:

- Deparaffinize slides and hydrate to distilled water.
- Stain in alcian blue solution for 30 minutes.
- Wash in running tap water for 2 minutes.
- Rinse in distilled water.
- Counterstain in nuclear fast red solution for 5 minutes.
- Wash in running tap water for 1 minute.
- Dehydrate and through 95% alcohol, 2 changes of absolute alcohol, 3 minutes each.
- Clear in xylene or xylene substitute.
- Mount with resinous mounting medium.

CONTROL: Small intestine, appendix, or colon.

INTEPRETATION:

Strongly acidic sulfated mucosubstances ---------- blue

Nuclei ---------- pink to red

Cytoplasm ---------- pale pink

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

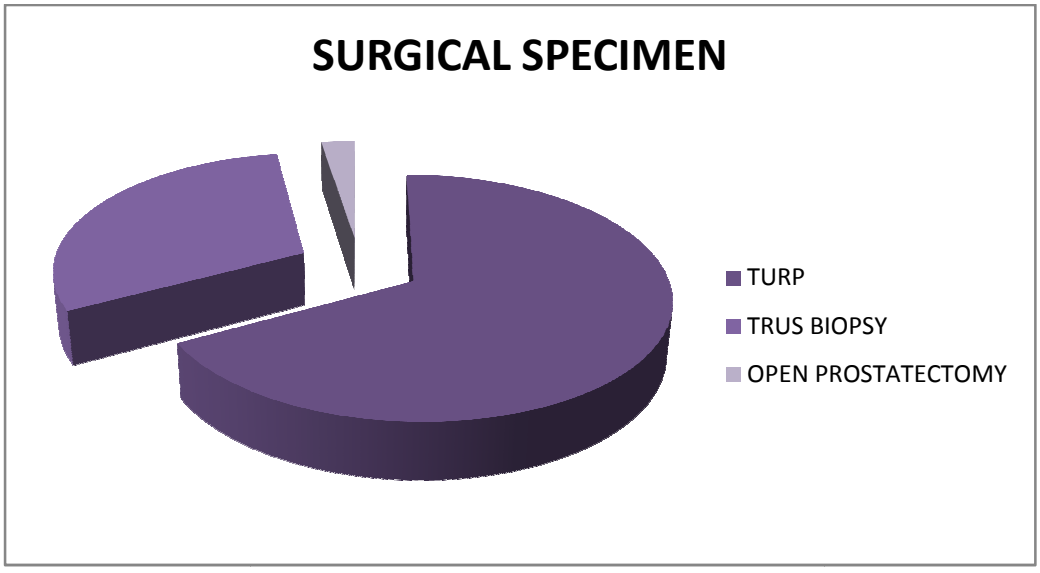
DISTRIBUTION OF CASES IN RELATION TO SURGICAL SPECIMENS

Out of total 328 cases analysed during the study period, 221 cases were TURP specimens, 99 cases were TRUS biopsy specimens and 8 cases of open prostectomy specimen.

TABLE 1: DISTRIBUTION OF CASES IN RELATION TO SURGICAL SPECIMENS:

SURGICAL SPECIMEN	TOTALNUMBER OF CASES	PERCENTAGE
TURP	221	67.07%
TRUS	99	30.46%
OPEN PROSTATECTOMY	8	2.46%

CHART 1: SHOWING DISTRIBUTION OF CASES AMONG SURGICAL SPECIMENS:



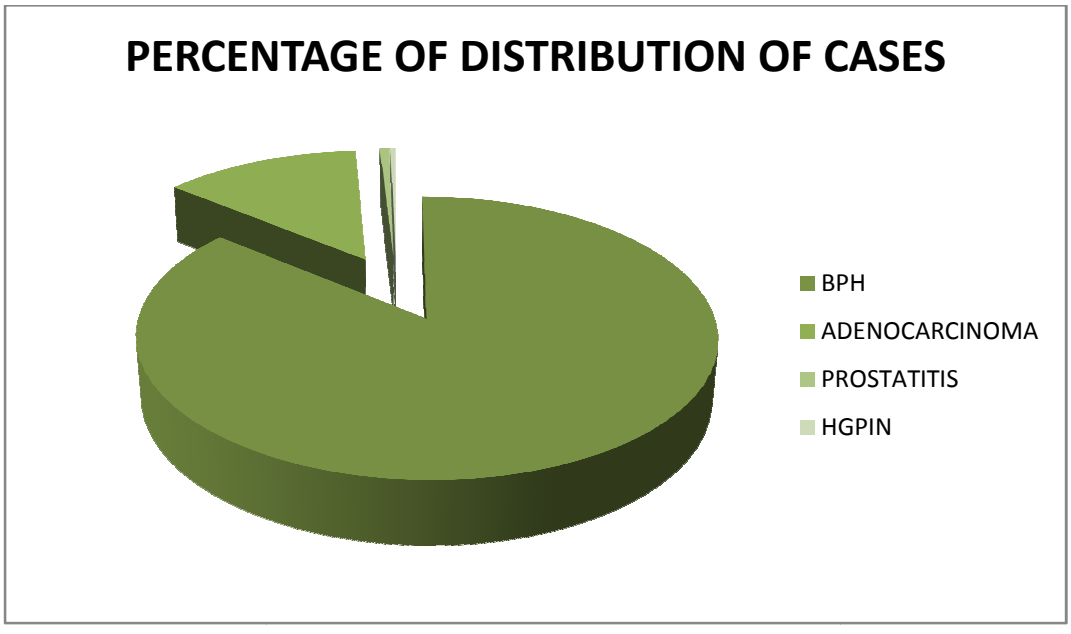
DISTRIBUTION OF PROSTATIC CASES BASED ON HPE DIAGNOSIS

Among the total 328 prostate cases in our department, 282 cases were benign prostatic hyperplasia followed by 43 cases of prostatic adenocarcinoma. Two cases were reported as prostatitis and one case of high grade prostatic intraepithelial neoplasia was reported.

TABLE 2: DISTRIBUTION OF PROSTATIC CASES BASED ON HPE:

HPE DIAGNOSIS	TOTAL NO OF CASES	PERCENTAGE
BPH	282	85.97%
ADENOCARCINOMA	43	13.10%
PROSTATITIS	2	0.6%
HGPIN	1	0.3%

CHART 2: SHOWING PERCENTAGE OF DISTRIBUTION OF CASES:



DISTRIBUTION OF CASES IN RELATION TO AGE

Among both BPH and prostatic adenocarcinoma, about 46.46% of patients were in the age group of 61-70 years followed by 22.15% of cases in the age group 51-60 years.

TABLE 3: DISTRIBUTION OF CASES IN RELATION TO AGE;

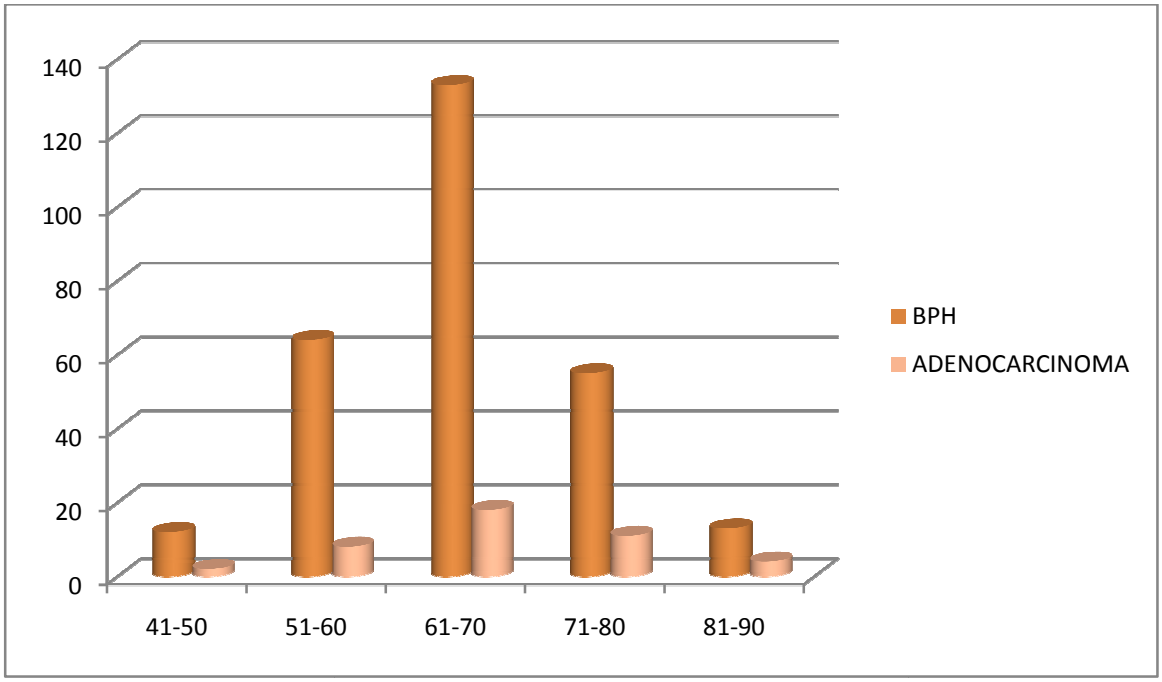
AGE	BPH	ADENOCARCINOMA	PERCENTAGE
41-50	12	2	4.30%
51-60	64	8	22.15%
61-70	133	18	46.46%
71-80	55	11	20.30%
81-90	13	4	5.2%
	274	43	

TABLE 4: DISTRIBUTION OF ADENOCARCINOMA CASES IN RELATION TO AGE:

AGE	ADENOCARCINOMA	PERCENTAGE
41-50	2	4.65%
51-60	8	18.60%
61-70	18	41.86%
71-80	11	25.58%
81-90	4	9.30%

Among prostatic adenocarcinoma cases, majority of the cases were seen in the age group of 61-70 years (41.86%) followed by age group of 71-80 years (25.58%).

CHART 3: SHOWING DISTRIBUTION OF CASES IN RELATION TO AGE:



DISTRIBUTION OF CASES BASED ON HPE DIAGNOSIS AND SURGICAL SPECIMEN

For BPH, TURP was the most commonly received specimen. Whereas, TRUS biopsy is the most common specimen received for diagnosis for prostatic adenocarcinoma.

TABLE 5: DISTRIBUTION OF CASES BASED ON HPE DIAGNOSIS AND SURGICAL SPECIMEN:

HPE DIAGNOSIS	TOTAL NUMBER OF CASES	TURP	TRUS	OPEN PROSTATECTOMY
BPH	282	210	64	8
ADENOCARCINOMA	43	16	27	-

TABLE 6: DISTRIBUTION OF ADENOCARCINOMA CASES IN RELATION TO SURGICAL SPECIMEN

SPECIMEN	NUMBER OF CASES	PERCENTAGE
TURP	16	37.20%
TRUS BIOPSY	27	62.79%

CHART 4: SHOWING DISTRIBUTION OF ADENOCARCINOMA CASES IN RELATION TO SURGICALSPECIMEN

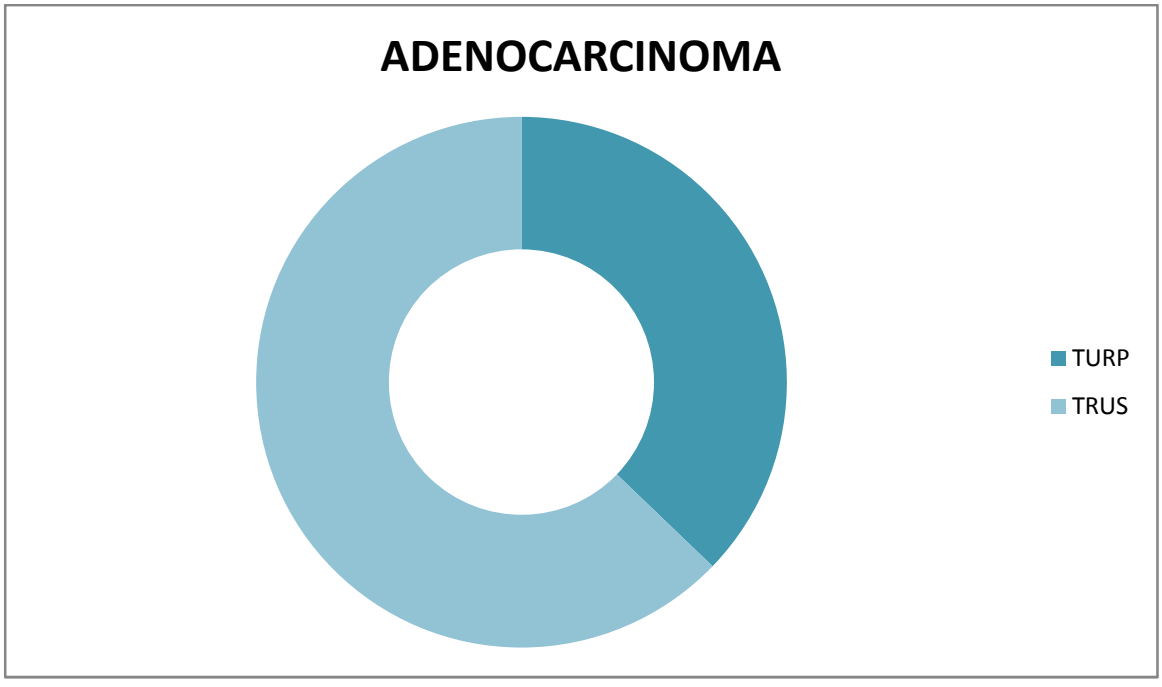
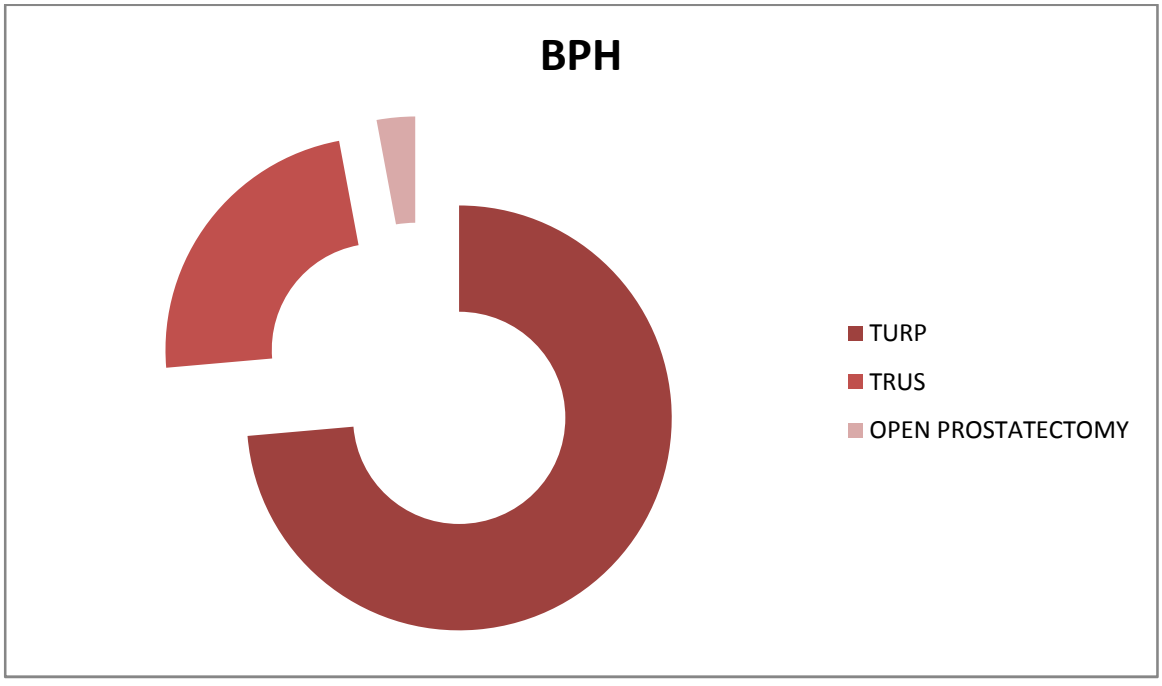


TABLE 7 : DISTRIBUTION OF BPH IN RELATION TO SURGICAL SPECIMEN

SPECIMEN	NUMBER OF CASES	PERCENTAGE
TURP	210	74.46%
TRUS	64	22.69%
OPEN PROSTATECTOMY	8	2.83%

CHART 5: SHOWING DISTRIBUTION OF BPH IN RELATION TO SURGICAL SPECIMEN



DISTRIBUTION OF ADENOCARCINOMA CASES SUBJECTED TO IHC AND ALCIAN BLUE IN RELATION TO GLEASON GRADING

Out of 43 cases of prostatic adenocarcinoma cases received during the study period, 25 cases were selected for IHC and Alcian blue staining. As Alcian blue staining differs with Gleason grading, the cases were selected according to their Gleason grading on H&E stain.

TABLE 8: DISTRIBUTION OF ADENOCARCINOMA CASES SUBJECTED TO IHC AND ALCIAN BLUE IN RELATION TO GLEASON GRADING

GLEASON GRADE	NO OF CASES	PERCENTAGE
I	1	4%
II	12	48%
III	4	16%
IV	4	16%
V	4	16%

RESULTS OF IHC STAINING

All of the 25 cases of BPH, subjected to p63 IHC staining, showed strong nuclear positivity in the basal layer of the hyperplastic glands. Whereas all of the adenocarcinoma cases were negative for p63 in the malignant glands indicating the absence of basal layer.

TABLE 9: EXPRESSION OF P63 AMONG BPH AND ADENOCARCINOMA CASES

HPE DIAGNOSIS	NO OF CASES POSITIVE FOR P63	PERCENTAGE
BPH	25	100%
ADENOCARCINOMA	0	0%

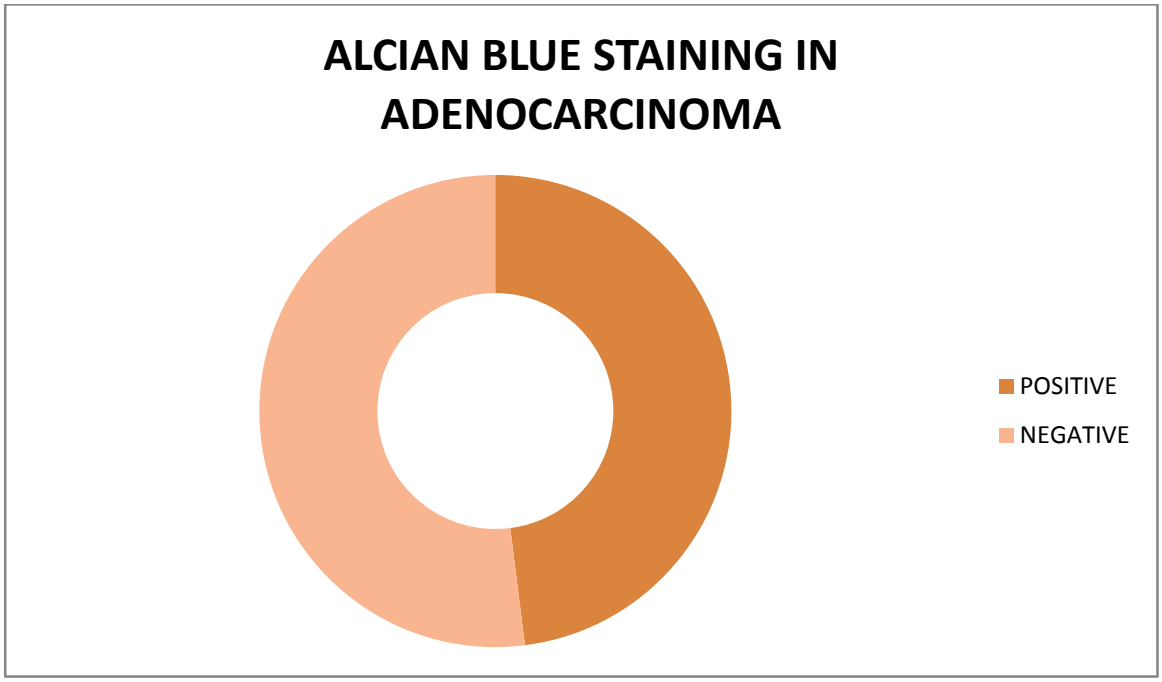
RESULTS OF ALCIAN BLUE STAINING

Alcian blue staining was seen in 13 cases of adenocarcinoma out of total 25 cases. Whereas, alcian blue staining was absent in all the cases of benign prostatic hyperplasia.

TABLE 10: ALCIAN BLUE POSITIVITY AMONG BPH AND ADENOCARCINOMA CASES.

HPE DIAGNOSIS	TOTAL NO OF CASES	NO OF CASES SHOWING ALCIAN BLUE POSITIVITY	PERCENTAGE
BPH	25	0	0%
ADENOCARCINOMA	25	12	48%

CHART 6: SHOWING ALCIAN BLUE STAINING IN ADENOCARCINOMA:



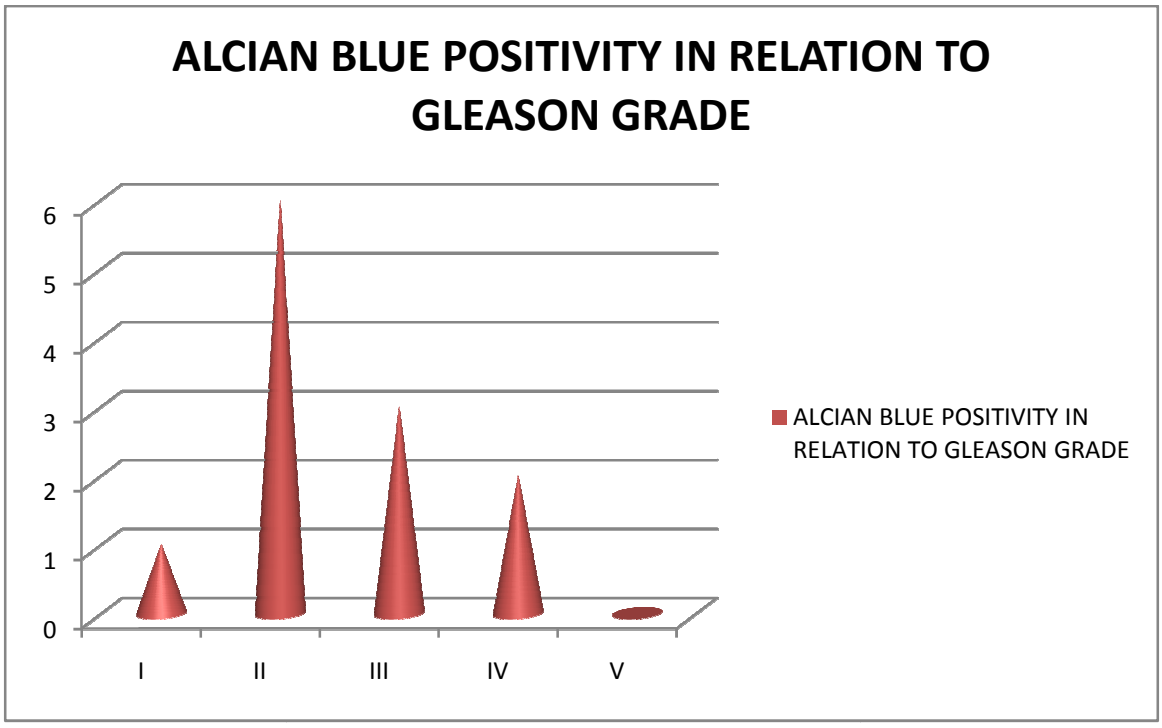
DISTRIBUTION OF ALCIAN BLUE POSITIVITY ACCORDING TO GLEASON GRADING IN ADENOCARCINOMA

Among various grades of adenocarcinoma,alcian blue staining was present in low and intermediate grades (grades I, II and III) and it was absent in higher grades(grades IV and V).

TABLE 11:ALCIAN BLUE POSITIVITY IN ADENOCARCINOMAS IN RELATION TO GEASON GRADING.

GLEASON GRADE	TOTAL NO OF CASES	NO OF CASES SHOWING ALCIAN BLUE POSITIVITY	PERCENTAGE
I	1	1	100%
II	12	6	50%
III	4	3	75%
IV	4	2	50%
V	4	0	0%

CHART 7: SHOWING ALCIAN POSITIVITY IN ADENOCARCINOMA IN RELATION TO GLEASON GRADE.



COLOUR PLATES

COLOUR PLATES

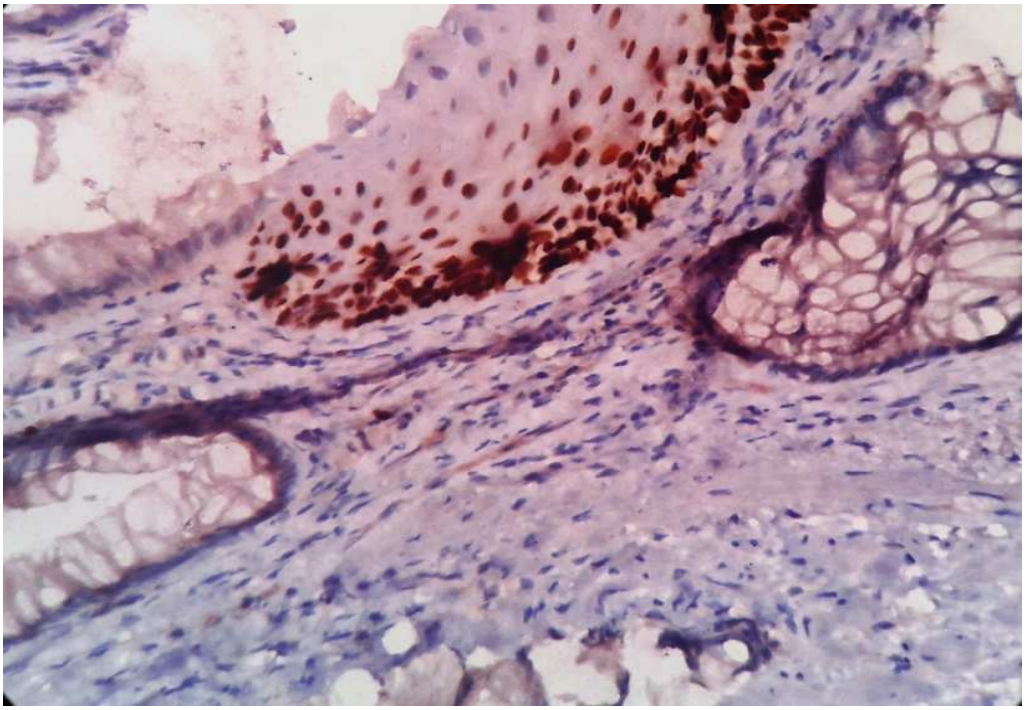


Fig 1A: IHC, 400X – showing absence p63 expression in malignant glands.

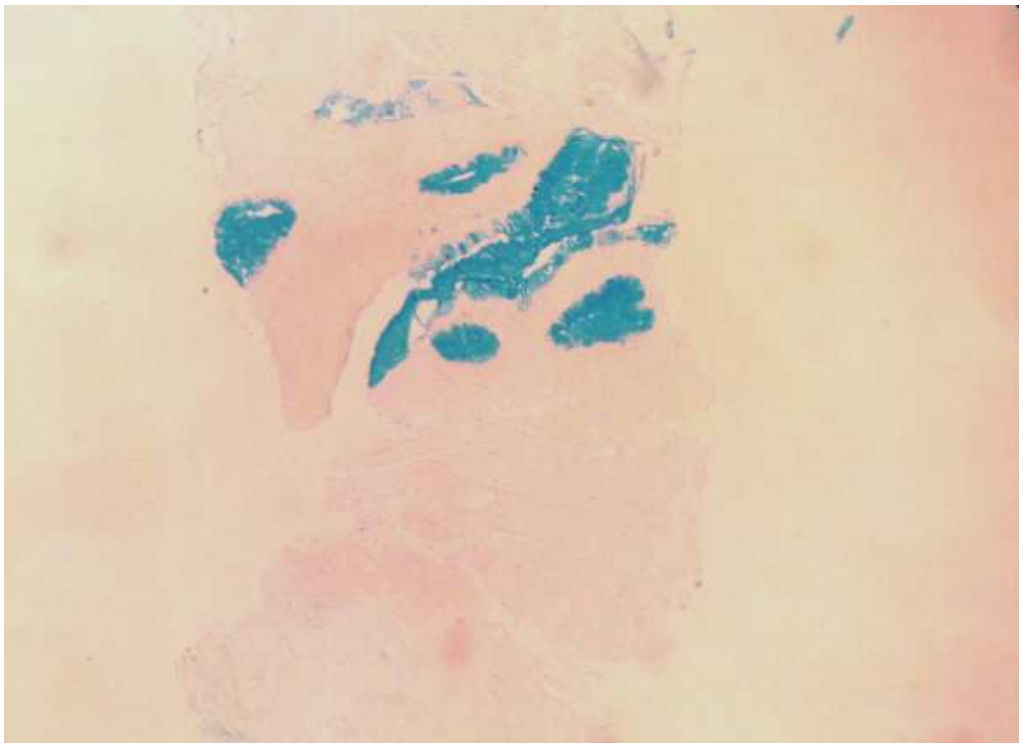


Fig 1B : 100X-showing alcian blue Positivity in malignant glands Gleason grade IV

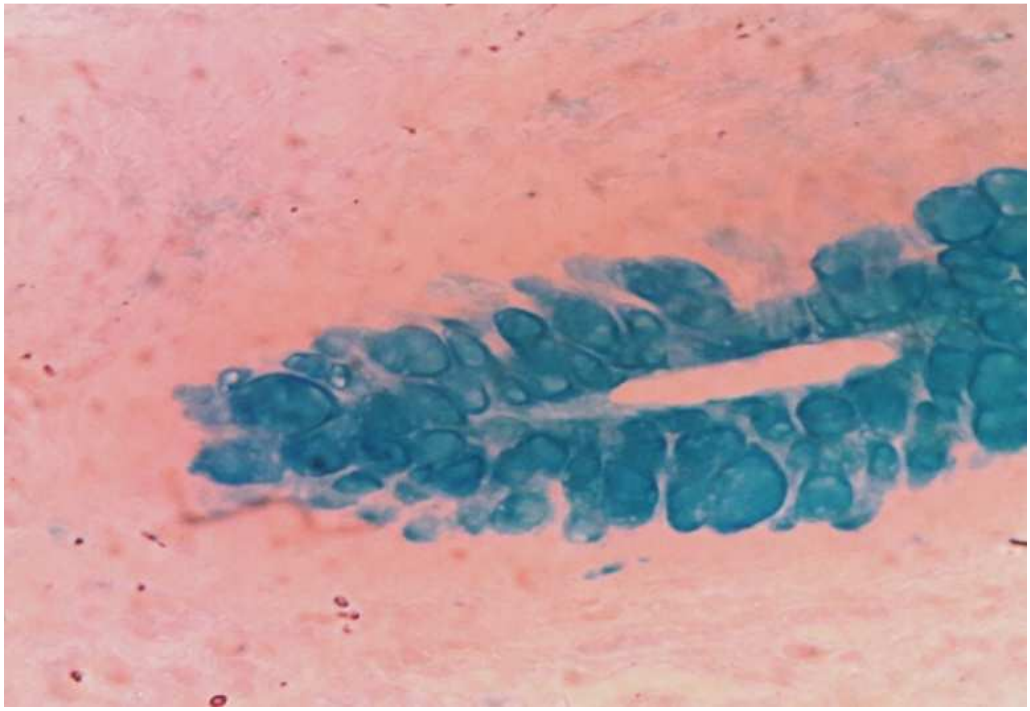


Fig 1C : 400X-showing alcian blue Positivity in malignant glands Gleason grade IV

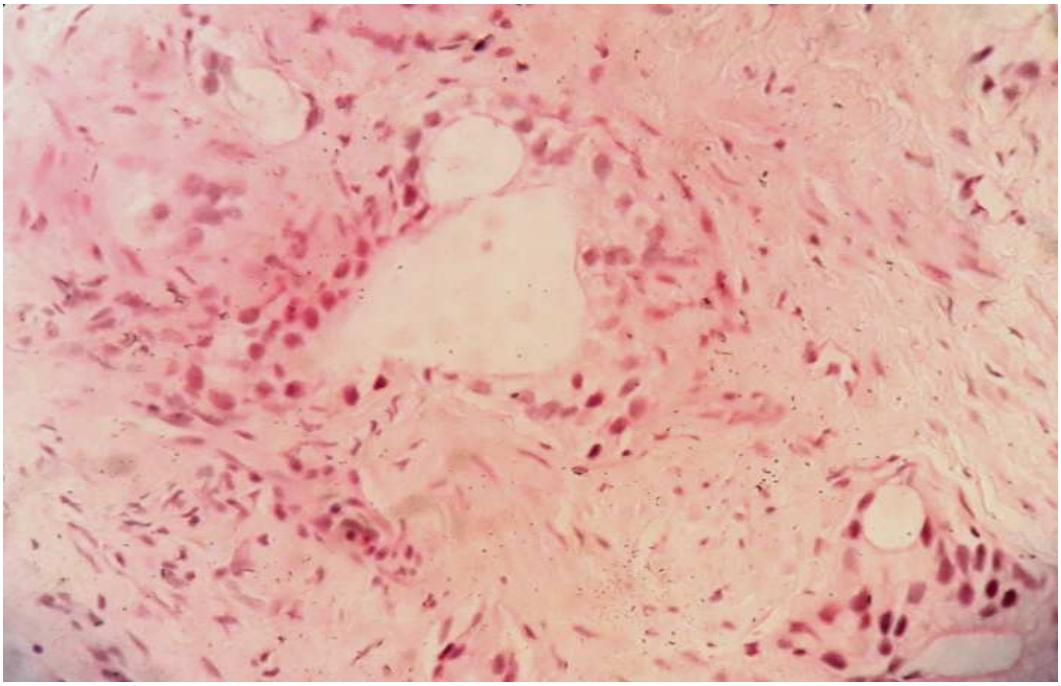


Fig 2A : H&E, 400X showing neoplastic gland

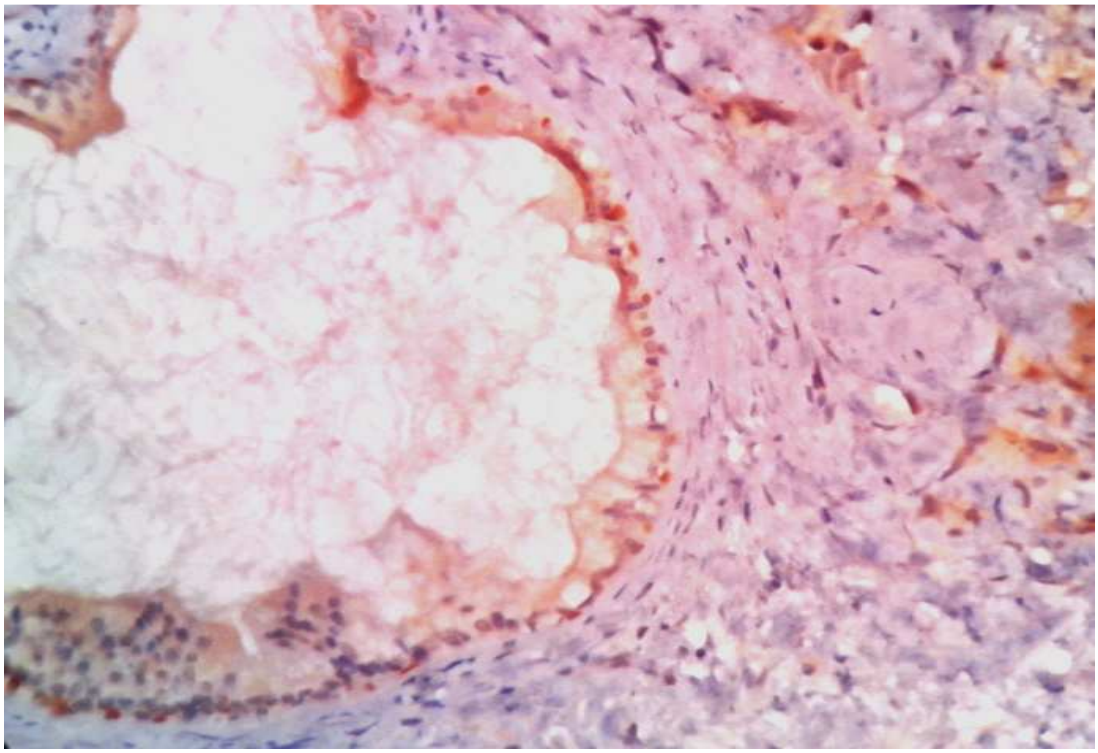


Fig 2B : IHC,400X showing discontinuous P63 staining.

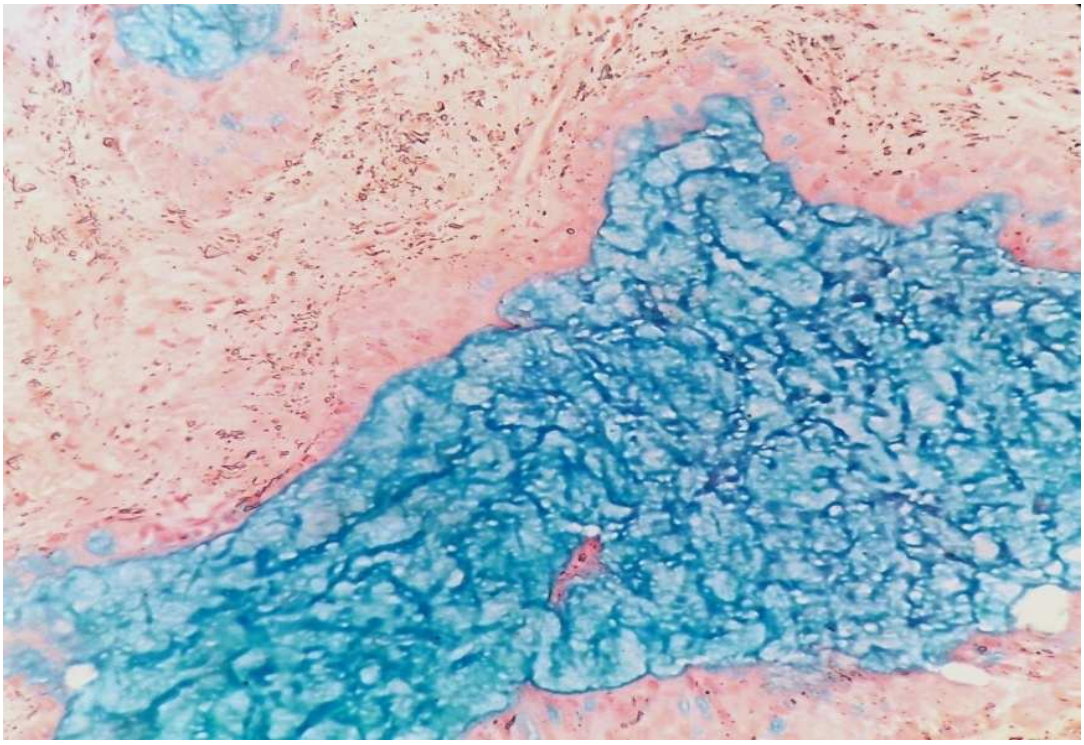


Fig 2C : 400X, showing alcian blue positivity in neoplastic glands Gleason grade II

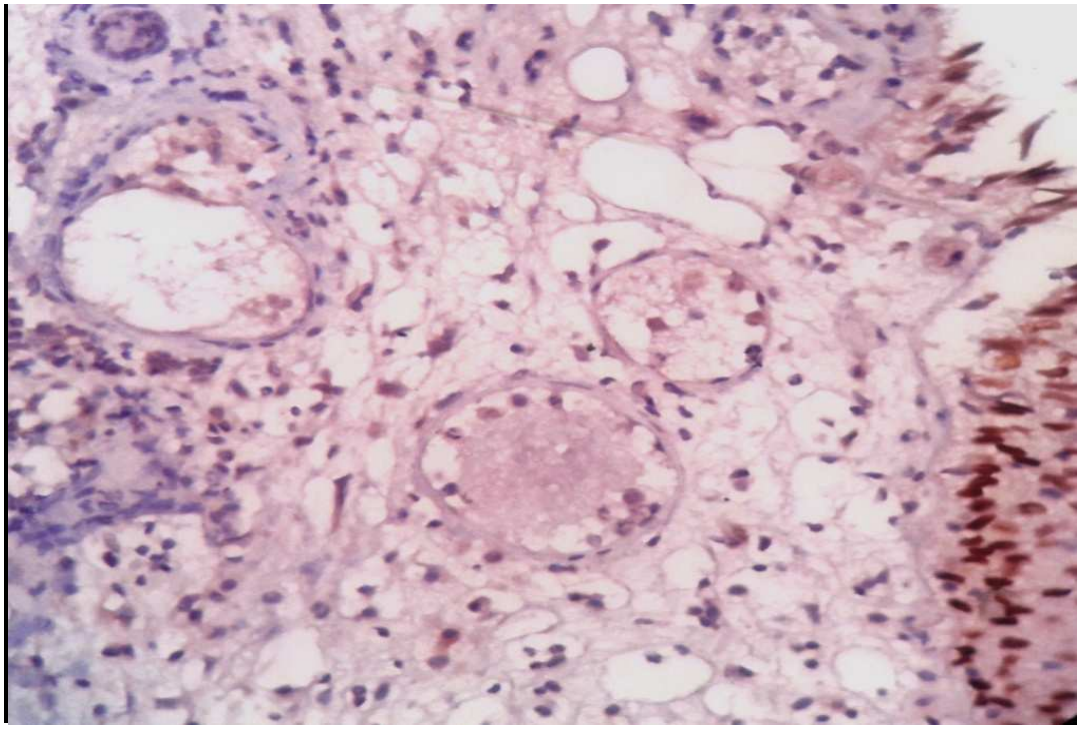


Fig 3A : IHC,400X showing P63 Negative (Gleason Grade I)

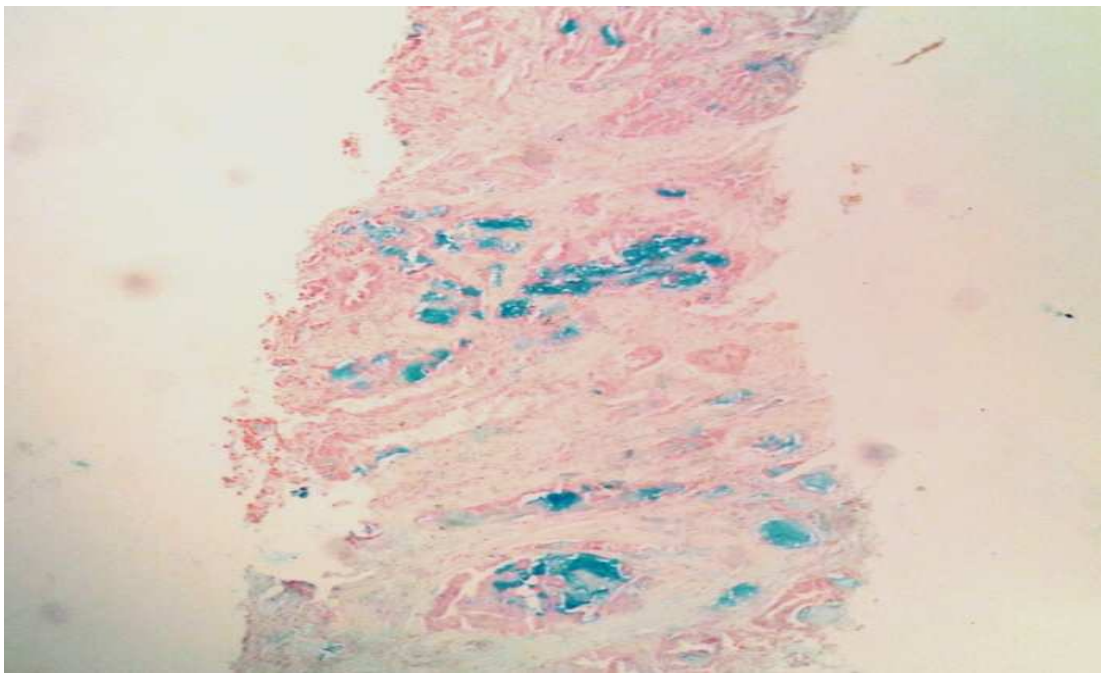


Fig 3B : 100X, Alcian blue positivity (Gleason Grade I)

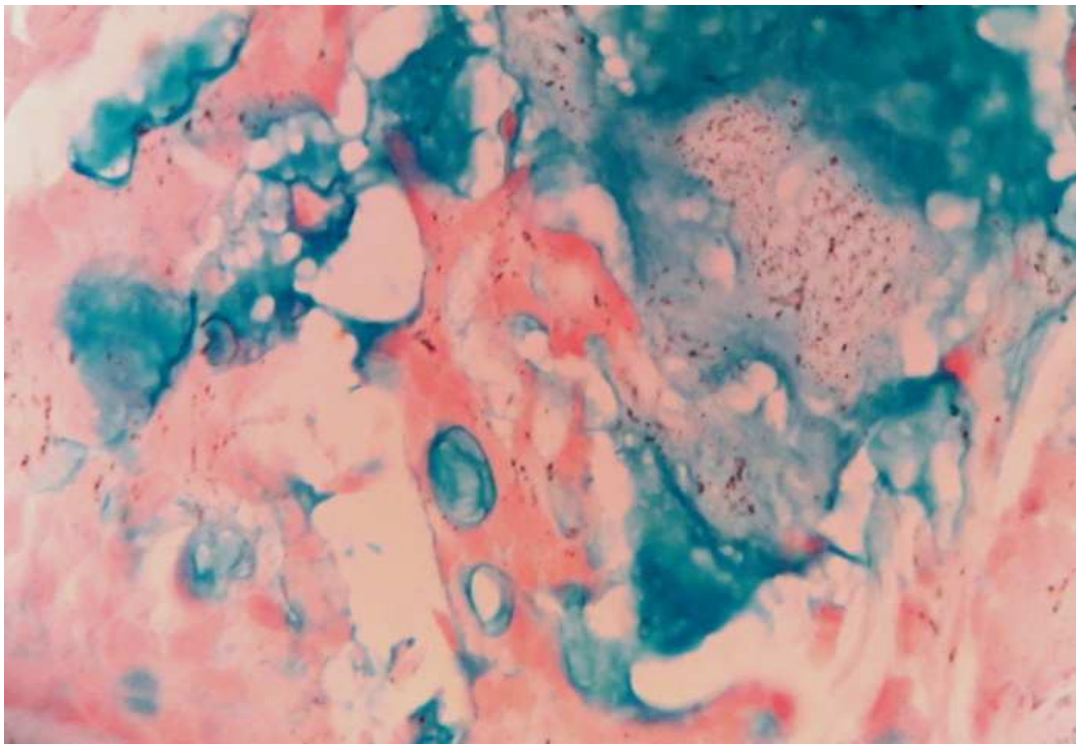


Fig 3C : IHC,400X Alcian blue positivity (Gleason Grade I)

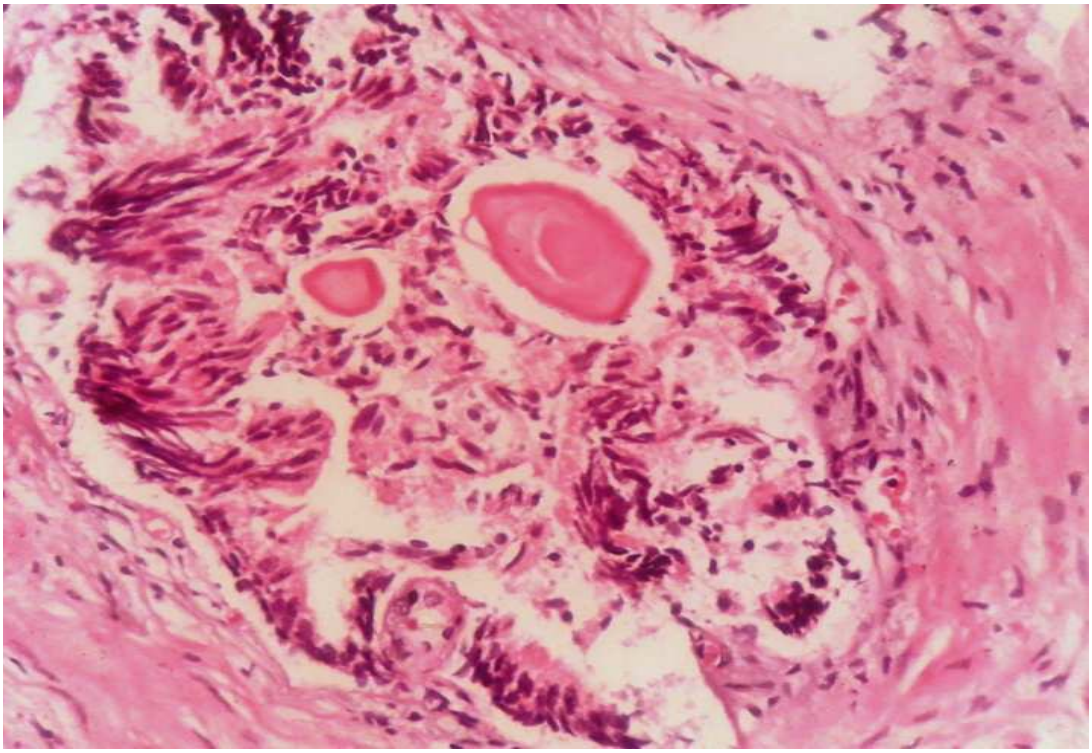


Fig 4A : 400X H & E Benign Prostatic hyperplasia showing intraluminal corpora amylacea

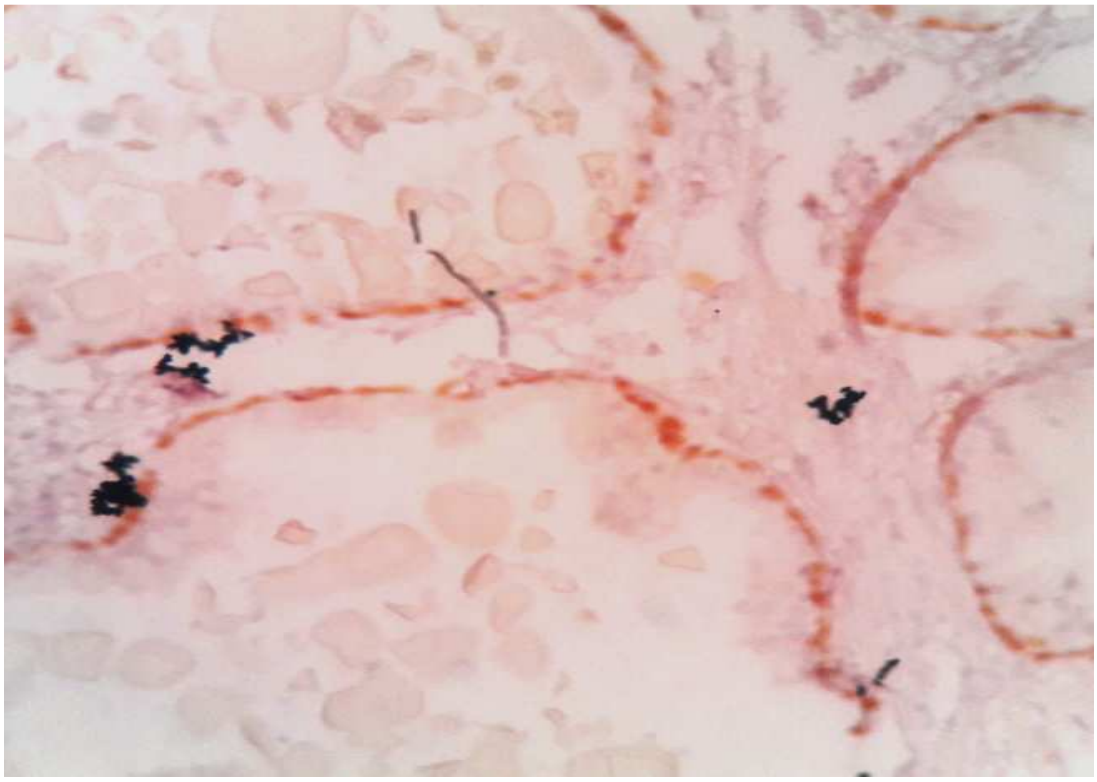


Fig 4B : 400X H & E IHC p63 positive in basal layer

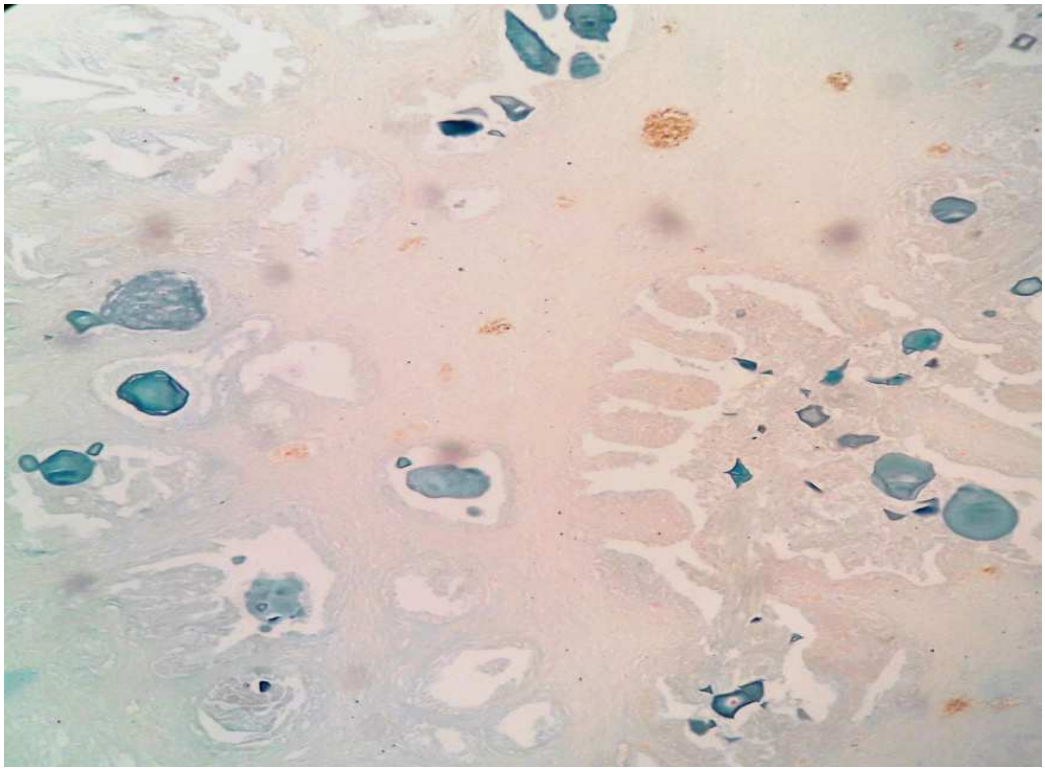


Fig 4C : 100X H & E Alcian Blue faint moderate positivity corpora amylacea

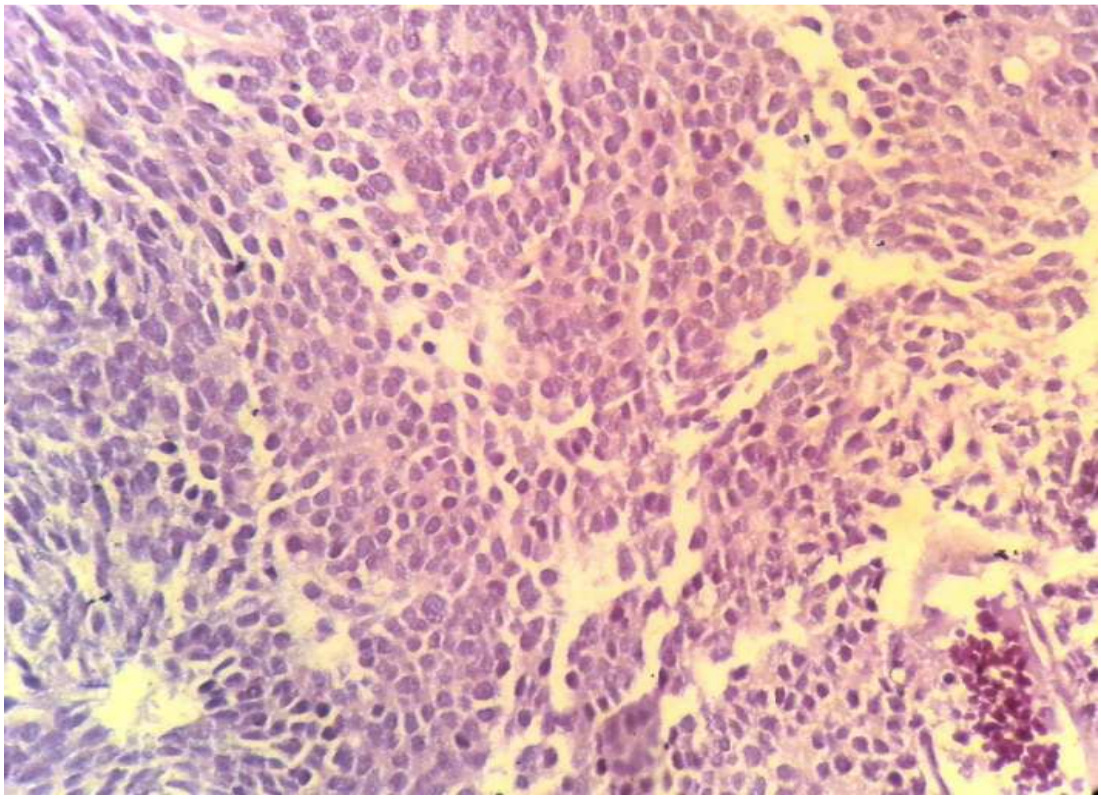


Fig 5A : H&E,400X,Adenocarcinoma (Gleason Grade V)

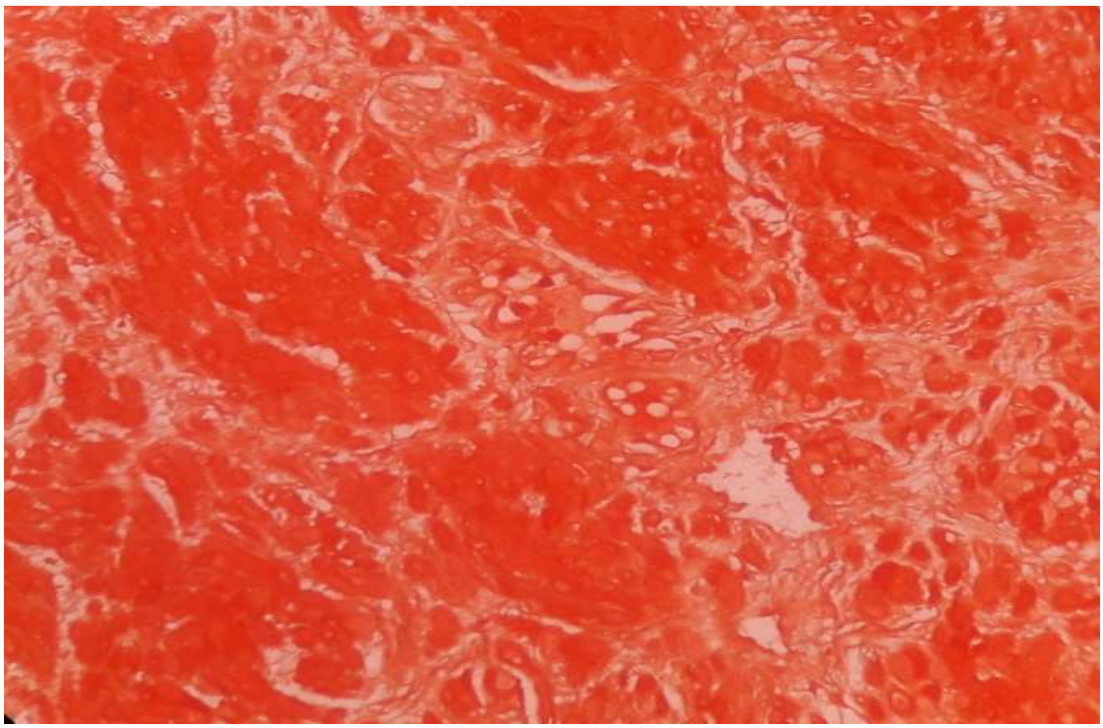


Fig 5B : 400X,alcian blue negative Gleason grade V

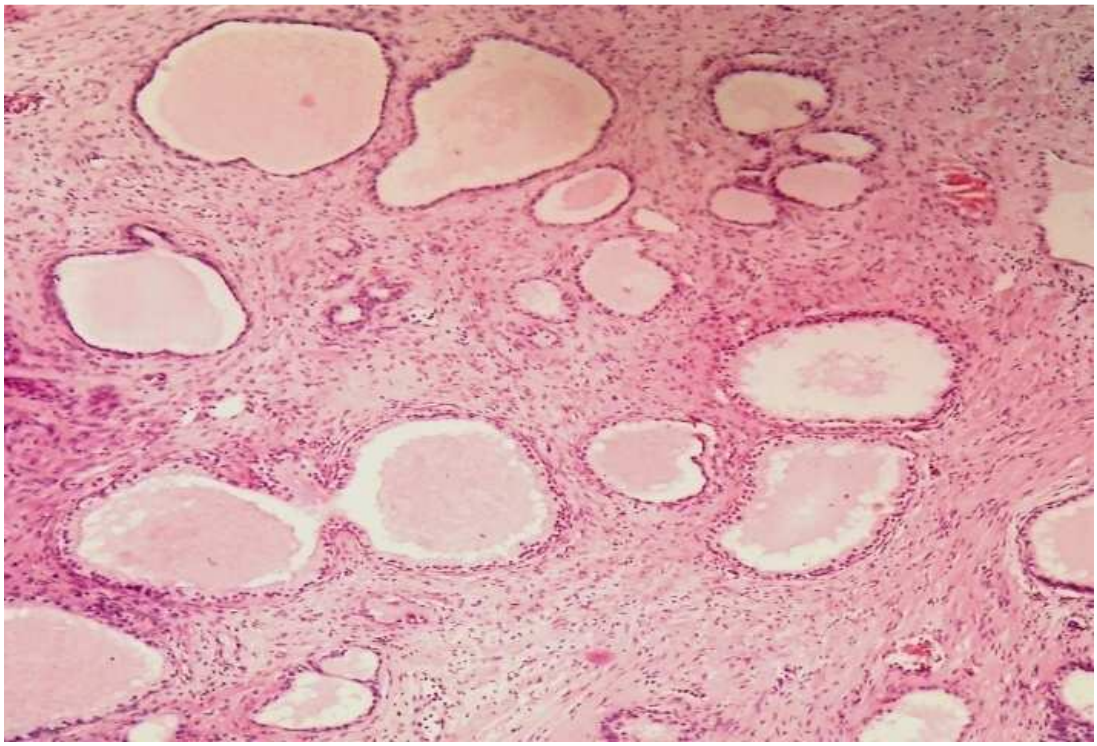


Fig 6 A : H&E,100X,benign prostatic hyperplasia.

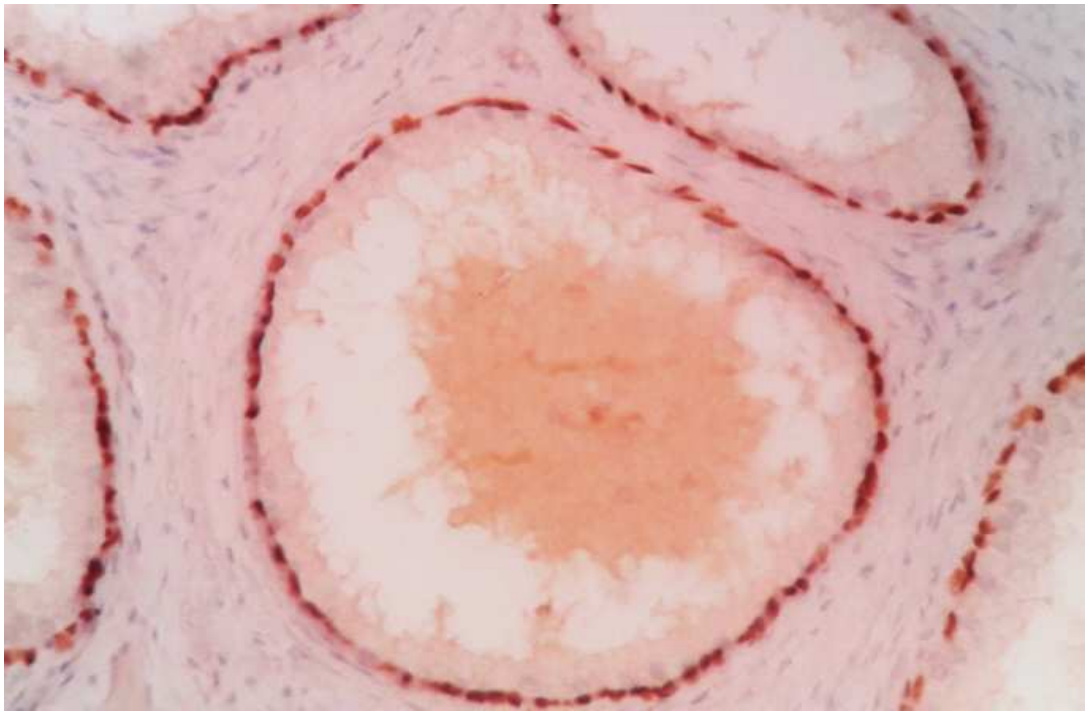


Fig 6 B : IHC, p63 positive in basal cells Glands lined by bilayered epithelium.

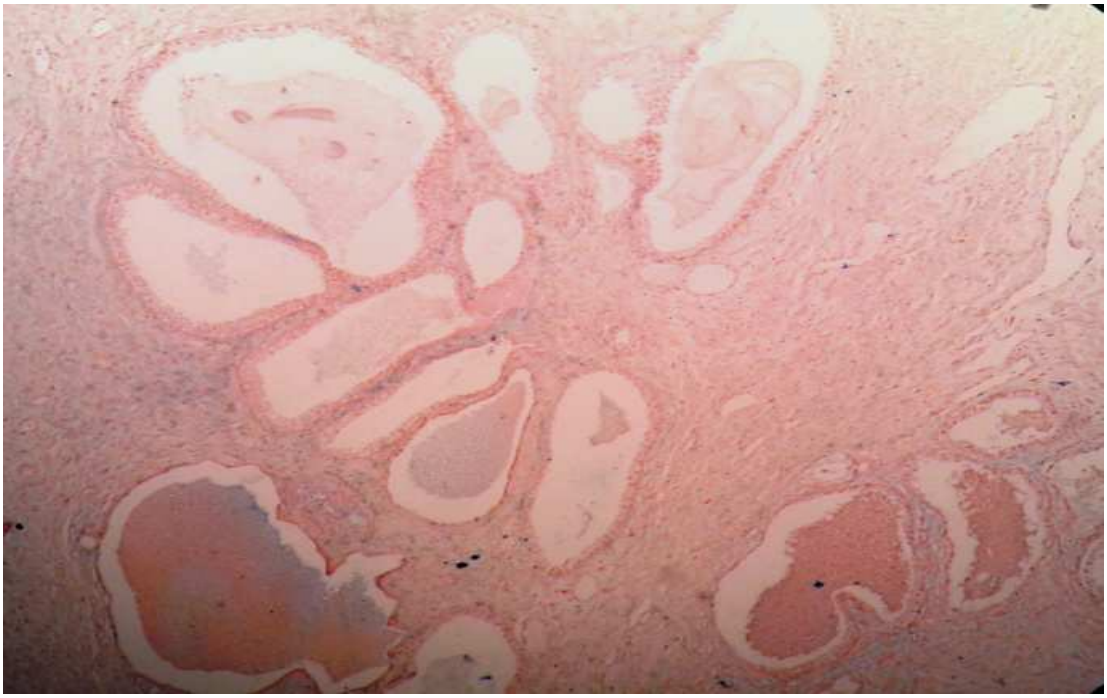


Fig 6C : 100X, alcian blue negativity in BPH.

DISCUSSION

DISCUSSION

Both benign prostatic hyperplasia and prostatic adenocarcinoma constitute more than 80% of prostatic diseases in older men. Prostatic adenocarcinomas exhibit wide spectrum of appearances ranging from well differentiated neoplasms to anaplastic tumors. Correct diagnosis of well differentiated adenocarcinoma from benign prostatic hyperplasia and other benign mimickers is a diagnostic enigma in surgical pathology.

Alcian blue can be used as an adjunctive in diagnosing prostatic adenocarcinoma in difficult situations and helps in differentiating adenocarcinomas from benign prostatic hyperplasia and other benign mimics.

INSTITUTIONAL STATISTICS;

In my study period of two years, total number of prostatic cases received in our institute was 328 cases. Among the received specimens, majority were TURP specimens(67.07%) followed by TRUS biopsy specimens(30.46%). Of which, about 98% is comprises of benign prostatic hyperplasia and prostatic adenocarcinoma.

AGE GROUP:

Both benign prostatic hyperplasia and prostatic carcinoma was more frequent in the age group 61-70 years(46.46%), followed by 51-60 years age group(22.15%). Among prostatic adenocarcinoma cases, 41.86% of cases were

seen in the age group of 61-70 years followed by 25.58% of cases in the age group of 71-80 years.

In a study by Agrawal et al, 53% of cases were above the age of 80 years. In an another study by Mathur et al, majority of prostatic adenocarcinoma case incidence was above 75 years of age.

ROLE OF MUCIN HISTOCHEMISTRY IN PROSTATE:

Alcian blue and PAS are the two mucin stains used in differentiating benign prostatic hyperplasia and prostatic adenocarcinoma.

PERIODIC ACID SCHIFF:

It is used for identification of neutral mucins. Neutral mucin is present in benign prostatic hyperplasia. PAS stain does not help in differentiating benign from malignant prostatic lesions. In a study by Anchit khanna, PAS staining was positive in 89.82% of BPH cases and 66.67% of carcinoma cases. Prostatic carcinomas with higher grades(4 and 5) was negative for PAS stain in the above indicating that PAS staining does not correlate withGleason grading.

ALCIAN BLUE STAINING:

Alcian blue stains acidic mucin and hence it is absent in benign prostatic hyperplasia whereas it will be positive in prostatic adenocarcinomas. It is also positive in atypical adenomatous hyperplasia and prostatic intraepithelial neoplasia indicating the evolution of prostatic adenocarcinoma from these premalignant lesions.But, with increasing Gleason grade,the ability of the

neoplastic cells to secrete acidic mucins decreases and hence Alcian blue will be negative. Alcian blue is relatively more specific stain in differentiating benign from malignant prostatic lesions.

ALCIAN BLUE STAINING RESULTS IN MY STUDY:

Among 25 cases of prostatic adenocarcinomas included in my study, 12 of the cases showed both intraluminal and intracytoplasmic positivity for Alcian blue indicating the presence of acidic mucin in neoplastic cells and in luminal secretion of neoplastic glands.The intensity of staining was different according to the amount of mucin present. Positivity of alcian blue was reducing with increasing gleason grading.

Among 25 cases of benign prostatic hyperplasia, none of the cases showed alcian blue positivity,indicating absence of acidic mucin in benign prostatic hyperplasia. Like in studies conducted by Frank et al[58] and Hukill et al[59], corpora amylacae present in benign prostatic hyperplasia showed weak to moderate positivity with alcian blue.

According to Anchit Khanna et al, [60] 66.67% of adenocarcinomas of the prostate showed alcian blue positivity.Positive staining was observed in both luminal and intracytoplasmic with intensity varying from deep blue in mucinous areas and light blue in non mucinous areas.

Pinder et al.[61] observed similar result of absence of Alcian blue staining in benign prostatic hyperplasia(0%), and McMahon et al.[62] obsevered 5% of

benign prostatic hyperplasia showing Alcian blue positivity. Arora et al. and Mathur et al.[65] reported it as 33.3% and 16% respectively.

Agarwal DN[63] reported positivity for acidic mucin in (46.66%) of prostatic adenocarcinomas. McMahon RF et al reported (50%) of positivity in prostatic adenocarcinoma; whereas Arora HL[64] reported it (60%) positivity in prostatic adenocarcinomas and Pinder et al. in (38%) of prostatic adenocarcinomas.

VARIOUS STUDIES	BENIGN PROSTATIC HYPERPLASIA	CARCINOMA PROSTATE
Mathur et al	16%	--
Arora et al	33.30%	60%
Pinder et al	0%	38%
Mc Mahon et al	5%	50%
Agrawal et al	0%	46.6%
Bastola et al	--	77.8%
Taylor et al	--	63%
My study	0%	48%

My study results are concordant with the above study results.

Luna –More S et al[66] studied the expression of neutral and acidic mucins in prostatic adenocarcinoma and atypical adenomatous hyperplasia (AAH) and found that acidic mucin positivity was higher in prostatic adenocarcinoma.

In a study by Bastola et al[68], Alcian blue positivity was seen in 77.8% of cases was seen in 100% cases of low grade and intermediate grade prostatic carcinoma (Gleason score of 2-7) and the positivity reduced to 50% in high grade prostatic carcinoma with Gleason score >8.

In a study by Taylor et al[67], 63 per cent of prostatic adenocarcinomas showed varying amounts of positive material in the lumens of the malignant glands. In a study by Escalona VR et al[69] , the positivity with alcian blue was 96.3% in well differentiated adenocarcinomas.

STUDIES	WELL DIFFERENTIATED AND INTERMEDIATE GRADE (GLEASON SCORE 2-6)	MODERATE-POORLY DIFFERENTIATED (GLEASON SCORE 7)	HIGH GRADE (GLEASON SCORE 8-10)
Escalona VR et al	96.3%	--	--
McMahon RF	100%	75%	38.40%
My study	100%	56.25%	25%
Bastola et al	100%	--	50%

My study results are concordant with the above study results.

P63 IMMUNOHISTOCHEMISTRY:

For confirming our diagnosis of benign prostatic hyperplasia and prostatic adenocarcinoma p63 immunohistochemistry was done. All the 25 cases of benign prostatic hyperplasia showed strong nuclear positivity in the basal layer of the glands.

Whereas, none of the prostatic adenocarcinomas showed p63 positivity, indicating absence of basal layer and confirming our diagnosis of adenocarcinoma. Basal cells are important for maintaining integrity of the duct and for proper differentiation of luminal cells. In prostate the basal cells are present on the basement membrane and express immunohistochemistry markers such as ck 14 and p63.

According to a study by Yank et al[70] in 1998 ,p63 is a homologue of p53 tumour suppressor gene. In a study by Totten et al [71],found that basal cells are were absent in prostatic adenocarcinoma.

p63 inhibits cell migration and as it is lost in prostatic adenocarcinoma, it leads to metastasis.[72] Increasing grades of prostatic intraepithelial neoplasm is associated with disruption of basal layer and hence shows absence of p63 staining or discontinuous p63 staining[73].

SUMMARY

SUMMARY

- Majority of prostatic diseases in our Institute is Benign prostatic hyperplasia constituting about 85.97%.
- Prostatic adenocarcinoma constitutes about 13.10% among all prostatic lesions in our Institute.
- About 67.07% of specimens received was TURP (Transurethral resection of prostate , 30.46% of specimens was TRUS guided biopsy and 2.46% of open prostatectomy specimens.
- 46.46% of prostatic lesions presented in the age group of 61-70 years followed by 22.15% of cases in the age group of 51-60 years.
- Among adenocarcinoma cases 41.86% of cases were seen in the age group of 61- 70 years followed by 25.58% in the age group of 71-80 years.
- In my study, 48% of adenocarcinomas was positive for acidic mucins (Alcian blue).
- None of the benign prostatic hyperplasia cases showed Alcian blue positivity (0%).
- Among different grades of prostatic adenocarcinoma (Gleason's grading), grade I tumours showed 100% positivity with decrease in Alcian blue staining with increase in grade. High grade prostatic adenocarcinomas Gleason Grade V were Alcian blue negative.
- Our diagnosis of benign prostatic hyperplasia and adenocarcinoma was confirmed by basal cell marker p63 immunohistochemistry.
- P63 immunohistochemistry was positive in 100% of benign prostatic hyperplasia and 100% negativity in prostatic adenocarcinoma.

CONCLUSION

In current scenario we have numerous immunohistochemical markers to differentiate benign from malignant prostatic lesions. Histomorphology and cytomorphology are still very important and indispensible in low socio economic centres where they cannot affort immunohistochemistry.

In such situations, mucin histochemistry will be an adjunct in differentiating benign and malignant prostatic lesions.it is also useful in differentiating benign prostatic lesions from premalignant conditions such as prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia.

Mucin histochemistry is cost effective and time saving when compared to immunohistochemistry.

Sensitivity of mucin histochemistry such as alcian blue in differentiating benign from malignant prostatic lesions increases when it is used along with immunohistochemistry.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Ferlay J, Shin HR, Bray F, *et al*. GLOBOCAN 2008 v 1.2, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10. Lyon, France: International Agency for Research on Cancer; 2010.
2. Lalitha K, Suman G, Pruthvish S, Mathew A, Murthy NS. Estimation of time trends of incidence of Prostate Cancer-an Indian scenario. Asian Pac J Cancer Prev 2012;13:6245-50.
3. 3.Yeole BB. Trends in the prostate cancer incidence in India. Asian Pac J Cancer Prev 2008;9:141-4.
4. 4.Swaminathan R, Shanta V, Ferlay J, Balasubramanian S, Bray F, Sankaranarayanan R, *et al*. Trends in cancer incidence in Chennai city (1982-2006) and statewide predictions of future burden in Tamil Nadu (2007-16). Natl Med J India 2011;24:72-7.
5. Hebert JR, Ghumare SS, Gupta PC. Stage at diagnosis and relative differences in breast and prostate cancer incidence in India: Comparison with the United States. Asian Pac J Cancer Prev 2006;7:547-55.
6. Huncharek M, Haddock S, Reid R, Kupelnick B. Smoking as a risk factor for prostate cancer: A meta - analysis of 24 prospective cohort studies. Am J Public Health 2010;100:693-701.
7. Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wolk A. Fatty fish consumption and risk of prostate cancer. Lancet 2001;357:1764-6.
8. Ali I, Waseem A, Wani, Saleem K. Cancer scenario in India with future perspective. Can Ther 2011;8:56-70.

9. Singh AN, Kirti, Dalela D, Sankhwar SN, Natu SM, Srivastava AN. Diagnosis and progression of Prostate Cancer in North Indian population: An affect of Body Mass Index and Age. J Adv Res Biol Sci 2013;5:256-9.
10. Amling CL, Riffenburgh RH, Sun L, Moul JW, Lance RS, Kusuda L, *et al*. Pathologic variables and recurrence rates as related to obesity and race in men with Prostate Cancer undergoing Radical Prostatectomy. J Clin Onco 2004;22:439-45.
11. Freedland SJ, Aronson WJ, Kane CJ, Presti JC Jr, Amling CL, Elashoff D, *et al*. Impact of obesity on biochemical control after Radical Prostatectomy for clinically localized Prostate Cancer: A report by the shared equal access Regional Cancer Hospital database study group. J Clin Oncol 2004;22:446-53.
12. Banerjee BD. Gene-environmental interaction and prostate cancer: The Indian scenario. Al Ameen J Med Sci 2011;4:305-8.
13. Sobti RC, Gupta L, Thakur H, Seth A, Singh SK, Kaur P. CYP17 Gene polymorphism and its association in north Indian prostate cancer patients. Anticancer Res 2009;29:1659-63.
14. Ganpule AP, Desai MR, Manohar T, Bapat S. Age-specific prostate specific antigen and prostate specific antigen density values in a community-based Indian population. Indian J Urol 2007;122-5.
15. Shah S, Jha B, Khanal MP. Effects of aging and ethinicity on serum free prostate specific antigen. Asian Pac J Cancer Prev 2011;12:2509-12.

16. Atish C, Prasad SD, Mukesh R, Surendran K, Sundaresan S, Thangapannerselvem T. A study on prostate specific antigen (PSA) with the ratio of free to total PSA. African J Biochem Res 2010;4:013-6.
17. Agnihotri S, Mittal RD, Kapoor R, Mandhani A. Raising cut-off value of prostatic specific antigen (PSA) for biopsy in symptomatic men in India to reduce unnecessary biopsy. Indian J Med Res 2014;139:851-6. *Epidemiology of prostate cancer in India*.
18. https://www.researchgate.net/publication/271332914_Epidemiology_of prostate_cancer_in_India
19. Gittes RG. Carcinoma of the prostate. N Engl J Med 1994, **324**: 236–245.
20. Babaian RJ, Mettlin C, Kane R, Murphy GP,Lee F, Drago JR, Chesley A. The relationship of prostate-specific antigen to digital rectal examination and transrectal ultrasonography. Findings of the American Cancer Society National Prostate Cancer Detection Project. Cancer 1992, **69**: 1195–1200.
21. Epstein NA. Prostatic biopsy. A morphologic correlation of aspiration cytology with needle biopsy histology. Cancer 1976, **38**: 2078–2087
22. Peller PA, Young DC, Marmaduke DP, Marsh WL, Badalament RA. Sextant prostate biopsies. A histopathologic correlation with radical prostatectomy specimens. Cancer 1995, **75**:530–538.

23. Rohr LR. Incidental adenocarcinoma in transurethral resections of the prostate. Partial versus complete microscopic examination. Am J Surg Pathol 1987, **11**: 53–58.
24. Hall GS, Kramer CE, Epstein JI. Evaluation of radical prostatectomy specimens; a comparative analysis of sampling methods. Am J Surg Pathol 1992, **16:** 315–324
25. Agoulnik I. U., Weigel N. L. Androgen receptor action in hormone-dependent and recurrent prostate cancer. J Cell Biochem. 2006;99: 362–372.
26. Edwards J., Bartlett J. M. The androgen receptor and signal-transduction pathways in hormone-refractory prostate cancer. Part 1: Modifications to the androgen receptor. BJU Int. 2005;95:1320–1326.
27. Chmelar R., Buchanan G., Need E. F., Tilley W., Greenberg N. M. Androgen receptor coregulators and their involvement in the development and progression of prostate cancer. Int J Cancer. 2007;120:719–733.
28. Denmeade S. R., Lin X. S., Isaacs J. T. Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. Prostate. 1996;28:251–265.
29. Chen C. D., Welsbie D. S., Tran C., et al. Molecular determinants of resistance to antiandrogen therapy. Nature Med. 2004;10:33–39.

30. Mackinnon AC, Yan BC, Joseph LJ, Al-Ahmadie HA. Molecular biology underlying the clinical heterogeneity of prostate cancer: an update.Arch Pathol Lab Med 2009, **133**: 1033–1040.
31. Hermans KG, van Marion R, van Dekken H, Jenster G, van Weerden WM, Trapman J.TMPRSS2:ERG fusion by translocation or interstitial deletion is highly relevant in androgen-dependent prostate cancer, but is bypassed in late-stage androgen receptornegative prostate cancer. Cancer Res 2006, **66**:10658–10663.
32. Ross JS, Sheehan C, Hayner-Buchan AM, Ambros RA, Kallakury BV, Kaufman R, Fisher HA, Muraca PJ. HER-2/neu gene amplification status in prostate cancer by fluorescence in situ hybridization. Hum Pathol 1997, **28**: 827–833.
33. Orteil H. Involutionary changes in prostate and female breast in relationship to cancer development. Can Med Assoc J 1926;16:237-41.
34. Amin MB, Ro JY. Ayala AG. Putative precursor lesions in prostatic adenocarcinoma: Fact or fiction? Mod Pathol 1993;6:478-83.
35. Drago JR, Mostofi FK, Lee F. Introductory remarks and workshop
36. McNeal JE, Bostwick DG. Intraductal dysplasia: A premalignant lesion of the prostate. Human Pathol 1986;17:64-71. summary. Urol 1989; 6:2-3.
37. .Mc Neal JE. Morphogenesis of prostatic carcinoma. Cancer 1985; 1659-66.

38. Rosai J. Male reproductive system. In: Rosai J, editor. Ackerman's Surgical pathology. 8th Ed. Missouri: Mosby-year book Inc 1996. p. 21-56.
39. Bostwick DG, Grignon DJ, Hammond ME, Amin MB, Cohen M, Crawford D, Gospadarowicz M, Kaplan RS, Miller DS, Montironi R, Pajak TF, Pollack A, Srigley JR, Yarbro JW. Prognostic factors in prostate cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000, **124**: 995–1000.
40. McNeal JE, Yemoto CE. Spread of adenocarcinoma within prostatic ducts and acini: morphologic and clinical correlations.Am J Surg Pathol 1996, **20**: 802–814.
41. Epstein JI, Carmichael M, Walsh PC.Adenocarcinoma of the prostate invading the seminal vesicle. Definition and relation of tumor volume, grade and margins of resection to prognosis. J Urol 1993, **149**: 1040–1045.
42. Villers A, McNeal JE, Freiha FS, Boccon-Gibod L, Stamey TA. Invasion of Denonvilliers' fascia in radical prostatectomy specimens. J Urol 1993, **149**: 793–798.
43. Harada M, Shimizu A, Nakamura Y, Nemoto R. Role of the vertebral venous system in metastatic spread of cancer cells to the bone. Adv Exp Med Biol 1992, **324**: 83–92.

44. Kothari PS, Scardino PT, Ohori M, Kattan MW,Wheeler TM. Incidence, location, and significance of periprostatic and periseminalv esicle lymph nodes in prostate cancer. Am J Surg Pathol 2001, **25**: 1429–1432.
45. Brawer MK, Peehl DM, Stamy TA, Bostwick DG. keratin immune reactivity in the being and neoplastic human prostate. Cancer Res. 1985;45:3663–3667.
46. Hedrick L, Epstien JI. Use of keratin 903 as an adjunct in the diagnosis of prostate carcinoma. Am J Surg Pathol. 1983;13:350–353.
47. O'Malley FP, Grignon DJ, shum DT. Use fullness of immune peroxides staining with high – molecular – weight cytokeratin in the differential diagnosis of small-acinar lesions of the prostate gland. Virchows Arch A Patathol Anat Histopathol. 1995;417:191–196.
48. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, et al. P63 is a prostate basal cell marker and is required for prostate development. Am J Pathol. 2000;157:1769–1775. Parsons GK,
49. Gage WR, Nelson WG, De Marzo AM. P63 protein expression is rare in prostate adenocarcinoma:implications for cancer diagnosis and carcinogenesis. Urology. 2001;58:619–624.
50. Gleason, D. F. (1977). "The Veteran's Administration Cooperative Urologic Research Group: histologic grading and clinical staging of prostatic carcinoma". In Tannenbaum, M. *Urologic Pathology: The Prostate*. Philadelphia: Lea and Febiger. pp. 171–198. ISBN 0-8121-0546-X.

51. Epstein JI, Allsbrook Jr WC, Amin MB, Egevad LL. ISUP Grading Committee. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol. 2005;29:1228–42.
52. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol. 2016;40(2):244–52.
53. Epstein JI, Zelefsky MJ, Sjoberg DD, et al. A contemporary prostate cancer grading system: A validated alternative to the Gleason score. Eur Urol (2015). Gleason
54. **.**DF**:** Histological grading and staging of prostaticcarcinoma. In Tannenbaum M (Ed): !e Prostate. Philadelphia, Lea and Febiger, 1977, 171
55. Blennerhassett JB, Vickery AL Jr. Carcinoma of the prostate gland. Cancer 1966, **19**: 980–984.
56. Kirchheim D, Niles NR, Frankus E, Hodges CV. Correlative histochemical and histological studies on thirty radical prostatectomy specimens. Cancer 1966, **19**: 1683–1696.
57. McNeal JE. Prostate. In Mills SE (ed.): Histology for pathologists, ed. 3. Philadelphia, 2007, Lippincott Williams & Wilkins, pp. 923–942

58. Franks LM, O'Shea JD, Thomson AE. Mucin in the Prostate: A Histochemical Study in Normal Glands, Latent, Clinical, and Colloid Cancers. Cancer. 1964;17:983–91. PubMed PMID: 14202599. Epub 1964/08/01
59. Hukill PB, Vidone RA. Histochemistry of mucus and other polysaccharides in tumors. II. Carcinoma of the prostate. Lab Invest. 1967;16(3):395–406. PubMed PMID: 4225797. Epub 1967/03/01 ;
60. Anchit Khanna, Rani Patil, Abhay Deshmukh, .Assessment Of The Potential Of Pathological Stains In Human Prostate Cancer. Journal of Clinical and Diagnostic Research 2014 124 – 128
61. Pinder SE, McMahon RF. Mucins in prostatic carcinoma. Histopathology 1990; 16:43-6.
62. McMahon RF, McWilliam LJ, Mosley S. Evaluation of three techniques for differential diagnosis of prostatic needle biopsy specimens. J Clin Pathol 1992;45:1094-8.
63. Agrawal DN, Zawar MP, Deshpande NM, Sudhamani S. The study of mucin histochemistry in benign and malignant lesions of prostate. J Sci Soc 2014;41:38-40.
64. Arora HL. Histochemistry of mucins in various human prostatic diseases. Indian J Patholo Microbiol 1979;22:353-8.

65. Mathur SK, Gupta S, Marwah N, Narula A, Singh S, Arora B. Significance of mucin stain in differentiating benign and malignant lesions of prostate. Indian J Pathol Microbiol 2003;46:593-5.
66. Luna –More S,Floez P,Ayala A,Diaz F,Santos A.Neutral and Acid mucins and eosinophil and argyrophil crystalloids in carcinoma and atypical adenomatous hyperplasia of prostate. Pathol Res Pract 1997;193:291-8.
67. Taylor NS.Histochemistry in the diagnosis of early prostatic carcinoma.Human Pathol 1979;10:513-20
68. Bastola et al .Evaluation of mucin histochemistry in benign and malignant prostatic lesion and their correlation PSA level.
69. Escalona VR, Estrada MNR **Mucin histochemistry demonstration for the differential diagnosis of prostate benign and malign lesions** MediSan 2017; 21 (04)
70. **Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Dotsch, V., Andrews, N. C., Caput, D. and McKeon, F.** (1998). p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol. Cell.* **2**,305 -316.
71. Totten RS, Heinemann NW, Hudson PB, Sproul EE, Stout AP. Microscopic differential diagnosis of latent carcinoma of the prostate. Arch Pathol Lab Med 1953; 55: 131–41.

72. Tucci P, M Agostini, F Grespi. Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. Proc. Natl. Acad. Sci. USA. 2012;109:15312–15317.
73. Bostwick DG, Brawer MK: Prostatic intraepithelial neoplasia and early invasion. Cancer 1987; 59:788-94
74. Wheater's Functional histology-A Text and Color Atlas 6E page 347
75. Argani, P. and J. I. Epstein . Inverted (Hobnail) high grade prostatic intraepithelial neoplasia (PIN): report of 15 cases of a previously undescribed pattern of high-grade PIN. Am J Surg Pathol 2001. 25:1534–1539
76. Melissari, M. , A. Lopez-Beltran , R. Mazzucchelli , E. Froio , D. G. Bostwick , and R. Montironi . High grade prostatic intraepithelial neoplasia with squamous differentiation. J Clin Pathol 2006. 59:437–439
77. Gaudin, P. B. , I. A. Sesterhenn , K. Wojno , F. K. Mostofi , and J. I. Epstein . Incidence and clinical significance of high grade prostatic intraepithelial neoplasia in TURP specimens. Urology 1997. 49:558–563.
78. Pacelli, A. and D. G. Bostwick . Clinical significance of high-grade prostatic intraepithelial neoplasia in transurethral resection specimens. Urology 1997. 50:355–359.

79. Bostwick, D. G. , M. B. Amin , P. Dundore , W. Marsh , and D. S. Schultz . Architectural patterns of high-grade prostatic intraepithelial neoplasia. Hum Pathol 1993. 24:298–310.
80. Reyes, A. O. , P.E.Swanson , J. M. Carbone , and P. A. Humphrey. Unusual histologic types of high-grade prostatic intraepithelial neoplasia. Am J Surg Pathol 1997. 21:1215–1222.
81. Berman, D. M. , J. Yang , and J. I. Epstein . Foamy gland high-grade prostatic intraepithelial neoplasia. Am J Surg Pathol 2000. 24:140–144.
82. Argani, P. and J. I. Epstein . Inverted (Hobnail) high-grade prostatic intraepithelial neoplasia (PIN): report of 15 cases of a previously undescribed pattern of high-grade PIN. Am J Surg Pathol 2001. 25:1534–1539
83. Alberto G. Ayala and Jae Y. Ro (*2007*) Prostatic Intraepithelial Neoplasia: Recent Advances. Archives of Pathology & Laboratory Medicine: August 2007, Vol. 131, No. 8, pp. 1257-1266.
84. Diagnostic surgical pathology,5E,pg 1880.
85. Al-Ahmadie HA, Tickoo SK, Olgac S, Gopalan A, Scardino PT, Reuter VE, Fine SW. Anteriorpredominant prostatic tumors: zone of origin and pathologic outcomes at radical prostatectomy. Am J Surg Pathol 2008, **32**: 229–235.
86. Garcia JJ, Al-Ahmadie HA, Gopalan A, Tickoo SK, Scardino PT, Reuter VE, Fine SW. Do prostatic transition zone tumors have a distinct morphology? Am J Surg Pathol 2008, **32**: 1709–1714.

87. Totten RS, Heinemann MW, Hudson PB, Sproul EE, Stout AP. Microscopic differential diagnosis of latent carcinoma of the prostate.
88. Kelemen PR, Buschmann RJ, Weisz-Carrington P. Nucleolar prominence as a diagnostic variable in prostatic carcinoma. Cancer 1990, **65**: 1017–1020. Arch Pathol 1953, **55**: 131–141.
89. Amin MB, Schultz DS, Zarbo RJ. Analysis of cribriform morphology in prostatic neoplasia using antibody to high-molecular-weight cytokeratins. Arch Pathol Lab Med 1994, **118**: 260–264.
90. Pacelli A, Lopez-Beltran A, Egan AJ, Bostwick DG. Prostatic adenocarcinoma with glomeruloid features. Hum Pathol 1998, **29**: 543–546.
91. Parwani AV, Kronz JD, Genega EM, Gaudin P, Chang S, Epstein JI. Prostate carcinoma with squamous differentiation: an analysis of 33 cases. Am J Surg Pathol 2004, **28**: 651–657
92. 92.Woods JE, Soh S, Wheeler TM. Distribution and significance of microcalcifications in the neoplastic and non-neoplastic prostate. Arch Pathol Lab Med 1998, **122**: 152–155.
93. Holmes EJ. Crystalloids of pro static carcinoma. Relationship to Bence-Jones crystals. Cancer 1977, **39**: 2073–2080.
94. Tran TT, Sengupta E, Yang XJ. Prostatic foamy gland carcinoma with aggressive behavior: clinicopathologic, immunohistochemical, and ultrastructural analysis. Am J Surg Pathol 2001, **25**: 618–623.

95. Levi AW, Epstein JI. Pseudohyperplastic prostatic adenocarcinoma on needle biopsy and simple prostatectomy. Am J Surg Pathol 2000, **24**: 1039–1046.

96. Dube VE, Farrow GM, Greene LF. Prostatic adenocarcinoma of ductal origin. Cancer 1973, **32**: 402–409.

97. Kuhajda FP, Gipson T, Mendelsohn G. Papillary adenocarcinomas of the prostate. Cancer 1984, **54**: 1328–1332.

98. Melicow MM, Pachter MR. Endometrial carcinoma of the prostatic utricle (uterus masculinus). Cancer 1967, **20**: 1715–1722

99. Cheville JC, Dundore PA, Bostwick DG, Lieber MM, Batts KP, Sebo TJ, Farrow GM. Transitional cell carcinoma of the prostate: clinicopathologic study of 50 cases. Cancer 1998, **82**: 703–707

100. Abrahamsson PA, Wadstrom LB, Alumets J, Falkmer S, Grimelius L. Peptide-hormone and serotonin-immunoreactive cells in normal and hyperplastic prostate glands. Pathol Res Pract 1986, **181**: 675–683.

101. Epstein JI, Lieberman PH. Mucinous adenocarcinoma of the prostate gland. Am J Surg Pathol 1985, **9**: 299–308.

102. Mai KT, Leahy CF. Squamous cell carcinoma occurring as a circumscribed nodule in the transition zone of the prostate: A case report and review of the literature. J Urol Pathol 1996, **5**: 85–92.

103. Guerin D, Hasan N, Keen CE. Signet ring cell differentiation in adenocarcinoma of the prostate. A study of five cases. Histopathology 1993, **22**: 367–371

104. Ali TZ, Epstein JI. Basal cell carcinoma of the prostate: a clinicopathologic study of 29 cases. Am J Surg Pathol 2007, **31**: 697–705.

105. Lauwers GY, Schevchuk M, Armenakas N, Reuter VE. Carcinoma of the prostate. Am J Surg Pathol 1993, **17**: 342–349.

106. Gretzer MB, Partin AW: PSA markers in prostate cancer detection. *Urol Clin North Am*. 30:677–686, 2003.

107. .Marchal C, Redondo M, Padilla M, et al: Expression of prostate specific membrane antigen (PSMA) in prostatic adenocarcinoma and prostatic intraepithelial neoplasia. *Histol Histopathol*. 19:715–718, 2004.

108. Bostwick DG, Pacelli A, Blute M, et al: Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: A study of 184 cases. *Cancer*. 82:2256– 2261, 1998.

109. Chuang AY, DeMarzo AM, Veltri RW, et al: Immunohistochemical differentiation of high-grade prostate carcinoma from urothelial carcinoma. *Am J Surg Pathol*. 31:1246–1255, 2007.

110. Jiang Z, Woda BA: Diagnostic utility of alpha-methylacyl CoA racemase (P504S) on prostate needle biopsy. *Adv Anat Pathol*. 11:316–321, 2004.

111. Wojno KJ, Epstein JI: The utility of basal cell-specific anticytokeratin antibody (34 beta E12) in the diagnosis of prostate cancer. A review of 228 cases. *Am J Surg Pathol*. 19:251–260, 1995.

112. Abate-Shen C, Shen MM, Gelmann E: Integrating differentiation and cancer: The Nkx3.1 homeobox gene in prostate organogenesis and carcinogenesis. *Differentiation*. 76:717–727, 2008.

113. McNeal JG. The prostate gland: morphology and pathobiology. Monogr Urol. 1983;4:3–33. 114.McNeal J. Pathology of benign prostatic hyperplasia. Insight into etiology. Urol Clin North Am. 1990; 17:477–86.

114. McVary KT, Roehrborn CG, Avins AL, Barry MJ, Bruskewitz RC, Donnell RF, et al. Update on AUA guideline on the management of benign prostatic hyperplasia. J Urol. 2011;185:1793–803

115. Stroup SP, Palazzi-Churas K, Kopp RP, Parsons JK. Trends in adverse events of benign prostatic hyperplasia (BPH) in the USA, 1998 to 2008. BJU Int. 2012;109:84–7.

116. Barry MJ, Fowler FJ, Jr, Bin L, Pitts JC, 3rd, Harris CJ, Mulley AG., Jr The natural history of patients with benign prostatic hyperplasia as diagnosed by North American urologists. J Urol. 1997;157:10–4.

117. Bosch JL, Tilling K, Bohnen AM, Bangma CH, Donovan JL. Establishing normal reference ranges for prostate volume change with age in the population-based Krimpen-study: Prediction of future prostate volume in individual men. Prostate. 2007;67:1816–24.

118. Loeb S, Kettermann A, Carter HB, Ferrucci L, Metter EJ, Walsh PC. Prostate volume changes over time: Results from the Baltimore Longitudinal Study of Aging. J Urol. 2009;182:1458–62.

119. Williams AM, Simon I, Landis PK, Moser C, Christens-Barry W, Carter HB, et al. Prostatic growth rate determined from MRI data: Age-related longitudinal changes. J Androl. 1999;20:474–80

120. Jin B, Turner L, Zhou Z, Zhou EL, Handelsman DJ. Ethnicity and migration as determinants of human prostate size. J Clin Endocrinol Metab. 1999;84:3613–9.

121. Pearson JD, Lei HH, Beaty TH, Wiley KE, Isaacs SD, Isaacs WB, et al. Familial aggregation of bothersome benign prostatic hyperplasia symptoms. Urology. 2003;61:781–5.

122. Sanda MG, Doehring CB, Binkowitz B, Beaty TH, Partin AW, Hale E, et al. Clinical and biological characteristics of familial benign prostatic hyperplasia. J Urol. 1997;157:876–9.

123. Rohrmann S, Fallin MD, Page WF, Reed T, Partin AW, Walsh PC, et al. Concordance rates and modifiable risk factors for lower urinary tract symptoms in twins. Epidemiology. 2006;17:419–27.

124. Partin AW, Page WF, Lee BR, Sanda MG, Miller RN, Walsh PC. Concordance rates for benign prostatic disease among twins suggest hereditary influence. Urology. 1994;44:646–50.

125. Kristal AR, Schenk JM, Song Y, Arnold KB, Neuhouser ML, Goodman PJ, et al. Serum steroid and sex hormone-binding globulin concentrations and the risk of incident benign prostatic hyperplasia: Results from the prostate cancer prevention trial. Am J Epidemiol. 2008; 168:1416–24

126. McConnell JD, Roehrborn CG, Bautista OM, Andriole GL, Jr, Dixon CM, Kusek JW, et al. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. N Engl J Med. 2003;349:2387–98.

127. Kumar R, Verma V, Sarswat A, Maikhuri JP, Jain A, Jain RK, et al. Selective estrogen receptor modulators regulate stromal proliferation in human benign prostatic hyperplasia by multiple beneficial mechanisms - Action of two new agents. Invest New Drugs. 2012;30:582–93

128. Haffner S, Taegtmeyer H. Epidemic obesity and the metabolic syndrome. Circulation. 2003;108:1541–5.

129. Meigs JB, Mohr B, Barry MJ, Collins MM, McKinlay JB. Risk factors for clinical benign prostatic hyperplasia in a community-based population of healthy aging men. J Clin Epidemiol. 2001;54:935–44.[PubMed]

130. Joseph MA, Harlow SD, Wei JT, Sarma AV, Dunn RL, Taylor JM, et al. Risk factors for lower urinary tract symptoms in a population-based sample of African-American men. Am J Epidemiol. 2003;157:906–14.

131. Parsons JK, Sarma AV, McVary K, Wei JT. Obesity and benign prostatic hyperplasia: Clinical connections, emerging etiological paradigms and future directions. J Urol. 2013;189:S102–6.

132. Parsons JK. Modifiable risk factors for benign prostatic hyperplasia and lower urinary tract symptoms: New approaches to old problems. J Urol. 2007;178:395–401

133. Sarma AV, Parsons JK, McVary K, Wei JT. Diabetes and benign prostatic hyperplasia/lower urinary tract symptoms – What do we know? J Urol. 2009;182:S32–7.

134. Parsons JK, Kashefi C. Physical activity, benign prostatic hyperplasia, and lower urinary tract symptoms. Eur Urol. 2008;53:1228–35.

135. Parsons JK. Modifiable risk factors for benign prostatic hyperplasia and lower urinary tract symptoms: New approaches to old problems. J Urol. 2007;178:395–401

136. Kristal AR, Arnold KB, Schenk JM, Neuhouser ML, Goodman P, Penson DF, et al. Dietary patterns, supplement use, and the risk of symptomatic benign prostatic hyperplasia: Results from the prostate cancer prevention trial. Am J Epidemiol. 2008;167:925–34.

137. Maserejian NN, Giovannucci EL, McVary KT, McKinlay JB. Dietary, but not supplemental, intakes of carotenoids and vitamin C are associated with decreased odds of lower urinary tract symptoms in men. J Nutr. 2011;141:267–73

138. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004;114:1752–61.

139. Sutcliffe S, Zenilman JM, Ghanem KG, Jadack RA, Sokoll LJ, Elliott DJ, et al. Sexually transmitted infections and prostatic inflammation/cell damage as measured by serum prostate specific antigen concentration. J Urol. 2006;175:1937–42.

140. Sutcliffe S, Rohrmann S, Giovannucci E, Nelson KE, De Marzo AM, Isaacs WB, et al. Viral infections and lower urinary tract symptoms in the third national health and nutrition examination survey. J Urol. 2007; 178:2181–5.

141. St Sauver JL, Jacobson DJ, McGree ME, Lieber MM, Jacobsen SJ. Protective association between nonsteroidal antiinflammatory drug use and measures of benign prostatic hyperplasia. Am J Epidemiol. 2006; 164:760–8.

142. Price H, McNeal JE, Stamey TA. Evolving patterns of tissue composition in benign prostatic hyperplasia as a function of specimen size. Hum Pathol 1990, **21**: 578–585.

143. Cordon-Cardo C, Koff A, Drobnjak M, Capodieci P, Osman I, Millard SS, Gaudin PB, Fazzari M, Zhang ZF, Massague J, Scher HI. Distinct altered patterns of p27KIP1 gene expression in benign prostatic hyperplasia and prostatic carcinoma. J Natl Cancer Inst 1998, **90**: 1284–1291.

ANNEXURES

INFORMATION SHEET

- We are conducting a study on benign prostatic hyperplasia and prostatic cancer among patients attending Government General Hospital, Chennai and for that your specimen may be valuable to us.

- The purpose of this study is to aid in differentiating prostatic cancer from benign prostatic hyperplasia easily with the help of certain special tests and immunohistochemical markers.

- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.

- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

ஆராய்ச்சி தகவல் தாள்

ஆராய்ச்சி தலைப்பு : **சுக்கியன் புற்றுநோய் மற்றும் சுக்கியன் மிகை வளர்ச்சியில் உயர் வேதியல் மற்றும் உயர் திசு சிறப்பு ஆராய்ச்சி முறையில் p63 வெளிபாட்டை கண்டறிதல்.**

ஆய்வாளர் : **மரு. ந.கிருத்திகா**
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை-600003.

தங்களது சுக்கியன் புற்றுநோய் மற்றும் சுக்கியன் மிகை வளர்ச்சியில் உயர் வேதியல் மற்றும் உயர் திசு சிறப்பு ஆராய்ச்சி முறையில் p63 வெளிபாட்டை கண்டறிதல்.

இராஜீவ்காந்தி அரசு பொதுமருத்துவமனைக்கு வரும் நோயாளிகளிடம் இருந்து பெறப்பட்ட வயிற்றுப் புற்றுநோய் கட்டிகளைப் பற்றிய ஒரு ஆராய்ச்சி இங்கு நடைபெற்றுவருகின்றது.

இந்த சுக்கியன் புற்றுநோய் மற்றும் சுக்கியன் மிகை வளர்ச்சியில் உயர் வேதியல் மற்றும் உயர் திசு சிறப்பு ஆராய்ச்சி முறையில் ி63 வெளிபாட்டை கண்டறிதலே எனது ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்தஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்தஆராய்ச்சியில் உங்களுடைய திசுக்களைஎடுத்து சில சிறப்புப் பரிசோதனைக்குஉட்படுத்திஅதன்தகவல்களைஆராய்வோம். அதனால்தங்களது நோயின்ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக்கொள்கிறோம்.

முடிவுகளைஅல்லதுகருத்துகளைவெளியிடும் போதோஅல்லதுஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லதுஅடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்தஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான்இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்தஆராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளைஆராய்ச்சியின் போதுஅல்லதுஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்தஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : மரு.B.அபிராமி, செல் : 90944 73433

பங்கேற்பாளர் கையொப்பம் .....இடம் : ..... தேதி : .....
பங்கேற்பாளர் பெயர் மற்றும் விலாசம் :

ஆராய்ச்சியாளர் கையொப்பம்.....இடம் : ..... தேதி : .....

INFORMED CONSENT FORM

Title of the study: A STUDY ON MUCIN HISTOCHEMISTRY AND p63 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS.

Name of the Participant : Dr.KIRUTHIKA.N
Name of the Principal(Co-Investigator) :
Name of the Institution : Madras Medical College
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read tome). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in "A STUDY ON MUCIN HISTOCHEMISTRY AND P63 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS".

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the resected tumors will be subjected to immunohistochemistry and histopathological examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understood that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

ஆராய்ச்சி ஒப்புதல்கடிதம்

ஆராய்ச்சி தலைப்பு : சுக்கியன் புற்றுநோய் மற்றும் சுக்கியன் மிகை வளர்ச்சியில் உயர் வேதியல் மற்றும் உயர் திசு சிறப்பு ஆராய்ச்சி முறையில் p63 வெளிபாட்டை கண்டறிதல்.

சென்னை மருத்துவக் கல்லூரி நோய்குறியியல் துறையில் பயிலும் முதுகலை மருத்துவர் மரு. ந.கிருத்திகா , அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்டவிஷயங்களை நான் புரிந்து கொண்டு நான்எனது சம்மதத்தைத்தெரிவிக்கிறேன்.

இந்தஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றிஎன் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான்இந்தஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால்எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் சுக்கியன் புற்றுநோய் மற்றும் சுக்கியன் மிகை வளர்ச்சியினை குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல்தாளைப் பெற்றுக்கொண்டேன்

நான்என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன்இந்த மருத்துவஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவை சிகிச்சை செய்யப்பட்டு நோய்க்குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்டமெழுகுக்கட்டிகளைவைத்துஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்து கொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம் .....இடம் : ..... தேதி : .....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் :

ஆராய்ச்சியாளர் கையொப்பம்.....இடம் : ..... தேதி : .....

MASTER CHART

S.NO	HPE NO	AGE	CLINICAL DIAGNOSIS	SPECIMEN TYPE	HPE DIAGNOSIS	GLEASON SCORE	GRADE	IHC-p63	ALCIAN BLUE
1	7682/16	61	carcinoma prostate	channel TURP	prostatic adenocarcinoma	3+4	gradeII	negative in neoplastic glands	positive
2	7979/16R2	67	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
3	8632/16A	73	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
4	10345/16	77	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
5	10659/16D	68	BPH	TURP	benign adenomyomatous hyperplasia of prostate			positive in basal layer of glands	negative
6	10881/16	64	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
7	11759/16	77	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
8	11866/16	70	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
9	11987/16	79	BPH	TURP	benign adenomyomatous hyperplasia of prostate			positive in basal layer of glands	negative
10	1074/15	75	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
11	1249/15	72	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
12	1319/15	74	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
13	1378/15	60	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
14	1462/15	67	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
15	1531/15	69	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
16	1572/15	70	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
17	1641/15	65	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
18	1690/15	68	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative

19	1836/15	70	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
20	1837/15	80	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
21	1864/15	60	BPH	TURP	benign adenomyomatous hyperplasia of prostate			positive in basal layer of glands	negative
22	1905/15	75	BPH with vesical calculous	TURP	benign adenomyomatous hyperplasia of prostate			positive in basal layer of glands	negative
23	1947/15B	75	obstructive LUTS	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
24	1967/15C	75	obstructive LUTS	biopsy	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
25	2022/15	76	BPH	TURP	benign adenomatous hyperplasia of prostate			positive in basal layer of glands	negative
26	5721/16D	70	?carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	4+3	GRADE III	neagative in neoplastic glands	positive
27	5805/16	67	?carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	grade II	negative in neoplastic glands	positive
28	6069/16	72	?carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	grade II	negative in neoplastic glands	negative
29	8412/16	79	?carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	gradeII	negative in neoplastic glands	negative
30	7810/16	80	?carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	gradeII	negative in neoplastic glands	negative
31	6899/16	66	carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	gradeII	negative in neoplastic glands	negative
32	7040/16	83	carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	grade II	negative in neoplastic glands	positive
33	6855/16G	70		TRUS biopsy	prostatic adenocarcinoma	3+4	gradeII	negative in neoplastic glands	negative
34	7640/16G	85	?carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	gradeII	negative in neoplastic glands	negative
35	8824/16H	79	carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	3+4	grade II	negative in neoplastic glands	positive
36	10198/16	77	carcinoma prostate	TRUS biopsy	prostatic adenocarcinoma	4+3	grade III	negative in neoplastic glands	positive
37	10098/16B	70	LUTS with hard nodule	TRUS biopsy	prostatic adenocarcinoma	4+3	grade III	negative in neoplastic glands	positive

38	**10239/16**	**75**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**5+4**	**grade V**	**negative in neoplastic glands**	**negative**
39	**11659/16E**	**56**	**nodular prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**4+4**	**gradeIV**	**negative in neoplastic glands**	**negative**
40	**10553/16**	**60**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**5+3**	**grade IV**	**negative in neoplastic glands**	**positive**
41	**10827/16**	**83**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**5+4**	**grade V**	**negative in neoplastic glands**	**negative**
42	**155/15C**	**80**	**carcinoma prostate**	**channel TURP**	**prostatic adenocarcinoma**	**3+4**	**gradeII**	**negative in neoplastic glands**	**positive**
43	**1761/15E**	**70**	**carcinoma prostate**	**blind prostate biopsy**	**prostatic adenocarcinoma**	**4+5**	**gradeV**	**negative in neoplastic glands**	**negative**
44	**3025/15B**	**80**	**carcinoma prostate**	**TURP with bilateral orchidectomy**	**prostatic adenocarcinoma**	**4+3**	**grade III**	**negative in neoplastic glands**	**negative**
45	**2679/15C**	**80**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**4+4**	**grade IV**	**negative in neoplastic glands**	**positive**
46	**1155/15A**	**66**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**3+4**	**grade II**	**negative in neoplastic glands**	**positive**
47	**3211/15D**	**64**	**carcinoma prostate**	**channel TURP**	**prostatic adenocarcinoma**	**5+5**	**gradeV**	**negative in neoplastic glands**	**negative**
48	**12679/15**	**83**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**4+4**	**grade IV**	**negative in neoplastic glands**	**negative**
49	**11526/16H**	**76**	**carcinoma prostate**	**TRUS biopsy**	**prostatic adenocarcinoma**	**3+3**	**grade I**	**negative in neoplastic glands**	**positive**
50	**5315/15**	**75**	**BPH**	**TURP**	**benign adenomyomatous hyperplasia of prostate**			**positive in basal layer of glands**	**negative**