ROLE OF EXPRESSION OF P63 AND CALPONIN IN PROSTATIC LESIONS

Dissertation submitted in

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M.D. DEGREE

in

PATHOLOGY – BRANCH III

THE TAMILNADU
DR. M.G.R. MEDICAL UNIVERSITY
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DECLARATION

I hereby declare that the dissertation entitled "**ROLE OF P63 AND CALPONIN IN PROSTATIC LESIONS**" was done by me in the Department of Pathology, Chengalpattu Medical College from June 2015 to May 2017 under the guidance and supervision of **Dr. S. Premalatha M.D**., Associate Professor, Department of Pathology, Chengalpattu Medical College.

This dissertation is submitted to the Tamil Nadu Dr.MGR Medical University, Chennai towards the partial fulfillment of the requirements for the award of M.D. Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

SREELA. S

Place:

Date:

CERTIFICATE

This is to certify that the dissertation entitled "**Role of Expression of P63 and Calponin in Prostatic lesions**" is a record of bonafide work done by **Dr.Sreela.S** in the Department of Pathology, Chengalpattu Medical College, Chengalpattu under the supervision of **Dr. S. Ravi M.D.,** Professor and Head, Department of Pathology and submitted in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai. This work has not formed the basis for the award of a degree or diploma, anytime before.

Dr. Usha Sadasivan,M.D.,
Dean,
Chengalpattu Medical College,
Chengalpattu

Dr. S. Ravi M.D.
Professor and Head,
Department of Pathology,
Chengalpattu Medical College
Chengalp attu

CERTIFICATE FROM THE GUIDE

This is to certify that the dissertation entitled, "**ROLE OF EXPRESSION OF P63 AND CALPONIN IN PROSTATIC LESIONS**" submitted by the candidate **Dr. SREELA. S** in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamil Nadu Dr.M.G.R. Medical University, Chennai is a bonafide research work done by her under my direct guidance and supervision, in the Department of Pathology, Chengalpattu Medical College, Chengalpattu. This work has not formed the basis for the award of any degree or diploma, anytime before.

Dr. S. PREMALATHA., MD.
Associate Professor
Department of Pathology,
Chengalpattu Medical College,
Chengalpattu

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INSTITUTIONAL ETHICAL COMMITTEE

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Title of Work	:	Role of expression of p63 and calponin in prostatic Lesions.
Principal Investigator	:	Dr.S.Sreela,
Designation	:	1st Year Post Graduate in Pathology
Co-Investigators	:	1. Dr.S.Premalatha,MD., Associate Professor, Department of Pathology.
Department	:	Pathology

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This is to certify that this dissertation work titled **"ROLE OF EXPRESSION OF P63 AND CALPONIN IN PROSTATIC LESIONS''** of the candidate **Dr. SREELA. S** with Registration Number **201513501** for the award of **Degree of M.D.** in the branch of **PATHOLOGY BRANCH – III.** I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** percentage of plagiarism in the dissertation.

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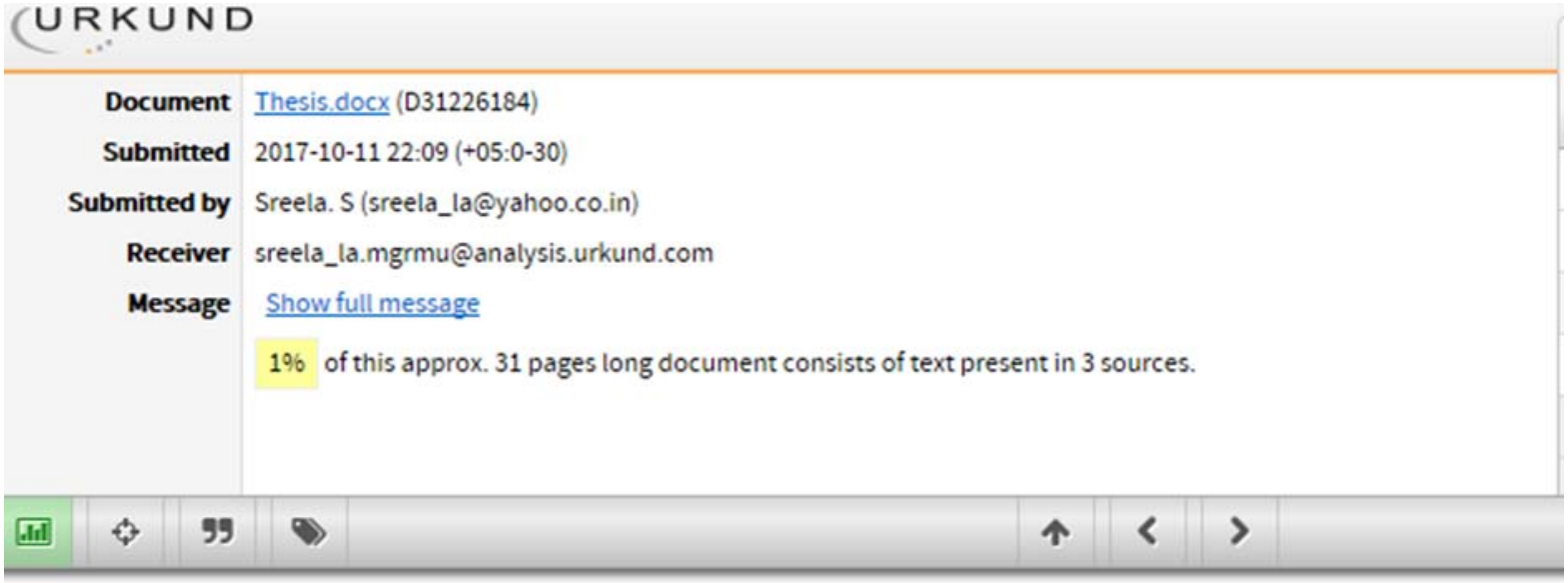

INTRODUCTION

Prostatic cancer is the sixth most common cause of cancer in the world and is a cause of significant morbidity and mortality in men (1) (2). It is the second most common cancer among men being leaded by lung carcinoma. In previous decades prostatic cancer was less among Asians, but due to influence of various dietary and environmental factors the incidence of prostatic cancer among Asians is on rise. The incidence of prostate cancer is expected to increase four times between 2020 and 2050 in men <65 years. (1)

There is an increase in diagnosis of prostate cancer due to increase in mass screening programme with use of PSA (Prostate Specific Antigen) along with DRE(Digital rectal examination) and imaging studies. (3) (4) (5)

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INTRODUCTION

Prostatic cancer is the sixth most common cause of cancer in the world and is a cause of significant morbidity and mortality in men(1) (2). It is the second most common cancer among men, being preceded by lung carcinoma.

In previous decades prostatic cancer was less among Asians, but due to influence of various dietary causes and environmental factors the incidence of prostatic cancer among Asians is on rise. The incidence of prostate cancer is expected to increase four times between 2020 and 2050 in men >65 years (1).

There is an increase in diagnosis of prostate cancer due to increase in mass screening programme with use of PSA (Prostate Specific Antigen) along with DRE(Digital rectal examination) and imaging studies (3) (4)(5).

Biopsies including needle core and TURP requires accurate interpretation for early diagnosis which is very challenging in view of small volume of biopsies and presence of benign mimickers. Mimickers include atrophy, atypical adenomatous hyperplasia, basal cell hyperplasia, atypical small acinar proliferation and high grade PIN. Further the threshold for detecting small foci of cancer in needle biopsies is very low.(3)

p63 is basal cell nuclear marker normally present in prostatic epithelium (1). The absence of this marker in prostate is more indicative towards malignancy. It is highly useful in case of ambiguous lesions of prostate or differentiating tangential sections from PIN/Prostatic carcinoma. Benign

mimickers will show strong positivity for p63 and premalignant lesions show patchy staining for the marker (6).

Normal prostatic ducts and acini are surrounded by fibromuscular stroma. In prostatic cancer this fibromuscular stroma is replaced by reactive stroma of wound repair type composed of myofibroblasts and fibroblasts and it is likely to promote tumor progression. This is done by promoting angiogenesis, tumor cell proliferation and invasion (7)(8)(9)(10)(11)(12)(13) . This reactive stroma appears to be associated with PIN and progresses to cancer.(8)

Calponin is an actin filament associated regulatory protein. It is predominantly a smooth muscle marker but also occurs in other tissues like epidermal keratinocytes, lung alveolar cell, fibroblasts. Generally it helps in cell proliferation, cell motility and cell adhesion. Only few studies have been done on role of this marker in prostate.

Various studies shows that Calponin is strongly expressed in normal prostate by the smooth muscle cells. In preneoplastic conditions like high grade PIN the expression of calponin with stromal cells is variable. The calponin expression in prostatic carcinoma varies from nil to scanty.

Based on this concept we are analysing the expression of p63, a diagnostic marker and calponin on various pathological lesions of prostate in prostatic biopsies – both needle core biopsy and transurethral resection of prostate.

AIM AND OBJECTIVES

1) To assess the diagnostic utility of p63 staining in prostatic lesions.

2) To assess the expression of Calponin in benign and malignant prostatic lesions

3) To determine the relationship between the immunostaining and histologic grade of prostatic carcinoma.

REVIEW OF LITERATURE

Embryology of prostate

Prostate develops during 3rd month of gestation due to evaginations of epithelium from prostatic urethra. Evaginations from proximal prostatic urethra give rise to simple periurethral glands (cervical glands of Alberian) (14).

Those evaginations arising near distal prostatic urethra give rise to five groups of tubules that grow later to become five different lobes of prostate mainly anterior, posterior, 2 lateral and one medial.

ANATOMY OF PROSTATE

The prostate is compound exocrine tubule-alveolar gland. Approximate weight of normal prostate is around 20g (14-26g) and dimensions are 4x3x2cm. It is an accessory gland of male reproductive system which also consists of paired seminal vesicles, paired bulbourethral glands. They produce secretory products that mix with sperm and produce semen. It is located in the true pelvis and is inverted cone in shape. Base is located superiorly and tapered apex is present inferiorly. Apex blends with the transverse urogenital diaphragm (15).

Prostatic urethra is about 3cm length and passes through prostate from apex of bladder trigone and continues as membranous urethra. Posterior wall of urethra shows urethral crest which is a longitudinal ridge lined by two prostatic sinuses. Prostatic ductules enter through these sinuses. Prostate is divided grossly into 5 lobes namely anterior, posterior, 2 lateral, one medial and three

zones mainly peripheral zone, central zone and transitional zone. Peripheral zone forms the bulk and is the most common site for carcinoma. This zone is also more prone for inflammation and atrophy (15). Central zone is inverted conical shape with base forming entire base of prostate. It lies posterior to transition zone and encircles ejaculatory duct. It is resistant to carcinoma and inflammation. Transition zone forms the least area and is the most common site for Benign Prostatic hyperplasia and Atypical adenomatous hyperplasia.(14)

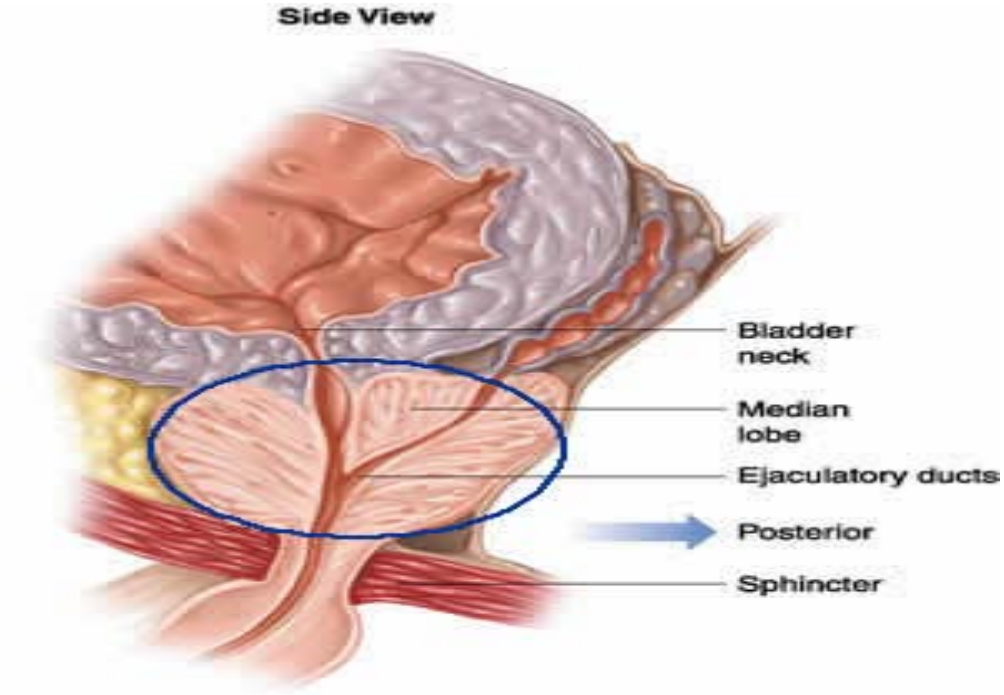


Fig1: Anatomy of prostate with prostatic lobes

Blood vessels and Nerve supply

Arterial supply is by Internal pudental artery, Inferior vesical artery and branches of middle rectal artery.

Venous drainage is by Vesicoprostatic plexus which drains in to Internal iliac vein.

Parasympathetic supplystimulates glandular activity and sympathetic supply mediate alpha-1 receptor mediated smooth muscle contraction. They penetrate capsule and enter prostate along with the blood vessels.

Lymphatics drain to Internal iliac lymph nodes, sacral nodes and partly to external iliac nodes.

HISTOLOGY

Prostate consists of small branched tubuloacinar prostatic glands lined by two layer of cells- luminal tall columnar secretory cells with basally located nucleus and abluminal flattened myoepithelial cells. The glands contain round pink concretions called as corpora amylacea.

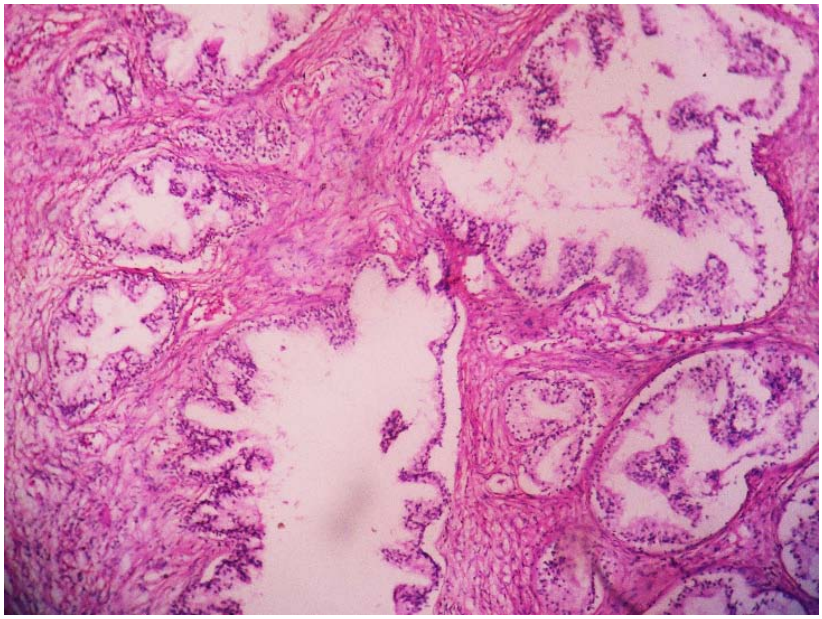


Fig2: Normal histology of prostate

ETIOPATHOGENESIS FOR PROSTATIC LESIONS:

The most important and proven risk factors for prostatic lesions are age, hormonal factors and family history. Considering the importance of prostatic malignancy as it is the second most common malignancy affecting elderly male and in view of its increasing incidence in present scenario, detailed analysis of risk factors of prostatic carcinoma are as detailed below:

AGE:

Risk for prostate cancer increases with increasing age. It begins to rise after 55 years and reaches peak at around 70 years and declines thereafter (16). According to United States of America statistics on prostatic cancer, it was estimated that every one in 10,000 men in their 40's and one in 15 men in their 60's will be affected by prostate cancer. In India the incidence is on rise due to

changing lifestyle, increased awareness, increased access to medical facility etc.

FAMILY HISTORY:

An individual with a positive family history has increased risk of developing prostate cancer. Families having a first degree relative suffering from prostatic cancer, the risk of developing cancer in future is increased 2 to 3 times. Risk is further increased if there is onset of cancer at an early age in first degree relative (17). Several genomic studies on familial prostate cancer describes the pattern of inheritance of high risk genes. It states that those high risk genes responsible for cancer follows Mendelian Autosomal dominant expression thereby resulting in early age of onset of the disease (18).

RACE:

Incidence rate of prostatic cancer in African American is much higher, around 60 fold when compared to men in Asian countries. This variations are mainly due to the food habits, screening programmes, environmental factors, diagnostic advancement and increased accessibility to total health care services. Migrants from Asian countries also shows similar incidence rate of prostatic cancer to that of Americans (19).

DIET:

Diet containing large amount of fat and increased calorie intake is associated with the increased risk of developing cancer along with reduced intake of antioxidants like selenium, vitamin C.

Vitamin D deficiency has also been identified as possible risk factors for developing prostatic carcinoma. Increased age, black race and northern latitudes which was proved as an independent risk factors, are all associated with Vitamin D deficiency.

Possible explanations of these factors are increased age is associated with decreased synthesis of Vitamin D and also due to decreased exposure to sunlight. Black race has more melanin pigments which can directly inhibit synthesis of vitamin D. In previous era, Asian men used to have a decreased incidence of prostatic cancer when compared to Americans due to their dietary habits. Asian diet is rich in fish that has higher amount of Vitamin D (20).

The changing food habits is one of the contributing factor leading to increase in incidence of prostate cancer in Asian population.

Other less important factors are anthropometric factors, hormonal profiles, environmental exposure to cadmium, rubber, textile, chemical, drug, fertilizer and atomic energy industries and other co-morbid health factors. There is also association with Xenotropic murine leukemia virus related virus

but it is controversial (15). They play a minor role in the development and progression of the disease.

ANTHROPOMETRIC FACTORS

Anthropometric factors like height and obesity and their association with prostate cancer risk has been extensively studied. It was hypothesized that adult height is due to the hormone Insulin like growth factor. This hormone carries significant risk for the development of prostate cancer. Regarding obesity, it was hypothesized that increased obesity reduces sex hormone binding globulins, therefore, availability of free sex hormones in the circulation which can stimulate cancer progression (21). But both the hypothesis has not been proved so far.

An increased level of IGF-1 that mediates the action of growth hormone was identified as an independent risk factor for prostate cancer. It was proven that administration of IGF-1 promotes growth of prostate and tumor development in animal models (22).

PATHOGENESIS:

It is the hormone dihydrotestosterone that plays a major role in the development of prostatic lesions including benign and malignant lesions.

Benign Prostatic Hyperplasia is characterized by increase in the epithelial and stromal cells commonly in the periurethral zone of prostate. This

increased cell number could be due to either increased proliferation of cells or decreased apoptosis (23).

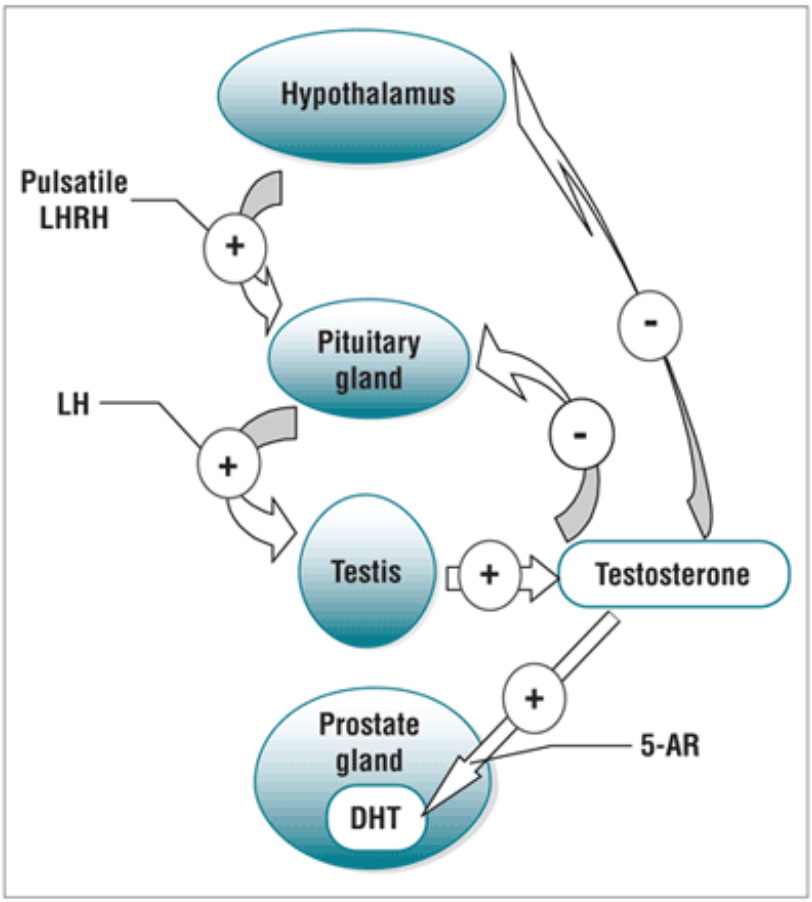


Figure 1. Hypothalamic-pituitary-testicular axis. DHT: dihydrotestosterone; 5-AR: 5-alpha reductase; LH: luteinizing hormone; LHRH: LH-releasing hormone.

Figure 3 :Pathogenesis of BPH

Dihydrotestosterone (DHT) is a active metabolite of hormone testosterone and is the main androgen responsible for development of BPH..

The stromal cells of prostate gland has an enzyme 5α reductase which helps in convertion of testosterone into DHT. This DHT is more potent than testosterone and has more affinity towards androgen receptor which is present

in epithelial and stromal cells thereby stimulating transcription of genes resulting in proliferation of epithelial cells and stromal cells.

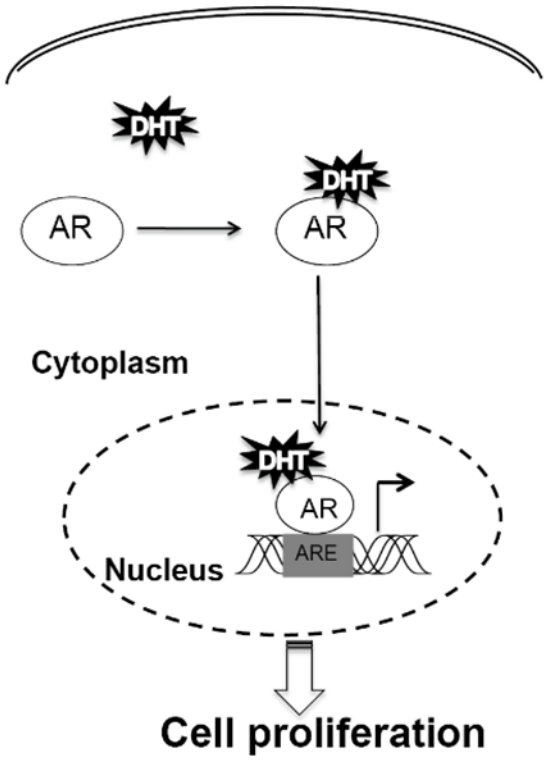


Figure 4: DHT and nuclear transcription

Though the pathogenesis for the BPH has been well understood, the exact triggering or inciting event is still not clear. One possible hypothesis is that prostatic inflammation could be a triggering factor for the cell proliferation (24) . Inflammatory cytokines such as Interleukin 2, Interleukin 6, interleukin 8, interleukin 15 and Interferon alpha causes tissue damage and oxidative stress to the stromal cells. This leads to compensatory cellular proliferation there by promoting the growth of the gland. (25).

In case of prostatic carcinoma these circulating androgens are essential for the onset of prostate cancer through their interactions with Androgen

receptor. Therefore surgical treatment like bilateral orchidectomy which is the source for androgens and antiandrogen drugs causes disease regression. But some of the tumors become androgen resistant by following mechanisms:

1) Androgen receptor gene (AR) amplification results in hypersensitivity to even low levels of androgens.

2) Mutation in androgen receptor gene causing ligand independent AR activation.

In addition to the androgens, prostatic cancer usually acquires large number of genetic alterations including point mutations, deletions, amplifications and translocations. There are prostate cancer specific chromosome rearrangements. It commonly involves E26 Transformation specific (ETS) gene family. ERG (ETS-related gene product) is the oncogene which belongs to the ETS family, fuses with Transmembrane protease serine 2 (TMPRSS2) resulting in Androgen independent tumor progression. This results in over expression of transcription factors which lead to up regulation of matrix metalloproteinase. Increased matrix metalloproteinase makes the malignant prostatic epithelial cells more invasive.(15)

Benign Prostatic Hyperplasia (BPH) and normal epithelium are negative for ERG rearrangements and fusion transcripts. TMPRSS2: ERG fusions are reported in 10 – 21% of high grade prostatic intraepithelial neoplastic lesions (26) and 29 – 59 % in hormone refractory and in metastatic prostatic carcinoma (27).

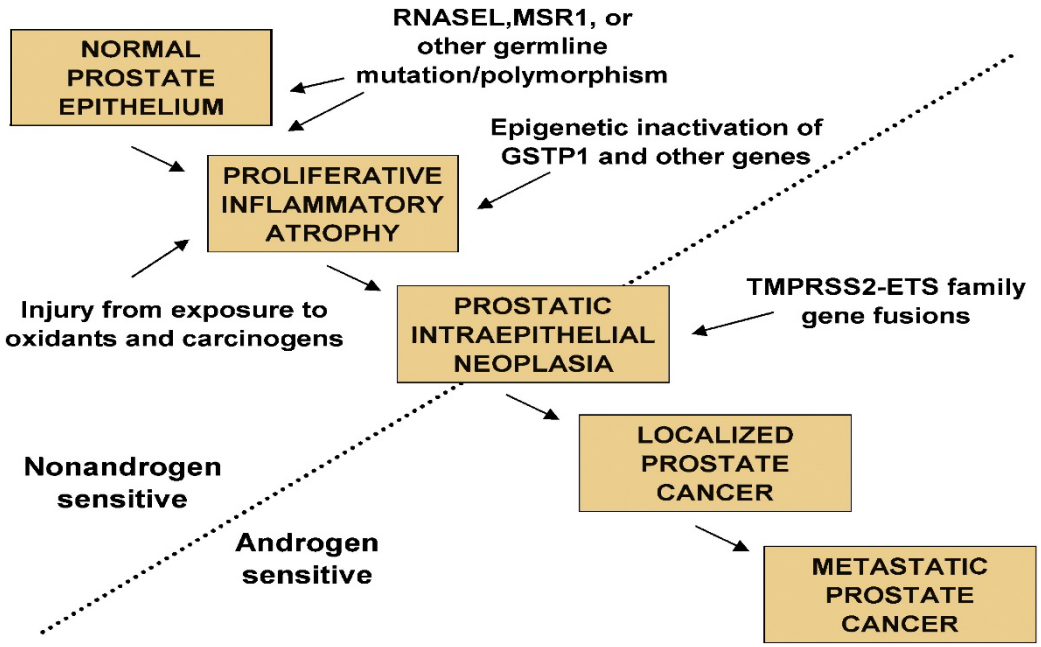


Figure 5: pathogenesis of prostatic carcinoma

Other common genetic alterations in prostate cancer includes mutation in BRCA 2 and PTEN tumor suppressor gene, MYC oncogene and at later stages TP53 and RB gene mutations.

EPIDEMIOLOGY OF PROSTATIC LESIONS

Prostate cancer is a major cause of morbidity and mortality in men and it stands fourth among most common cancer in world and second most common among men being preceded by lung cancer (1). Literature shows that incidence of prostatic carcinoma in 2012 was 1.1million which accounted for about 8% among all cancer and 15% cancer among men (28). The worldwide prostate cancer burden is expected to grow to 1.7 million new cases and 4,99 000 new deaths by 2030 simply due to the growth and aging of the global population. The lifetime risk for developing prostate cancer is 1 in 7 men. About 161,360

new cases have been diagnosed with prostatic cancer based on 2017 statistics in United states.(29)

The incidence rates of prostate cancer were considered low in Asian and North African countries previously. The prevalence of prostate cancer in India is far lower as compared to the western countries but with the increased rate of urbanisation, changing food habits and sophisticated lifestyle and easy access to medical facility, more cases of prostate cancer are being diagnosed nowadays. The data regarding the exact incidence of prostate cancer in India is limited mainly because of the fact that it is not a notifiable disease and only limited population based cancer registries are available in India.

In India, when comparing the incidence rates of different cancers, oral cancer being most common and prostate cancer has lower incidence when compared to other cancers (28). The estimated incidence of prostate cancer in India is around 3.75/100,000 persons. According to Population Based Cancer Registries (PBCR) , prostate cancer has ranked among top 10 cancers . It is second most common site of cancer in cities like Delhi, Kolkata, Nagpur and Thiruvananthapuram. The incidence rate is increasing in major cities compared to smaller cities. It is highest in the metrocities like in Delhi, the rate being 10.9%, in Chennai it is 4.2% and lowest in northeast India like in Manipur the rate being 0.8% (23). It is hypothesized that Indian men are genetically more predisposed to develop aggressive prostate cancer. Research shows that people of South Asian descent have 40% more risk to die from prostate cancer compared to people of other races. (23)

Benign prostatic hyperplasia is the most common benign lesion of prostate of aging men. It poses major public health problem causing significant morbidity thereby affecting quality of life of aging men.

The prevalence of BPH increases with increasing age. It is about 8% in the age group of 30 to40 years and 50% and 80% in the 8^{th} and 9^{th} decade respectively. The risk of developing BPH in men aged 70 -79 years is 4.6 times higher than those of 40 – 49 of age. It has been estimated that the doubling time of BPH growth is 4.5 years for the age group of 35- 50 years and 10 years for the age range of 51 – 70 years(23)

Specimen types and sample handling:

Most commonly received specimens in the laboratory are TURP (Trans Urethral Resection of Prostate) and Needle core biopsies. Others are simple and Radical Prostatectomy. In my study I have taken only needle core biopsies and TURP.

- Needle Core Biopsies

It is usually done when there is a suspicion for prostatic cancer either clinically or radiologically such as nodular mass on Digital Rectal Examination (DRE), Imaging shows any hypoechoic lesion or increased serum PSA levels. It is done under guidance of Transrectal Ultrasound by using 18G needle. It can be done as an OP procedure also. Most common route of biopsy is Transrectal route, can also be done by transurethral or perineal route.

The needle core biopsy is divided into three main types based on number of cores obtained:

a) Sextant biopsy

This is the most commonly performed procedure in most hospitals nowadays, Here 6 cores of tissue are taken ,two each from bilateral base, mid gland and apex.

b) Extended biopsy

It has more sensitivity than sextrant in diagnosing cancer. It can be done as an initial procedure in suspicious cases. In this procedure 6 cores as that of sextant biopsy is taken along with cores from mid and lateral peripheral zone of prostate. Transition zone is not included in extended biopsy.

c) Saturation biopsy

This should not be done as a preliminary procedure. It should be done only when suspicious of carcinoma and previous biopsies show negative reports inspite of persistently elevated serum PSA (Prostate specific Antigen) levels. Here transition zone is also included in biopsy.

These cores taken should be submitted entirely with only maximum 3 cores per block, otherwise they may hinder evaluation.

Fixative preferred is formalin as, Bouin’s fixative if used may enhance appearance of nucleoli even in benign glands. When section cutting is done

unstained slides are cut and kept for IHC in order to look for atypical foci. Levels 1,3,5 are used for H&E study whereas 2,4,6 are used either for IHC or H&E. Multiple sections should be present in each slide as it increases the probability of detecting lesions.

- TURP (Transurethral resection of Prostate)

It is usually done in cases of Benign prostatic hyperplasia where rubbery prostatic chips are taken from transition zone and areas around proximal prostatic urethra. If≤ 12g is received submit fully in 6-8 cassettes. If >12g is received, initial 12g is submitted fully followed by 1 cassette for each 5g. Incidentally prostatic carcinoma is identified in around 10% of the cases. If it is identified rest of the tissue has to be submitted for examination (15).

COMMON PROSTATIC LESIONS

Prostatic lesions are broadly classified into inflammatory lesions which could be infectious and non infectious, benign hyperplasia, benign tumor like conditions and neoplasms which include intraepithelial lesions (carcinoma in situ), frank carcinoma and its variants.

BENIGN PROSTATIC HYPERPLASIA

Size and weight of prostate gradually increases till puberty and then there is a rapid increase in growth. By around 21-30yrs prostate reaches its normal weight of 20g. After that it remains stable. But as age increases in majority of population, the prostatic size again increases due to proliferation

which is designated as Benign Prostatic hyperplasia. In elderly age group it is the most common disorder causing urinary obstruction in men. By around 60yrs of age more than 50% men have BPH, this percentage increases and by around 90yrs more than 90% patients have BPH. So due to this increased frequency in elderly men it is thought to be a part of normal ageing process. The prostatic stroma and glands are responsive to androgen hormone. So this hyperplasia occurs only in patients having testes and not in patients who are castrated. This hyperplasia is considered to be due to hormonal imbalance that alters the normal balance of proliferation and cell death both in glandular and stromal counterpart (30).

p27 protein is considered as negative regulator of cell cycle and present in normal prostate but absent in hyperplastic conditions. It is expressed by both stroma and glandular epithelium. Initial stages in BPH the proliferation and hyperplasia occurs in stromal component and later expands to glandular component. This proliferation starts in periurethral and transition zone.

Grossly the prostate shows nodularity, some nodules may protrude into bladder lumen also. These nodules are situated more laterally. Less commonly they may involve peripheral zone also.

In microscopy there is proliferation of both glandular and stromal components. The glandular components may be sometimes very closely packed when it is termed as adenosis and some may show papillary projections into the lumen which is normal. Some of the glands may be cystically dilated and show

corpora amylacea which are inspissation secretion of glycoprotein mature which may sometimes show calcifications. The glands are lined by 2 layer of epithelium- inner luminal/secretory layer containing columnar cells with pale cytoplasm and regular centrally placed nuclei and inconspicuous nucleoli. The abluminal myoepithelial cells are usually flat. The stroma contain both smooth muscle and fibrous component. In TURP specimens, BPH can be reported as such but in needle biopsies it should be reported only as ‘Benign prostatic tissue” as most needle biopsies sample might not be taken from representative area in case of BPH i.e transition zone (30) . Few inflammatory cells such as lymphocytes and plasma cells are seen surrounding glands which may be due the hyperplasia and vise versa. It may also shows elevations in PSA but should not be over diagnosed as acute or chronic prostatitis in absence of symptoms.

INFLAMMATORY CONDITIONS

A) Acute and chronic prostatitis

Acute prostatitis in biopsy examination is very uncommon as most of the lesions get resolved by antibiotics. It shows presence of abundant neutrophils both within and surrounding the acini, desquamated cellular debris within ducts, stromal edema and hyperemia. Sometimes it may lead to formation of prostatic abscess, usually due to coliform organisms associated with bladder obstruction. Similarly chronic inflammation is associated with presence of lymphocytic infiltrates around ducts admixed with plasma cells. Some cases of BPH also shows chronic inflammatory cell infiltrate. Therefore

prostatitis must be diagnosed only in correlation with clinical symptoms and serum PSA levels. Otherwise it should just be commented as acute and chronic inflammation. The reactive changes seen following inflammation may mimic carcinoma (31).

B) Granulomatous inflammation

It is further classified into infectious granulomas, non infectious granulomatous prostatitis, post biopsy granulomas and systemic granulomatous prostatitis. Infectious causes are most commonly due to mycobacterial and mycotic lesions. Mycobacterial prostatitis can occur as a result of hematogenous dissemination as well as a result of complication of BCG (Bacillus calmette Guerin) immunotherapy for superficial bladder carcinoma. It shows presence of both large caseating and small non-caseating granulomas mainly involving peripheral zone of prostate, may also occur in central and transitional zone, these granulomas usually surround intact acini.

Non specific granulomatous prostatitis is the most commonly diagnosed granulomas which is seen in elderly adults. It presents as an indurated prostate therefore clinically and by rectal ultrasound is suspicious for carcinoma. Early lesions show dilated ducts filled with neutrophils, debris, foamy histiocytes and desquamted epithelial cells. Rupture of ducts and acini show localised granulomas and chronic inflammatory infiltrate composed of mixed inflammatory infiltrate mainly lymphocytes, plasma cells, eosinophils and histiocytes. Post biopsy granulomas are seen sometimes following TURP and

occasionally needle core biopsies. They are reaction to altered epithelium and stroma from trauma of previous cautery. It can occur between 9 days to 52 months following biopsies. It is composed of central region of fibrinoid necrosis surrounded by palisading histiocytes. They could be necrobiotic and non specific foreign body giant cell like granuloma. If previously another biopsy has been done few eosinophils are seen surrounding the granuloma. Systemic granulomatous prostatitis consists of cases with tissue eosinophilia such as allergic granulomatous prostatitis and Churg-strauss syndrome and those without eosinophilia like Wegner's granulomatosis.

PREMALIGNANT LESIONS OF PROSTATE

A) Prostatic Intraepithelial Neoplasia

This lesion consists of architecturally benign ducts and acini lined by cytologically atypical cells. It is also called as Prostatic duct dysplasia. It is further classified into low grade and High grade PIN (Prostatic intraepithelial neoplasia). The difference between both is the presence of prominent nucleoli in High Grade PIN at 20x magnification. Low grade PIN shows stratification of nuclei, mild nuclear enlargement and no prominent nucleoli. The reproducibility in reporting low grade PIN is very less and its association with prostatic carcinoma is questionable. It is usually reported as Benign prostatic tissue without mentioning about PIN. High grade PIN is associated with prostatic carcinoma. It is usually more commonly seen in peripheral zone which is also a common site for prostatic carcinoma. It shows TMPRSS-ERG

fusion similar to prostatic carcinoma in many cases. Similarly seen are aneuploid DNA, numerical chromosomal alterations in chromosome 7,8,10,12 and Y chromosome, Deletions of chromosome 8p, increased expression of p16, p53, AMACR, Bcl-2 and MYC genes and Hypermethylation of Glutathione S-transferase (31).

Incidence of HGPIN(High grade PIN) in needle core biopsies is 16% and in TURP is 1-5% but is seen in 80-100% prostate glands harbouring adenocarcinoma and more commonly seen in elderly people mainly by 8^{th} decade. It is an incidental finding and may show abnormal serum PSA level. Microscopy shows prostatic ducts and acini showing architectural complexity lined by crowded epithelial cells showing abnormal cytological feature like, stratification, hyperchromasia, enlarged monomorphic nuclei, prominent nucleoli, amphophilic cytoplasm with preserved basal layer demonstrated either by light microscopy or by IHC. 4 patterns of HGPIN are identified mainly- **Tufted, Micropapillary, Cribriform and flat** of which tufted pattern is more common. Flat pattern shows nuclear atypia without epithelial hyperplasia. Other uncommon variants are signet ring, mucinous, foamy, inverted or hobnail, small cell neuroendocrine. The nuclei towards centre of gland shows more bland cytology compared to nuclei arranged peripherally against basement membrane. The grade of PIN is assessed based on nuclei situated peripherally against basement membrane.

MIMICKERS OF PIN

- Central zone of gland

Mimics PIN with complex architecture with glandular infolding lined by tall pseudostratified epithelium with eosinophilic cytoplasm. They may have cribriform and roman bridge patterns but there is no nuclear atypia and it has a prominent basal layer.

- Clear cell cribriform hyperplasia

Nodular or diffuse growth of glands showing cribriform pattern with cells having clear cytoplasm. But it is more common in transition zone, shows no nuclear atypia and at least some glands show well defined basal layer.

- Basal cell Hyperplasia

There is proliferation of small glands more commonly in transition zone, there is presence of solid nests of cells, may show prominent nucleoli along with mitotic activity (32)(33). Because of presence of prominent nucleoli, these may be mistaken for High grade PIN. The nuclei will be more round to form solid basaloid nests but HGPIN will have pseudostratified and columnar cells but glandular lumina will be preserved. In basal cell hyperplasia atypical cells are seen undermining the overlying benign secretory cells and the cells stream parallel to basement membrane. In HGPIN the nuclei are perpendicular to basement membrane. In IHC- Basal markers show multi-layered positivity in basal cell hyperplasia but HGPIN shows patchy staining

- Acinar (Usual) Adenocarcinoma

Cribriform High grade PIN may resemble cribriform Gleason pattern 3 adenocarcinoma. Mostly it is diagnosed by the presence of infiltrating malignant glands in stroma. In absence of infiltrating glands, infiltrating cribriform carcinoma is diagnosed only in presence of large cytologically atypical glands with back to back arrangement, or outside of the prostate such that they are inconsistent with cribriform PIN. In IHC, if a negative reaction is obtained by numerous atypical glands for basal cell marker a diagnosis of carcinoma can be made. Absence of basal cell markers in few glands is not diagnostic as sometimes normal benign glands may itself show absence of marker sometimes. In such cases it should be reported as “Focus of atypical cribriform glands” and a repeat biopsy done which may reveal carcinoma. In other scenario when there are few atypical cells seen adjacent to High grade PIN, it is difficult to distinguish acinar adenocarcinoma from tangential sectioning or outpouching of small glands (34). These foci are called PINATYP. The PINATYP glands can be called adenocarcinoma only if they lack basal layer in more number of glands or is far away from HGPIN focus. If the HGPIN focus shows a continuous basal cell layer, then the adjacent glands without basal layer can be considered to have focus of carcinoma.

- Ductal Adenocarcinoma

They are aggressive tumors arising from transition zone, so can be seen in TURP specimen. The glands may be either large or cystic and may have

back to back arrangement. They show true papillary fronds with fibrovascular core and show central comedonecrosis. Basal cell markers are inconclusive as both show patchy and irregular staining. Too crowded and too many glands with absent basal cell marker go more towards ductal adenocarcinoma rather than high grade PIN (31).

B) INTRADUCTAL ADENOCARCINOMA

It is an atypical glandular lesion involving the entire gland but the architecture is maintained. It is added as a new entity in the recent WHO 2016 classification of prostatic cancers (35)(36)(37) .

Criteria for diagnosing atypical intraductal carcinoma of prostate:

- Major criteria

Large glands size which is twice the normal gland size, presence of basal cells, cells involving whole glandular lumen with cytological atypia and mitosis, comedonecrosis.

- Minor criteria

Round and smooth gland contour with right angle gland branching containing 2 population of cells solid or extensive cribriform architecture.

Follow up of cases may show invasive carcinoma on re-biopsy. The TMPRSS-ERG fusion is more commonly seen in Intraductal carcinoma of prostate. Close differential diagnosis is Infiltrating cribriform adenocarcinoma

(Gleason pattern 4 or Gleason pattern 5 with comedonecrosis). The contour, branching pattern and presence of basal cell layer demonstrated by basal cell marker p63 goes in favour of IDC-P (Intraductal adenocarcinoma of prostate). If infiltrating high grade carcinoma is seen associated with IDC-P, It is reported as infiltrating carcinoma with intraductal spread. If in core biopsies if only lesion suggestive of IDC-P is seen without infiltrating carcinoma, use of basal cell markers is indicated. If IDC-P is associated with Gleason pattern 3 adenocarcinoma, a note should be left as 'IDC-P is typically associated with High grade carcinoma which may have been missed due to sampling error. It is important to distinguish between HGPIN and IDC-P as former doesn't need any definitive therapy. Both share many common cytological features like nucleomegaly, hyperchromasia and prominent nucleoli but patterns like solid, dense cribriform are not seen in High grade PIN. Other patterns like loose cribriform and micropapillary patterns are seen in both and IDC-P can be diagnosed only by the presence markedly enlarged nuclei and comedonecrosis (38).

Dawkins et al (39) studied about allelic instability in prostatic cancers to demonstrate role of IDC-P in progression of prostate cancer. It was proved that 29% Gleason pattern 4 cancer and 60% of IDC-P demonstrated Loss Of Heterozygosity (LOH) of certain microsatellite marker but was not observed in HGPIN and Gleason pattern 3. Another study showed presence of ERG rearrangement in 75% IDC-P but was absent in HGPIN. Also cytoplasmic PTEN loss was seen in 84% of intraductal carcinoma and absent in High grade

PIN (40)(41). Loss of PTEN is associated with aggressive behaviour and poor prognosis. All IDC-P detected by biopsies are followed by definitive therapy i.e radical prostatectomy or radiation therapy even in absence of documented infiltrating cancer.

PROSTATIC CANCER

Prostatic carcinoma causes significant morbidity among elderly males. It has been classified by WHO 2016 as follows.

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF PROSTATE (2016)

EPITHELIAL TUMOURS

Adenocarcinoma (acinar)

- Atrophic
- Pseudohyperplastic
- Foamy
- Colloid
- Signet ring
- Microcystic variant
- Pleomorphic giant cell adenocarcinoma
- Sarcomatoid carcinoma

Prostatic intraepithelial neoplasia (PIN), High grade

Intraductal carcinoma NOS

Ductal adenocarcinoma

- Cribriform
- Papillary
- Solid

Urothelial tumors

Urothelial carcinoma

SQUAMOUS TUMORS

- Adenosquamous carcinoma
- Squamous cell carcinoma

BASAL CELL TUMOURS

- Basal cell adenoma
- Basal cell carcinoma

NEUROENDOCRINE TUMOURS:

- Adenocarcinoma with Neuroendocrine differentiation
- Well differentiated neuroendocrine tumor

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

MESENCHYMAL TUMOURS

Stromal tumor of uncertain malignant potential

Stromal sarcoma

Leiomyosarcoma

Rhabdomyosarcoma

Chondrosarcoma

Angiosarcoma

Synovial sarcoma

Inflammatory myofibroblastic tumour

Osteosarcoma

Undifferentiated pleomorphic sarcoma

Hemangioma

Chondroma

Leiomyoma

Granular cell tumor

Solitary fibrous tumor

Solitary fibrous tumor, Malignant

HEMATOLYMPHOID TUMORS

Diffuse Large B cell lymphoma

Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma

Follicular lymphoma

Mantle cell lymphoma

Acute myeloid leukemia

B cell lymphoblastic leukemia/Lymphoma

MISCELLANEOUS TUMOUR

Cystadenoma

Nephroblastoma

Rhabdoid tumor

Germ cell tumor

Clear cell adenocarcinoma

Melanoma

Paraganglioma

Neuroblastoma

SECONDARY/METASTATIC TUMOUR

NEWER ENTITY ADDED IN WHO 2016:

Intraductal carcinoma

New variants of acinar adenocarcinoma

Microcystic variant

Pleomorphic large cell variant

Large Cell carcinoma of prostate

Though there are many subclassification of prostate cancer, **the term prostate cancer usually refers to Prostatic Adenocarcinoma.**

Majority of prostatic cancer is diagnosed in asymptomatic patients due to early detection programs by using PSA levels and Digital rectal examination. Increased PSA levels and Abnormal DRE is an indication to do prostatic biopsy. When symptomatic it usually indicates an advanced disease. Symptoms are obstructive bladder symptoms, pelvic pain, bone pain, tenderness, spinal cord compression or adenopathy. Advanced diseases show rarely symptoms like Disseminated Intravascular Coagulation, Non bacterial endocarditis, ascites or pleural effusion. Small cell carcinoma variant may sometimes cause paraneoplastic symptoms (15).

Prostatic carcinoma doesn’t show any distinguishable gross mass lesion. Grossly evident tumors are usually more than 1cm in size with indurated

yellow to yellow tan homogenous areas which are denser and firmer than the surrounding normal spongy parenchyma. The tumor lacks areas of haemorrhage and necrosis and blends with normal parenchyma without any sharp delineating borders. Tumors are usually seen in posterior and posterolateral aspect of prostate corresponding to peripheral zone. The anterior tumors are mostly admixed with benign prostatic hyperplasia, therefore are difficult to identify (15).

The diagnosis of prostatic cancer requires combination of architectural, nuclear, cytoplasmic and intraluminal features as presence of individual features may also be seen in benign conditions. Architectural features include presence of uniform, small crowded glands infiltrating into stroma in well differentiated carcinoma. Less differentiated tumors show poorly formed, fused or large cribriform glands. Poorly differentiated carcinomas show infiltrative pattern of growth as single cells and solid sheets. The nuclear features include enlarged hyperchromatic nuclei, prominent nucleoli, uniform non pleomorphic, mitotic figures and apoptotic bodies. Cytoplasm will be amphophilic with sharp luminal borders and lack of lipofuscin. Presence of corpora amylacea indicates benign lesion. In malignancy lumen may contain blue tinged mucinous secretion, pink amorphous secretion and presence of crystalloids and intraluminal necrosis in high grade tumors.(31)

The features that are pathognomonic of prostatic adenocarcinoma and not seen in benign lesions are perineural invasion, mucinous fibroplasia (collagenous micronodules) and glomerulations. Mucinous fibroplasia is

focally organised mucinous secretion characterised by loose fibrous tissue with in growth of fibroblasts (42). These secretions can displace epithelium leading to atrophic cytoplasm and small pyknotic nuclei. Glomerulations consists of glands with a cribriform proliferation attached to one end of gland resembling glomerulus. Perineural invasion is said only when nerve is partially encircled by glands which shows some atypical features. If it is the key diagnostic feature used then nerve should be completely encircled by glands. Perineural indentation must be differentiated in which nerve is intended by benign glands (43).

VARIANTS OF CARCINOMA MIMICKING BENIGN GLANDS

A) ATOPHIC VARIANT

Rare variant and is usually not associated with history of hormonal therapy (44)(45). There is infiltration of small atrophic glands with cytological features of malignancy between larger benign glands. It is usually admixed with non atrophic prostatic carcinoma.

B) PSEUDO HYPERPLASTIC VARIANT

The glands are large dilated with branching and papillary infolding (46)(47). There will be numerous closely packed glands lined by tall columnar cells with pale luminal cytoplasm and basally located nuclei with features of malignancy. Lumen may show crystalloids. ISUP (International society for urologic pathology) recommends this a grade of 3+3=6 or if it is circumscribed 3+2=5.(47)

C) **FOAMY (XANTHOMATOUS) VARIANT**

Prostatic carcinoma with foamy cytoplasm with infiltrative pattern of distribution. Nuclei is small and pyknotic and malignant features not seen. They are graded as 3+3=6. (48)

D) MUCINOUS (COLLOID) ADENOCARCINOMA

More than 25% of the tumor should have aggregates of tumor cells floating in mucin lakes. No significant intracellular mucin is seen. This variant has to be differentiated from metastasis before diagnosing the same. These tumors are given Gleason grade 4 and considered as aggressive variant. In needle biopsy it is called as 'Prostatic carcinoma with mucinous features'

E) SIGNET RING CELL VARIANT

At least 25% of tumor shows signet ring cells containing optically clear vacuoles displacing nuclei to periphery and they are infiltrative. It is associated with high grade prostatic carcinoma. It is assigned Gleason grade 5 according to ISUP (International Society of Urologic Pathology).

F) MICROCYTSIC VARIANT:

It is composed of cystically dilated glands with gland diameter 10 times more than that of malignant acinar glands. Nuclear atypia is difficult to assess in atrophied cells due to cystic dilatation of the glands. It has been graded as Gleason 2 or 3, with favorable prognosis

G) PLEOMORPHIC GIANT CELL VARIANT:

It is the highly aggressive variant of adenocarcinoma composed of giant cells with marked pleomorphism, lack of cohesiveness and areas of extensive necrosis (49). It has a poor outcome and generally assigned high Gleason score 9 or 10.

Other rare variants are Prostatic carcinoma with Paneth cell like differentiation, Lymphoepithelioma like variant, Oncocytic variant.

GRADING OF PROSTATIC ADENOCARCINOMA

The grading system for prostatic carcinoma was developed by Donald. F.Gleason in1966 solely based on architectural patterns (50)(51).

Then it underwent several modifications and popularly called as modified Gleason's grading system. It was first modified by International Society of Urological Pathology (ISUP) consensus meeting in the year 2005 (52). Again in the year 2014 ISUP Prostate Cancer Grading Panel consensus meeting made further alterations.

Table 1: Gleason's microscopic grading system of prostatic carcinoma

STAGE		DESCRIPTION
1		Circumscribed nodule of tightly packed , uniform, round to oval well formed glands with no/minimal infiltration to adjacent parenchyma.
2		Nodular with minimal peripheral infiltration of less uniform , more loosely arranged glands.
3	a	Single, separate and variable glands which are closely packed or irregularly separated, ragged with poorly defined edge.
	b	Very small glands or tiny cell clusters with features of 3a
	c	Sharply and smoothly circumscribed rounded masses of papillary or loose cribriform tumor. (papillary intraductal tumor)
4	a	Fused raggedly outlined, raggedly infiltrating glands.
	b	Same features of 4a with hypernephroid pattern composed of solid sheets of large pale cells with optically clear cytoplasm
5	a	Sharply circumscribed,rounded masses of solid cribriform tumor, usually with central comedonecrosis.
	b	Ragged masses of anaplastic carcinoma with only enough gland formation to call as adenocarcinoma.

Post hormonal and post radiotherapy tumours are excluded from Gleasons grading.

Based on Gleasons score prostatic adenocarcinoma is divided into well differentiated, moderately differentiated and poorly differentiated tumours.

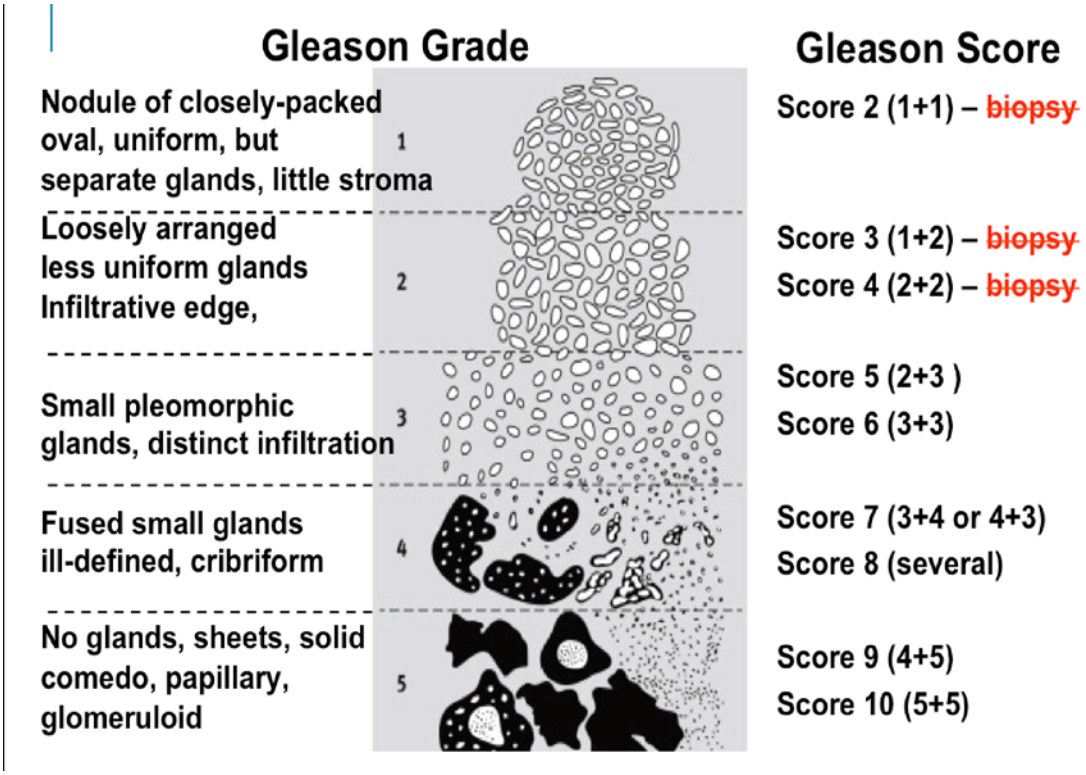


Fig6: Modified gleason grade

Gleasons score 2- 6: Well differentiated tumors, with excellent prognosis.

Gleasons score 7 (3+4): Moderately differentiated tumors.

Gleasons score 7(4+3): Moderately to poorly differentiated tumors.

Gleasons score 8 – 10: Poorly to undifferentiated tumors, aggressive in nature.

In prostatectomy specimen Gleason score is sum of primary and secondary pattern. Primary pattern is the most common pattern and secondary

pattern is the second most common pattern. In biopsy samples the score should start from 3. Minimum score will be 3+3=6 (14).

ISUP (International society of Urological pathology) 2005 has done several modifications in reporting.

- In needle core biopsies, transurethral resections and simple prostatectomy specimen tertiary pattern is also included if it is of a higher grade than secondary pattern.
- In high grade cancers the lower grade pattern need not be involved if it is <5%
- In Radical prostatectomy specimen include primary and secondary pattern for Gleason score and separately mention tertiary pattern.
- In multifocal tumors the dominant nodule has to be given separate Gleason score.
- Individualised Gleason score given for some morphological variants.

Gleason in original grading included cribriform architecture in both pattern 3 and 4 but didn't give any strict criteria to differentiate between both. The 2005 ISUP modified Gleason's score gave a strict criteria to differentiate between both.(52)(53)

Cribriform Grade 3 pattern: It includes small round glands with round contours with evenly spaced round lumens.

Cribriform Grade 4: It includes irregular gland with irregular contours with irregular lumens or slit like lumens.

At 2014 ISUP consensus meeting again made modification in cribriform pattern of prostatic carcinoma. They concluded that all cribriform pattern are now considered under pattern 4.

Newer modifications in Gleasons grading system in WHO 2016 are as follows:

1. All cribriform patterns are grouped under pattern 4.
2. Glomeruloid pattern gouped under pattern 4.
3. Mucinous adenocarcinoma may be pattern 3 or pattern 4.
4. Intraductal carcinoma should not be graded.

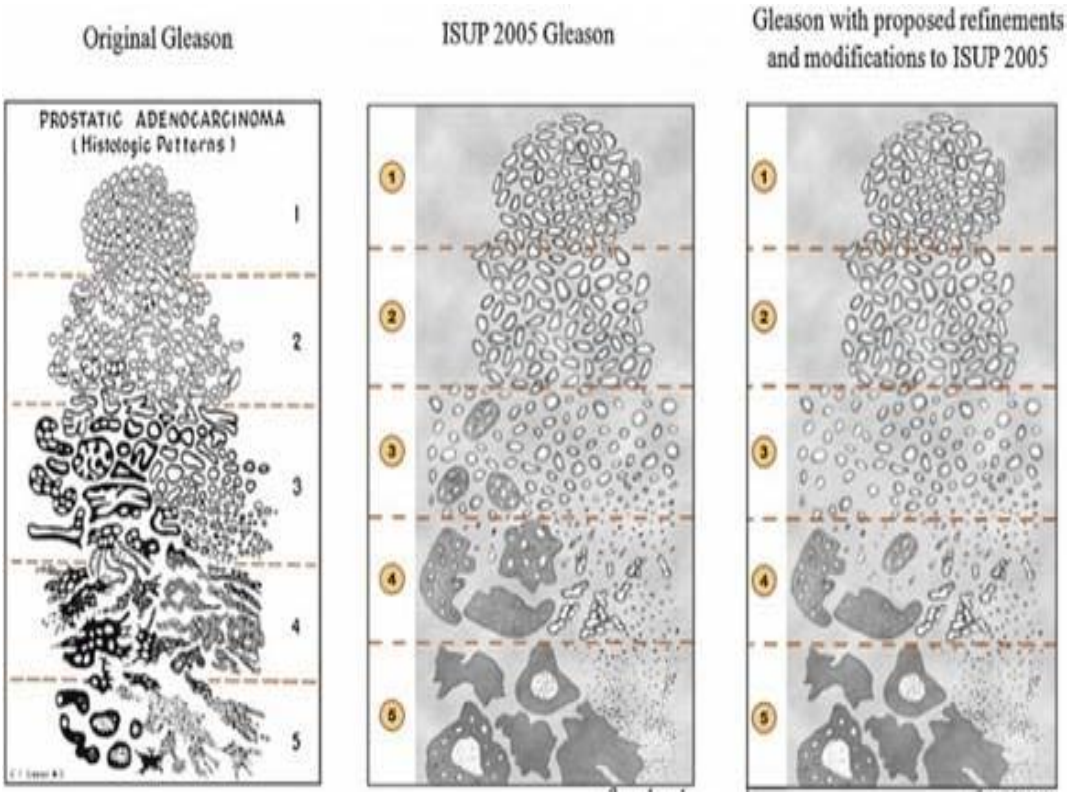


Fig 7: Evolution of Gleason grading

Gleason 3 is most common pattern seen in 87.7% (31). Grading should be done first at scanner and 10x followed by high power to prevent overdiagnosis.(54)(55)(56)(57)(52)

Recently based on Gleasons score prostatic carcinoma is divided into 5 prognostic groups by ISUP (International Society of Urologic Pathology). It was proposed by John Hopkins Hospital in the year 2013. It is a multi-institutional study including Johns Hopkins Hospital, Memorial Sloan-Kettering Cancer Center (MSKCC), University of Pittsburgh, Cleveland Clinic, and the Karolinska Institute which includes 20,845 cases, and all were followed for a period of 3 years for analyzing risk free survival.

The prognostic groups are as follows:

Grade group 1 (Gleason score 3 + 3 = 6): Only individual discrete well-formed glands **Grade group 2** (Gleason score 3 + 4 = 7): Predominantly well-formed glands with lesser component of poorly formed / fused / cribriform glands.

Grade group 3 (Gleason score 4 + 3 = 7): Predominantly poorly formed/ fused / cribriform glands with lesser component of well-formed glands.

Grade group 4 (Gleason score 8) - Only poorly formed/fused/cribriform glands or Predominantly lacking glands and lesser component of well-formed glands

Grade group 5 (Gleason scores 9–10): Lack of gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands.

They analysed that the 5 year risk free survival for grade groups 1 to 5 are 96%, 88%, 63%, 48% & 26% respectively .

RISK STRATIFICATION

Based on triad of serum PSA level, Gleason score and clinical stage of localized prostate cancer, D'Amico et al in the year 1998 proposed risk stratification that was included in the WHO 2016 Blue book. They provide a better options for treatment recommendatrions than just using stage of cancer

alone. It forms the basis for initial treatment for men with prostate cancer and it avoids overtreatment of early stage cancer. Recommendations for disease monitoring, treatment of recurrent disease, and systemic therapy for metastatic castration-recurrent prostate cancer also are included in risk groups and are as follows:

Low risk - Diagnostic PSA <10.0 ng/ml and highest biopsy Gleason score <6 and clinical stage T1c or T2a.

Intermediate risk - Diagnostic PSA >10.0 ng/ml but <20ng/ml or highest biopsy Gleason score =7 or clinical stage T2b.

High risk - Diagnostic biopsy >20 ng/ml or highest biopsy Gleason sc ore >8 or clinical stage T2c /T3.

Thus according to ISUP recommendations It was decided that any biopsy report for prostate carcinoma it is mandatory that it should contain modified Gleason scoring system and Prognostic groups. Treatment options are categorized according to risk stratification.

MIMICKERS OF ADENOCARCINOMA OF PROSTATE

There are several mimickers of prostatic adenocarcinoma and this is classified according to gleason score.

A) Benign Mimickers of Gleason score 2-6 Adenocarcinoma

- Atrophy(T=N)*
- Radiation atypia (T=N)

- Verumontanum mucosal gland hyperplasia(T=N)
- Adenosis (Atypical adenomatous hyperplasia) (T>N)
- Basal cell hyperplasia(T>N)
- Nephrogenic adenoma(T>N)
- Seminal vesicles (N>T)
- Colonic mucosa(N)
- Cowpers gland(N)

*T-TURP, N-Needle biopsy

Atypical Adenomatous hyperplasia (Adenosis)

Seen in 2.2% to 19.6% of TURP specimen (58). Crowded benign glands which show patchy staining may mimic adenocarcinoma (59). It is a hyperplastic condition occurring in transition zone, so it is most commonly seen in TURP specimen than needle core biopsies. Microscopy shows well circumscribed nodule with proliferation of small to medium sized glands. Some glands may occasionally infiltrate surrounding stroma blending along with benign glands. Dilated parent glands may be centally located. High power shows small glands with pale to clear cytoplasm, round uniform nuclei and inconspicuous nucleoli. Basal cells are present but are patchy or fragmented and require basal markers like p63 for identification.

The features that are common for both adenosis and carcinoma are presence of crowded glands, crystalloid, minimal infiltration at periphery and medium sized nucleoli and expression of AMACR (59). Presence of

macronucleoli goes in favour of adenocarcinoma. The diagnostic feature differentiating both is the presence of basal layer in adenosis which is demonstrable by markers like p63. Adenosis is more closer to benign prostatic hyperplasia than carcinoma as far as proliferation rate is considered (60)(61).

Atrophy

Mainly affects elderly, it is also seen 70% of men between 19-29 yrs (62) . It presents as an induration or as a hypoehoic lesion in ultrasound thereby necessitating biopsy. It is age related and could be associated with androgen deprivation and radiotherapy. BPH can also compress peripheral zone leading to atrophy. Also follows ischaemia and inflammation. It mainly affects peripheral zone. Microscopy shows prostatic acini with loss of cytoplasm indicating increased nucleocytoplasmic ratio. The glands will be crowded and hyperchromatic giving a basophilic appearance. The atrophy can be diffuse or focal. Focal atrophy shows admixture of patterns including simple atrophy, simple atrophy with cyst formation, Post atrophic hyperplasia.and partial atrophy . In simple atrophy glands are spaced similar to normal glands. Simple atrophy with cyst formation show large dilation of atrophic acini.

Postatrophic hyperplasia shows proliferative atrophic acini surrounding central large dilated ducts (Feeder duct). The glands may appear infiltrative but not as individual glands infiltrating in between larger benign glands. Longitudinal tangential section may show them as cords of cell thereby

mimicking cancer. PAH some times may also show prominent nucleoli but basal cell marker p63 will be uniformly positive.

Partial atrophy retain lobular pattern and is less basophilic as nuclei are more widely spaced. Glands have undulating luminal surfaces with papillary infoldings. Cells are benign without prominent nucleoli. The presence of crowded glands with pale cytoplasm may mislead to diagnosing it as low grade adenocarcinoma. Partial atrophy shows patchy staining with basal cell markers and also express racemase (62).

Basal cell Hyperplasia

Also called “fetalisation” or “Embryonal hyperplasia of prostate. It consists of tubules or glands lined by basal cell layer (63)(64)(65). Florid basal cell hyperplasia may be confused with prostatic carcinoma. Features favouring basal cell hyperplasia are multilayering of cells, solid nests, cells with scant cytoplasm, pseudocribriform glands, well-formed lamellar calcifications, intracytoplasmic eosinophilic globules. Basal cell hyperplasia mainly occurs in transition zone and therefore is seen mainly in TURP specimen (66). The cells will show intense positivity for p63 and high molecular weight cytokeratin and negative for AMACR (66).

Colonic Mucosa

Very rare and is seen in transrectal biopsies. Features mimicking prostatic carcinoma include distorted architecture, blue tinged intraluminal

mucinous secretions, prominent nucleoli, mitosis and negative stains for basal markers. Points that help in differentiation are presence of lamina propria, presence of rectal tissue in a detached fragment, goblet cells, associated inflammation and muscularis propria.

Cowper glands

Seen in TUR and rarely in needle biopsies (67). They resemble foamy gland carcinoma. Cowpers glands show non infiltrative lobular pattern of dimorphic population of ducts and mucinous acini in cowper. They are composed of rounded cells that are distended to such an extent that glandular lumina is occluded.

Mesonephric remnant hyperplasia

Seen usually in elderly patients mostly in anterior fibromuscular stroma and adjacent anterolateral periprostatic tissue, posterior aspect of base and around seminal vesicle. Consists of tubules arranged in lobular distribution and lumen contain dense eosinophilic colloid like material. IHC shows PSA, p63 negative staining, focal positivity for racemase and HMW cytokeratin. It also shows diffuse positivity for PAX8.

Nephrogenic adenoma

It is located in suburethral prostatic stroma. Patients may have a history of past surgery, calculi or trauma. It consists of tubulo papillary proliferation along the transitional epithelium of urethra sometimes extending into prostatic

fibromuscular stroma thereby mimicking low grade prostatic adenocarcinoma. The tubules are lined by cuboidal, flattened or hobnail cells with moderate eosinophilic to clear cytoplasm and nuclei showing minimal atypia which is usually degenerate, inconspicuous nucleoli and absent to rare mitosis. They are PAN-CK and PAX2 positive and PSA/PSAP negative. Prostatic adenocarcinoma shows AMACR, PSA/PSAP positivity and PAX2/PAX8 negativity.

Seminal Vesicle

Incidence of presence of seminal vesicles in TUR specimen is 3% but it is more likely to be misdiagnosed as carcinoma in needle biopsy specimen due to less amount of tissue obtained. In biopsies it shows dilated irregular glandular lumen surrounded by many small glandular diverticula resembling adenocarcinoma. The cells show nuclear hyperchromasia, marked pleomorphism and some may show prominent nucleoli but there is no mitotic activity (31). The atypia is thought to be degenerative. Also contain lipofuscin pigment which is typical of seminal epithelium. Prostatic carcinoma shows only mild to moderate atypia. Immunohistochemistry shows negative staining for PSAP(Prostatic specific Acid Phosphatase). High molecular weight cytokeratin stains the basal cells around seminal vesicle.

Verumontanum Mucosal Gland Hyperplasia

Small acinar proliferation involving verumontanum are seen in radical prostatectomy and rarely in prostatic needle biopsies (68). The small crowded

verumontanum glands may mimic prostatic adenocarcinoma especially when multiple cores or extensive involvement of single core. The glands are not infiltrative among benign glands. It is seen contiguous and adjacent to urothelium. Corpora amylacea and brown orange green concretions are seen in lumen. These glands are similar to prostatic acini and by immunohistochemistry luminal cells stain positive for PSA and basal stain are positive for p63 and HMW cytokeratin.

B) MIMICKERS OF GLEASON SCORE 7 TO 10 ADENOCARCINOMA

- Nonspecific granulomatous prostatitis
- Paraganglia
- Clear cell cribriform hyperplasia
- Sclerosing Adenosis
- Xanthoma
- Signet ring cell lymphocytes.

Non specific granulomatous prostatitis

In this condition there will be elevation of serum PSA level along with an abnormal DRE (69). 4% of granulomatous prostatitis closely mimic carcinoma. Presence of other inflammatory cells and multinucleated giant cells to be noted which might be absent in needle biopsy. NSGP is localised initially near ruptured ducts and acini. To differentiate from poorly differentiated adenocarcinoma, IHC may be used. The epitheloid cells will be negative for PSA, PSAP and PANCK and positive for histiocytic markers.

Clear cell cribriform hyperplasia

It is seen in transition zone so will be present in TUR specimen. Consists of numerous cribriform glands separated by stromal proliferation like nodular hyperplasia (70). The cells have small bland nuclei with inconspicuous nucleoli and clear cytoplasm, with no pleomorphism. At least some of glands show prominent basal layer which is absent in carcinoma.

Sclerosing Adenosis

Relatively circumscribed lesion seen in TUR specimen. The glands resemble adenosis with pale to clear cytoplasm and bland appearing nuclei which may occasionally show prominent nucleoli. They have prominent basal layer. They have a dense spindle cell component composed of plump cells with amphophilic cytoplasm and shows focal myxoid areas(31). There is hyaline sheath like structure surrounding glands which is absent in adenocarcinoma

Signet ring lymphocytes

Usually seen in TURP specimen composed of aggregates of degenerated lymphocytes with a signet ring appearance (31). It is associated with thermal injury so not seen in needle biopsy or prostatectomy.

Xanthoma

Rare but can be confusing in needle biopsies especially when it is present as cords and individual cells infiltrating prostatic stroma (31). The cells

have abundant vacuolated cytoplasm with small benign nuclei. It will be positive for CD68 and negative for CAM5.2.

IMMUNOHISTOCHEMISTRY IN PROSTATIC CARCINOMA

In H&E sections, evaluating architectural patterns and cytological feature itself helps a lot in differentiating benign from malignant lesions. But immunohistochemistry proves to be helpful in categorising ambiguous lesions. Also helps in detecting carcinoma in biopsy material showing atypical glands. Also helps in diagnosing whether a lesion is prostatic or not.

Principles of IHC in prostatic carcinoma diagnosis:

- Confirm the presence of basal cell layer in lesions. Carcinomas shows absence of basal layer. The basal cell markers used are p63 and High molecular weight Cytokeratin.

- Demonstration of proteins that are upregulated in prostatic carcinoma (eg.AMACR)

Dual chromogen Antibody cocktail is more useful where we combine 1 or 2 basal cell marker with AMACR (Alpha-methylacyl-CoA-racemase). It is very useful when foci of carcinoma is small and tissue preservation is important.

AMACR is a marker overexpressed in malignancy and show negative or weak cytoplasmic staining in benign glands. AMACR is a mitochondrial and peroxisomal enzyme that is involved in beta-oxidation of branched-chain fatty

acids. It is highly sensitive (75-95%) but not specific as it is expressed in other malignancies. It is upregulated in HGPIN and carcinomas.

p63 and HMW cytokeratin are the commonly used basal cell markers that that stain the basal cells in prostate. HMCK (High molecular weight cytokeratin) stains intermediate filaments in basal cytoplasm and doesn't stain secretory cells. p63 is a nuclear marker and few studies like that of Michael et al (71) show that p63 is a more sensitive marker compared to HMCK. In present study, the expression of p63 and calponin is studied on prostatic biopsies including TUR and needle core.

Prostate lineage specific markers like PSA, newer markers like prostate specific membrane antigen (PSMA) and proPSA helps in detecting prostatic origin of lesion and helps in excluding non prostatic lesions that act as mimickers of adenocarcinoma eg: seminal vesicle. In case of metastasis with unknown primary, if PSA is positive it helps in confirming prostatic origin.

In case of distinguishing from poorly differentiated prostatic carcinoma and poorly differentiated urothelial carcinoma prostatic carcinoma shows positivity for PSA, PSAP and negative for thrombomodulin and Uroplakin. Others newer marker used in diagnosing prostatic origin are Prostein and NKX3.1

In this study, two markers p63 and calponin are used and their expression in prostatic lesions is studied. Numerous studies have been done on p63 expression on prostate. Much studies have not been done in literature on

calponin. Jennifer et al in his study says that there is development of a reactive stroma environment in many prostatic cancers and this is likely to induce tumorigenesis (8).

p63 in prostate

p63 also known as TP63 (transformation related protein 63) is a member of p53 family. p63 is located on chromosome 3 and contain 15 exons. It has two isoforms namelyTAp63 and delta Np63. DeltaNp63 involved in stem cell regulation and skin, cervix, breast and urogenital tract epithelial development. Knock out of this gene leads to failure of development of breast, salivary and lacrimal gland tissue (71). Tap63 is restricted to apoptotic function and is found to maintain oocyte integrity (2). p63 is expected to maintain stem cell integrity in those organs in which it is present. (72) p63 plays good role in epithelial cell development and differentiation, also affects cell proliferation, apoptosis and senescence. Those having germline mutation in p63 is more prone to develop cancers.

In prostate p63 is selectively expressed in basal cells and absent in secretory and neuroendocrine cells (71). DeltaNp63 is the isoform mainly expressed by basal cells of prostate (72). p63 is a nuclear marker and is taken up by nucleus of basal cells. Preneoplastic lesions like PIN show patchy p63 staining, here no invasion is seen. Infiltration to stroma indicates disruption of basal cell membrane and is frank evidence of carcinoma. p63 is strongly and diffusely expressed in normal prostate and other benign lesions and is absent in

malignancy as malignancy is devoid of basal cells. This negative immunostaining in carcinoma can be used to detect benign mimickers. Study by Shah et al showed that use of a antibody cocktail increases the sensitivity of detection. Literature shows aberrant cytoplasmic positivity in some prostate cancers and it is associated with increased mortality. (73)

In present study we examine the expression of p63 in prostate both benign and malignant lesions of prostate and also on ambiguous lesions and thereby categorising it.

Calponin

Calponin is an actin filament associated cytoplasmic protein. It has three isoforms namely h1, h2 and h3. h1 is the basic isoform and is smooth muscle specific (74). h2 is neutral calponin and is found in both smooth muscle and non smooth muscle cells including epidermal keratinocytes, lung alveolar cells, endothelial cells, fibroblasts and myeloid blood cells (74). h3 calponin is acidic calponin first discovered in brain and thought to involve in neuronal regeneration and growth.

h1 calponin in prostate was expressed in fibromuscular stroma by smooth muscle cells in normal prostate. It is a cytoplasmic marker. Previous studies show that level of calponin was decreased or absent in cancerous tissue and had a correlation with metastasis and prognosis. This decreased expression indicates destruction of smooth muscle like stroma. This is as a result of stromal reaction or desmoplasia. This has been studied mainly in two organs

that is breast and colon (75). This is called reactive stroma which is composed of fibroblasts, myofibroblasts, endothelial cells and immune cells. This stroma is thought to enhance tumor progression by stimulating angiogenesis and by promoting cancer cell survival, proliferation and invasion (75). Ayala et al studied about the correlation between reactive stroma and biochemical recurrence. This reactive stroma in itself is considered to be an independent risk factor for tumor progression. Previous studies have been conducted on expression of other markers like vimentin and smooth muscle actin on reactive stroma.

In present study we study the expression of calponin in benign, premalignant and malignant lesions of prostate.

MATERIALS AND METHODS

Study Place: Department of Pathology, Chengalpattu Medical College and Hospital, Chengalpattu.

Study Design: The present study is a retrospective observational study conducted in the Department of Pathology during the period of June 2015 to May 2017.

Ethical clearance for the study was obtained from the Institutional Ethics Committee of Chengalpattu Medical College, Chengalpattu.

A total sample of 60 cases of prostatic lesions was analyzed during the period of June 2015 to May 2017.

Selection of study population

INCLUSION CRITERIA

Tissue blocks of patients who are diagnosed as having benign and malignant prostatic lesions.

EXCLUSION CRITERIA

- Tissue blocks of patients who are diagnosed as prostatic carcinoma and underwent preoperative Radiotherapy or Chemotherapy.
- Poorly preserved or poorly fixed tissues.

During the period of June 2015 to May 2017, as per the inclusion and exclusion criteria, biopsies including needle core and TURP specimen received in the Department of Pathology were included.

History written in the histopathology request form was recorded on predesigned and pretested proforma (Annexure I).

MATERIALS USED

Tissue sections (5microns) prepared from paraffin embedded formalin fixed tissues.

Haematoxylin and eosin staining kit

p63 and calponin (h1) monoclonal antibody kit

Secondary antibody kit

Positive control

Negative control

METHOD:

Formalin fixed paraffin embedded blocks and haematoxylin eosin stained sections of 60 prostatic biopsies which included both TURP and needle core are taken up for the study. Based on histopathological examination, they were categorized as follows:

1. Benign prostatic hyperplasia,

2. Prostatic intraepithelial neoplasia

3. Prostatic adenocarcinoma.

Prostatic adenocarcinoma was assigned Gleason grade ranging from grade 1 to grade 5 according to modified Gleason grading system. Immunohistochemistry was performed on the tissue sections taken from the blocks along with positive and negative control.

Immunohistochemistry

Procedure

1. 5µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.

2. The slides were incubated at 58ºC for overnight.

3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.

4. Rehydrated through descending grades of alcohol as follows:

   (i) Absolute alcohol x 2 changes 5 minutes each

   (ii) Washed in distilled water 2 changes, 2 minutes each

5. Heat induced antigen retrieval was done with pressure cooker with Tris buffer for 15 to 20 minutes.

6. Then cooled for 10 minutes.

7. Washed in distilled water 2 changes, 2 minutes each.

8. Washed in Tris Buffer Saline (TBS) for 2 minutes.

9. Endoperoxidase blocking was done by adding hydrogen peroxide on the section and kept for 10 minutes.

10. Washed in the wash buffer for 2 minutes twice.

11. Primary antibody p63/ Calponin (Mouse monoclonal; prediluted) was added and kept for 40 minutes in a moist chamber.

12. Then washed in wash buffer 2 minutes 2 times each.

13. Poly excel target binder reagent was added and kept for 15 minutes.

14. Washed in two changes of buffer 2 minutes each.

15. Poly excel HRP (Horse Radish Peroxidase) was added and incubated for 15 minutes.

16. Washed with buffer – 2 minutes, 2 changes.

17. Working DAB (Dichromogen (1ml DAB buffer + 1 drop chromogen, mix well) was added and kept for 2-5 minutes.

18. Then washed in distilled water.

19. Counter stained with hematoxylin for 30 seconds.

20. The slides were washed in running tap water for 3 minutes.

21. The slides were air dried, cleared with xylene and mounted with DPX.

Positive control included blocks containing normal prostatic gland-internal control.

Negative control included Primary antibody replaced by PBS.

The IHC sections were viewed for expression of p63 and calponin.

p63 Expression

It was expressed as either positive or negative. It was considered positive when nucleus of basal cell layer took brown stain with negative staining of stroma and luminal epithelium of prostatic acini (A12).

The positive staining was further categorized into focal (+), Strong(+++).

Negative staining was taken into consideration only when it failed to stain any cells in the focus thought to be malignant and good positive internal and external control staining was present.

Calponin Expression

Calponin expression was analysed and reported as positive or negative. It was considered positive when it showed cytoplasmic staining of stromal cells. The percentage of positive staining cells and staining intensity was scored in a scale of 0-3 (7).

Staining Percentage (7)

Score 0 – 0% positive cells

Score 1 – 1-33% positive cells

Score 2 - 34-66% positive cells

Score 3 – 67-100% positive cells

Staining intensity:

Score 0 – No staining

Score 1 – Staining obvious only at 400X

Score 2 – Staining obvious at X100 but not X40

Score 3 – Staining obvious at 40X

Staining Index = Staining percentage x Staining intensity

0 – Zero

1-2 – Low

3-4 – Moderate

6-9 - High

STATISTICAL ANALYSIS

The primary data was entered in MS Excel and analysed using SPSS 20v. The results were presented in terms of tables and graphs. The descriptive statistics frequency and percentages were calculated.

The association between the categorical variables were analysed by chi square test with 5% level of significance.

OBSERVATION AND RESULTS

On observation of 60 cases, it was noted that 30 cases belonged to the neoplastic group and 30 to non-neoplastic group.

Among the 30 cases of BPH, of which 2 showed BPH with prostatitis features and one was adenosis, The rest 30 cases were neoplastic lesions of which 28 were from prostatic adenocarcinoma and 2 were from Prostatic intraepithelial neoplasia accounting for 47% and 3% respectively. The distribution of cases is depicted below:

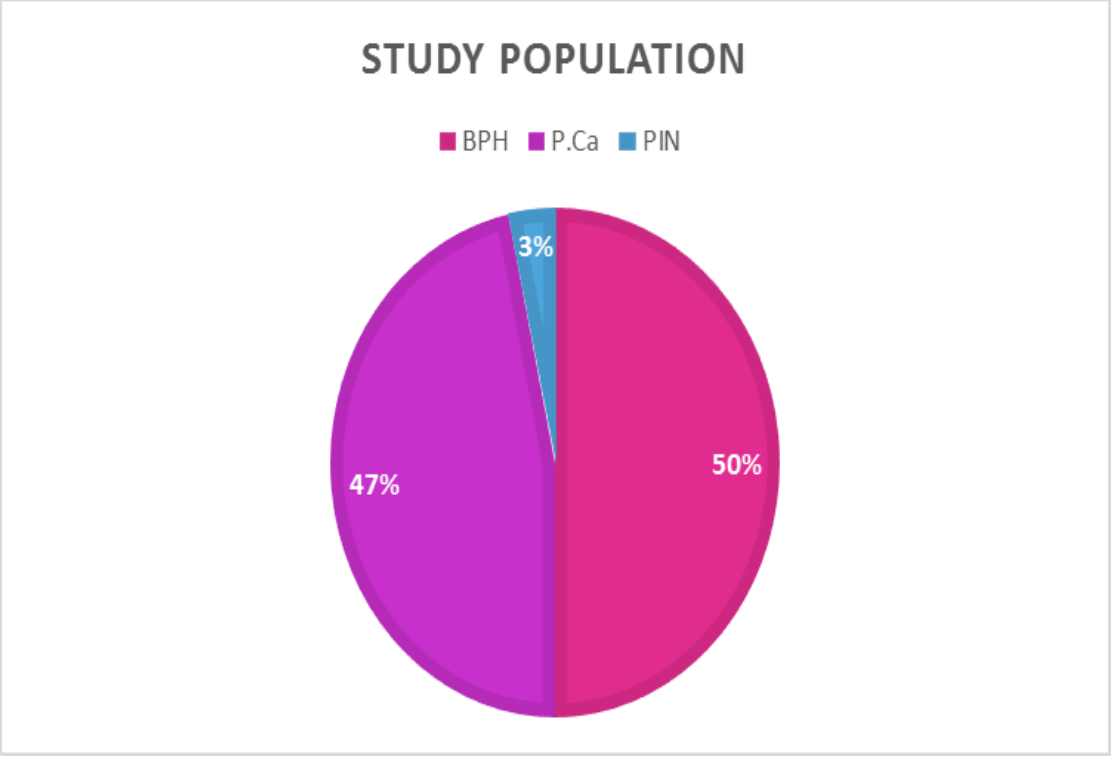


Chart 1: Case distribution of prostatic lesions

AGE WISE DISTRIBUTION OF PROSTATIC LESION

Out of the 30 cases studied of Benign Prostatic Hyperplasia majority of cases belonged to the age group of 61-70 years. 2 cases belonged to age group <50years. No benign cases were there above 80 years of age.

Among the 28 Prostatic carcinoma, studied majority of the cases were in the age group of 61-70 years accounting for 37% of total cases of Prostatic carcinoma. 2 cases were from patients below 50 years of age and 3 cases in patients in age group of 81-90 years accounting for 11% of total number of cases. There were two cases of prostatic intraepithelial neoplasia both of which were in the age group of 71-80 year.

Table2: Age wise distribution of prostatic lesions

Age (years)	BPH	%	Prost.Ca	%	PIN	%	Total
<50	2	6.5	2	7.4	0	0	4
51-60	5	19.4	4	11.1	0	0	9
61-70	12	38.7	10	37.0	0	0	22
71-80	11	35.5	9	33.3	2	100	22
81-90	0	0.0	3	11.1	0	0	3
Total	30	100.0	28	100.0	2	100	60

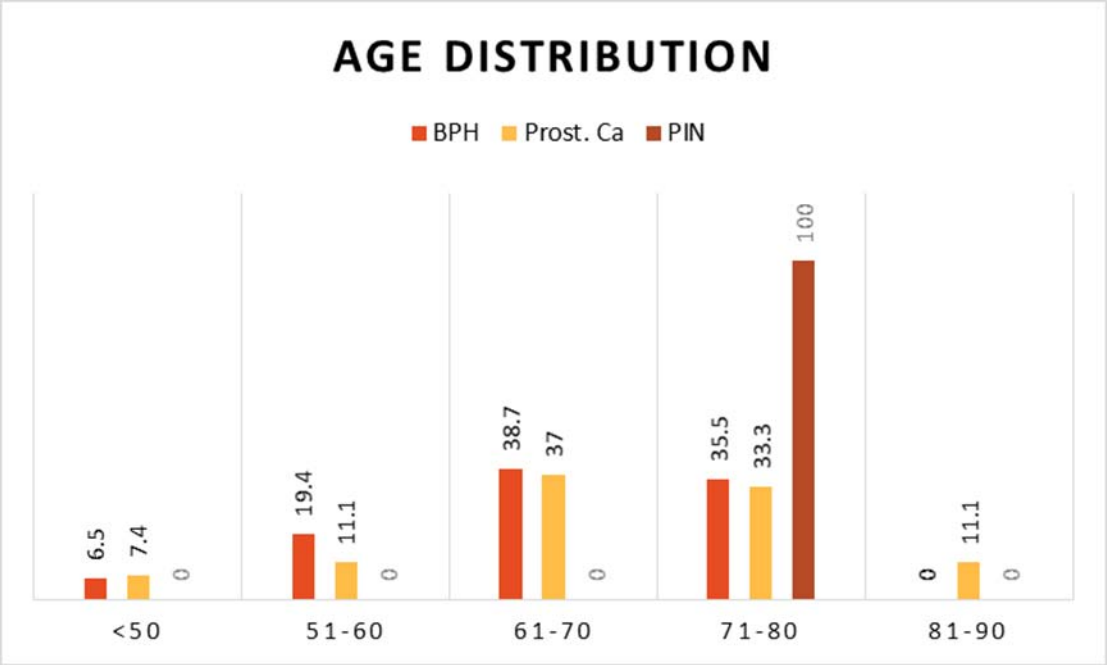


Chart 2: Age distribution of prostatic lesions

The mean age group of 30 cases of Benign Prostatic Hyperplasia was 67 years. The mean age group of Prostatic Intraepithelial Neoplasia was 76.5 years and mean age group of Prostatic Adenocarcinoma is 69 years.

NATURE OF SPECIMEN DISTRIBUTION

Table3: Distribution of specimen type

Nature of Specimen	Benign/Premalignant	Prostatic carcinoma	Total
TURP	30	10	40
Needle biopsy	2	18	20
Total	32	28	60

Out of the 60 specimen received, 40 were TURP and 20 were needle core biopsies. Out of the 40 TURP specimen, 30 were from benign/ precancerous lesion/ suspicious of carcinoma, 10 were from cases which were diagnosed as prostatic carcinoma by histopathological examination.

Among the 20 needle core samples received 2 cases which were suspected to be prostatic carcinoma based on clinical and biochemical examination turned out to be benign prostatic hyperplasia . The remaining 18 cases were malignant.

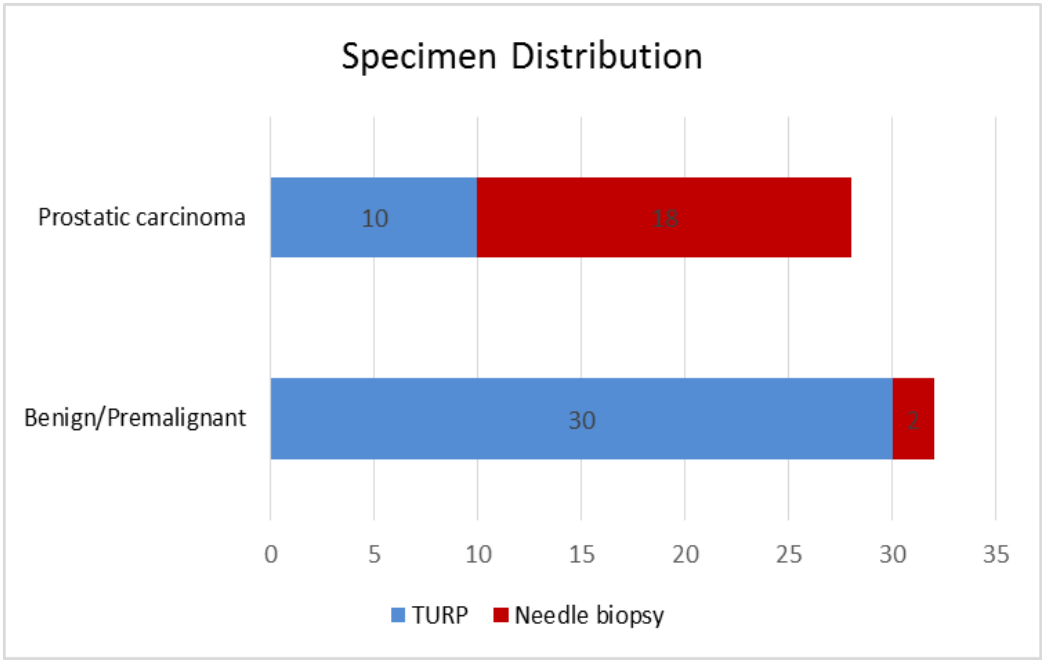


Chart 3: Distribution of nature of specimen

The maximum TURP specimens had come for BPH and highest number of needle core biopsies received were for Prostatic carcinoma.

GLEASON SCORE AND PROSTATIC ADENOCARCINOMA

The Prostatic carcinoma cases were scored according to Gleason score as 3+3, 3+4, 4+3, ≥4+4.

Table 4: Prostatic carcinoma case distribution according to Gleason score

Gleason Score	Prostatic Adenocarcinoma	%
3+3	9	36
3+4	6	24
4+3	4	16
≥ 4+4	6	24
Total	25	100

Out of the 28 cases studied only 25 were given Gleason scoring. One case being microinvasive carcinoma and other two being suspicious for carcinoma were not given any Gleason grading.

Their distribution as follows- 36% came under Gleason score 3+3, 24% under Gleason score 3+4, 16% under Gleason score 4+3, 24% had Gleason score ≥4+4

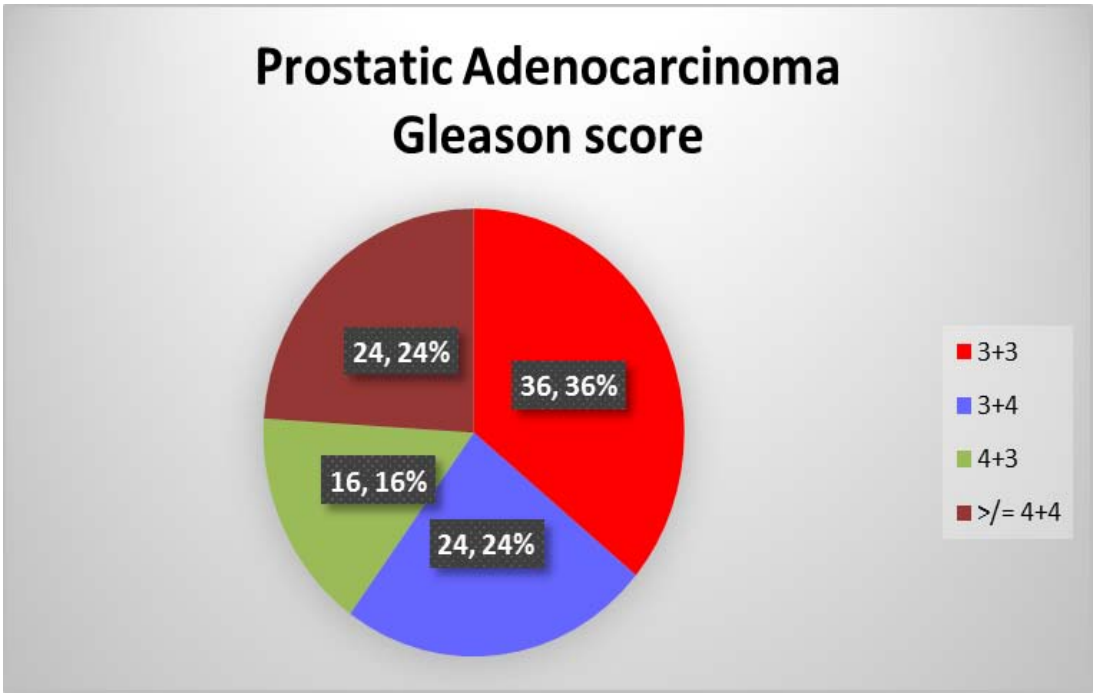


Chart 4: Case distribution according to Gleason score

Gleason score of 3+3 had maximum number of cases (n=9) closely followed by score of ≥4+4(n=7)

CORRELATION WITH SERUM PSA LEVEL

Table 5: Serum PSA level and prostatic lesions

Serum PSA	BPH	Carcinoma	PIN	Total	chi sq	p
<10ng/ml	4	1	0	5	10.47	**0.03**
11.-20ng/ml	9	3	0	12		
>20ng/ml	17	24	2	43		
Total	30	28	2	60		

The benign and malignant prostatic lesions were correlated with serum PSA levels.

Among the 30 cases of BPH, 17 had serum PSA level >20ng/ml. The serum PSA level in both cases of PIN were >20ng/ml.

Of the 28 malignant cases 24 had serum PSA >20ng/ml and in one case serum PSA was <10ng/ml.

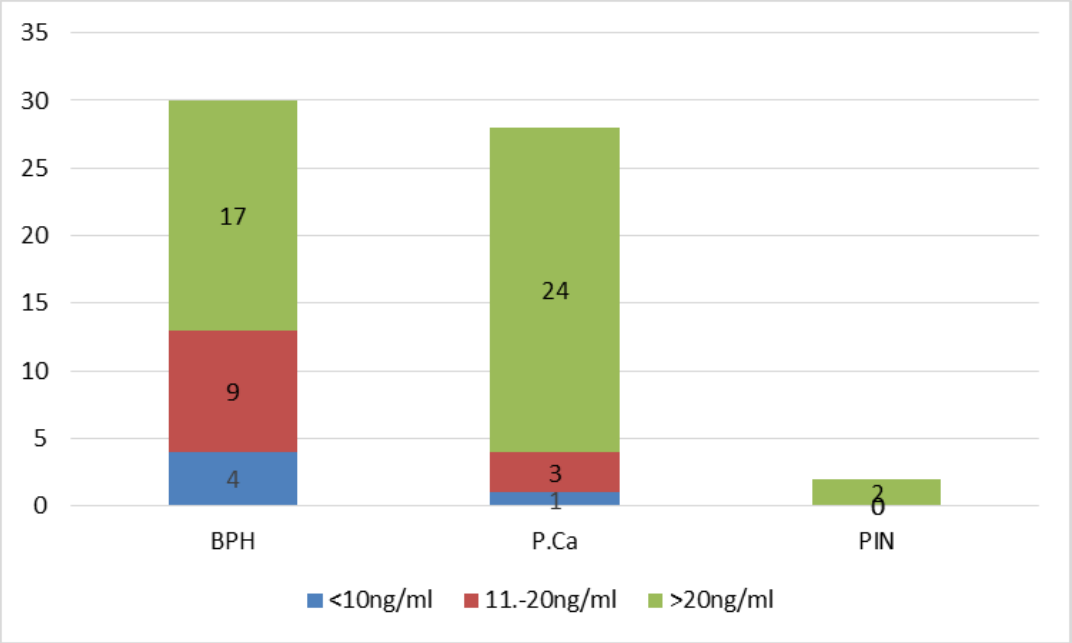


Chart 5: Case distribution of prostatic lesions based on serum PSA level

The incidence of highly increased serum PSA level (>20ng/ml) was more in patients with prostatic carcinoma than in patients with BPH. The correlation between serum PSA level and BPH, Prostatic carcinoma and Prostatic Intraepithelial Neoplasia is statistically significant ($p<0.03$).

SERUM PSA LEVEL AND GLEASON SCORE

Analysing serum PSA levels with Gleason score we had the following results.

Table 6: Serum PSA level and Gleason score

Serum PSA level	Gleason Score								
	Nil*	**<3+3**	**%**	**3+4**	**%**	**4+3**	**%**	**≥4+4**	**%**
<10ng/ml	4	1	4	0	0	0	0	0	0
10-20ng/ml	12	0	0	0	0	0	0	1	4
>20ng/ml	19	8	32	6	24	4	16	5	20
Total	35	9	36	6	24	4	16	6	24
p value								0.17	

*- Includes BPH, PIN and one microinvasive carcinoma and 2 suspicious case of carcinoma.

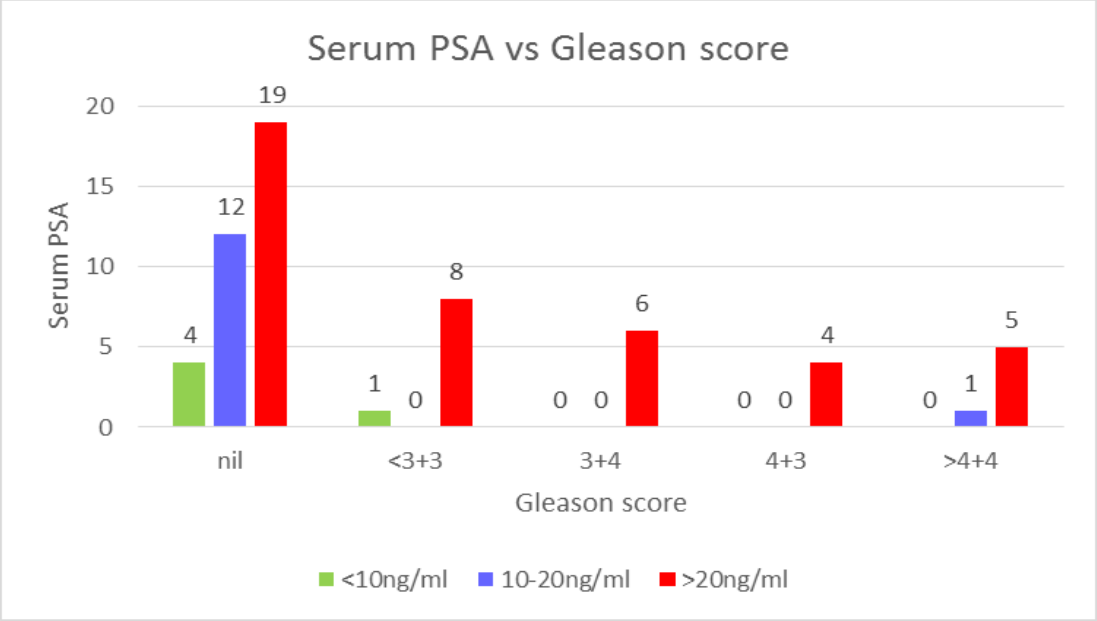


Chart 6: Serum PSA and Gleason score case distribution

In our study, there were only 4% cases that had serum PSA level<10ng/ml. All the cases had a Gleason score of 3+3. In cases having a serum PSA level between 10-20ng/ml 4% cases had a Gleason score of 4+4 or above it. In cases with serum PSA level >20ng/ml, majority of cases belonged

to Gleason score 3+3 accounting for 32% of total cases. Gleason score 3+4 and ≥4+4 accounted for 24% each of the total number of cases. Score 4+3 showed 16% of cases.

p value for correlation between serum PSA level and Gleason score was>0.05 hence statistically not significant.

AGE AND GLEASON SCORE:

Table 7: Age wise distribution of Gleason score

Age Distribution	Gleason score							
	3+3	%	4+3	%	3+4	%	≥4+4	%
</=50yrs	2	22	0	0	0	0	0	0
51-60yrs	0	0	1	17	1	25	1	17
61-70yrs	3	33	2	33	2	50	2	33
71-80yrs	4	44	2	33	1	25	1	17
81-90yrs	0	0	1	17	0	0	2	33
Total	9	100	6	100	4	100	6	100
Chi square-21.13				P value- 0.2				

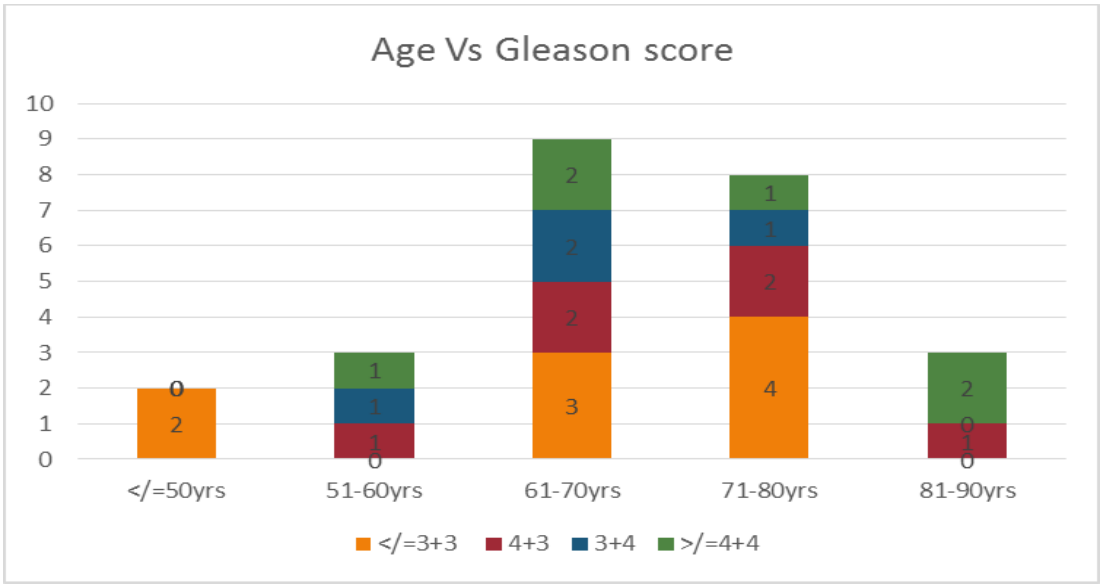


Chart 7: Age wise distribution of Gleason score

In the present study we analysed the distribution of Gleason score with respect to age.

Gleason score 3+3 was seen predominantly in the age group of 71-80 years constituting 44%. Gleason score 4+3 was seen in highest frequency in age group 61-80 years constituting 66% respectively.

For Gleason score 3+4 and ≥4+4 was commonly seen in age group of 61-70 years constituting 50% and 33% respectively.

IMMUNOHISTOCHEMICAL EXPRESSION OF p63 IN BENIGN, PREMALIGNANT AND MALIGNANT GLANDS

Table 8:Expression of p63 in prostatic lesions

P63	BPH	%	Prost. Ca	%	PIN	%	Total	Chi sq	p
Strong basal nuclear positivity	24	80	0	0	0	0	24	67.55	**0.001**
Patchy positivity	3	10	0	0	2	100	5		
Cytoplasmic positivity	0	0	14	50	0	0	14		
Negative	3	10	14	50	0	0	17		
Total	30	100	28	100	2	100	60		

In our study we analysed the expression of p63 in all 60 cases which included 30 cases of benign lesions, 28 cases of prostatic carcinoma and 2 cases of prostatic intraepithelial neoplasia.

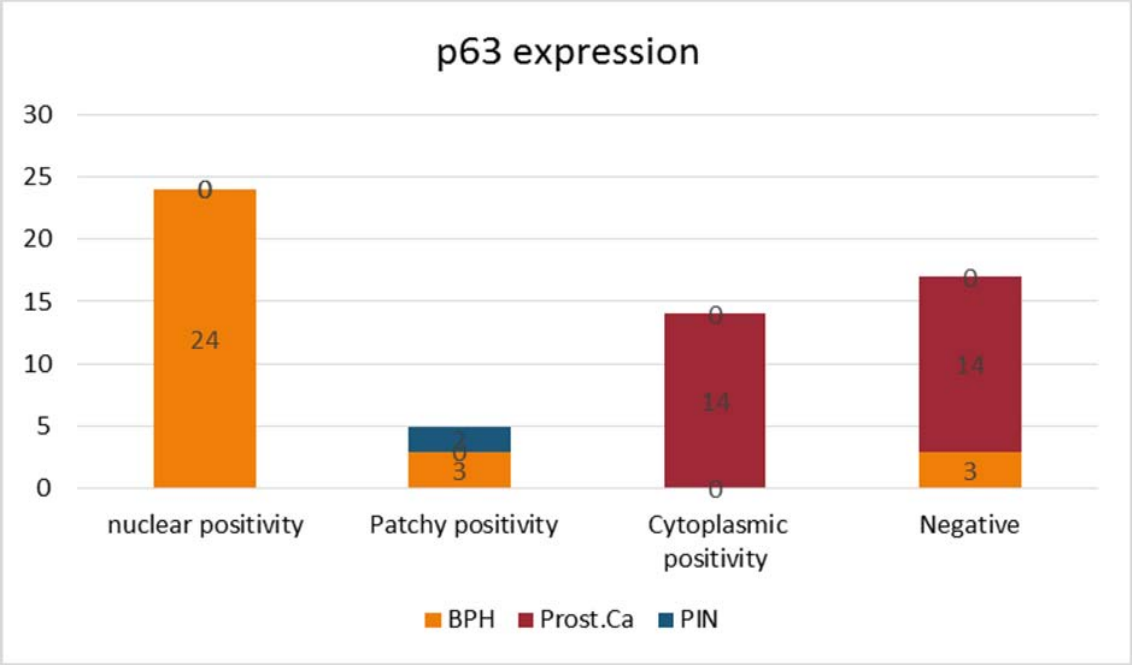


Chart 8: Expression of p63 in BPH, PIN and prostatic carcinoma

Out of the 30 cases of BPH, 27 showed positive immunostaining which comprised 90% of cases. 3 cases (10%) were not informative with p63 staining as it showed negative staining even though histopathology clearly showed it as benign. Of the positive cases 24 showed strong basal nuclear positivity(80%) and 3 showed focal positivity (10%).

Results for Prostatic carcinoma- Among the 28 cases carcinoma cases all were negative for basal cell staining (100%). Of this 14 showed total negative staining whereas other 14 showed cytoplasmic positivity of luminal cells. Cytoplasmic staining is a very rare pattern of expression for a protein which normally should show nuclear positivity of basal cell. Sensitivity of p63 was 90% and specificity was 100%.

The expression of p63 in BPH and Prostatic carcinoma is statistically highly significant. (p=0.001)

CALPONIN EXPRESSION IN BPH, PIN AND PROSTATIC CARCINOMA

Table 9: Calponin expression in benign, premalignant and malignant lesions

Calponin (% staining)	BPH	%	Carci-noma	%	PIN	%	Total	Chi sq	p
0% positive cells (0)	0	0.0	2	7.1	0	0	2	41.05	**0.001**
1-33% positive cells(1)	1	3.3	19	67.8	0	0	20		
34-66% positive cells(2)	4	13.3	4	14.2	2	100	10		
67-100% positive cells(3)	25	83.3	3	10.7	0	0	28		
Total	30	100	28	100	2	100	60		

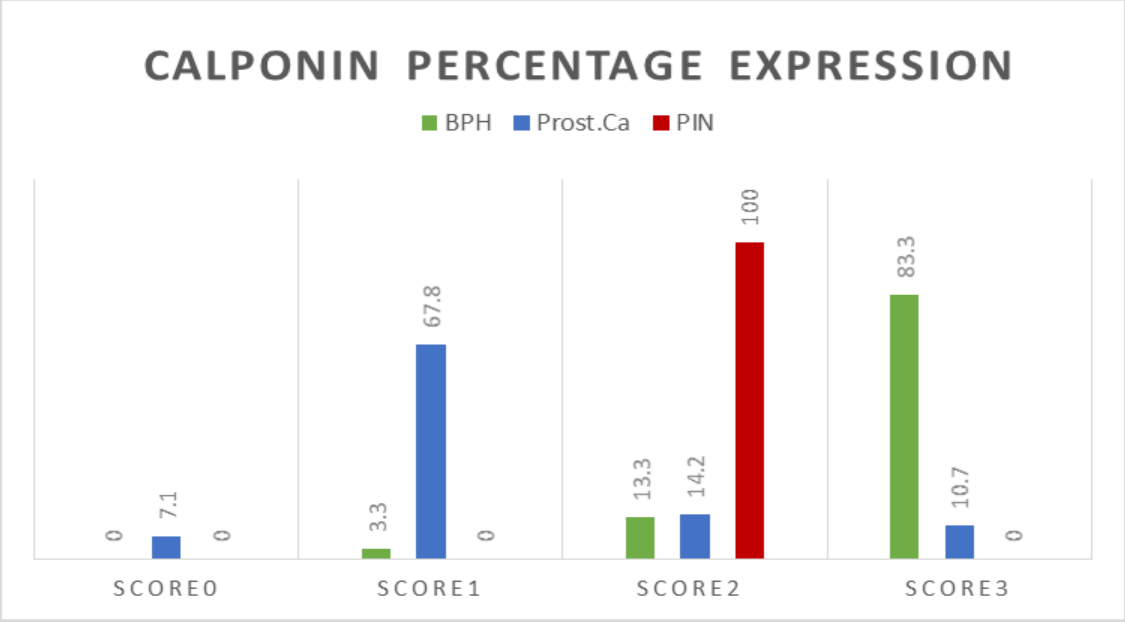


Chart 9: Percentage expression of Calponin in benign, premalignant and malignant lesions

We studied about expression of Calponin in BPH and Prostatic carcinoma. Both factors namely the percentage of staining and intensity of

staining were was studied. In BPH among 30 cases 25 showed intense staining (83.3%), 4 showed moderate staining (13.3%) and 1 showed low staining(3%).

Out of 28 Prostatic carcinoma cases ,19 showed low positivity(67.8%), 4 cases showed moderate and 3 cases showed intense staining accounting for 25% and 10.7% respectively. 2 cases showed no staining at all (7.1%). 2 cases of PIN showed grade 3 positivity. This showed the Calponin showed only low to no staining in Prostatic carcinoma indicating presence of reactive stroma. The relationship of percentage of staining of calponin in BPH and malignancy was highly significant statistically.(p=0.001)

INTENSITY OF CALPONIN EXPRESSION IN PROSTATIC LESIONS

Table 10: Calponin intensity score in BPH, PIN and Prostatic adenocarcinoma

Intensity	BPH	%	Carci-noma	%	HGPIN	%	Total	Chi sq	p
No staining	0	0	2	7.4	0	0	2	24.47	**0.001**
staining obviously only at 400x (Score1)	0	0	2	7.4	0	0	2		
Staining obvious at X100 but not X40 (Score 2)	3	9.7	15	55.6	0	0	18		
Staining obvious at 40X (Score3)	27	90.3	9	29.6	2	100	38		
Total	30	100	28	100	2	100	60		

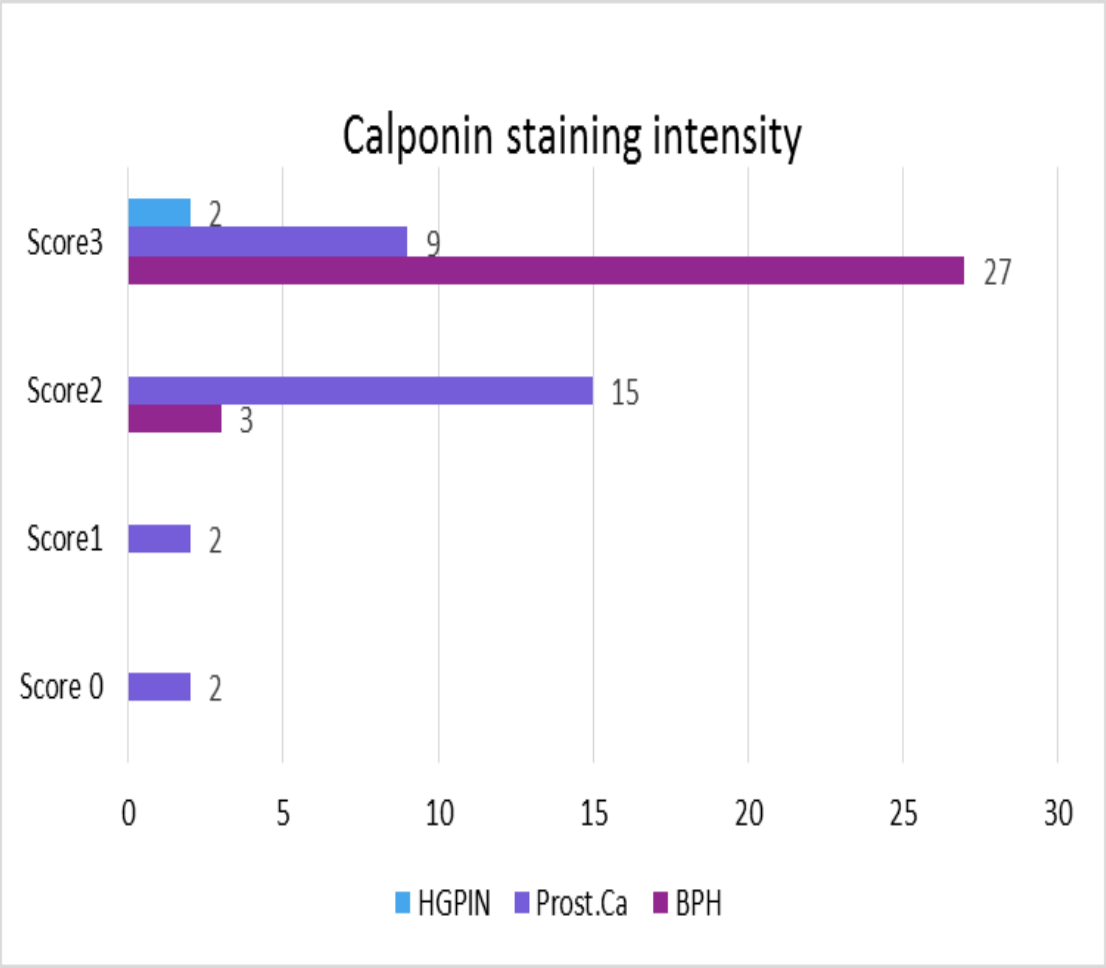


Chart 10: Staining intensity of Calponin in benign, premalignant and malignant lesions

Out of 30 BPH cases 27 showed score 3 staining and 3 showed score 2 staining. Among 28 cases of prostatic carcinoma 15 showed score 2 staining, 9 showed score 3, 2 showed score1 and score 0 respectively. $P<0.01$ hence statistically significant.

STAINING INDEX FOR CALPONIN

Table 11: Staining index of Calponin in BPH, PIN and Prostatic adenocarcinoma

Index	BPH	%	Prost.Ca	%	HGPIN	%	Total	Chi sq	p
0	0	0.0	2	7.4	0	0	2	49.22	**0.0001**
1	0	0.0	2	7.4	0	0	2		
2	0	0.0	12	42.8	0	0	9		
3	1	3.2	5	18.5	0	0	6		
4	1	3.2	4	14.2	0	0	6		
6	5	19.4	1	3	2	100	10		
9	23	74.2	2	7.4	0	0	25		
Total	30	100.0	28	100.0	2	100	60		

Staining index is obtained by multiplying score for percentage staining of Calponin and score for intensity of Calponin. It ranges from 0 to 9. Score between 0-2 is called low staining index, 3-4 called moderate staining index and 6-9 high staining index.

Out of 28 cases of Prostatic carcinoma 16 cases (57%) showed low staining index (0-2), 9 cases (32%) showed moderate staining index (3-4) and 5 cases (17%) showed high staining index (6-9). Majority of cases came under low staining index.

Among 30 cases of BPH 28 cases (93.6%) showed high staining index and 2 cases (6.4%) showed moderate staining index and none showed low staining index.

There is an association between staining index and benign and malignant lesions which were statistically significant (p<0.01)

GLEASON GRADE AND p63 EXPRESSION

Table 12: Comparison of Gleason grade and p63 expression

P63	Nil*	(3+3)	(3+4)	(4+3)	>(4+4)	Total	chi sq	p
Strong basal nuclear positivity	24	0	0	0	0	24	46.79	**0.001**
Patchy positivity	5	0	0	0	0	5		
Cytoplasmic positivity	1	3	4	3	3	14		
Negative	5	6	2	1	3	17		
Total	35	9	6	4	6	60		

*- Includes BPH, PIN and one microinvasive carcinoma and 2 suspicious case of carcinoma.

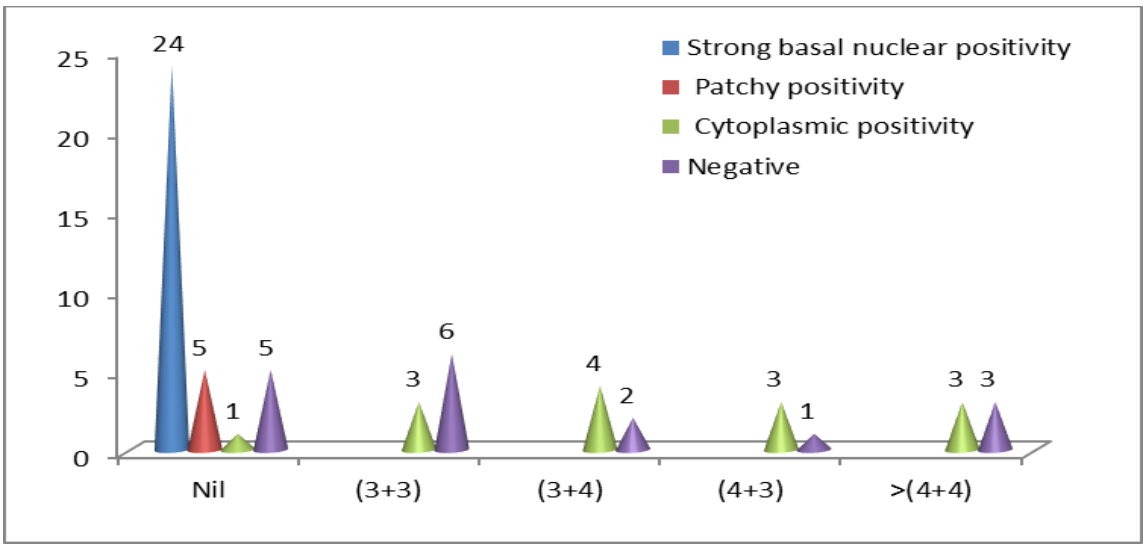


Chart 11: p63 expression in different Gleason grade

Out of 28 prostatic carcinoma cases one was BPH with microinvasive carcinoma and two were suspicious of carcinoma in HPE diagnosis, all showed negative basal staining with p63. Of the 25 cases which had a Gleason score, 9 cases showed a score of 3+3 of which 3 showed aberrant cytoplasmic positivity. 6 cases showed a Gleason score of 3+4 of which 4 showed cytoplasmic positivity. Similarly Gleason score 4+3 and≥ 4+4 had 4 and 6 cases respectively , 3 cases showed aberrant cytoplasmic positivity in each of them. The sensitivity of p63 was 90% and specificity was 100%.

GLEASON GRADE AND CALPONIN EXPRESSION

Table 13: Comparison of Calponin expression and Gleason grade

Calponin	Nil*	(3+3)	(3+4)	(4+3)	>(4+4)	Total	chi sq	p
Score0	0	1	0	0	1	2	47.28	**0.001**
Score1	2	6	4	3	5	20		
Score2	6	2	1	1	0	10		
Score3	27	0	1	0	0	28		
Total	35	9	6	4	6	60		

*- Includes BPH, PIN and one microinvasive carcinoma and 2 suspicious case of carcinoma.

Among the 28 carcinoma cases, 2 cases showed negative staining of the stroma, 19 cases showed score 1 staining (1-33% cytoplasmic staining), 4 cases

showed moderate staining (34-67%) and 3 cases showed score3 staining(68-100%). When Calponin expression was compared with Gleason score majority of cases belonged to 3+3 score. Score 1 staining was seen in decreasing order in 3+3 followed by≥ 4+4, 3+4 and 4+3. 2 cases showed negative staining and belonged to scores 3+3 and ≥4+4 respectively.

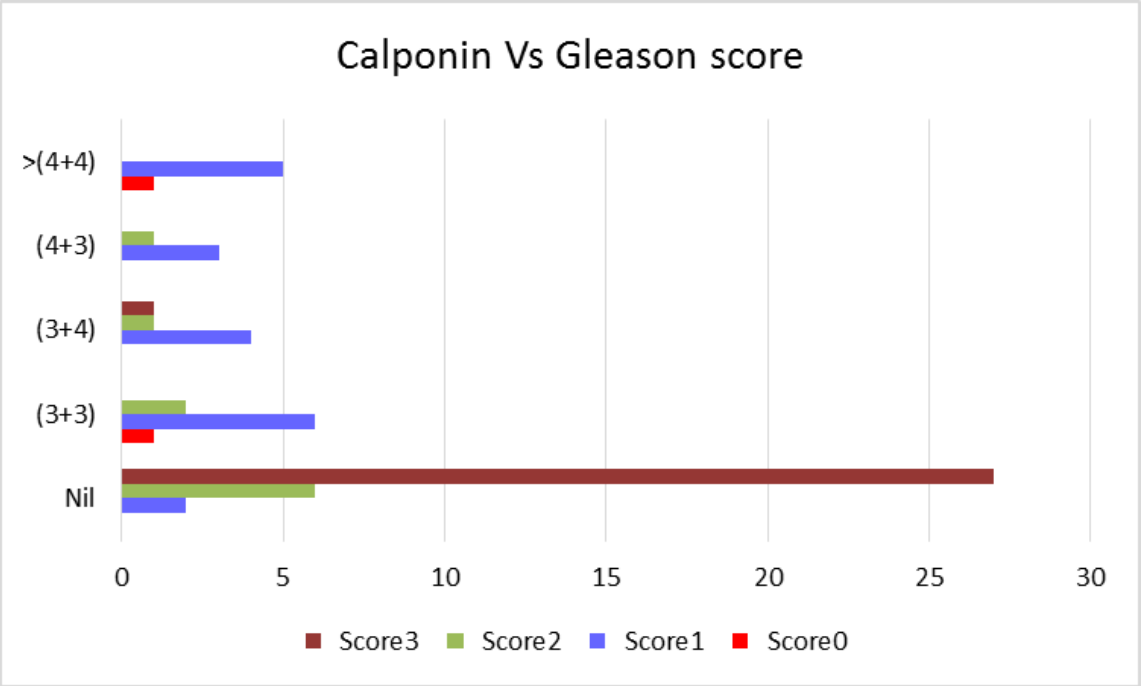
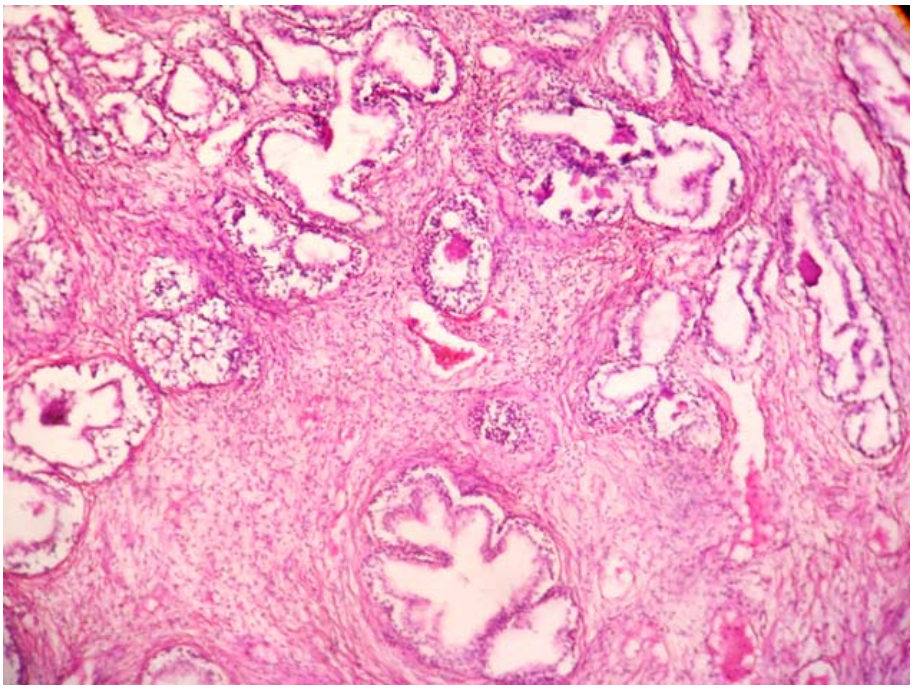
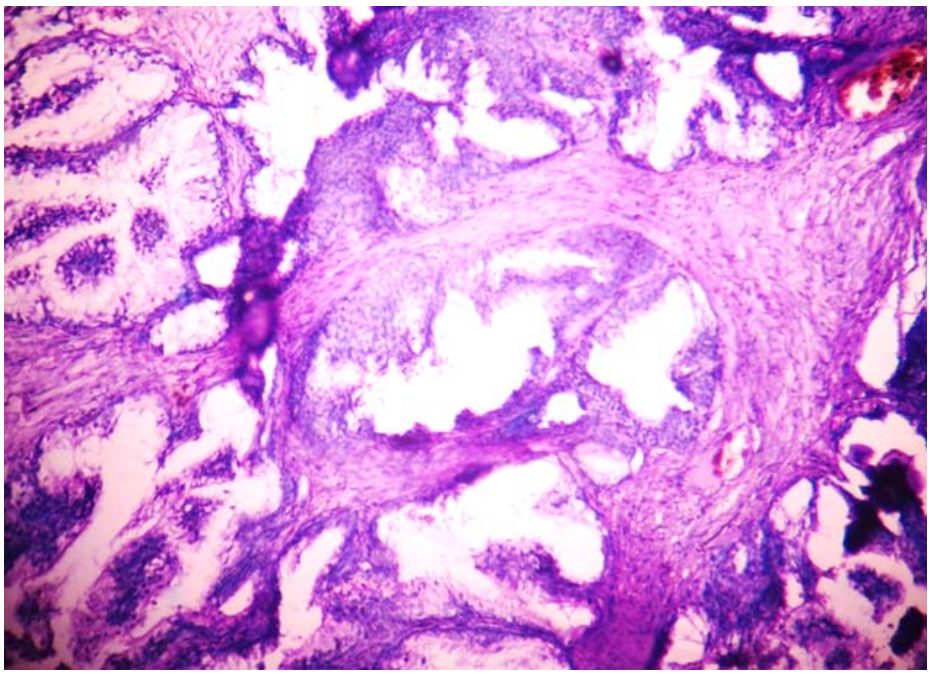


Chart 12: Calponin expression in different Gleason grade

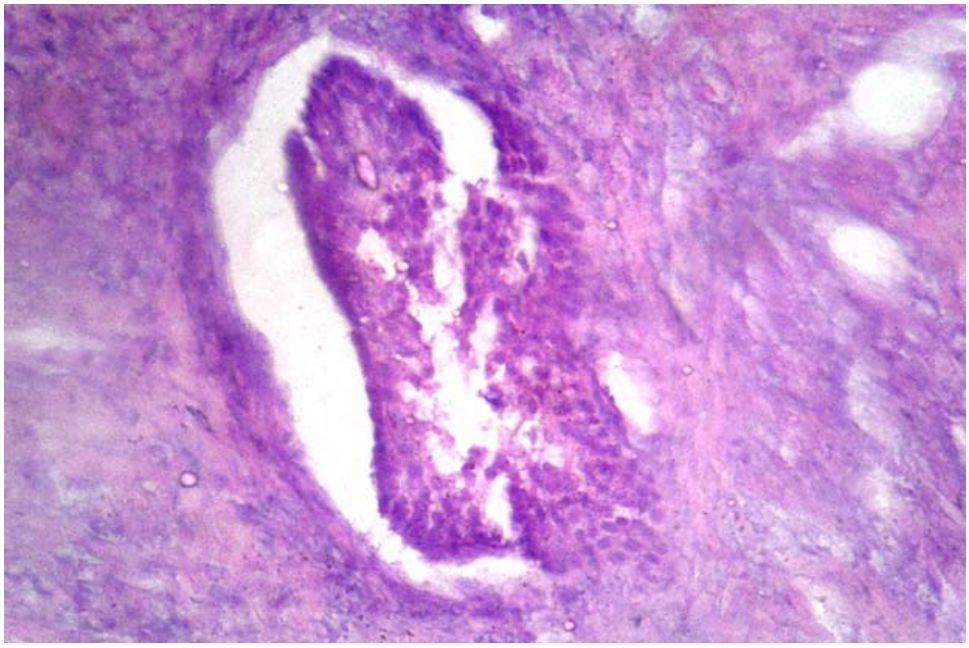
COLOUR PLATES



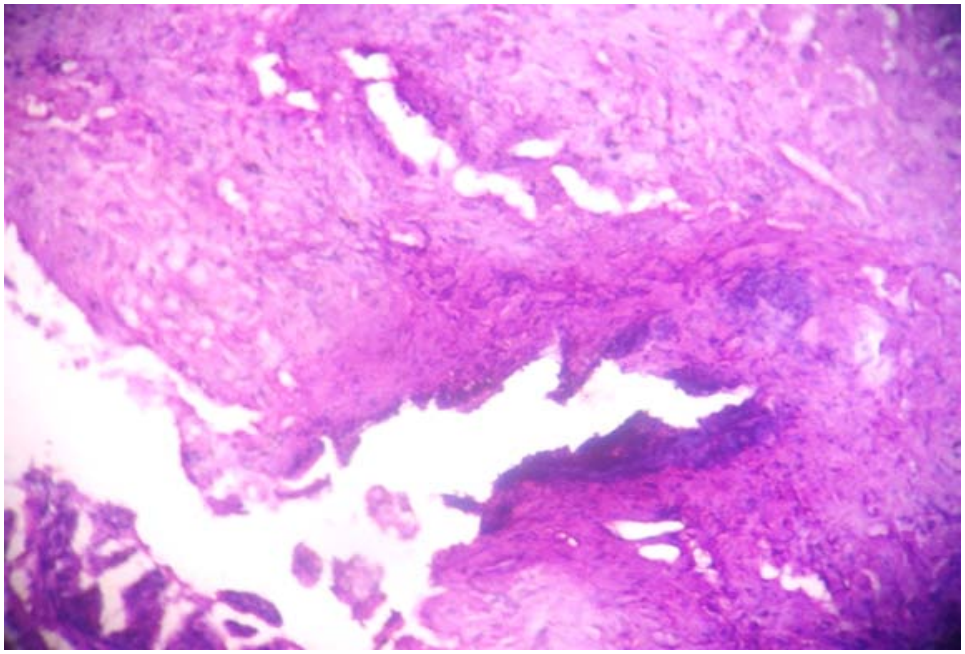
Benign Prostatic Hyperplasia H&E (10x)



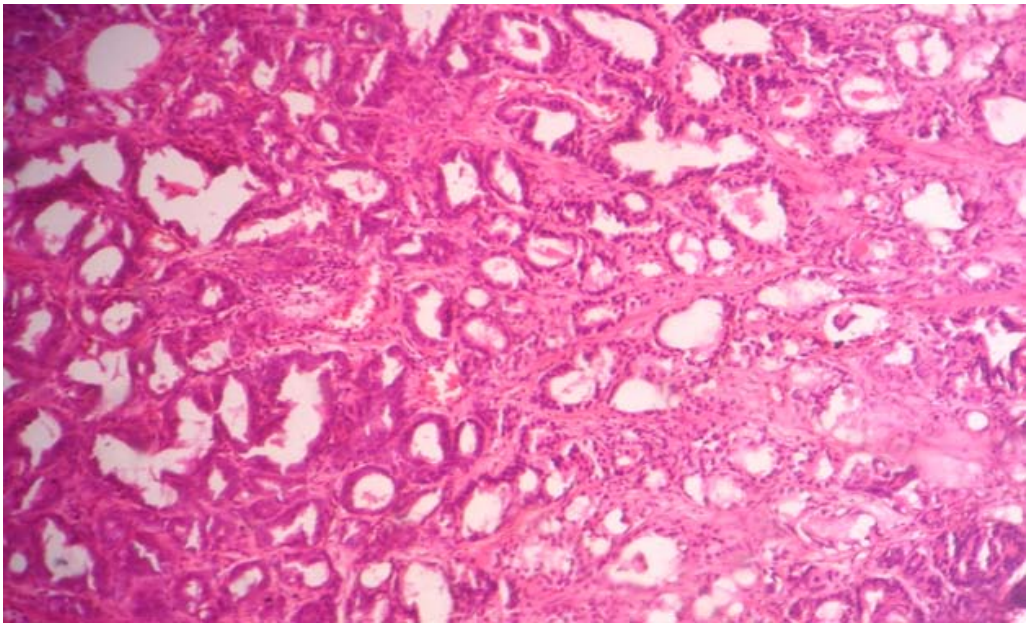
High Grade Prostatic Intraepithelial Neoplasia H&E (10X)



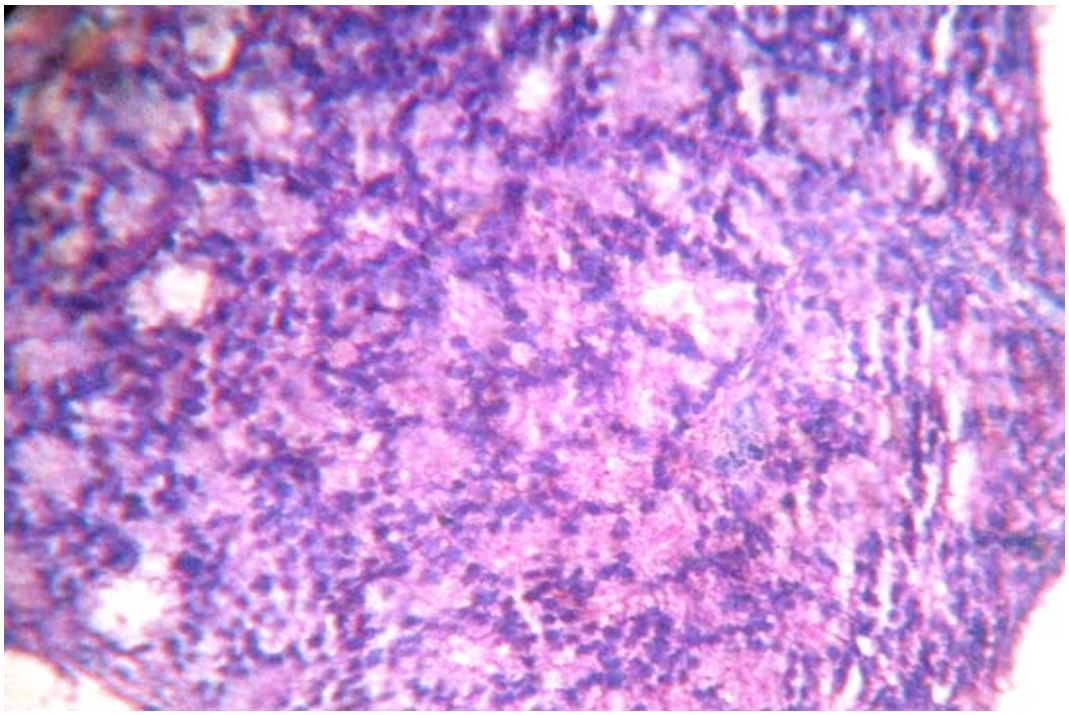
High Grade Prostatic Intraepithelial Neoplasia H&E (40x)



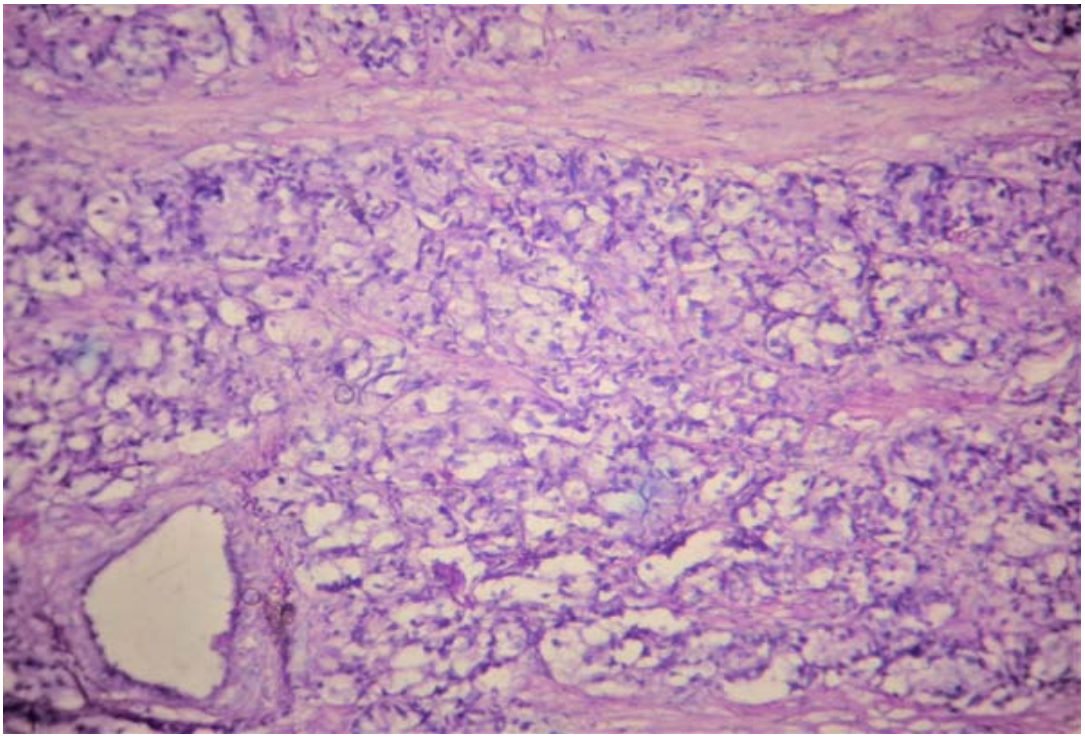
Microinvasive prostatic carcinoma H&E(40x)



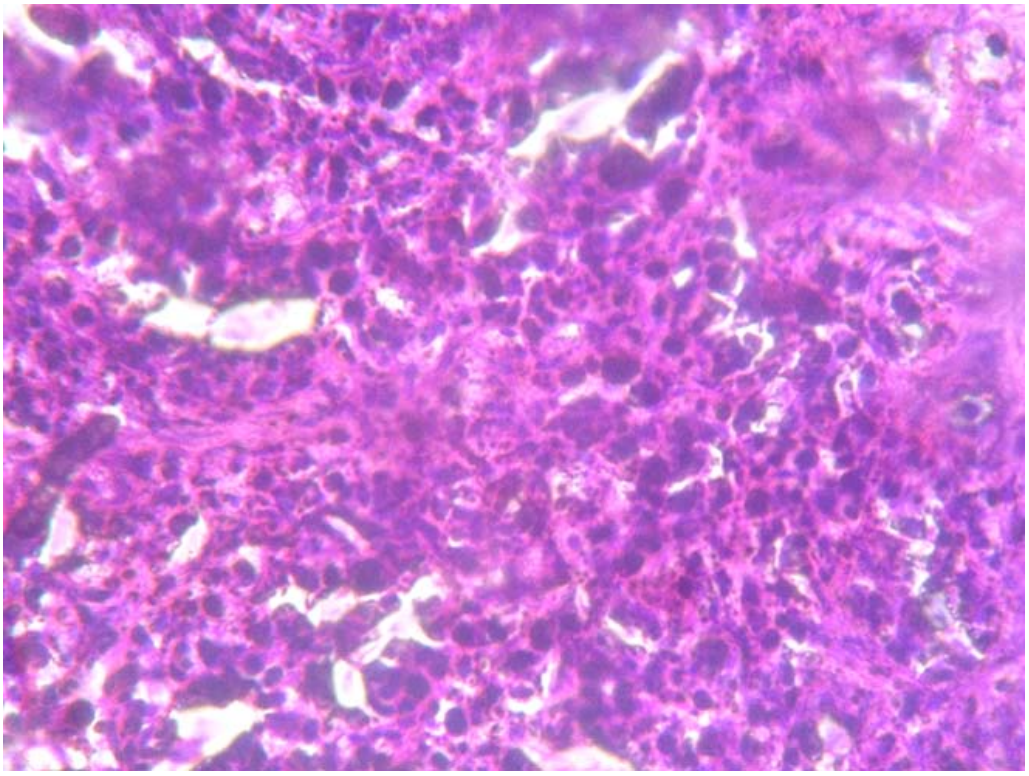
Gleason pattern 3 H&E (10x)



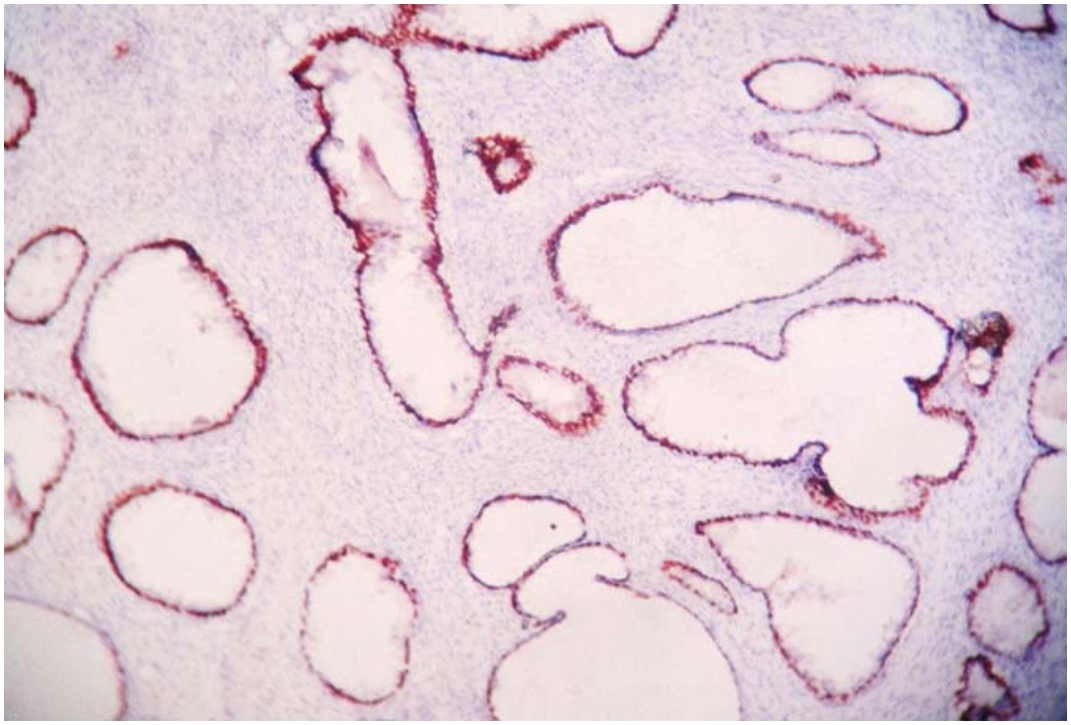
Gleason pattern 4- Cribriform pattern H&E (40x)



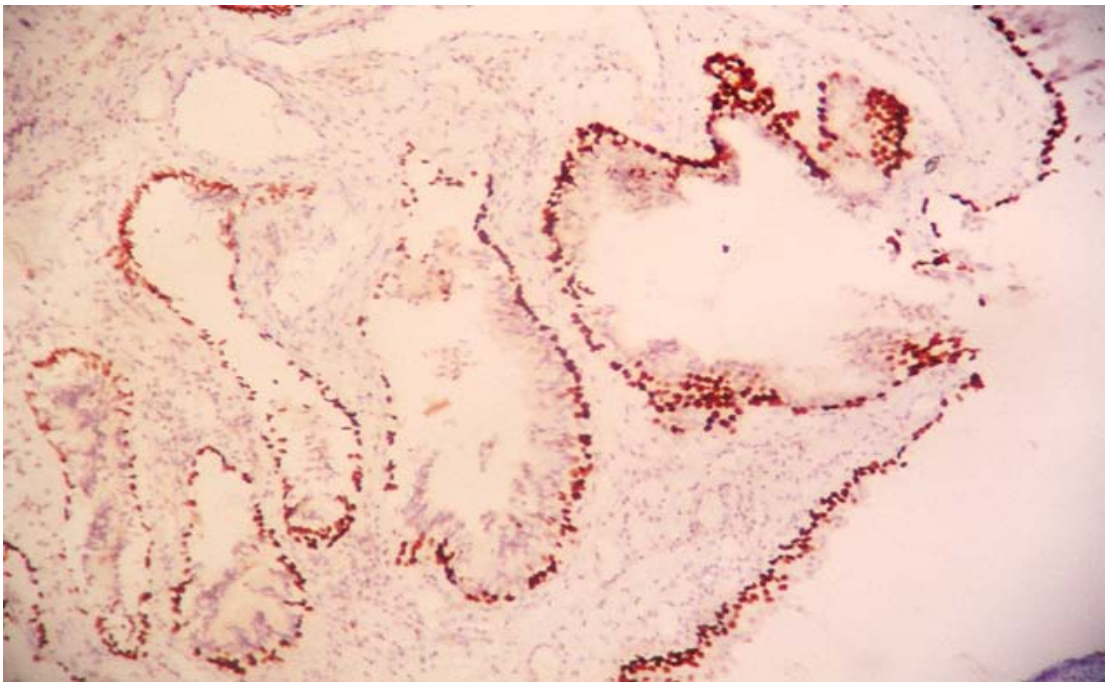
Gleason pattern 4 H&E(Hypernephroid pattern)-10x



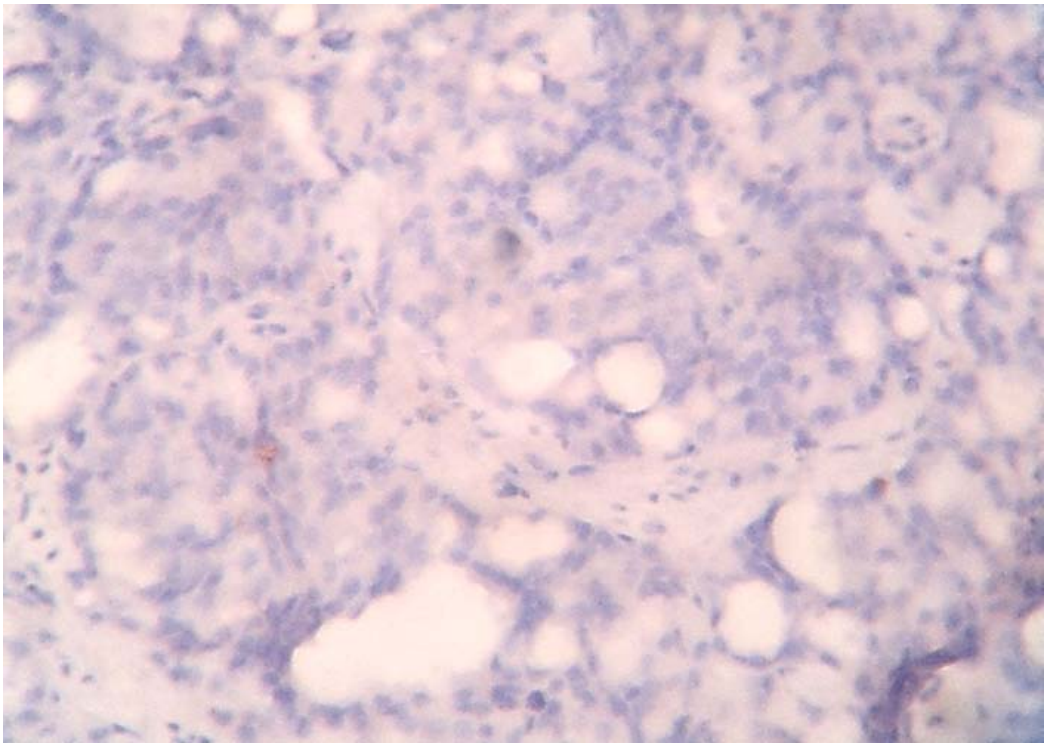
Gleason pattern 5-H&E (40x)



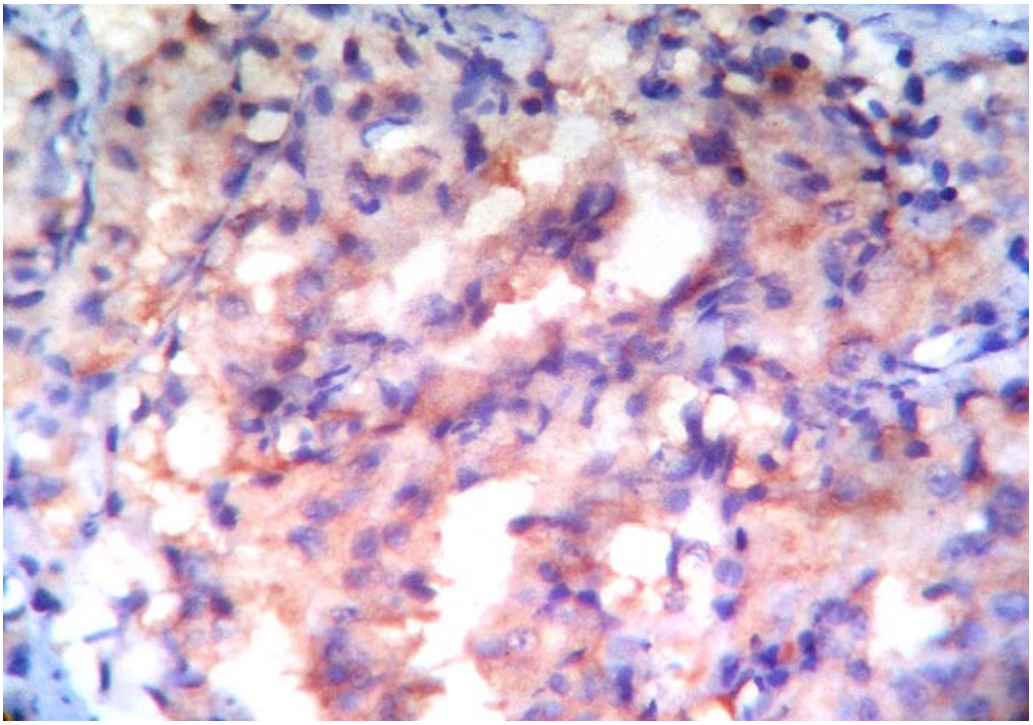
p63 strong basal nuclear positivity (10X)



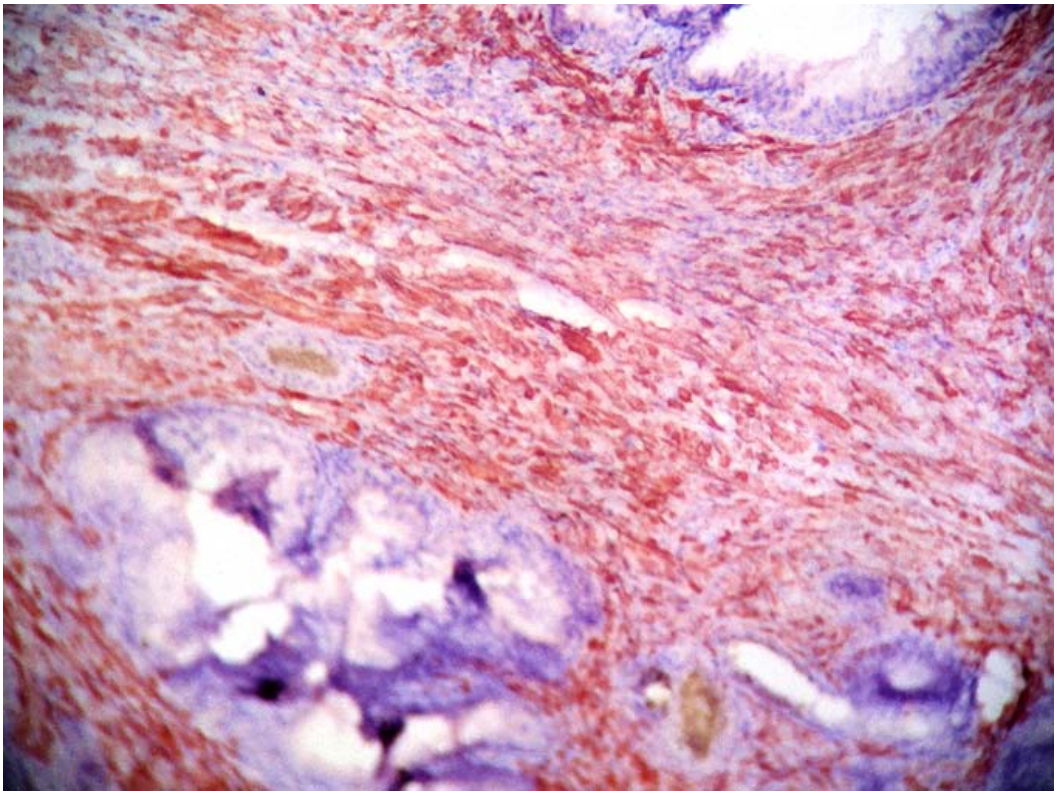
p63 positive in basal cells(40x)



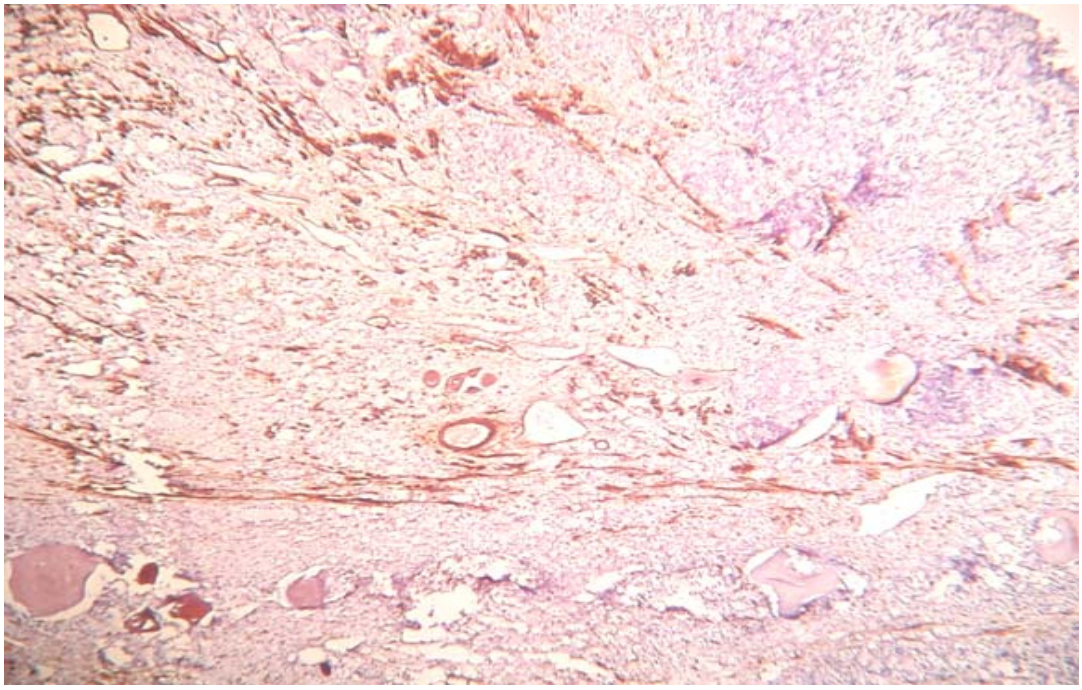
p63 negative staining(40x)



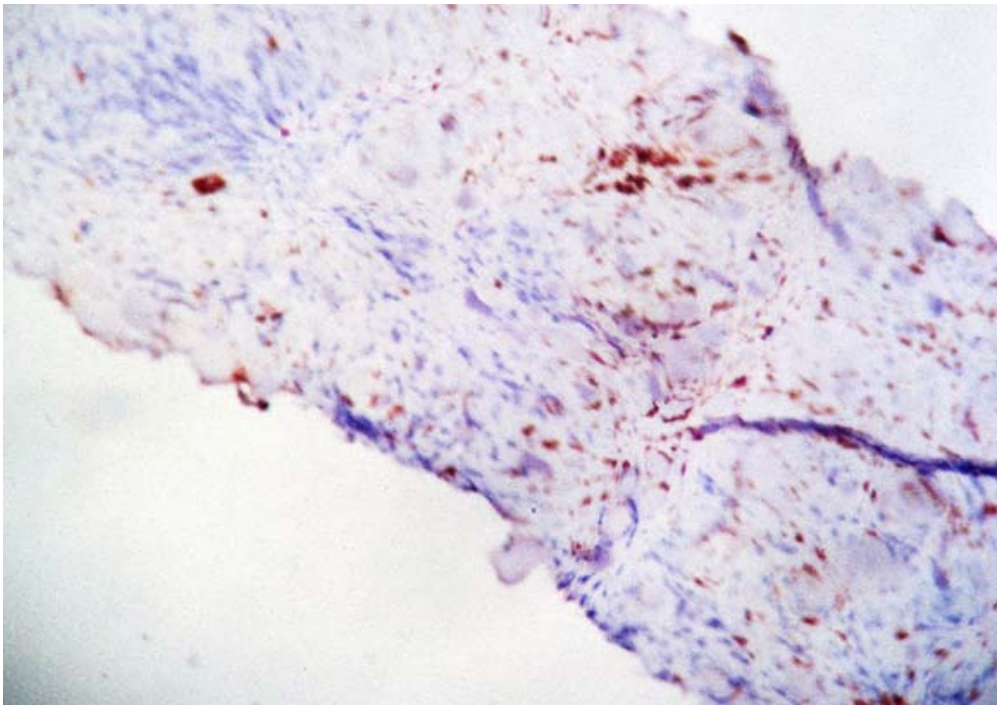
Cytoplasmic positivity of p63(40x)



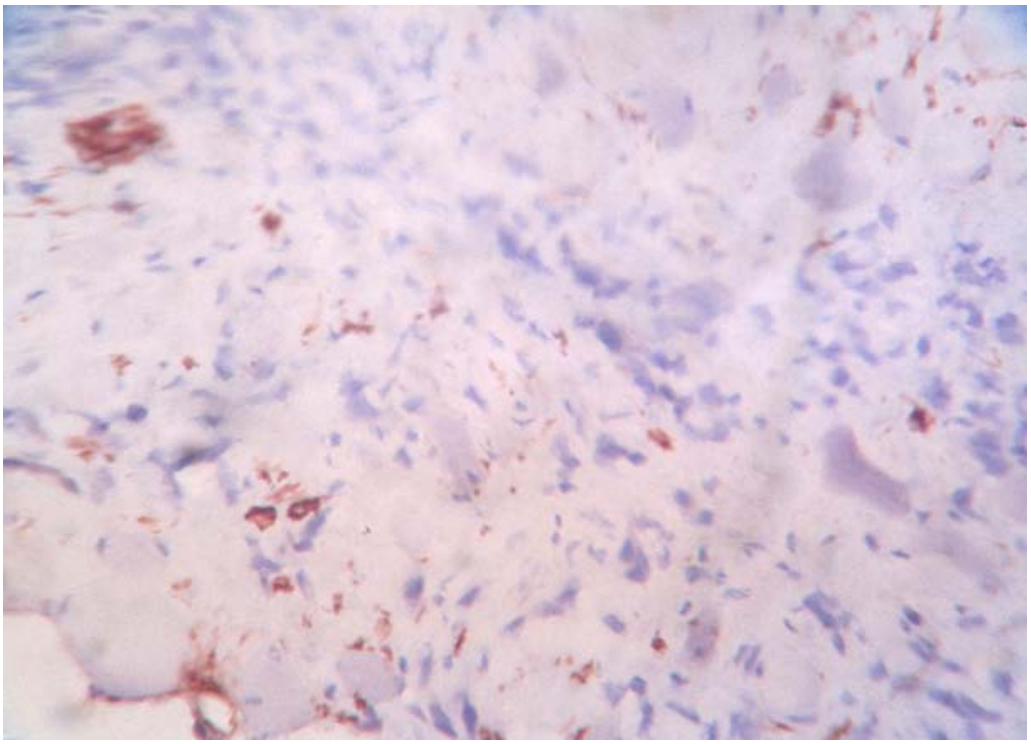
Calponin high index of staining(40x)



Calponin moderate staining (10x)



Calponin low staining (10x)



Calponin low staining(40x)

DISCUSSION

Prostatic carcinoma is currently emerging as one of the leading causes of cancer in the world in developing and developed countries. So it is important to differentiate prostatic carcinoma from its mimickers and BPH.

In present study we have used a well known marker p63, which takes up basal cells in prostate. As prostatic carcinoma shows absence of basal cells there is no staining of p63. This concept has been of immense help in differentiating suspicious and ambiguous lesions.

One study by Fahd Al Qahtani et al (80) showed age distribution of both BPH and prostatic carcinoma.

Table 14: Comparison of age distribution in different studies

STUDY GROUP	Age distribution	BPH	Carcinoma/PIN
Our Study	<50yrs	2(6.5%)	2(6.5%)
	50-70yrs	17(56.6%)	14(46.6%)
	70-90yrs	11(36.6%)	14(46.6%)
Fahd et al	50-70yrs	18(36%)	12(24%)
	70-90yrs	32(64%)	38(76%)

In study done by Fahd et al the majority of cases belonged to age group 70-90 years in both BPH and Prostatic carcinoma accounting for 64 and 76%

respectively.(80) This was in contrast to our study in which majority of cases were in age group of 60-70 years in both BPH and Prostatic carcinoma.

In our study, most cases including both benign and malignant lesions had PSA level >20ng/ml, when serum PSA level is compared to Gleason score of prostatic carcinoma, majority of the cases belong to Gleason score 3+3. The level of PSA in BPH ranged from 6-46ng/ml and had a mean of 25.7ng/ml. The level of PSA in Prostatic carcinoma ranges from 8-99ng/ml and had a mean of 50.89ng/ml. The mean PSA level of Prostatic intraepithelial neoplasia is 36.5ng/ml. In study done by Fahd et al the level of PSA in BPH ranges from 2-5 ng/ml with mean of 2.5ng/ml and level of PSA in Prostatic carcinoma ranges from 7-84ng/ml with a mean of 34 ng/ml.

We studied expression of two immunohistochemical markers p63 and Calponin in 60 cases. Totten et al observed that basal cells were invariably lacking in prostatic carcinoma (76). He also stated that basal cells were not always present on benign prostatic epithelium on H&E stained slides. With advent of immunohistochemistry those cases in which basal cells were not identified in H&E sections were diagnosed as benign lesions based on basal cell positivity for p63. In our study we analysed the expression of p63 in benign, premalignant, malignant and lesions which were suspicious of carcinoma by H&E.

Signoretti et al in their study reported that(72) all BPH showed basal p63 positivity. 97% of prostatic carcinoma was negative for p63, only <1% of

cells showed positivity for p63 which is a rare expression pattern. Weinstein et al also in his study have noted that p63 is positive only in benign lesions. It is sensitive and specific. False negativity is very less so chance of false positive diagnosis of malignancy is very rare. Leong et al (77) in a study on 247 biopsy samples found that out of 138 cases of BPH 128 cases showed strong intense basal nuclear staining and 106 out of 113 samples showed negative for malignancy. Our study results coincides with that of Shiran et al (78) who studied on 72 biopsies, 43 being BPH and 29 Prostatic carcinoma , out of this 38 cases of BPH showed positive staining and all malignancy showed negative staining for p63. In their study p63 showed a sensitivity of 83.37%, specificity of 100% and positive predictive value of 100%. In study by Fahd et al 49 out of 50 BPH (98%) showed p63 positivity and 48 out of 50 Prostatic carcinoma shows negative staining (96%).

Our study is in concordance with all these studies. It showed that benign lesions i.e BPH showed strong basal nuclear positivity of p63. But 3 cases of BPH didn't show any positive staining accounting for 10%. This finding appears similar to that as with Fahd et al. This negativity could be explained by prolonged fixation in formalin as the antigenicity will be masked by prolonged fixation or could be due to cautery artefact also. 3 cases showed only patchy or focal staining. It also helped in clearing suspicion about few ambiguous. In both cases of Prostatic Intraepithelial Neoplasia there was only patchy nuclear staining.

Among the 28 Prostatic carcinoma studied none showed positive nuclear staining for p63.About 14 cases showed aberrant cytoplasmic positivity. It is a rare expression pattern since in adenocarcinoma p63 is usually absent. In most of the cases we could see the malignant glands infiltrating benign glands indicated by presence of islands of entrapped benign glands indicated by presence of p63 strong basal positivity among the negatively staining malignant glands. About 2 cases which showed suspicious foci of malignancy in H&E were diagnosed as malignant with the help of negative p63 staining in immunohistochemistry. Sensitivity of p63 was 90% and specificity was 100%

About 14 Prostatic carcinoma cases showed aberrant cytoplasmic positivity which coincides with the study conducted by Dhillon et al (73)in which he studied 298 prostatic cancer cases and found a positive correlation between aberrant cytoplasmic p63 expression and number of cancer deaths. In their study they also studied Ki67 activity which showed high proliferation rates and decreased apoptosis. This cytoplasmic staining of p63 instead of the usual nuclear pattern indicates an altered and potentially oncogenic function of the mislocalised protein in the tumor progression and survival. Bismar et al also did a similar study in which cases showing abnormal p63 expression was correlated with increased PSA levels and recurrence during follow up period. (79). This cytoplasmic expression of p63 could be a real shift of protein from nucleus to cytoplasm that may play a significant role in cancer progression and identifying high risk patients. In our study we were not able to follow up patients to know the mortality or recurrence rate.

p63 is a nuclear transcriptional factor belonging to p53 gene family based on structural similarities. The alterations in the protein expression will disrupt the checkpoints in cell cycle and apoptosis and will provide a very important role in progression of cancer.

Only less studies have been conducted on expression of Calponin in prostatic lesions. The main concept is based on formation of reactive stroma in prostate cancer that will enhance tumor progression. The purpose of this is to analyse whether reactive stroma is a part of prostatic carcinoma. This reactive stroma is a part of wound repair like stroma composed of myofibroblasts rather than the fibroblasts and smooth muscle cells forming the normal stroma in prostatic glands and in benign lesions. Chemical mediator TGF-beta plays an important role in transforming fibroblasts to myofibroblasts. Calponin(h1) is a smooth muscle marker and will be expressed in the normal and benign prostatic stroma. Due to presence of reactive stroma the Calponin expression will be diminished to absent in prostatic carcinomas.

In our study out of the 30 BPH cases 29 showed moderate to strong cytoplasmic positivity for calponin accounting for 96% of cases. This is in concordance with study conducted by Jennifer et al(7) in normal prostate, PIN and prostate cancer for presence of reactive stroma. In that study they did immunohistochemistry with the combination of vimentin, smooth muscle actin and calponin to find the reactive stroma. In our study only one case showed weak expression of calponin in BPH.

We excluded cases who took prior radiotherapy or androgen manipulation as both results in mesenchymal reaction that affects the results. In prostatic carcinoma 74.9% cases showed low to zero cytoplasmic staining in stroma which coincides with that of Jennifer et al which showed 78% of prostatic carcinoma cases showing low to zero staining. The 2 cases of PIN showed moderate staining. Sensitivity of staining for calponin is 96% and specificity is 75%. Jennifer et al stated that reactive stroma formation starts at prostatic intraepithelial neoplasia itself and further diaplaces the normal stroma and progresses to cancer. The neoplastic cells and reactive stroma displaces the normal tissue and ultimately form the tumor.

SUMMARY

Prostatic carcinoma is one of the leading cause of cancer worldwide and cause significant morbidity and mortality. It has to be distinguished from benign lesions as well as benign mimickers to provide optimal treatment.

Our objective was to 1) Analyse the expression of p63 in prostatic lesions. 2) To differentiate any suspicious foci to benign and malignant. 3) To Study expression of calponin in benign and malignant lesions 4) To correlate the expression with histopathological grading.

A total of 60 cases which included 40 cases of TURP and 20 cases of needle biopsy were studied retrospectively in Chengalpattu Govt. Medical College for a period of 3 years from june 2015 to may 2017 was done. The lesions were further grouped as benign, premalignant and malignant. The age wise distribution was analysed and in our study the most common age group for both benign and malignant lesions were 60-70 years. Correlation of serum PSA level was done with both type of lesions and Gleason score. The serum PSA level was >20ng/ml for most of the cases of malignancy. In our study most number of carcinoma cases had Gleason score 3+3. Immunohistochemistry was done on paraffin embedded blocks of prostatic lesion by 2 markers p63 and Calponin and its expression was analysed. The expression of these markers were analysed on all prostatic lesions studied.

In our study on expression of p63 in benign, malignant and premalignant lesions, majority of benign lesions showed basal nuclear positivity of p63. All

cases of malignancy showed negative staining of basal cells for p63. Few of the cases of Prostatic carcinoma showed aberrant cytoplasmic positivity of p63 which could indicate increased chance of cancer mortality according to previous studies. Suspicious foci of carcinoma were resolved using the p63 staining. The premalignant lesions showed patchy staining by p63. The sensitivity of p63 was 90% and specificity was 100%.

Study on expression of Calponin showed that all benign lesions showed high index of cytoplasmic staining. 75% malignant lesions showed absent to low index of staining. Few cases showed moderate index of staining. This decrease of staining is thought to be due to formation of reactive stroma which replaces the normal fibromuscular stroma to a stroma composed of fibroblasts and myofibroblasts predominantly and is thought to induce tumor progression according to literature.

CONCLUSION

Immunohistochemical p63 staining is diagnostically reliable in identifying basal cells in prostate needle biopsies and TURP specimens. P63 is a valuable tool with high sensitivity and specificity in differentiating BPH from Prostatic carcinoma.

The decreased or absent stromal staining of Calponin which indicates a reactive stroma in malignancy can be used alone or in conjunction with p63 for confirmation of diagnosis of Prostatic carcinoma. In future specific studies will be directed towards identifying specific markers of reactive stroma which will aid in predicting the rate of cancer progression and possibility of recurrence. The future holds promise for novel therapeutic drugs targeting the specific components of reactive stroma.

The aberrant cytoplasmic staining of p63 which is associated with increased mortality could not be studied due to loss of follow up which is a limitation in this study.

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ANNEXURE - I

PROFORMA

Date:

1. Name : OP/IP No :
2. Age :
3. Sex : Male Female
4. History :
5. Serum PSA level :

Clinical Diagnosis :

Histopathological confirmation and grading of H&E stained section:

1. Benign prostatic hyperplasia,
2. Benign prostatic hyperplasia with prostatitis
3. Prostatic intraepithelial neoplasia high grade and low grade
4. Prostatic adenocarcinoma

    Gleason grade 2

    Gleason grade 3

    Gleason grade 4

    Gleason grade 5

p63 expression

Negative

Positive- Basal nuclear positivity

Focal positivity

Cytoplasmic positivity

Calponin:

Negativity

Low positivity:

Moderate positivity:

Strong positivity**:**

ANNEXURE II

KEY TO MASTER CHART

1. AGE

1. <50

2. 51-60

3.61-70

4.71-80

5.81-90

2. SEX

1. Male

3. HPE DIAGNOSIS:

1. Benign prostatic hyperplasia

2. Benign prostatic hyperplasia with prostatitis

3. Prostatic intraepithelial neoplasia.

4. Prostatic adenocarcinoma.

4. GLEASONS SCORE:

1.3+3

2. 3+4

3. 4+3

4.≥4+4

4. IHC DIAG- p63 EXPRESSION:

1. Strong basal nuclear positivity

2. Patchy positivity

3. Cytoplasmic positivity

4. Negative

5.IHC – CALPONIN EXPRESSION

Percentage staining- 0- 0% staining

1-1-33% staining

2-34-66% staining

3-67-100% staining

Staining intensity:

0 – No staining

1 – Staining obvious only at 400X

2 – Staining obvious at X100 but not X40

3 – Staining obvious at 40X

Staining Index = Staining percentage x Staining intensity

0 – Zero

1-2 – Low

3-4 – Moderate

6-9 - High

6.PRE OPERATIVE SERUM PSA LEVEL:

1.< 10ng/ml

2.10 – 20 ng/ ml

3.>20ng/ml

ANNEXURE – III

GLOSSARY

HGPIN	:	High grade Prostatic intraepithelial neoplasia.
DAB	:	Di amino benzidine
H&E	:	Haematoxylin & Eosin
IHC	:	Immunohistochemistry
PBS	:	Phosphate buffer solution
TBS	:	Tris buffer solution
BPH	:	Benign prostatic hyperplasia
PIN	:	Prostatic Intraepithelial neoplasia
DHT	:	Dihydrotestosterone
PSA	:	Prsotate Specific Antigen
IDC – P	:	Intraductal carcinoma prostate
AMACR	:	Alpha-methylacyl-CoA racemace
WHO	:	World Health Organisation
ISUP	:	International society of Urologic Pathology
DRE	:	Digital rectal examination.
NSGP	:	Non specific Granulomatous prostatitis.
PANCK	:	Pancytokeratin

Master Chart

S.No	HPE No:	AGE	SEX	Age group	Clinical diagnosis	Specimen type	HPE diag	Gleason Score	Serum PSA level	IHC Diagnosis			
										p63	calponin %staining	Intensity	Index=M2*N2
1	G924/16	75	M	4	BPH	TURP	BPH	nil	2	4	3	3	9
2	G962/16	65	M	3	BPH	TURP	BPH	nil	3	1	3	3	9
3	G750/16	65	M	3	BPH	TURP	BPH	nil	1	1	3	3	9
4	G162/17	65	M	3	?Ca Prostate	Needle core	BPH	nil	3	1	3	3	9
5	G273/16	78	M	4	BPH	TURP	BPH	nil	2	2	3	3	9
6	G154/17	76	M	4	BPH	TURP	BPH	nil	2	1	3	3	9
7	G1559/17	67	M	3	BPH	TURP	BPH	nil	3	1	3	3	9
8	G244/17	68	M	3	BPH	TURP	BPH with prostatiti	nil	3	2	2	3	6
9	G1154/17	70	M	3	BPH	TURP	BPH	nil	1	1	3	3	9
10	G101/17	58	M	2	BPH	TURP	BPH	nil	3	1	2	2	4
11	G1503/16	65	M	3	BPH	TURP	BPH	nil	1	1	3	3	9
12	G278/17	79	M	4	BPH	TURP	BPH	nil	3	1	3	3	9
13	G247/17	40	M	1	BPH	Needle biopsy	BPH	nil	2	2	2	3	6
14	G1502/17	70	M	3	BPH	TURP	BPH	nil	2	4	1	3	3
15	G1116/17	72	M	4	BPH	TURP	BPH	nil	3	1	3	3	9

16	G156/17	60	M	2	BPH	TURP	BPH	nil	3	1	3	3	9
17	G1115/17	68	M	3	BPH	TURP	BPH with prostatiti	nil	3	1	3	2	6
18	G1118/17	75	M	4	BPH	TURP	BPH	nil	3	1	3	2	6
19	G900/17	60	M	2	BPH	TURP	BPH	nil	1	1	3	3	9
20	G1113/17	80	M	4	BPH	TURP	BPH	nil	2	1	3	3	9
21	G923/17	72	M	4	BPH	TURP	BPH	nil	3	1	3	3	9
22	G964/17	78	M	4	BPH	TURP	BPH	nil	3	1	3	3	9
23	G1013/17	75	M	4	BPH	TURP	BPH	nil	2	1	3	3	9
24	G1012/17	60	M	2	BPH	TURP	BPH	nil	3	4	3	3	9
25	G1015/17	63	M	3	BPH	TURP	BPH	nil	3	1	3	3	9
26	G1014/17	63	M	3	BPH	TURP	BPH	nil	3	1	3	3	9
27	G97/17	50	M	1	BPH	TURP	BPH	nil	2	1	3	3	9
28	G16/17	71	M	4	BPH	TURP	BPH	nil	2	1	3	3	9
29	G213/17	52	M	2	BPH	TURP	BPH	nil	3	1	3	3	9
30	G756/17	70	M	3	BPH	TURP	BPH	nil	2	1	3	3	9
31	G1367/13	75	M	4	BPH	TURP	HGPIN	nil	3	2	2	3	6
32	G1769/14	78	M	4	BPH	TURP	HGPIN	nil	3	2	2	3	6
33	G61/17	65	M	3	BPH	TURP	Prostatic Ca	(4+4)	3	4	0	0	0
34	G151/17	60	M	2	Prostatic Ca	Needle core	microinvasive ca	nil	2	3	2	3	6
35	G152/17	82	M	5	Prostatic Ca	TURP	Prostatic Ca	(4+4)	3	4	1	1	1
36	G163/17	65	M	3	Prostatic Ca	Needle core	Prostatic Ca	(3+3)	1	4	1	2	2
37	G1534/16	70	M	3	Prostatic Ca	Needle core	Prostatic Ca	(3+3)	3	4	1	2	2
38	G601/17	75	M	4	Prostatic Ca	TURP	Suspicious of P.Ca	nil	2	4	1	2	2
39	G1030/17	75	M	4	Prostatic Ca	Needle core	Prostatic Ca	(3+4)	3	4	1	2	2
40	G1787/16	40	M	1	Prostatic Ca	TURP	Prostatic Ca	(3+3)	3	4	1	2	2
41	G1654/17	75	M	4	?Prostatic Ca	Needle core	Prostatic Ca	(3+3)	3	3	2	3	6
42	G885/17	78	M	4	Prostatic Ca	Needle core	Prostatic Ca	(3+4)	3	3	2	2	4

43	G1025/16	50	M	1	Prostatic Ca	TURP	Prostatic Ca	(3+3)	3	4	0	0	0
44	G859/17	83	M	5	Prostatic Ca	Needle core	Prostatic Ca	(3+4)	3	3	1	3	3
45	G1095/17	53	M	2	Prostatic Ca	Needle core	Prostatic Ca	(3+4)	3	3	3	3	9
46	G960/16	86	M	5	Prostatic Ca	Needle core	Prostatic Ca	(5+3)	3	3	1	2	2
47	G197/16	67	M	3	Prostatic Ca	Needle core	Prostatic Ca	(4+5)	3	3	1	3	3
48	G564/17	60	M	2	Prostatic Ca	TURP	Prostatic Ca	(4+3)	3	3	1	3	3
49	G1087/17	65	M	3	Prostatic Ca	Needle core	Prostatic Ca	(3+4)	3	4	1	3	3
50	G842/16	59	M	2	Prostatic Ca	Needle core	Prostatic Ca	(4+4)	2	3	1	2	2
51	G977/15	80	M	4	BPH	TURP	Prostatic Ca	(5+3)	3	4	1	2	2
52	G1875/15	70	M	3	?Prostatic Ca	Needle core	Prostatic Ca	(3+4)	3	3	1	2	2
53	G1317/13	75	M	4	BPH	TURP	Prostatic Ca	(4+3)	3	3	1	2	2
54	G316/17	65	M	3	Prostatic Ca	Needle core	Prostatic Ca	(4+3)	3	4	2	2	4
55	G680/14	65	M	3	Prostatic Ca	Needle core	Prostatic Ca	(4+3)	3	3	1	3	3
56	G1093/16	70	M	3	BPH	TURP	Suspicious of P.Ca	nil	3	4	3	3	9
57	G1182/14	66	M	3	Prostatic Ca	TURP	Prostatic Ca	(3+3)	3	3	1	1	1
58	G61/13	80	M	4	Prostatic Ca	Needle core	Prostatic ca	(3+3)	3	3	1	2	2
59	G1371/13	75	M	4	Prostatic Ca	Needle core	Prostatic ca	(3+3)	3	4	1	2	2
60	G1342/16	78	M	4	Prostatic Ca	Needle core	Prostatic ca	(3+3)	3	4	2	2	4