DISSERTATION

On

TO DETERMINE THE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PROSTATIC ADENOCARCINOMA FROM CANCER MIMICKERS

submitted in partial fulfillment of the requirements for the degree of

Doctor of Medicine

(BRANCH-III)

M.D. PATHOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

TIRUNELVELI MEDICAL COLLEGE
TIRUNELVELI
MAY 2018

CERTIFICATE

This is to certify that the dissertation titled **"TO DETERMINE THE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PROSTATIC ADENOCARCINOMA FROM CANCER MIMICKERS",** is a bonafide work done by **Dr.R.ILAKKIARANI** Post Graduate Student, Department of Pathology, Tirunelveli Medical College, Tirunelveli – 627011, in partial fulfilment of the university rules and regulations for the award of MD DEGREE in PATHOLOGY BRANCH-III, under my guidance and supervision, during the academic period from 2015-2018.

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I hereby certify that this dissertation entitled **"TO DETERMINE THE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PROSTATIC ADENOCARCINOMA FROM CANCER MIMICKERS"** is a record of work done by **Dr.R. ILAKKIARANI**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2015- 2018. This work has not formed the basis for previous award of any degree.

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DECLARATION

I solemnly declare that the dissertation titled **"TO DETERMINE THE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PROSTATIC ADENOCARCINOMA FROM CANCER MIMICKERS"** was done by me at Tirunelveli Medical College, Tirunelveli–627011, during the period 2015 to 2018 under the guidance and supervision of **Prof.Dr.S.VASUKI MD**, to be submitted to The Tamil Nadu Dr. M.G.R. Medical University towards the partial fulfilment of requirements for the award of MD DEGREE in PATHOLOGY BRANCH-III.

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ABBREVIATIONS :

AAH	Atypical adenamatous hyperplasia
AMACR	Alpha mehyl acyl co-A racemase
ASAP	Atypical small acinar proliferation
BCH	Basal cell hyperplasia
BPH	Benign prostatic hyperplasia
CCCH	Clear cell cribriform hyperplasia
CK	Cytokeratin
DAB	Diamino benzidine
DRE	Digital rectal examination
EDTA	Ethylene diamine tetra acetate
HGPIN	High grade prostatic intra epithelial neoplasia
HMWCK	High molecular weight cytokeratin
IHC	Immuno histochemistry
LGPIN	Low grade prostatic intra epithelial neoplasia
PAA	Prostatic acinar adenocarcinoma
PAH	Post atrophic hyperplasia
PC	Prostate cancer
PIA	Proliferative inflammatory atrophy
PIN	Prostatic intra epithelial neoplasia
TURP	Trans urethral resection of prostate
VMGH	Verumontanum mucosal gland hyperplasia

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REF NO:829/GS/2016

PROTOCOL TITLE: TO DETERMINE THE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PROSTATE ADENOCARCINOMA FROM CANCER MIMICKERS.

PRINCIPAL INVESTIGATOR: Dr.R. ILAKKIARANI, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN PATHOLOGY

DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

Dear , Dr. R. Ilakkiarani, MBBS.,. The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 05.08.2016.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented.
   d. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
   e. Approval for amendment changes must be obtained prior to implementation of changes.
   f. The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

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CERTIFICATE - II

This is certify that this dissertation work titled **"TO DETERMINE THE EFFECTIVENESS OF IMMUNOHISTOCHEMISTRY IN DIFFERENTIATING PROSTATIC ADENOCARCINOMA FROM CANCER MIMICKERS"** of the candidate **Dr.R.ILAKKIARANI**, with registration Number **201513304** for the award of **M.D.** Degree in the branch of **PATHOLOGY (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 page and result shows **1 PERCENTAGE** of plagiarism in the dissertation.

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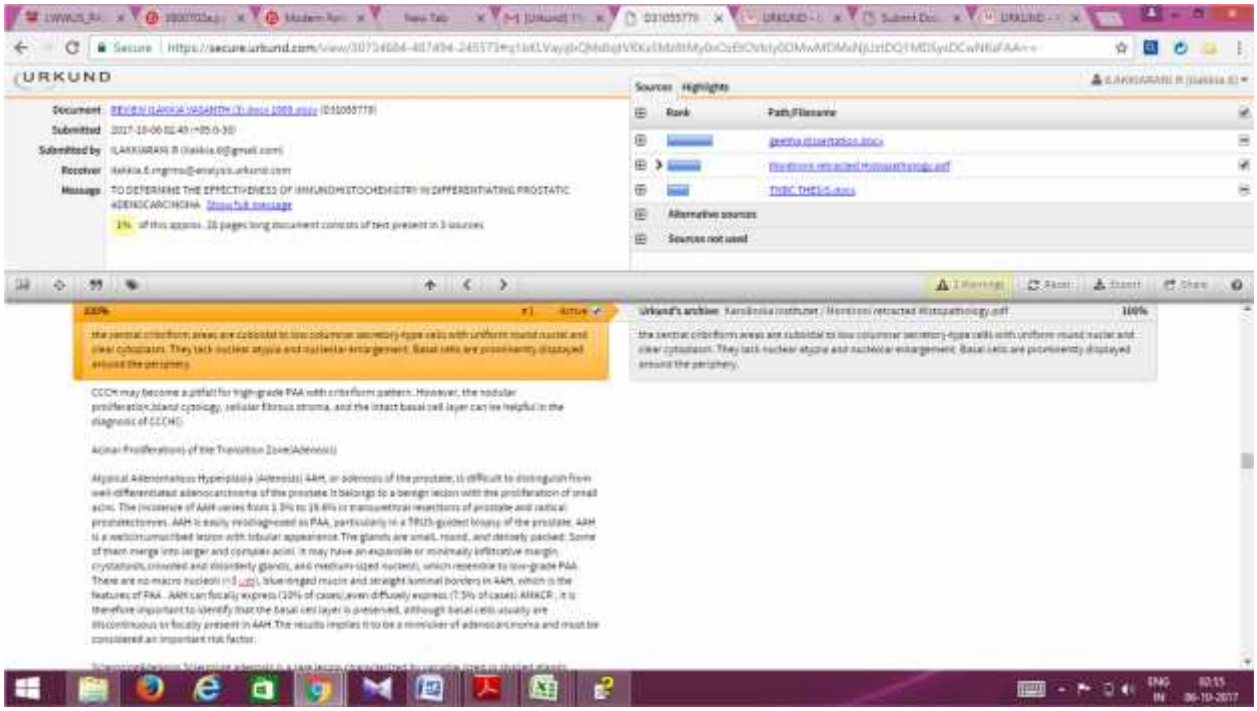
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the central cribriform areas are cuboidal to low columnar secretory-type cells with uniform round nuclei and clear cytoplasm. They lack nuclear atypia and nucleolar enlargement. Basal cells are prominently displayed around the periphery.
CCCH may become a pitfall for high-grade PAA with cribriform pattern. However, the nodular proliferation,Island cytology, cellular fibrous stroma, and the intact basal cell layer can be helpful in the diagnosis of CCCHO.
Acinar Proliferations of the Transition Zone(Adenosis)
Atypical Adenomatous Hyperplasia (Adenosis) AAH, or adenosis of the prostate, is difficult to distinguish from well-differentiated adenocarcinoma of the prostate. It belongs to a benign lesion with the proliferation of small acini. The incidence of AAH varies from 1.3% to 19.4% in transurethral resections of prostate and radical prostatectomies. AAH is easily misdiagnosed as PAA, particularly in a TRUS-guided biopsy of the prostate. AAH is a well-circumscribed lesion with lobular appearance.The glands are small, round, and densely packed. Some of them merge into larger and complex acini. It may have an expansile or minimally infiltrative margin. crystalloids, crowded and disorderly glands, and medium-sized nucleoli, which resemble to low-grade PAA. There are no macro nucleoli (<3 um), blue-tinged mucin and straight luminal borders in AAH, which is the features of PAA . AAH can focally express (10% of cases),even diffusely express (7.5% of cases) AMACR , it is therefore important to identify that the basal cell layer is preserved, although basal cells usually are discontinuous or focally present in AAH.The results implies it to be a mimicker of adenocarcinoma and must be considered an important risk factor.

CONTENTS

INTRODUCTION

Prostate cancer is the second most common cause of cancer and the sixth leading cause of cancer related death in men worldwide[1]. It is essentially a disease of elderly men more than 65 years of age. Previously it was thought that prevalence of prostate cancer in India is far lower as compared to the western countries but with the increased migration of population from rural to urban, increase in awareness, and accessibility to medical care, more new cases of prostate cancer are being picked up and it becomes vivid that we are not much behind to the rate from western countries. As the most frequent histological subtype, prostatic acinar adenocarcinoma has number of benign mimickers including prostatic or non-prostatic lesions and normal structures which may lead to erroneous diagnosis and inappropriate treatment. It is very important to be aware of the existence of these mimickers and to recognize their histological features. Differentiation of prostatic adenocarcinoma from benign prostatic lesions and hyperplasia sometimes can't be done on the sole basis of morphologic findings. In these cases diagnosis can be made according to the presence or absence of basal cell layer considering the fact that in prostatic adenocarcinoma there is no basal cells but benign lesion shows encirclement by this basal cell layer. Hence, using basal cell immunohistochemistry markers like p63 seems to be useful in distinguishing these two important categories of prostatic lesions. Another useful marker detectable by

immunohistochemistry is alpha methyl acylcoA racemase(AMACR), an enzyme selectively expressed in neoplastic glandular epithelium.

Cocktails of antibodies directed against basal cell markers and AMACR are particularly useful in evaluating small foci of atypical glands, and substantiating a diagnosis of minimal adenocarcinoma. The present study is carried out with the aim to evaluate the utility and expression of immunohistochemistry markers in differentiating prostatic adenocarcinoma from benign mimickers and in resolving morphologically suspicious foci on TURP and needle core biopsies.

AIM

To determine the effectiveness of immunohistochemistry in differentiating prostatic adenocarcinoma from cancer mimickers.

OBJECTIVES

1. To study the expression of antibodies against p63 and AMACR using immunohistochemistry and to determine the effectiveness of these markers in differentiating prostatic adenocarcinoma from cancer mimickers.
2. To find out the correlation of AMACR expression with Gleason grading of prostatic adenocarcinoma cases.
3. Also to find out the expression of p63 and AMACR antibodies in benign glands and premalignant lesions.

REVIEW OF LITERATURE

ANATOMY OF PROSTATE

The human prostate gland is a part of male reproductive system. It is a walnut-shaped gland, measures about 5 cm x 4 cm x 3 cm and weighs 20 gms upon maturity. The gland is found low in the pelvis minor, surrounds the bladder neck and the first part of the urethra, and is posterior to the symphysis pubis. The gland lies ventral to the ampulla of the rectum, where the posterior portion of the prostate can be easily palpated.

The prostate has three major anatomical zones: the peripheral zone (PZ), the central zone (CZ) and the transition zone (TZ), occupying 65%, 25% and 10% respectively, of the prostate volume[2]. The biology of these regions differs, which is important for the development of cancer & other histological lesions. The central zone is located at the base of the prostate and surrounds the ejaculatory ducts, extending out from the verumontanum in a wedge-shaped fashion. The transition zone, which surrounds the proximal prostatic urethra, and is found at the junction of the proximal and distal segments of the urethra. The transition zone is important in that it can undergo hyperplasia, resulting in the formation of nodules known as benign prostatic hyperplasia which may lead to clinical symptoms of prostatism such as urinary frequency, hesitancy and dribbling. The largest zone, which is the peripheral zone, completes the remaining part of the prostate, and is also where carcinoma of the prostate predominantly occurs.

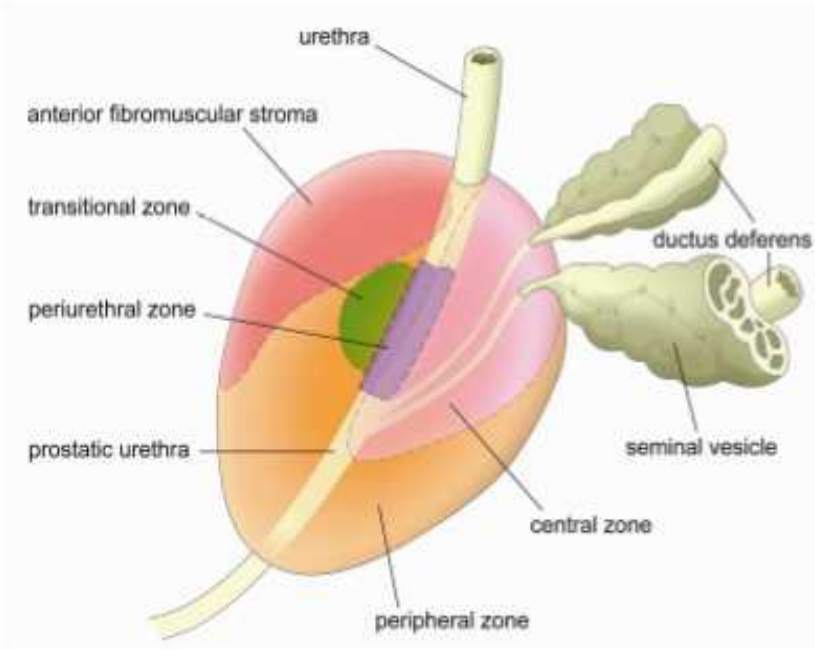


Figure:1 Anatomy of prostate

PHYSIOLOGY

The main function of the prostate is the secretion and storage of slightly alkaline seminal plasma that is added to semen upon ejaculation. A large part of the ejaculate consists of prostatic fluid, which serves to nourish and protect the semen. Prostatic secretions contain numerous enzymes and substances including proteases such as Prostatic specific antigen, acid phosphatase, potassium, zinc, citric acid, spermine, amino acids, and prostaglandins. These secretory products enhance fertility by promoting sperm viability and motility. An antimicrobial role has been suggested for a few of these substances such as zinc, spermine, and proteases. Testosterone, which is secreted by the testis, is metabolised to dihydrotestosterone by 5-alpha reductase in the prostate.

HISTOLOGY

Microscopic study of, adult prostate in men in between third to fifth decades comprises of branching duct-acinar glandular system that is embedded in a dense fibromuscular stroma.

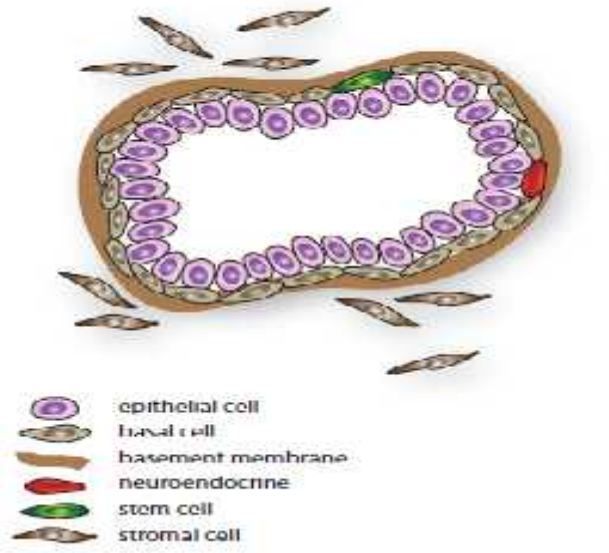


Figure: 2 Histology of Prostate

The normal epithelium of the prostate is classically defined as having two cell layers: a luminal or secretory cell layer requiring androgens for growth and survival and an androgen insensitive basal cell layer. The basal cell layer separates the secretory cells from the basement membrane and is nearly continuous which is a diagnostic criterion for benign conditions. For the diagnosis of invasive carcinoma, the complete absence of basal cells is an important finding. The third cell type in normal prostatic epithelium are neuroendocrine cells (NE). Stroma contains cells such as

skeletal cells, smooth muscle cells and also fibroblasts and endothelial cells.

EMBYOLOGICAL DEVELOPMENT

Androgens are necessary for the development and function of the prostate, that includes i) testosterone, synthesized in the testes ii) dehydroepiandrosterone, synthesized in the adrenal glands and iii) dihydrotestosterone, that is converted from testosterone within the prostate. The prostate is developed from the urogenital sinus and is recognizable at the period of 9 to 10 weeks during embryological development.

There is an infantile resting period after birth until the age of 10 to 12 years and then a pubertal maturation period until age 18 years. Eventhough there is no change in size of prostate gland during the resting period, duct formation and solid budding continues. The pubertal period is marked by substantial androgen-driven increase in gland size, further branching and differentiation of immature prostatic epithelium into the adult-type basal and secretory cells. It has been proposed that the central zone of the prostate is of mesodermal Wolffian duct origin. In this sense, the prostate gland is of dual embryonic derivation.

PROSTATE CANCER

Cancer development

Prostate cancer development and progression is a multistep process. Prostate cancer development occurs due to the loss of balance between cell proliferation rate and programmed cell death (apoptosis).

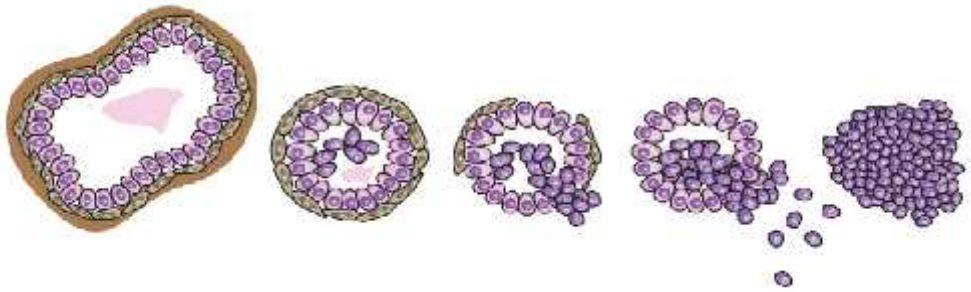


Figure:3 Tumour Progression

(This figure shows the gradual transformation of a normal gland into a more undifferentiated structure. Basal cells tend to disappear, the glands become smaller, lumina are not well defined and eventually there are only cancer cells, scattered or forming solid sheets)

In Prostate cancer, tumour cells have been believed to originate from luminal epithelial cells since they are dependent on androgens and express luminal cell markers. The development of prostate cancer (PC) occurs through the accumulation of genetic and epigenetic changes, leading to an inactivation of tumor suppressor genes and activation of oncogenes[3]. These alterations most likely take several decades and cancer development can be considered a continuous transformation from benign cells, cancer precursors and finally malignant cells[4].

Important premalignant lesions of prostatic adenocarcinoma which includes: prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA). Among these two lesions, PIN exhibits more convincing correlation with cancer[5,6,7,8]. PIN is considered to be an intermediate stage from benign epithelium to carcinoma. Histologically PIN is very similar to prostate cancer, with the exception that the basal layer is discontinuous but still present[9,10]. Prominent nucleoli are vital for the diagnosis. PIN is classified into low-grade and high-grade PIN (LGPIN and HGPIN)[5,11].

These lesions are converted into malignant neoplasms through many sequence of changes. Initially they are limited to the prostate, after that infiltrates the prostate capsule, involve the surrounding tissues and finally form metastases.

PIA consists of proliferative epithelial cells without the ability to differentiate into common secretory cells[2,4]. It is not yet clear that whether there is any significant correlation between PIA and prostatic adenocarcinoma, eventhough some relationship between PIA and cancer is observed.

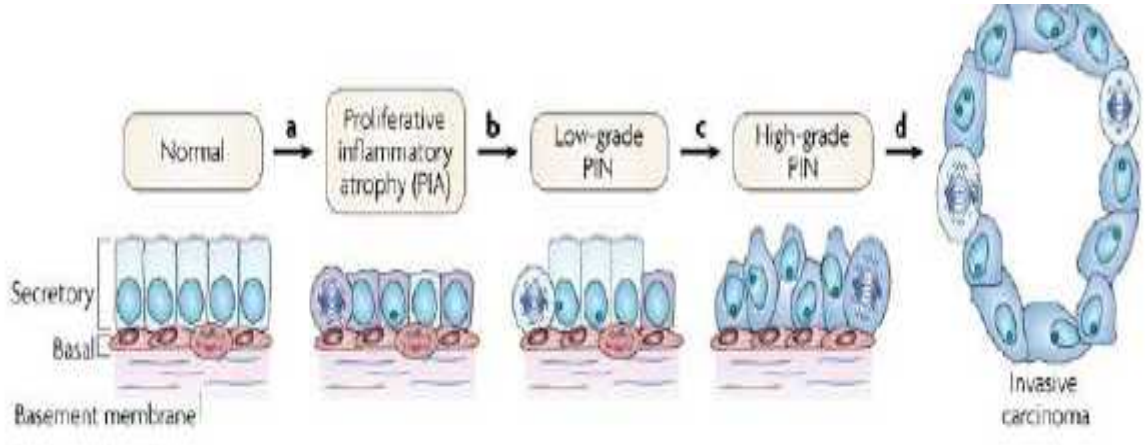


Figure: 4 Progression and Development of prostatic carcinoma[3]

PIA is more common in the peripheral zone[12,2,13]. PIA is often seen close to PIN or cancerous lesions[12,13,14]. PIA is associated with chronic inflammation, which is known to have a role in cancer development[15]. However, whether PIA is a premalignant lesion or not, remains to be further elucidated, as there are conflicting reports[16,17,18].

Risk factors

Age, family history and race are some of the definitive risk factors in the development of prostate cancer .

Clinically, carcinoma of the prostate is most often detected in men over 60 years of age and it is rare before 40 years of age.

The second most important risk factor is a family history of the patient. About 25% of men with prostate cancer have a known positive family history. The degree of risk is related to the age of the relatives at diagnosis and the number of relatives affected. The risk of developing PC is known to be hereditary, with a reported estimated risk of 27-42 % in

studies on monozygotic twins[19,20,21]. The hereditary pattern most likely follows an autosomal dominant inheritance[22]. It has been suggested that patients with familial PC have a worse prognosis than sporadic cases.

Race is a definite risk factor for prostate cancer incidence and detection. Variations in gene promoter hypermethylation may potentially cause racial differences in prostate cancer pathogenesis. Promoter regions of genes which are normally unmethylated become methylated in cancer cells which is caused by 5'- cytosine of the dinucleotide pair (CpG) being covalently bound by methyl group, resulting in silencing of the tumor suppressor and regulator gene expression.

Probable risk factors for development of prostate cancer include diet and steroid hormones. An interesting hypothesis exists about zinc and prostate cancer according to which uptake of zinc may be different in racial groups. This suggestion is based on the evidence that the normal prostate contains high amounts of free zinc ions that are secreted into the seminal fluid. In other words, the loss of ability to retain normal intracellular levels of zinc is an important factor in the development and progression of prostate cancer. It is also noted that the dietary zinc supplements are mostly nontoxic.

The strongest dietary link to prostate cancer development is high fat intake[23]. Dietary fat could increase PC risk via one or more mechanisms, such as lipid peroxidation leading to the production of DNA-damaging free

radicals and alteration of serum sex hormone levels. Increased dietary fats and calories leads to secretion of growth hormone and insulin causing Insulin growth factor synthesis that result in reduced cell apoptosis and increase in cellular proliferation.

In contrast, intake of fruits and vegetables, particularly tomatoes, may decrease the risk of development of prostate cancer[24]. Lycopene, a carotenoid antioxidant, is an agent in tomatoes that has been associated with this diminished risk[25].

Intake of soya bean containing isoflavones can be correlated with decreased risk of prostate cancer. The mechanism of action of these isoflavones shown in some experimental studies is by inhibiting tyrosine kinase enzymes, which play a part in cell proliferation and angiogenesis[26].

Selenium and vitamin E are other micronutrients being studied that have been shown to reduce the risk of prostate cancer[27].

Steroid hormones are considered to be an important risk factor in prostate carcinoma which includes sex hormones particularly testosterone and vitamin D. Increased serum vitamin D levels can lower the risk of development of prostate cancer especially in tumours with high grade[28].

DIAGNOSIS

1. Digital rectal examination

The initial examination of the prostate, in patients with urogenital symptoms, is often digital rectal examination(DRE). This is a simple diagnostic method with minimal complications[29]. Although the detection rate of tumors using DRE is not as high as for serum PSA measurements, a combination of the two methods has higher detection rate than each alone[30]. Tumors discovered through DRE are often more advanced than PSA-detected cancers[30].

2. Tissue biopsy

Tissue biopsy is necessary to find out Gleason score which is essential for choosing the appropriate treatment. Biopsy is performed on the basis of abnormal serum PSA or DRE[31]. Most studies show a higher detection rate with more number of biopsies[32]. Often the procedure is repeated at intervals if PSA levels indicate that cancer may be present although biopsies are negative. Known complications of transrectal biopsies include infection, bleeding, haematuria, haemospermia and urinary difficulties[33,34] . Recently, Trans rectal ultrasound (TRUS)-guided core biopsies have emerged as a method choice for PC diagnosis. Core biopsies can be combined with immunohistochemistry which increases the diagnostic accuracy and adds clinically important information on extent of disease and Gleason score.

Gleason Grading System

The Gleason grading system used for prostate cancer was developed in 1966 by Donald F.Gleason, whereby the cancer was graded based on the morphology of the tumour.

Gleason grading system includes 5 grades given to each primary and secondary histological pattern. Each grade describes a glandular pattern, with grade 1 being the best differentiated and grade 5 representing the worst or least differentiated pattern. The two predominant patterns are graded and added to obtain Gleason score. It has minimum score of 2and maximum score of 10, with 5-8 range being the commonest.

A study reported that low Gleason grade tumours were usually located in the transition zone of the prostate gland, and higher Gleason grade tumours were often found in the peripheral zone and associated with worse prognosis[35].

Gleason grade 1 tumours consist of circumscribed nodules of uniform, single glands which are closely packed. The glands in Gleason grades 1 and 2 are also larger than those of higher Gleason grades. Gleason grade 1 and 2 patterns are associated with cells with abundant and pale cytoplasm[36].

Gleason grade 2 tumours are rather well circumscribed but tend to infiltrate beyond the lobular margins into the nearby non-neoplastic gland. The glands are loosely arranged and less uniform that those in grade 1.

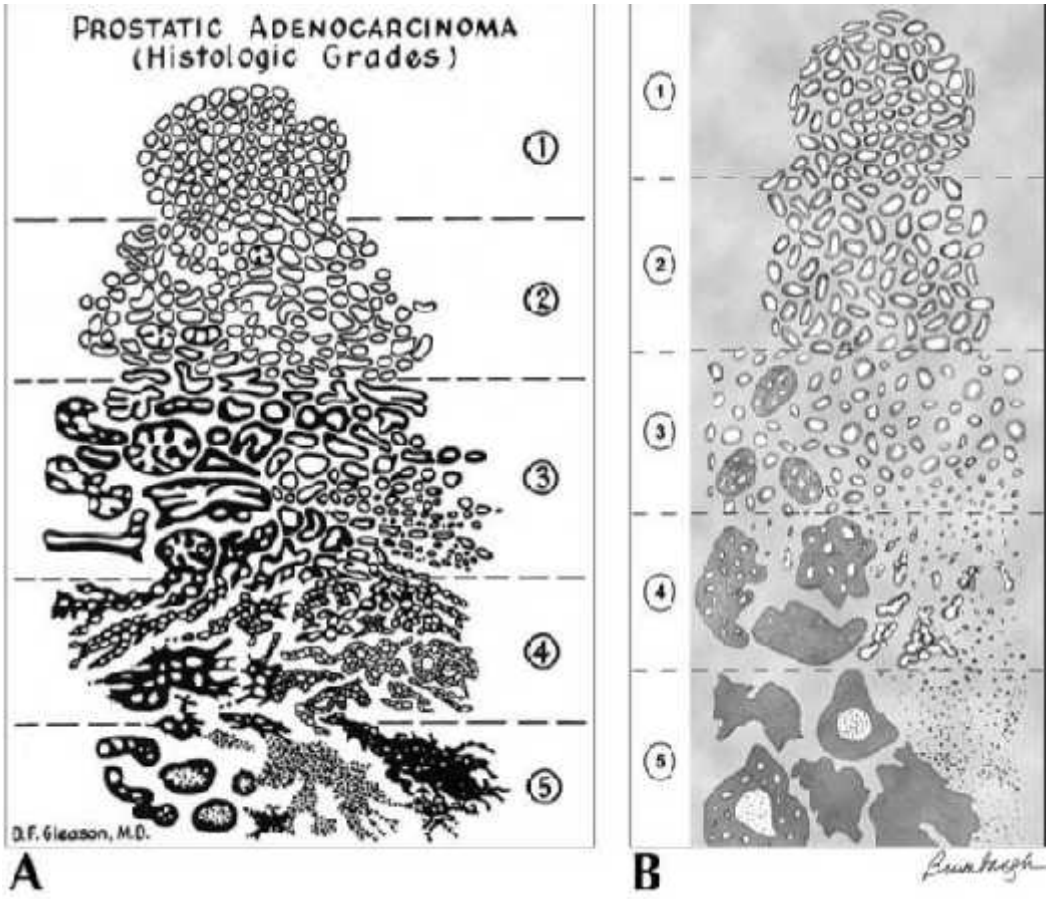


FIGURE 5-Gleason grading

Gleason grade 3 tumours infiltrate within non-neoplastic prostatic lobules. The sizes and shapes of the glands are more variable. The glands

can be large and cribriform, and are considered Gleason grade 3 as long as the glands are not coalescent and still maintain their rounded contours.

Gleason grade 4 glands, on the other hand, are coalescent and fused, with some abortive glandular profiles. Cribriform patterns can be seen but the contours are now irregular and the glandular outlines larger. These cells may have pale to clear cytoplasm.

Gleason grade 5 glands are made up of sheets, cords, single cells or solid nests. The glands have sparse or no lumina. Comedonecrosis is also seen in Gleason grade 5[37].

The critical importance of pathologic assessment of prostate cancer for treatment and prognostication calls for reproducibility, consistency and consensus on Gleason grading.

Prostate Specific Antigen (PSA) Screening and Pitfalls

For many years now, serum PSA has been the method of choice for screening the prostate cancer. And it has also been widely accepted that a serum PSA level of more than 4ng/ml is usually indicative of probable prostate cancer[38].

There is still controversy regarding the age to begin and stop PSA screening, the threshold value to consider biopsy, screening a population who are at higher risk to develop the cancer, and also other existing

diseases that might affect the PSA levels. Some of these issues remain unresolved. As such, overdiagnosis may occur, resulting in unnecessary aggressive treatment or invasive procedures, which could affect the patient's overall well being in addition to the possible financial and psychological burden.

It has also been mentioned that total PSA is not a "classic" tumour marker because an increase in level of PSA is not directly correlated with worse stages or grades[38]. As PSA is more prostate specific, rather than cancer specific, a mild to moderate increase can be commonly present in benign conditions like prostatitis and in benign prostatic hyperplasia (BPH).

In addition, a number of prostate cancers are present in patients with PSA values within normal range[39]. There have also been reports that PSA levels can decrease with increasing Gleason scores[38].

Serum PSA tests may also be used to monitor various treatments of prostate cancer in deciding whether post-therapy biopsies are needed. Elevated serum PSA levels following radical prostatectomy (0.2 ng/mL) indicate recurrent or persistent disease.

PREMALIGNANT LESIONS OF PROSTATE

Prostatic intraepithelial neoplasia is the most common precursor lesion of prostatic carcinoma. The only method of detection is biopsy. Prostatic intraepithelial neoplasia (PIN) does not significantly elevate serum prostate-specific antigen concentration and cannot be detected by ultrasonography.

As most cases of PIN progresses to adenocarcinoma, its presence needs repeated biopsy for diagnosing invasive carcinoma. PIN, patient age and serum PSA concentration were jointly highly significant predictors of cancer, with PIN providing the highest risk ratio. Carcinoma will develop in most patients with PIN within 10 years. PIN can be classified into low grade PIN and high grade PIN.

The incidence and extent of PIN appear to increase with patient age. In young men, mostly the PIN foci are low grade. With advancing age, the frequency of HGPIN increases. The PIN is found predominantly in the peripheral zone of the prostate (75%-80%), rarely in the transition zone (10%-15%), and extremely rare in the central zone (<5%).

PIN may involve part of the lumen of a duct or the entire unit. PIN is characterized by cellular proliferations within preexisting ducts and acini with cytologic changes mimicking cancer, including nuclear and nucleolar enlargement. At the onset, the epithelial proliferation is manifest

as increased cellularity and pseudostratification, but as the process progresses, intraluminal papillae may develop.

1. Low grade prostatic intraepithelial neoplasia(LGPIN)

Low grade prostatic intraepithelial neoplasia is quite difficult to recognize, as it has common features with normal and hyperplastic epithelium. It has the similar morphology as HGPIN, but most of the cells lacked prominent nucleoli. More prominent nucleoli, when observable, comprise less than 10% of dysplastic cells[40,41]. The basal cell layer normally surrounding secretory cells of ducts and acini remains intact[42,43]. The distinction between HGPIN and LGPIN is based primarily on the extent of cytological abnormalities (that is prominence of the nucleoli) and secondarily on the degree of architectural complexity[44,45]. Immunostaining studies of microvessel density may help to differentiate HGPIN from LGPIN[46].

2. High grade prostatic intraepithelial neoplasia(HGPIN)

Morphologically, there are four basic patterns of HGPIN: flat, tufting, micropapillary and cribriform[47]. These patterns often merge with each other. Other unusual patterns of PIN include the signet ring cell pattern, small cell neuroendocrine pattern, mucinous pattern, microvacuolated (foamy gland) pattern, and inverted (hobnail) pattern. The most common are the papillary and tufting patterns, less frequent is the

cribriform pattern. Other than diagnostic utility, these architectural patterns have no known clinical significance.

Several studies indicate that HGPIN is the most likely precursor lesion of PC[40,42,43,48] because of the similarities between them:

1 Age. The frequency of HGPIN and PC increase with age[49]

2 Coexistence. HGPIN often coexist with PC in the same samples[40].

3 HGPIN is predominantly located in the peripheral zone, the zone in which most clinically important prostate carcinomas are found[49].

4 Morphological similarities. HGPIN is characterized by cellular crowding and stratification. There is inequality in cell and nuclear size. Hyperchromatism is frequently seen with an enlarged nucleus, often containing prominent nucleoli. These changes are also seen in Gleason grade 1-4 PC[50].

5 Histologically, the atypia observed in HGPIN is virtually indistinguishable from that of PC except that in HGPIN the basal membrane is still intact[51]**.** As HGPIN progresses, the likelihood of basal cell layer disruption increases. In PC, there is complete loss of the basal cell layer.

6 Both in HGPIN and PC, collagenase type IV expression is increased. This enzyme is responsible for basal membrane degradation and thus facilitates invasion[52,53]**.**

7 Molecular and genetic similarities- Several genetic changes encountered in PC cells can be found in HGPIN[54]**.**

8 PC and HGPIN have similar proliferative and apoptotic indices[55].

Atypical Glands Suspicious for Malignancy

The term atypical small acinar proliferation (ASAP) is used to describe atypical glandular proliferations that do not reach the threshold to be diagnosed as cancer either qualitatively or quantitatively. ASAP is the most frequent indication found by immunohistochemistry in prostatic needle biopsies. This diagnosis does not imply a specific pathologic entity, but only implies that the glandular changes are suspicious for malignancy. Lesions that may be given such a diagnosis are a focus of small glandular proliferation with lack of prominent nucleoli, lack of nuclear enlargement, artefactual distortion, too few glands to be sure, depletion of tissue, inability to do basal cell stains, and so forth[56-59]. The frequency of this diagnosis in contemporary large prostate biopsy series is quite variable, ranging from less than 1% to 23%, with an average around 5%. In one study indicate that invasive carcinoma was identified in 48.9% of patients on repeat biopsy when the patient had a diagnosis of atypical glands suspicious for malignancy. Immunohistochemical staining with basal cell markers(34βE12 and p63) and AMACR is very useful.

PROSTATIC ADENOCARCINOMA

Carcinomas may arise in any zone of the prostate, but the distribution is different in each zone; 60% arise in the peripheral zone, 24% in the transtition zone and 8% in the central zone[60]. Adenocarcinomas rarely can arise from ectopic prostate tissue[61].

Adenocarcinoma is the most common malignancy of the prostate gland, accounts for more than 25% of all malignancies in men, and is the second leading cause of death after lung carcinomas[62]. The majority are multifocal (60-90%)[63] and exhibit an acinar or mixed acinar and ductal growth pattern.

The rule of "three toos" (too small glands, too crowded glands with back-to-back arrangement and too clear glands) is very useful in identifying prostatic carcinoma in biopsy and TURP specimens. To confirm the diagnosis of carcinoma, three diagnostic criteria including nuclear enlargement, prominent nucleoli and lack of basal cells should be present. In carcinoma, the size of the nucleoli is often at least 1 micron in diameter.

Several other features have been shown to be helpful for diagnosis of carcinoma - intraluminal crystalloids, blue mucin, glomerulations, mucinous fibroplasia (collagenous micronodules) and circumferential perineural invasion.

There are several benign conditions which mimic adenocarcinoma. In these situations, overexpression of AMACR by tumour cells and complete loss of basal cells which are evaluated by HMWCK(34βE12), CK5/6 and p63 immunohistochemistry provide confirmatory evidence for the diagnosis.

Variants of Prostatic adenocarcinoma

Variants of prostatic adenocarcinoma account for 5% to 10% of all adenocarcinomas. Recognition of these variants is important because many have a poorer prognosis than conventional acinar prostate adenocarcinoma.

1. Mucinous adenocarcinoma

The criteria for primary mucinous adenocarcinoma of the prostate are (a) at least 25% of the tumor should show aggregates of cells floating in lakes of extracellular mucin, (b) No signet ring component or significant intercellular mucin should be present, (c) extraprostatic primary tumor sites should be ruled out or tumor cells should be positive for PSA and Prostatic acid phosphatase (PAP) immunostaining. When adhering to these criteria, this is an extremely rare tumor. No pure mucinous adenocarcinoma of the prostate has been reported so far.

2. Signet Ring Cell Carcinoma of the Prostate

Primary signet ring cell carcinoma of the prostate is extremely rare. While Gleason grade 5 carcinomas can show single cells with signet ring morphology, signet ring cells should comprise more than 25% of neoplastic

cells to render the diagnosis of signet ring cell carcinoma. It is imperative that metastatic involvement from another site (i.e. stomach, bladder, colon, etc.) to be ruled out.

3. Ductal Adenocarcinoma

It is Previously known as “Endometrioid adenocarcinoma”

Histologically these tend to show two main patterns. One consists of true papillary fronds with well established fibrovascular cores lined by high columnar cells exhibiting a variable degree of cytologic atypia and prominent nucleoli. Second pattern consists of an intraductal proliferation of large, back to back glands imparting a somewhat cribriform appearance. In both cases the surrounding stroma often appears altered or fibrotic. The main differential diagnosis is high grade prostatic intraepithelial neoplasia (HGPIN).

4. **Pseudohyperplastic and foamy gland carcinoma**

These variants of acinar adenocarcinoma are best characterised by striking tendency to mimic benign processes such as adenosis or foci of crowded benign glands. So it is helpful to perform immunoperoxidase stains to confirm diagnosis.

Pseudohyperplastic carcinoma is composed of glands showing features commonly associated with benignity including large, branched glands with papillary infolding and even corpora amylacea. Clues which

identify the lesion as malignant are nuclear enlargement, macronucleoli, mitoses, intraluminal crystalloids, and sometimes the presence of adjacent PIN.

In contrast, foamy gland carcinoma often has a worrisome, infiltrative pattern with rather bland cytology. Foamy gland carcinomas are characterised by xanthomatous cytoplasm because of accumulation of lipids and small hyperchromatic nuclei with inconspicuous nucleoli. The behaviour of foamy gland carcinomas is often aggressive, even in the presence of deceptively innocuous microscopic features.

5. Prostatic adenocarcinoma with atrophic features

This variant mimics a benign hyperplastic change and composed of tumor cells with an attenuated cytoplasm and nuclei occupy almost the entire cell height. These cells are identifiable as malignant because of their infiltrative pattern of growth, nuclear enlargement, macronucleoli.

6. Small Cell Carcinoma of the Prostate

Small cell carcinoma arising primarily from prostate is very rare. It is a highly aggressive malignant tumour. Small cell carcinoma is composed of an infiltrate of small uniform cells showing nuclear molding, stippled chromatin, and inconspicuous nucleoli. Frequent mitotic figures are noted.

7. Sarcomatoid carcinoma

Sarcomatoid carcinoma of the prostate is another uncommon variant of adenocarcinoma with a biphasic appearance, containing carcinoma and a spindle or pleomorphic sarcomatoid component. Majority of patients have metastatic disease at the time of diagnosis, and 50% have a history of prostate cancer treated with radiation or hormonal therapy. Sarcomatoid carcinoma has poorer prognosis.

8.Lymphoepithelioma like carcinoma

Lymphoepithelioma like carcinoma is an extremely rare variant of prostate carcinoma. It shows a very close similarity with undifferentiated nasopharyngeal carcinoma, thats why it is called as"lymphoepithelioma." As such, the microscopic picture is that of nests, sheets, cords, or single malignant cells having large vesicular nuclei with prominent nucleoli in a background of dense lymphoid population (host reaction) which can sometimes obscure the carcinomatous nature of the lesion.

9. Squamous/adenosquamous carcinoma

The histology of prostatic squamous carcinoma is composed of infiltrating nests, strands and sheets of cells with cytologic atypism. The hallmark features of squamous differentiation includes: individual cell keratinization, intercellular bridges, and/or keratin pearl formation.

Adensquamous carcinomas most often show a transition between glandular and squamous differentiation. Other primary sites should be excluded.

10. Basal cell/adenoid cystic carcinoma

This neoplastic process resembles adenoid cystic carcinoma of the salivary gland. The key microscopic features are expansile pattern of growth, multinodularity, a cribriform architecture with luminal-basal lamina-like material, a surrounding fibromyxoid stroma, common occurrence of squamous differentiation, and merging with foci of basal cell hyperplasia. The differential diagnosis includes basal cell hyperplasia, acinar adenocarcinoma with cribriform pattern of growth, basaloid carcinoma and true adenoid cystic carcinoma.

Problems associated with diagnosis.

The most of prostate adenocarcinomas can be easily diagnosed; but difficult cases do exist. First is in the differentiation between well-differentiated adenocarcinoma from its many benign mimickers and atypical small-gland proliferation. Second is the threshold for identifying very small foci of tumor in needle biopsies.

Eventhough numerous criteria that have been validated for the diagnosis of prostate cancer in previous studies, it is still very difficult in applying such criteria on dealing with less number of glands with atypia.

Hence a meticulous systematic approach is necessary.

The process should involve evaluation of (a) architectural features; (b)cytologic features; (c) clues that may assist in the diagnosis,such as bluish acid mucin, crystalloids, collagenous micronodules, glomerulation, and circumferential perineural invasion, as mentioned before; and (4) presence of associated high-grade PIN. Caution is warranted if marked inflammation, budding from apparent benign glands, and artifacts of crushing or thick sections are present.

Benign mimickers of prostatic adenocarcinoma

Prostate cancer is the most frequent malignant and heterogeneous neoplasm in males[64]. Among its all subtypes, prostatic acinar adenocarcinoma (PAA) is the most common and mostly the cases are without any symptoms but show an elevated prostate-specific antigen (PSA) or with symptoms only when the patients have locally advanced or metastatic lesions[65]

Generally, it is easy to make a diagnosis of Prostatic acinar adenocarcinoma by its typical histological and cytological characteristics. However, sometimes the diagnosis becomes a challenge because numerous benign or malignant prostatic or non prostatic lesions and normal structures can be very similar to PAA, especially in a small piece of tissue from transrectal ultrasound (TRUS)-guided needle biopsy or transurethral resection of the prostate, which may lead to an erroneous diagnosis and

inappropriate treatment. So it is very important to be aware of the existence of these mimickers.

CLASSIFICATION OF BENIGN MIMICKERS OF ADENOCARCINOMA

1.Atrophy

Simple
Cystic
Post atrophic hyperplasia
Partial atrophy

2.Prostatic hyperplasia

Basal cell hyperplasia
Benign nodular hyperplasia-
(small gland pattern)
Clear cell cribriform hyperplasia
Sclerosing adenosis

3.Inflammation

Usual prostatitis with preservation artifacts

Nonspecific Granulomatous prostatitis

Xanthogranulomatous Prostatitis(xanthoma)

Malakoplakia

4. Atypical Adenomatous Hyperplasia (Adenosis)

5.Reactive atypia

Inflammatory
Ischemic
Radiation

6.Metaplasia

Mucinous
Nephrogenic adenoma

7.Histoanatomic structures

Seminal vesicle
Cowper's gland
Paraganglion
Verumontanum gland (hyperplasia)
Mesonephric gland remanants (hyperplasia)

Benign mimickers of gleason score2-6 prostatic adenocarcinoma

Atrophy, Adenosis, Basal cell hyperplasia, Radiation atypia, Nephrogenic adenoma, Seminal vesicle, Cowper glands,Verumontanum hyperplasia and colonic mucosa are considered as benign mimickers of prostatic adenocarcinoma with gleason score of 2-6.

Benign mimickers of gleason score 7-10 prostatic adenocarcinoma

Nonspecific granulomatous prostatitis, Clear cell cribriform hyperplasia, Sclerosing adenosis, xanthoma, Paraganglia and Signet ring cell lymphocyte are considered as benign mimickers of prostatic adenocarcinoma with gleason score of 7-10.

I.Prostatic Atrophy

Prostatic atrophy is a common lesion involving the elderly population. Amongst the mimickers, atrophy and partial atrophy are commonly misdiagnosed as Prostatic carcinoma. So far the etiology of atrophy is largely unknown. It is supposed that prostatic atrophy is caused by age-related physiological changes, nonspecific inflammation, nutrient deficiency, local compression and anemia, hormonal or radiotherapy action, and so on. It is usually located in the peripheral zone but may be present in the transition and central zones as well.

Microscopically, atrophy can be diffuse or focal. Diffuse atrophy is typically seen in patients with androgen deprivation therapy. In contrast, focal atrophy is often sporadic, is sometimes associated with inflammation, and occurs as heterogeneous patches.

Prostatic atrophy is divided into four categories: simple atrophy, cystic atrophy, post-atrophic hyperplasia (PAH) and partial atrophy[66]. Simple or cystic atrophy and PAH usually involve an entire lobule.

The size and shape of cells are similar to normal cells, except parts of cells in cystic and dilated glands are flat. PAH is characterized by a coexistence of atrophic and hyperplastic glands[67]. Its typical features are a pile of tightly arranged acini budded from the small atrophic glands. Commonly, PAH does not show local infiltration or confluent glands.

Microscopically, partial atrophy may be mistaken as PAA. In contrast to above mentioned atrophic subtypes, partial atrophy shows lobular or diffused pattern of growth. The cells usually have relatively scant cytoplasm and irregularly crinkled nuclei without basophilic appearance at low magnification. Moreover, in about half of the cases, basal cells are very difficult to be noted or even absent. Apart from, it is necessary to note that whether the basal cell layer is intact in the atrophic glands using IHC markers as those basal cells show positivity for high molecular weight cytokeratin (HMWCK- 34βE12), CK5/6 and p63 marker.

In cases with partial atrophy the glands may show AMACR expression, similar to PAA and HGPIN[68].

II. Prostatic Hyperplastic Lesions

1.Benign prostatic hyperplasia (BPH)

Benign prostatic hyperplasia is a very common benign lesion with increasing volume of the prostate. It mainly locates in the transitional and periurethral zone. BPH commonly presents as large and discrete nodules caused by hyperplasia of both glandular and stromal components[69] .

Histologically, the glands vary from small and crowded glands to large glands with cystic dilatation and exhibit complicated growth pattern, including papillary infoldings and branching structure[70]. It is noted that medium to large sized glands present in BPH may mimic pseudohyperplastic adenocarcinoma, which is a variant of PAA, especially in the tissues obtained from needle core biopsy. However, the tumor cells of adenocarcinoma usually have malignant nuclear features and the neoplastic glands lack basal cell layer.

2.Basal Cell Hyperplasia

Basal cell hyperplasia (BCH) of the prostate gland is a relatively common lesion in hyperplastic prostates being examined in TURP specimens. BCH occurs in about 23% of whole prostatic tissues and10% of peripheral zone by needle core biopsy[71]. BCH usually coexists with BPH and presents as wellcircumscribed lobules with smooth borders

Conventional BCH show the features that glands with basal cells that may be multilayered or forming solid nests of basaloid cells. The basal cells are showing basophilic cytoplasm and bland nuclei without any nucleoli or features of pleomorphism. We can easily differentiate this condition from adenocarcinoma. But the unusual subtypes of this condition, florid BCH and atypical BCH may simulate PAA.

Microscopically florid BCH shows vast production of basal cells affecting greater than hundred small packed acini and create a nodule formation. Atypical BCH is characterised by increased generation of basal cells which are having nuclei with conspicuous nucleoli. In both these conditions we can appreciate the features of atypical nuclei, secretions in the lumina, hyaline globules within the cytoplasm, even very few mitosis[72,73]. Infrequently, the packed glands may exhibit features of infiltrative growth pattern.

Inspite of all this, the following features such as multilayered cells or solid nests of basal cells, calcifications, and cellular fibrous stroma may be helpful to find out this condition. It is evident that IHC staining may be useful adjunct to confirm this hyperplastic basal cells and exhibits negative expression of AMACR, PSA and prostatic specific acid phosphatase (PSAP) markers[73,74].

3.Clear Cell Cribriform Hyperplasia

Clear cell cribriform hyperplasia (CCCH) of the prostate is a rare form of BPH. It consists of enlarged glands filled with anastomosing clear cells, which form a cribriform growth pattern. The cells comprising the central cribriform areas are cuboidal to low columnar secretory-type cells with uniform round nuclei and clear cytoplasm. They lack nuclear atypia and nucleolar enlargement. Basal cells are prominently displayed around the periphery. CCCH may become a pitfall for high-grade PAA with cribriform pattern. However, the nodular proliferation, bland cytology, cellular fibrous stroma, and the intact basal cell layer can be helpful in the diagnosis of CCCH[75].

4. Sclerosing Adenosis

Sclerosing adenosis is a rare lesion characterized by variable sized or shaped glands disorderly embed into prominent sclerotic stroma. Sclerosing adenosis of prostate is very similar to that of breast. It is of both practical and academic importance to recognize sclerosing adenosis, because of its remarkable resemblance to adenocarcinoma on histologic examination and its myoepithelial differentiation. Sclerosingadenosis is not a premalignant condition and is localized to the transition zone.

It is a benign condition caused by hyperplasia of both glandular and stromal components. The lesion presents as nodular with well defined boundary, but without capsule[76].There are both clear secretory cells and

amphophilic basal cells in the hyperplastic glands. The cells may have conspicuous nucleoli and intraluminal acid mucin. Sclerosing adenosis should be differentiated from small acinar adenocarcinoma.

III. Inflammation

1.Ordinary prostatitis

Occasionally, needle biopsies with prostatitis of the usual type may cause diagnostic problems[77-79]. This is especially true when there is poor preservation and mechanical (crush) artifacts. In few cases, immunohistochemical stains (such as keratins, leukocyte common antigens) are needed for resolving the differential diagnosis.

2.Non-specific granulomatous prostatitis

Granulomatous prostatitis commonly results in a prostate gland that feels firm to hard and clinically simulates carcinoma. In biopsy samples, especially needle biopsies, florid nonspecific granulomatous prostatitis may simulate carcinoma[80,81].

The association of the inflammation with ducts may be absent and when the inflammatory process is diffuse, high-grade (Gleason 5) carcinoma needs to be considered. The problem is amplified if poor preservation or mechanical artifacts are present. The recognition of the inflammatory nature of the cells along with the association of giant cells and fibrosis are helpful features. In diagnostically difficult cases,

immunohistochemical staining with cytokeratins, prostatic epithelial markers and lymphohistiocytic markers may be helpful.

3. Xanthogranulomatous prostatitis (xanthoma)

Collections of lipid-laden macrophages in the prostate may cause diagnostic confusion with the hypernephroid pattern of adenocarcinoma (Gleason4)[82,83]. Xanthomatous histiocytes have small uniform nuclei with inconspicuous nucleoli. They are frequently admixed with other type of inflammatory cells. In few situations, we can appreciate distinct population of foam cells which can create confusion in diagnosis. The problem is compounded by the fact that some hypernephroid carcinomas do not show the typical nuclear features of malignancy. They may have small dark nuclei without prominent nucleoli. In certain cases, to resolve the diagnostic dielemma, immunohistochemistry using stains for epithelial and prostatic cells, and histiocytes may be helpful.

4.Malakoplakia

Malakoplakia of the prostate is a rare infiltrative lesion characterized by diffuse sheets of histiocytes, usually admixed with other inflammatory cells including lymphocytes, plasma cells and neutrophils. When von Hansemann histiocytes predominates during the early stage of malakoplakia, this can simulate carcinoma particularly of Gleason pattern4. The lack of any acinar differentiation and admixed inflammatory infiltrate

along with the typical Michaelis–Gutmann bodies will lead to a correct diagnosis. Loss of expression of cytokeratins and prostatic epithelial markers and positive staining of CD68 may be helpful indifficult diagnostic situations.

IV. Acinar Proliferations of the Transition Zone(Adenosis)

Atypical Adenomatous Hyperplasia (Adenosis) AAH, or adenosis of the prostate, is difficult to distinguish from well-differentiated adenocarcinoma of the prostate. It belongs to a benign lesion with the proliferation of small acini. The incidence of AAH varies from 1.5% to 19.6% in transurethral resections of prostate and radical prostatectomies. AAH is easily misdiagnosed as PAA, particularly in a TRUS-guided biopsy of the prostate.

AAH is a well circumscribed lesion with lobular appearance.The glands are small, round, and densely packed. Some of them merge into larger and complex acini. It may have an expansile or minimally infiltrative margin, crystalloids, crowded and disorderly glands, and medium-sized nucleoli, which resemble to low-grade PAA.

There are no macro nucleoli (>3 μm), blue-tinged mucin and straight luminal borders in AAH, which is the features of PAA. AAH can focally express (10% of cases), even diffusely express (7.5% of cases) AMACR[76] , it is therefore important to identify that the basal cell layer is preserved, although basal cells usually are discontinuous or focally present

in AAH. The results implies it to be a mimicker of adenocarcinoma and must be considered an important risk factor.

V. Reactive atypia

Medium to large glands may display reactive atypia in the setting of inflammation, ischemia and radiation. Such processes may lead to glandular distortion and nuclear atypia which sometimes results in a pattern that may be confused with prostatic intraepithelial neoplasia (PIN) and large gland patterns of adenocarcinoma.

The important points to differentiate reactive atypia from malignant condition is the identification of following features that includes inflammation, infarction, and intact basal cell layer. The atypia associated with reactive conditions may result in nuclei that appear hyperchromatic and somewhat degenerate. In some cases, the nucleolar enlargement associated with the reactive state may be more prominent and more uniform than that seen with adenocarcinoma. Presence of a residual basal cell layer sometimes requiring confirmation with the 34βE12 stain are the best clues to the benign nature of this condition.

VI. Prostatic metaplasic lesions

1.Mucinous metaplasia

Mucinous metaplasia usually occurs in the peripheral zone closed to normal glands of the prostate. The glands that are lined by mucin abundant tall columnar cells and small basal nuclei. It is necessary to find out

mucinous metaplasia in order not to confuse it with intraluminal mucin which is often identified with PAA[85]. The metaplastic cells are positive for mucin staining (PAS, mucicarmine, and Alcian blue), and IHC staining of PSA and PSAP.

2. Nephrogenic Adenoma

Nephrogenic adenoma is a benign lesion of the urothelial lined organs from the renal pelvis to the urethra. Nephrogenic adenoma may be observed in TURP specimens as urethral mucosa, and suburethral tissue may be sampled during the TURP procedure. When nephrogenic adenoma is present in the prostatic urethra and involves suburethral tissue and seemingly infiltrates the prostatic parenchyma proper, it may potentially be confused with a prostatic adenocarcinoma since proliferation of closely packed small glands is the common histologic manifestation in this condition.

3. Paneth cell-like metaplasia

Paneth cell-like metaplasia often represents a series of differentiation, such as neuroendocrine differentiation, exocrine differentiation, or intestinal Paneth cell differentiation. It is frequently associated with HGPIN and PAA, particularly in the patients who underwent radiotherapy or hormone therapy. It has lately been found that AMACR may be strongly positive in benign prostatic acini with Paneth

cell-like change[86]. To a certain extent, it is necessary to pay attention to avoid misdiagnosis.

VII. Normal histoanatomic structures and non-neoplastic lesions that may simulate adenocarcinoma of the prostate

1.Ejaculatory Ducts and Seminal Vesicle Tissue

Tissue fragments derived from seminal vesicles and ejaculatory ducts are occasionally observed during examination of TURP or prostatic needle biopsy specimens.

Epithelium of the ejaculatory duct and seminal vesicle contains coarsely granular, yellow-brown lipofuscin pigment which is more characteristic. It must be remembered, that prostatic adenocarcinoma also rarely contains intracytoplasmic lipofuscin pigment which is finer and less refractile when compared to seminal vesicle pigment.

The epithelial cells in the seminal vesicle and ejaculatory duct often have large atypical, hyperchromatic nuclei; These structures often show small glandular structures arranged in a back-to-back pattern, and therefore they may be confused with a small acinar carcinoma.

2. Cowper gland and paraganglionic tissue

Tissue from bulbourethral Cowper glands may occasionally present in TURP or needle biopsy specimens from the apex. Cowper gland may be confused with a well-differentiated adenocarcinoma of the prostate; however, the superficial similarity of Cowper gland to salivary glands, the

bland nuclear features, loss of prominent nucleoli, increased intracytoplasmic mucin, and negative immunoreactivity for PSA and PAP suggests the correct diagnosis.

3.Hyperplasia of mesonephric remnants

Hyperplasia of mesonephric remnants is a rare yet small glandular proliferation within the prostate gland, the severity of the diagnostic pitfall being exemplified by a case misdiagnosed as cancer that resulted in a radical prostatectomy that did not show evidence of cancer.

Microscopically, it is characterized by a lobular proliferation of small tubular structures lined by a single layer of epithelium or by infiltrating glands between muscle bundles and prostatic acini without a stromal desmoplastic response. Variation in the size of the tubules is seen, with occasional cyst formation, intratubular papillary proliferation, and eosinophilic secretions within dilated glands. The acini are lobular, but can be infiltrative and may be architecturally mistaken for adenocarcinoma.

4.Verumontanum Mucosal Gland Hyperplasia

Verumonatum mucosal gland hyperplasia (VMGH) is a small-gland proliferation in the verumontanum,misdiagnosed as carcinoma.

VMGH has an expansile circumscribed growth pattern with glands of small caliber arranged in a back-to-back fashion. Distinction from cancer is usually not a problem when attention is paid to the cytologic features at higher power. The glands have a layer of basal cells and

corpora amylacea or orange-brown secretions, and the nuclei lack nuclear enlargement or conspicuous nucleoli.

ROLE OF IMMUNOHISTOCHEMISTRY IN PROSTATIC LESIONS

The pathological process which affect prostate gland with sufficient frequency are inflammation, benign nodular hyperplasia & tumours. Prostatic lesions on routine hematoxylin & Eosin staining sometimes cause diagnostic dilemma between benign and malignant lesions and especially in premalignant lesions like atypical adenomatous hyperplasia and prostatic intraepithelial neoplasia. There are number of benign small acinar lesions in the prostate gland that may be difficult to differentiate from small acinar adenocarcinoma. An important diagnostic criterion in the differentiation is the loss of basal cell layer in adenocarcinoma and its presence in the benign lesions. Several immunohistochemical stains have been used to stain basal cells of prostate against their markers eg. high molecular weight cytokeratin(34βE12), p63.

I. Basal cell –associated markers

Basal cell associated markers highlight basal cells present in benign prostate glands and related benign, but architecturely atypical, proliferations. Under hematoxylin & eosin staining, basal cells may be

mimicked by prostatic stromal cells juxtaposed to the glandular basement membrane, or by endothelial cells of blood vessels closely situated to acini,and by tangentially sectioned neoplastic cells. Generally employed basal cell markers are HMWCK (34βE12) and p63.

1. High molecular weight cytokeratin

High molecular weight cytokeratin (34βE12) is a cytoplasmic marker that highlights intermediate cytokeratin filaments in glandular basal cells and is specific for basal cells in prostate. It is also known CK903 and targets CK1, CK2, CK10 and CK14[87]. There are some disadvantages of 34βE12 includes that (a) long term formalin fixation and formalin fixation interval may affect its antigenicity. (b) Antigen retrieval methods may affect its expression. (c) In Kalantari et al study, reported that the sensitivity of 34βE12 is lower than p63 for differentiation of benign lesions from adenocarcinoma[88]. Staining with HMWCK may vary between glands of a benign glandular proliferation and the staining pattern may not be cicumferential. So it has been used in combination with prostate cancer specific marker α-methylacyl coenzyme A racemase or with other basal cell associated markers rather than used alone.

2. p63

P63 is a basal cell marker and targets the p63 nuclear protein, which is homologous to the TP53 tumour suppressor gene. It has been proven

that it selectively stains the basal cell nuclei. It has been proven to regulate growth and development in epithelium of the skin, cervix, breast and urogenital tract as well as prostate.

P63 has similar applications to those of high molecular weight cytokeratins in the diagnosis of prostatic adeno-carcinoma, but with certain advantages. The advantages are (a) It stains a subset of 34βE12 negative basal cells, (b) Less susceptible to the staining variability of 34βE12 (particularly in transurethral resection of prostate (TURP) specimens with cautery artifact), and (c) It is easier to interpret because of its strong nuclear staining intensity and low background. It seems to be more sensitive in basal cell detection than 34βE12. However, false negative p63 stainings can occur and sometimes basal cells could be absent in small foci due to the cutting procedure. Aberrant P63 expression may also be noted in some prostatic adeno-carcinomas with unusual features, most representing entrapped benign glands or intraductal spread of carcinoma with residual basal cells.

3. CK5/6 basal cell marker

Another HMWCK is CK 5/6. It was reported to be a very sensitive and specific marker of prostatic basal cells, with fewer unsatisfactory results. It is normally expressed by complex epithelium and a marker of mesothelial cells and malignant mesothelioma as well as pancreatic, bile tract and

mammary carcinomas. It reacts with prostatic basal cells not with tumour cells or HGPINs. It is more effective than 34βE12 for ambiguous lesions.

Basal cell associated marker cocktails

Eventhough the use of single basal cell marker is sufficient for diagnosing morphologically difficult prostatic lesions, p63 and HMWCK 34βE12 or CK5/6 cocktail provide intense positivity and highlight both nuclei and cytoplasm[89].

II. Epithelial markers

1.Cancer specific marker-AMACR

AMACR, an α-methylacyl coenzyme A racemase is also known as P504S and it is involved in β-oxidation of branched-chain fatty acids and fatty acid derivates. It is located in mitochondria and peroxisomes. AMACR is generally expressed in the cytoplasm of cancer epithelial cells and is normally negative in benign tissue. However it can be expressed in HGPIN as well as in benign lesions such as atrophy and adenosis. AMACR reactivity can also be seen in secondary tumours involving the prostate such as urothelial carcinoma and colonic adenocarcinoma. AMACR expression may be negative in 5 to 25% of prostate carcinomas[90]. In some variants of adenocarcinoma such as atrophic carcinoma, foamy gland carcinoma and pseudohyperplastic carcinoma, AMACR expression can be

negative[91]. So positive AMACR staining doesnot always indicate carcinoma and negative staining does not rule out carcinoma.

Currently, AMACR is used as an additional IHC marker in combination with p63 and high molecular-weight cytokeratins for the diagnosis of prostate cancer. Interestingly, observed association of decreased AMACR expression in localized PC with the worse disease outcome and PSA recurrence, suggesting its possible use as a marker of prognosis.

Treatment options and prognosis of prostatic adenocarcinomas and benign lesions differ significantly, so they must be diagnosed with accuracy. This requires application of immunohistochemical stains for basal cells especially in morphological ambiguous cases (p63 shows nuclear staining in basal cells of benign prostate lesions and no staining in prostatic adenocarcinoma). A double immunohistochemical staining with combination of p63 and AMACR has a very important diagnostic utility.

2.Cytokeratins

The cocktail of AE1 and AE3 detects both acidic (CK14-16 and CK 19) and basic (CK1-6 and CK8) cytokeratins. It is the most commonly and universally used epithelial marker. It is useful in differentiating nonspecific granulomatous prostatitis, crushed or marked inflammation and xanthoma cells from high grade prostate cancer. In post therapy cases, CK AE1/AE3

can be used in highlighting atrophic prostate cancer cells since it is not suppressed by therapy.

III. Prostate lineage –specific markers

PSA and prostate-specific acid phosphatase (PSAP) are used to confirm a prostatic acinar cell origin. It is useful (1) To rule out non prostatic carcinoma mimics such as seminal vesicle/ejaculatory duct, cowper gland, hyperplastic mesonephric glands, nephrogenic adenoma and paraganglionic tissue. (2) To differentiate unusual variants of prostatic carcinoma such as ductal, mucinous and signet ring carcinoma (which show positive staining for PSA and PSAP) from secondary tumours involving the prostate (which are negative for these markers).

Antibody cocktails

Recently, the cocktails combining basal cell associated markers and AMACR are used in diagnosing morphologically difficult cases. The antibody cocktails used are (a) AMACR , p63 and HMWCK 34βE12. (b) AMACR/P63/CK5/6. (c) AMACR/p63. The triple or PIN cocktail combines AMACR, p63 and HMWCK using 2 chromogens, red for AMACR and brown for HMWCK and p63. This 3-antibody, 2- chromogen cocktail has been considered as a simple and easy assay for routine use. It has many advantages which include greater sensitivity for basal cells, easier evaluation of atypical acini (due to different colored chromogen) and

minimizing the potential loss of representation from evaluating a single slide[92,93]. The PIN 4 cocktail which has the combination of AMACR/HMWCK(CK5 and CK14) and p63, may be useful in diagnosing prostatic intraepithelial neoplasia (PIN), especially in morphologically difficult and limited tissue cases. P504S stains cytoplasm in prostatic adenocarcinoma and atypical adenomatous hyperplasia whereas p63 and HMW CKs stain normal and benign prostate glands.

MATERIALS AND METHODS

Present study is a retrospective study to determine the effectiveness of Immunohistochemistry in differentiating prostatic adenocarcinoma from premalignant lesions and benign mimickers of prostatic adenocarcinoma. The study was carried out in the department of pathology, Tirunelveli medical college over a period of 2015-2017. Study material includes about 54 cases of prostatic lesions diagnosed in TURP specimens and prostatic needle biopsies were collected. Since it is a retrospective study, blocks and slides of prostatic lesions from 2013 to 2017 were collected.

Inclusion criteria

1 Cases that were diagnosed as prostatic adenocarcinoma, premalignant lesions of prostate (High grade intraepithelial neoplasia, Low grade intraepithelial neoplasia), benign mimickers of prostatic adenocarcinoma (includes atrophy, adenosis, basal cell hyperplasia, chronic prostatitis) in TURP specimens and prostatic needle biopsies.

2 Cases which had suspicious atypical foci or prostatic intraepithelial neoplasia.

Exclusion criteria

1. Inadequate biopsy tissue sample
2. Poorly processed material
3. Autolysed specimen

Materials Required

1 Blocks which contains formalin fixed paraffin embedded tissue of TURP and prostatic needle biopsy specimens, which were diagnosed as prostatic adenocarcinoma, premalignant lesions of prostate (High grade intraepithelial neoplasia, Low grade intraepithelial neoplasia), benign mimickers of prostatic adenocarcinoma(includes atrophy, adenosis, basal cell hyperplasia, chronic prostatitis)

2 Hematoxylin and eosin stained tissue sections made from the blocks.

3 Postively charged slides for holding tissue sections for IHC

4 Chemicals for preparing antigen retrieval solutions and for wash buffers

5 Microoven for antigen retrieval.

6 Kit for performing immunohistochemistry which includes primary antibody (P63 and AMACR) and universal kit

7 Microscope used for making gleason grading of prostatic adenocarcinoma in hematoxylin and eosin stained slides and grading of IHC slides.

METHODOLOGY

I.Collection of donor blocks and slides

The haematoxylin and eosin stained sections which were prepared from formalin fixed paraffin embedded blocks of prostatic lesions diagnosed in TURP and prostatic needle biopsies are collected. The following cases are selected

1 Slides which contain prostatic adenocarcinoma, premalignant lesions of Prostate (High grade intraepithelial neoplasia, Low grade intraepithelial neoplasia), benign mimickers of prostatic adenocarcinoma(includes atrophy, adenosis, basal cell hyperplasia, chronic prostatitis)

2 Slides which contain suspicious atypical foci and features of prostatic intraepithelial neoplasia.

II.Preparation of haematoxylin and eosin slides

All the TURP and prostatic needle biopsy specimens were fixed in 10% formalin and were subjected to histopathological examination. Formalin fixed paraffin embedded blocks were made. Sections of 2-4 micron thickness were made and routine staining with hematoxylin and eosin was done.

Cases were selected after examining the slides. Gleason grading was done for all prostatic adenocarcinoma cases according to the histological pattern. Immunohistochemitry using basal cell specific markers p63 and prostate

cancer specific marker AMACR was done for all 54 cases including Prostatic adenocarcinoma, Prostatic intraepithelial neoplasia, benign mimickers of prostatic adenocarcinoma. Statistical analysis was done to rule out expression of p63 and AMACR.

III.Immunohistochemistry

1.Section cutting

Sections were taken at 5 microns thickness on the surface of the APES (3-aminopropyltriethoxysilane) coated slides. This was followed by incubation of slides at 58-60^{0}c for one hour.

2.Antigen retrieval solution

We used antigen retrieval solution and a wash buffer as prescribed by the manufacturer (PATH INSITU).

1 Tris EDTA at a pH of 9 .

2 Tris wash buffer at pH of 7.6.

3.Antigen retrieval

Many methods have been used for antigen retrieval which includes Microwave method, and water bath, autoclave, proteolytic enzyme and pressure cooker method. In our institution we followed antigen retrieval by using microwave method as it produces even heating and less time consuming with lesser disadvantages as compared to other methods.

4. Procedure for immunohistochemistry as given by manufacturer

1 Section cutting and incubation is followed by Xylene wash (3 changes) for 10minutes each.

2 Rehydrated in graded alchohol containing 100%, 80%, 70% for five minutes each.

3 Rinsed in distilled water for 2minutes.

4 Antigen retrieval for 15-20 minutes in Tris-EDTA buffer.

5 Cooling for 15minutes.

6 Washed in TBS wash buffer- 3 changes 5minutes each.

7 Treated with endogeneous peroxide block for 7-10minutes.

8 Washed in TBS wash buffer- 3 changes 10minutes each.

9 Application of primary antibody (p63/AMACR) – 30 mins.

10 Washed in TBS wash buffer- 3 changes 10minutes each.

11 Add Target binder for 15 mins

12 Washed in TBS wash buffer- 3 changes 10minutes each

13 Application of HRP POLYMERASE for 15 mins.

14 Washed in TBS wash buffer- 3 changes 10minutes each.

15 Application of Diamino-benzidine tetrachloride(DAB) chromogen (1 drop)and DAB buffer (1ml) for 5 mins.

16 Washed in distilled water – 2 changes.

17 Counterstaining with Harris Hematoxylin – 1dip/30seconds to impart

background staining.

18 Wash in running tap water.

19 Place in xylene – 2 changes 5 minutes each.

20 Dehydrate in 100% alcohol – 5 minutes.

21 Mount the section with Dextrene phthalate xylene

22 Observation and grading under light microscope.

5. Grading of IHC stained sections:

After immunohistochemistry using p63 and AMACR was done, the slides were examined under all the magnification with the help of light microscopy and grading was done.

Interpretation of p63 immunostaining

Immunostaining of p63 was interpreted as positive/negative. Positive staining was graded as mild, moderate and strong according to percentage of basal cells showing nuclear positivity. Positive staining was defined as positive staining of nuclei of basal cells. Positive staining in the foci in question, was taken as benignity and negative staining of an entire suspicious focus was taken as presumptive evidence of malignancy. Pattern of positive staining considered as continuous or discontinuous was also recorded to see the difference in various non-malignant conditions.

Table 1: Grading of p63 staining[88]

Percentage of cells with positivity	Result	Grading
0%	Negative	Negative
<5%	Mild	1+
5-75%	Moderate	2+
>75%	Strong	3+

Interpretation of AMACR staining

AMACR staining were considered positive, in case of circumferential, dark, diffuse or granular, cytoplasmic or luminal staining. The percentage positivity was graded from 0 to 3+. IHC results were negative if there was an absence of staining or if only focal weak non-circumferential fine granular staining was seen with absence of staining in the adjacent benign glands.

Table 2 : Grading of AMACR staining[94]

Percentage of positivity	**Result**	**Grading**
0%	Negative	Negative
1-10%	Mild	1+
11-50%	Moderate	2+
$\geq$ 51%	Strong	3+

OBSERVATION AND RESULTS

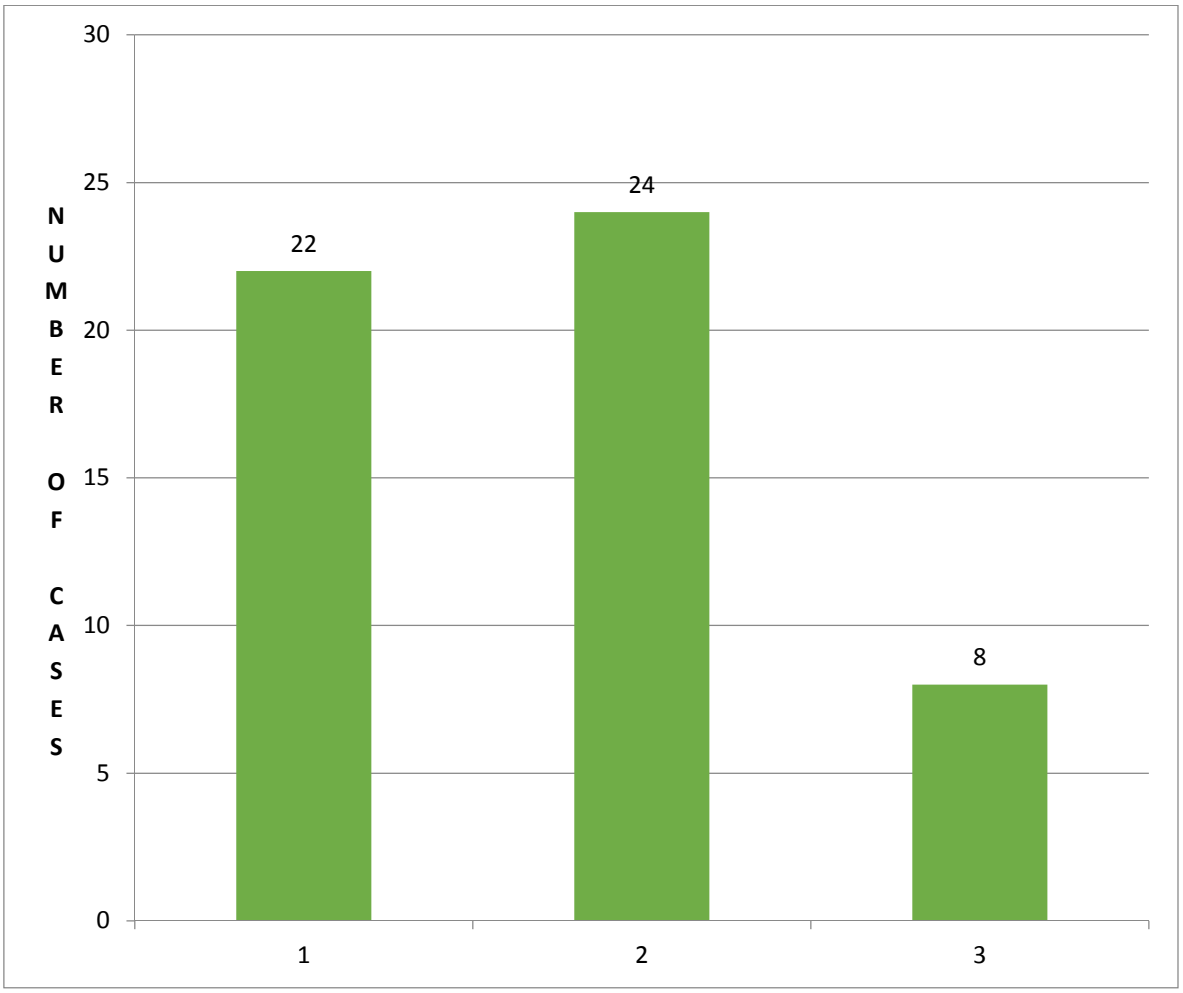
In this study there were total of 54 cases, out of these 45 were TURP specimens and 9 were prostatic needle biopsies . Out of 54 cases, 24 cases were prostatic adenocarcinoma , 19 cases were low grade PIN, 3 cases were HGPIN, 2 cases were basal cell hyperplasia, 2 cases were atrophy, 2 cases were adenosis and 2 cases were chronic prostatitis.

Table-3 Distribution of samples based on histopathological diagnosis

Histopath diagnosis	Frequency	Percent
Adenocarcinoma	24	44.44%
Prostatic intraepithelial neoplasia	22	40.74%
Cancer mimickers	8	14.81%
Total	54	100.00%

This table shows that out of 54 cases, 24 cases are adenocarcinoma, 22 cases are prostatic intraepithelial neoplasia (19 Low grade PIN and 3 High grade PIN) and 8 cases are benign mimickers of adenocarcinoma (includes 2 cases of basal cell hyperplasia, 2 cases of atrophy, 2 cases of adenosis, 2 cases of chronic prostatitis).

Chart 1- Distribution of samples based on histopathological diagnosis



In this chart all 54 cases are divided into 3 categories.

(1) indicates prostatic intraepithelial neoplasia cases which are about 22.

(2) indicates adenocarcinoma cases which are about 24.

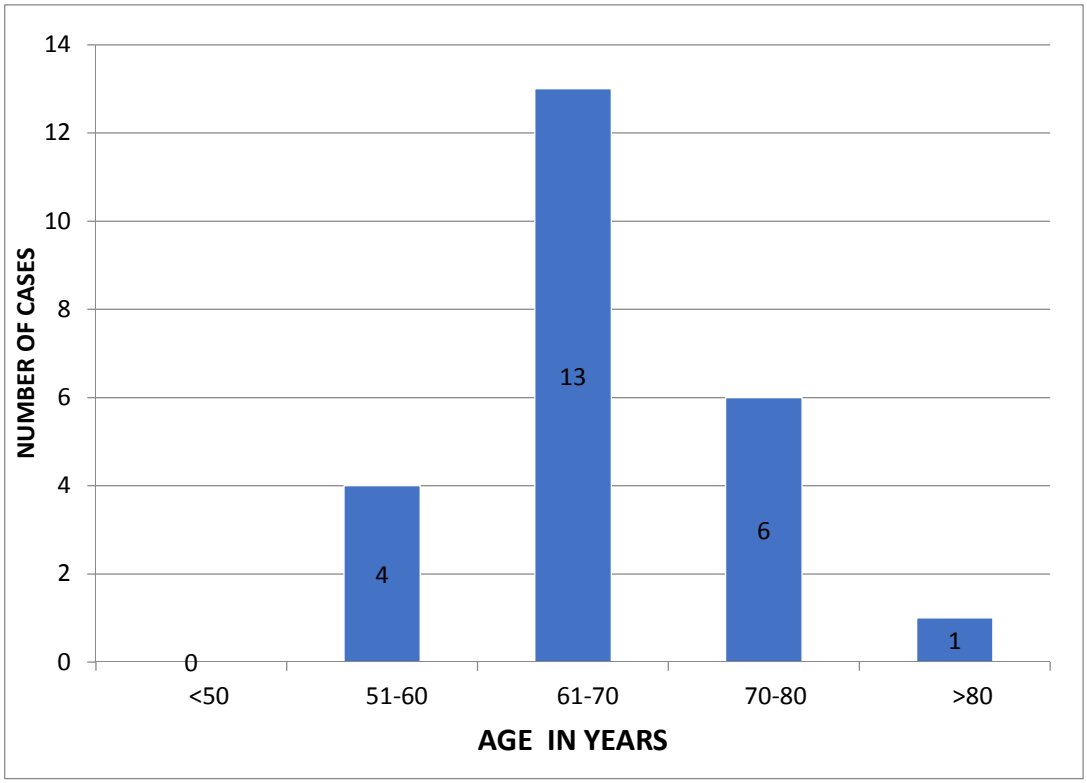
(3) indicates benign mimickers of adenocarcinoma which are about 8.

Table 4- Age distribution in adenocarcinoma cases

S.NO	Age (In years)	No of adenocarcinoma Cases
1	<50	0
2	51-60	4
3	61-70	13
4	70-80	6
5	>80	1

This table shows that out of 24 prostatic adenocarcinoma cases, 20 cases are above 60 years of age with peak incidence in between 60 -70 years. Also there are no cases below the age group of 50 years.

Chart 2- Age distribution in adenocarcinoma cases



This chart shows that all the prostatic adenocarcinoma cases are above 50 years of age with peak incidence in between 61 to 70 years.

Table 5-Age distribution in all cases (n=54)

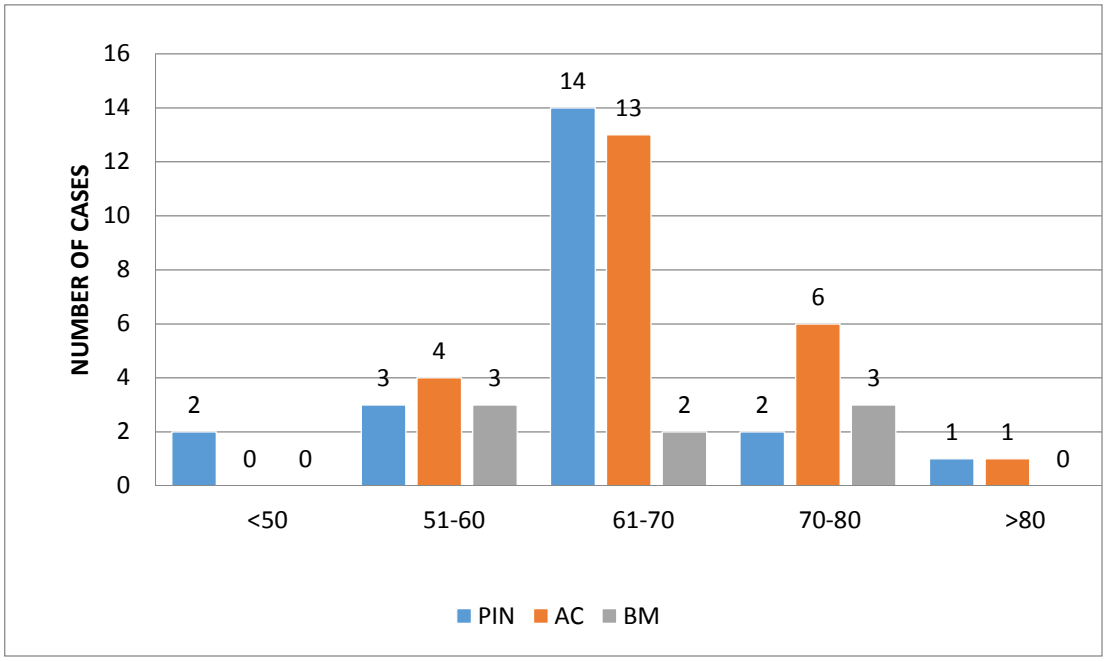
(Age in years)

Histopathological Diagnosis	**<50**	**51-60**	**61-70**	**71-80**	**>80**
Low gradePIN	2	3	11	2	1
High grade PIN			3		
Adenocarcinoma		4	13	6	1
Basal cell hyperplasia		1	1		
Atrophy		1		1	
Adenosis		1		1	
Chronic prostatitis			1	1	

This table shows that all the cases are above the age group of 50 years except 2 cases with peak incidence in between 60-70 years.

In our present study, mean age of all the cases is 66 $\pm$ 8.18

Chart-3 Age distribution in all cases (n=54)



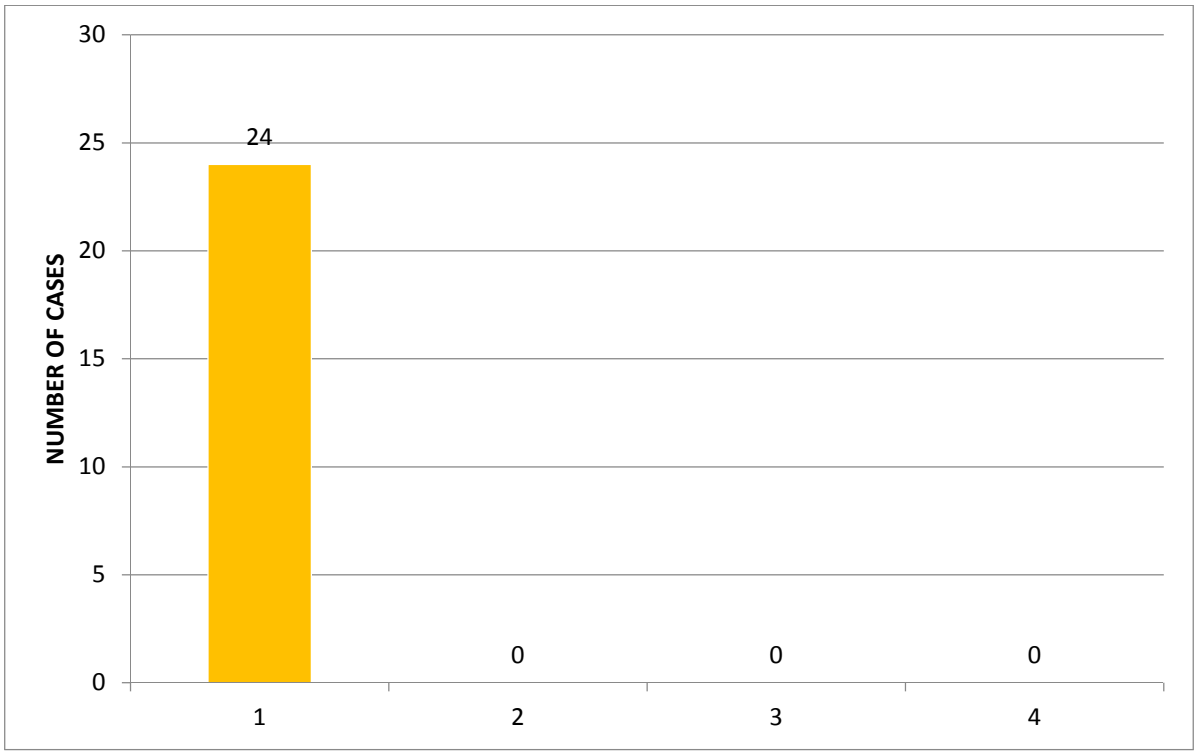
This chart explains that out of 54 cases , 29 cases are in the age group of 61-70 years of age. It also indicates that all the prostate adenocarcinoma(AC), prostatic intraepithelial neoplasia (PIN) and benign mimickers of prostatic adenocarcinoma (BM) cases are above 50 years of age with less incidence (2 cases) below 50 years.

Table-6 p63 immunoreactivity in adenocarcinoma of prostate and cancer mimickers

Histopathological diagnosis	No.of.cases	Grading of p63 positivity			
		0% Negative	<5% Mild	5-75% Moderate	>75% Strong
Adenocarcinoma	24	24	0	0	0
Low grade PIN	19	0	0	6	13
High grade PIN	3	1	0	2	0
Basal cell hyperplasia	2			1	1
Atropy	2			2	
Adenosis	2				2
Chronic prostatitis	2			2	

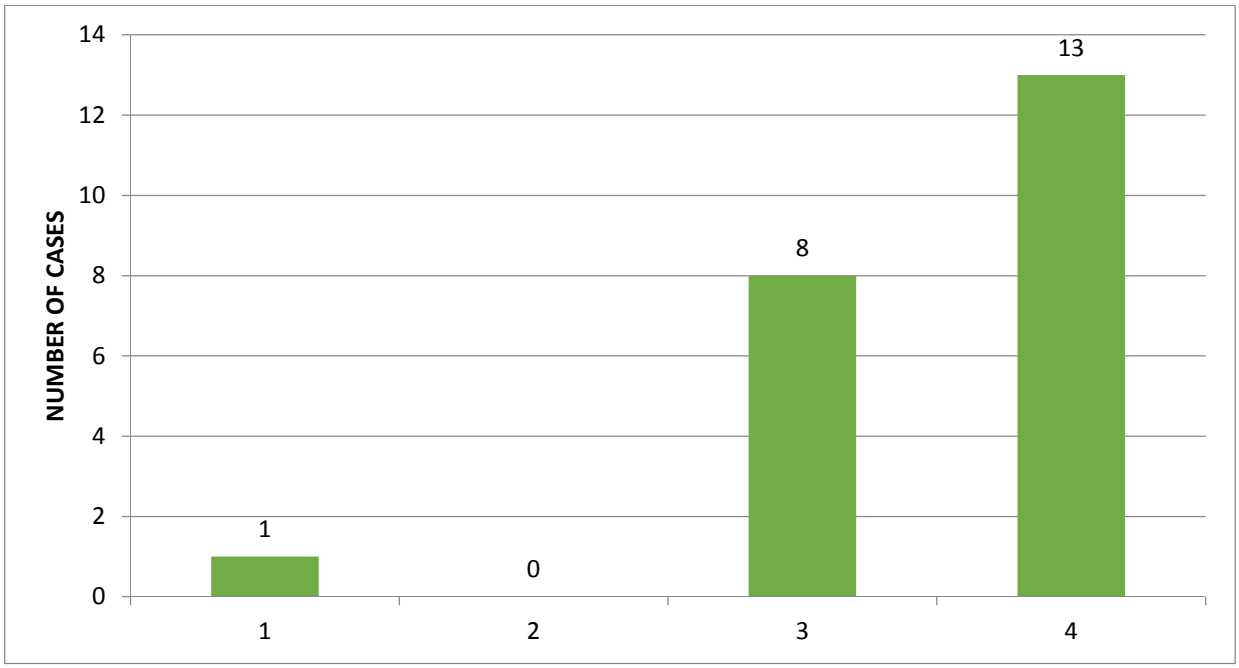
This table shows that all 24 adenocarcinoma cases show negative staining for p63. All LGPIN cases show moderate to strong p63 positivity for basal bells. Out of 3 HGPIN cases, 1 case shows negative staining and 2 cases show moderate p63 expression. All benign mimickers of adenocarcinoma cases show moderate to strong positive p63 expression. In our study 1 HGPIN case showed patchy basal cell staining for p63.

Chart -4 Expression of p63 in adenocarcinoma cases



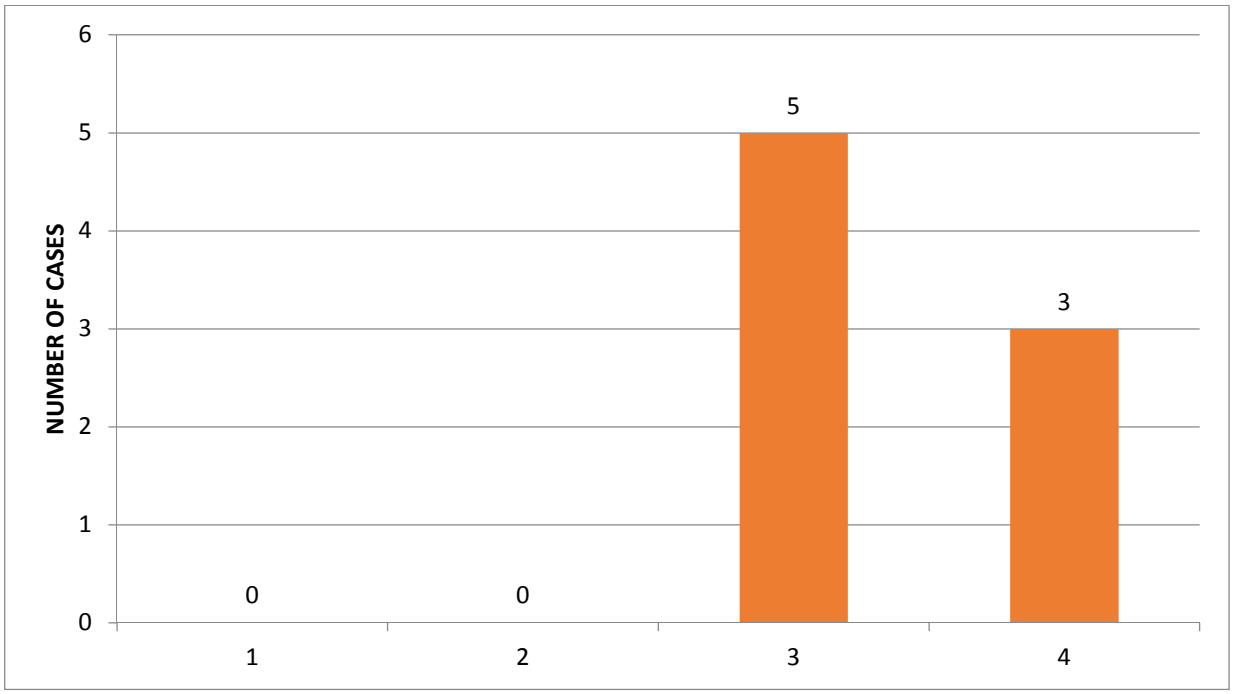
This chart explains that all 24 adenocarcinoma cases show negative staining for p63 (1-Negative, 2-Mild positive , 3-moderate positive, 4-strong positive)

Chart-5 p63 expression in prostatic intraepithelial neoplasia cases



This chart shows that out of 22 cases(19 LGPIN and 3 HGPIN) , 13 cases show strong positivity, 8 cases show moderate positivity and 1 case shows negative staining for p63. In this study one HGPIN case shows patchy basal cell staining for p63 in the PIN foci. (1-Negative, 2-Mild positive , 3-moderate positive, 4-strong positive)

Chart-6 p63 expression in benign mimickers of adenocarcinoma cases



This chart shows that out of 8 benign mimickers of adenocarcinoma cases, 5 cases show moderate degree of positivity and 3 cases show strong positivity for p63. (1-Negative, 2-Mild positive, 3-moderate positive, 4-strong positive)

Table 7- Relationship between p63 expression and cases

Histopathological Diagnosis	P63 expression		p value
	Negative	Positive	
Non cancerous prostatic Lesions	1	29	**<0.0001**
Adenocarcinoma	24	0	

Statistical analysis was done using Pearson chi square test to find out the p value which showed significant value of about <0.0001. It indicates that there is a significant association between p63 expression and noncancerous lesions of prostate.

We also measured the sensitivity and specificity of p63 expression in dignosing prostatic adenocarcinoma and cancer mimickers cases.

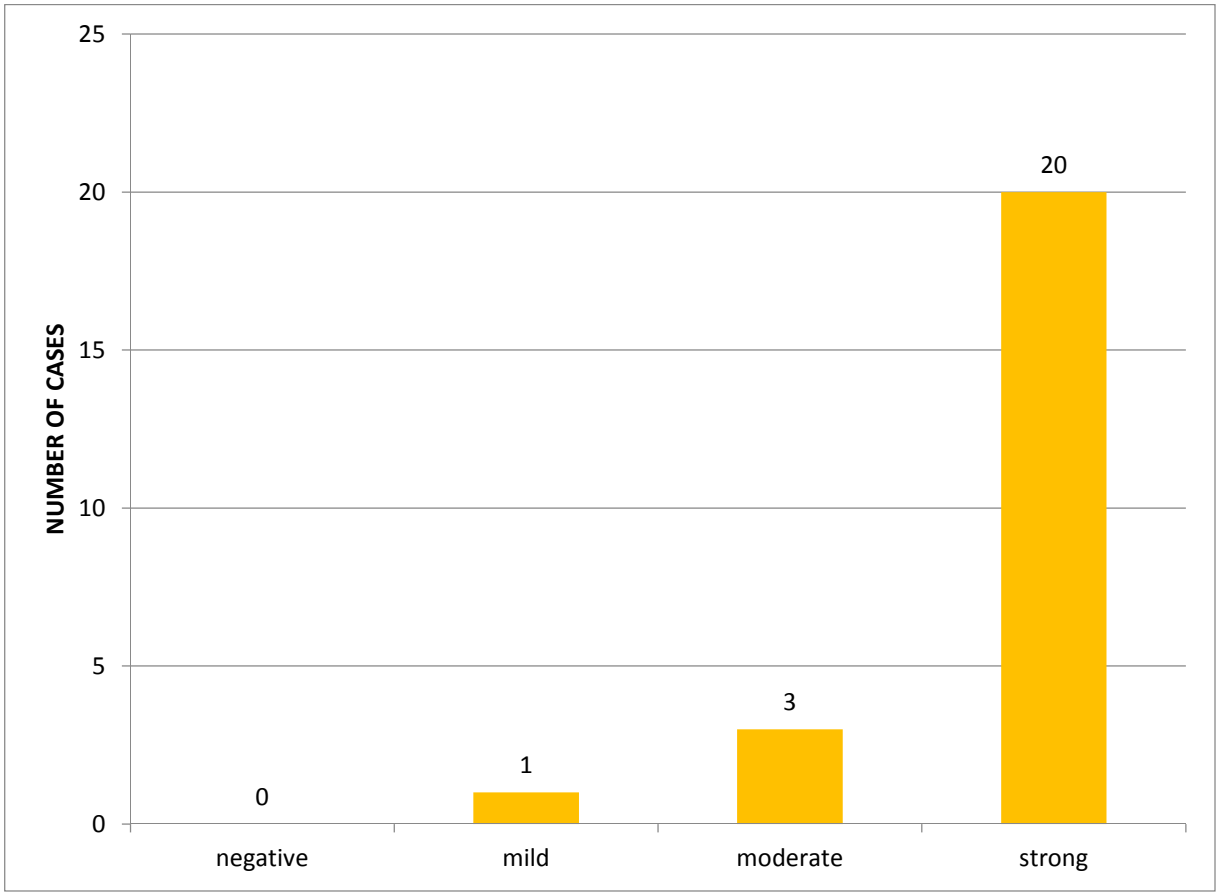
Sensitivity	Specificity	PPV	NPV
100%	100%	100%	100%

Table 8- AMACR immunoreactivity in adenocarcinoma and cancer mimickers

Histopathological diagnosis	No.of. cases	Grading of AMACR expression			
		0% Negative	1-10% Mild	11-50% Moderate	≥51% Strong
Adenocarcinoma	24	0	1	3	20
Low grade PIN	19	19			
High grade PIN	3	3			
Basal cell hyperplasia	2	1			1
Atrophy	2	2			
Adenosis	2	2			
Chronic prostatitis	2	2			

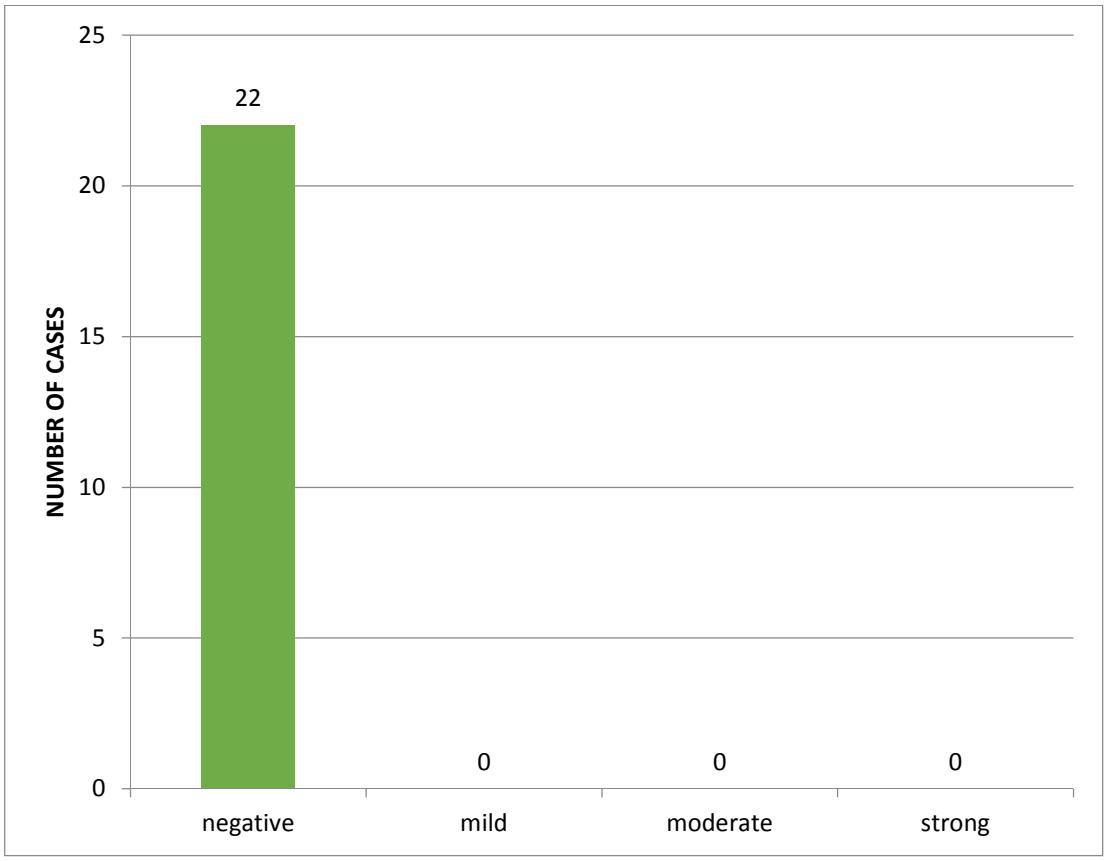
This chart explains that all 24 adenocarcinoma cases show AMACR positivity and all PIN cases show negative staining. Out of 8 benign mimickers of prostatic adenocarcinoma cases, one basal cell hyperplasia case shows strong AMACR expression and remaining 7 cases are negative for AMACR expression.

Chart-7 AMACR expression in Prostatic adenocarcinoma cases



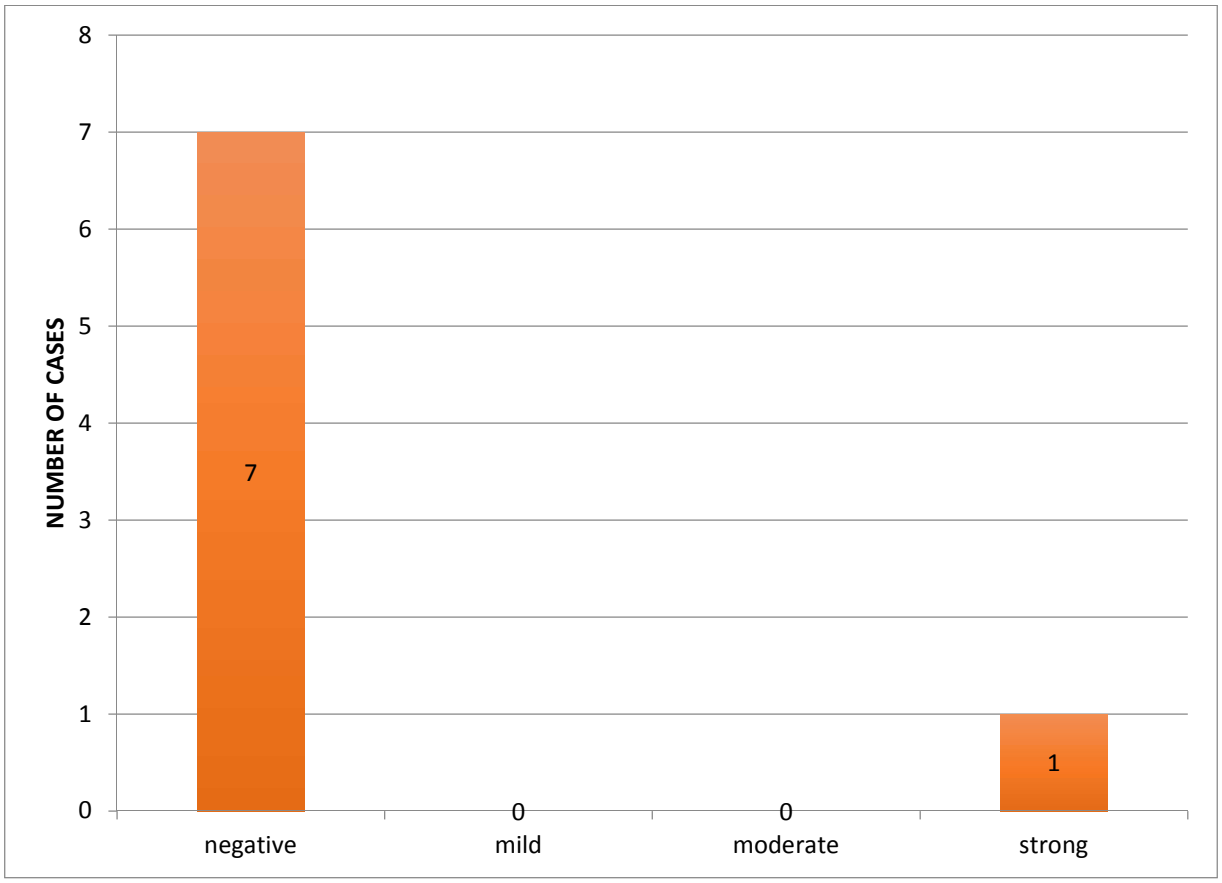
This chart explains that out of 24 adenocarcinoma cases ,20 cases show strong AMACR expression, 3 cases exhibits moderate degree of AMACR expression and 1 case shows mild AMACR expression.

Chart-8 AMACR expression in cases with Prostatic intraepithelial neoplasia



This chart shows that out of 22 Prostatic intraepithelial neoplasia cases (19 Lowgrade PIN Cases and 3 High grade PIN cases), all cases show negative AMACR expression.

Chart-9 AMACR expression in benign mimickers of Prostatic adenocarcinoma



This chart shows that out of 8 benign mimickers of adenocarcinoma cases (2 Basal cell hyperplasia cases, 2 cases with atrophy, 2 cases with adenosis and 2 cases with chronic prostatitis), 1 basal cell hyperplasia case exhibits strong AMACR Expression and all other cases show negative staining for AMACR.

Table 9- Relationship of AMACR expression with cases

Histopathological Diagnosis	AMACR Expression		p value
	Negative	Positive	
Non cancerous prostatic Lesions	29	1	**<0.0001**
Adenocarcinoma	0	24	

The association between AMACR Expression and adenocarcinoma cases was assessed using chi square test that showed p value of about <0.0001 which is statistically significant.

We also measured the sensitivity and specificity of AMACR expression in dignosing prostatic adenocarcinoma and cancer mimickers.

Sensitivity	Specificity	PPV	NPV
100%	88%	96%	100%

Correlation of Gleason grade and AMACR expression in prostatic adenocarcinoma cases

In this study, prostatic adenocarcinoma cases are divided into 5 distinct groups according to Gleason grading[95].

Group 1=Gleason score $\leq$ 6,

Group 2=Gleason score 3+4=7,

Group 3=Gleason score 4+3=7,

Group 4=Gleason score 8,

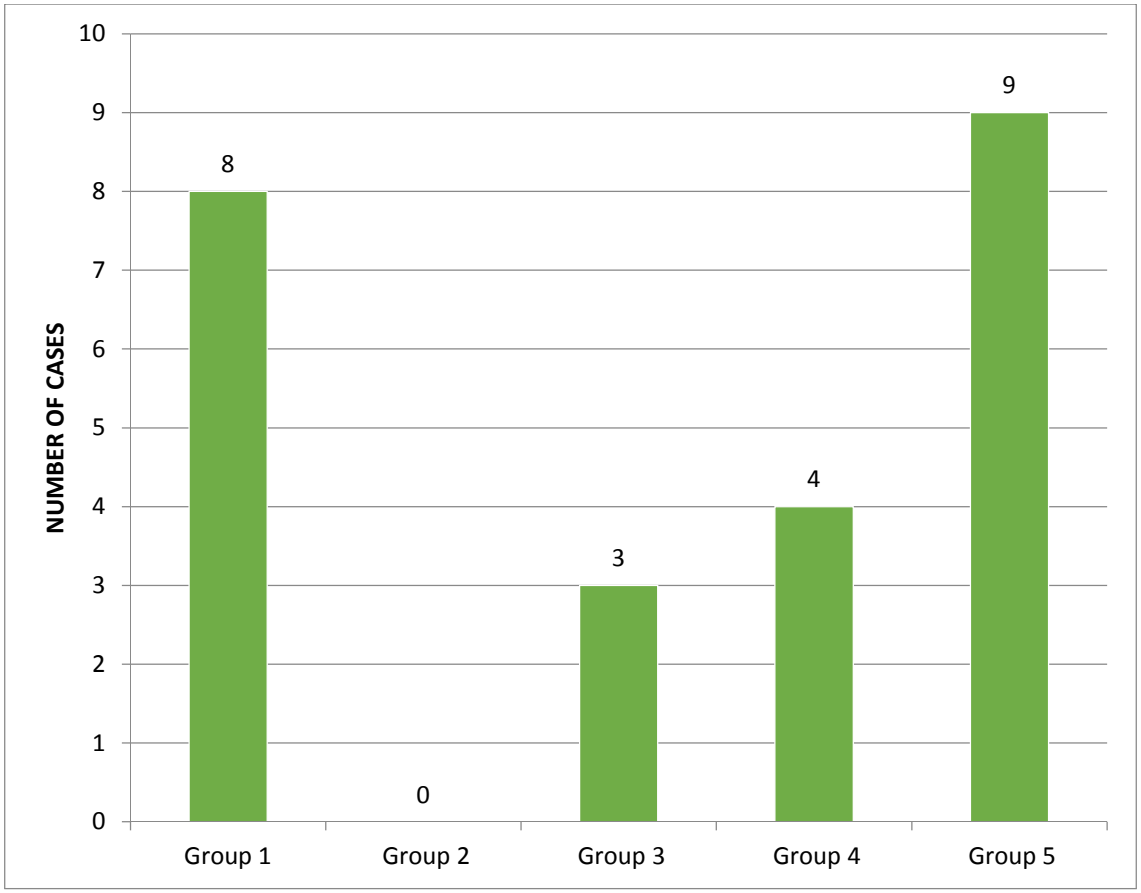
Group 5=Gleason score 9 & 10.

Table-10 Distribution of adenocarcinoma cases based on Gleason score

Gleason score		Frequency
≤ 6	Group 1	8
3+4	Group 2	0
4+3	Group 3	3
8	Group 4	4
9 and 10	Group 5	9

This table shows that out of 24 prostatic adenocarcinoma cases, 8 cases are in group1, no cases in group 2, 3 cases in group 3, 4 cases in group 4 and 9 cases in group 5.

Chart-10 Distribution of adenocarcinoma cases based on Gleason grading.



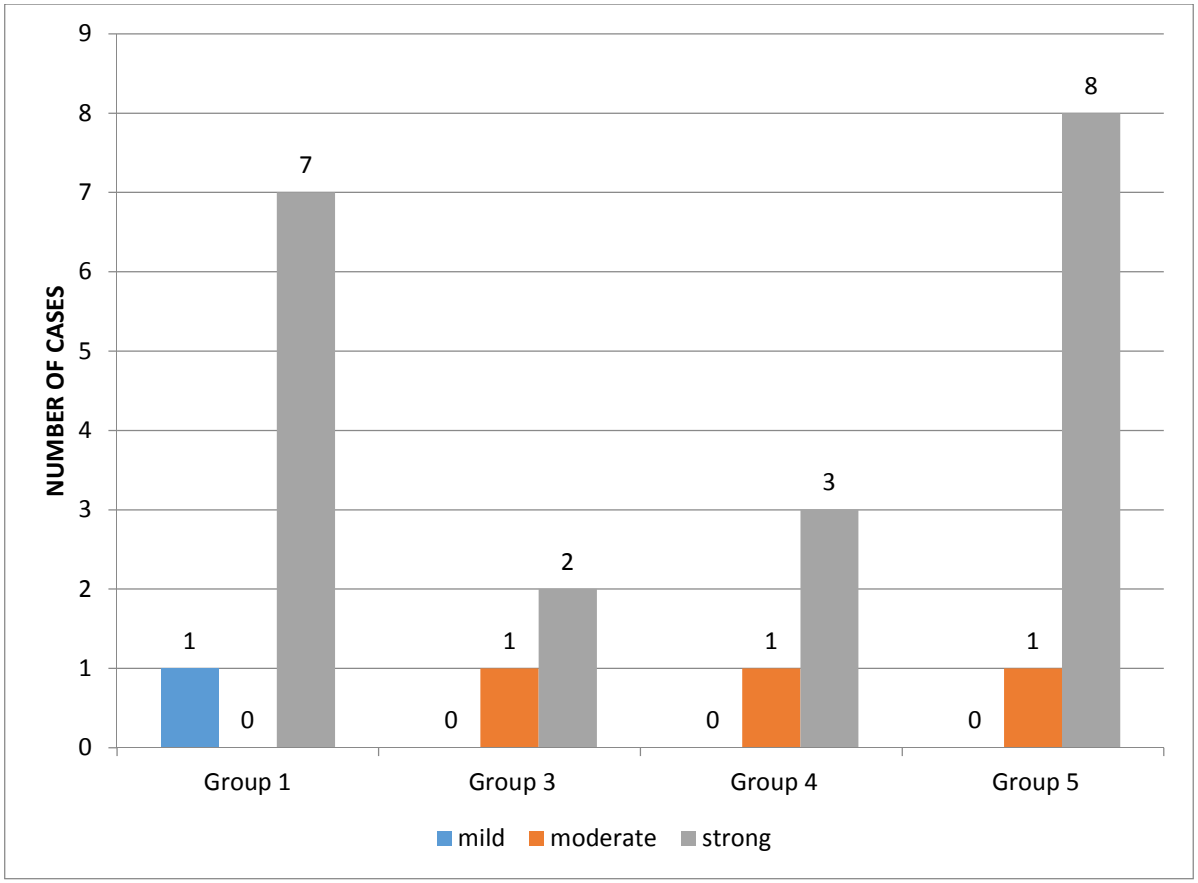
This chart explains that all 24 adenocarcinoma cases are divided into 5 groups based on Gleason score. Group 1 has 8 cases, Group 3 has 3 cases, Group 4 has 4 cases and Group 5 has 9 cases.

Table 11-Correlation of Gleason score and AMACR expression

Gleason score	Number of cases	AMACR expression		
		Mild	Moderate	Strong
≤6	8	1		7
4+3=7	3		1	2
8	4		1	3
9 &10	9		1	8

This table shows that out of 24 prostatic adenocarcinoma cases,8 cases are in group1, no cases in group 2, 3 cases in group 3, 4 cases in group 4 and 9 cases in group 5. Of these, 19 cases showed strong AMACR expression, 3 cases with moderate degree of expression and 1 case with mild degree of expression. This table also indicates that strong AMACR expression is noted irrespective of Gleason grade, which explains that there is no correlation between AMACR expression and Gleason grading.

Chart-11 Correlation of Gleason score grouping and AMACR expression



This chart explains that correlation between AMACR expression and Gleason grade. Out of 9 cases with high Gleason score (group5), 8 cases show strong AMACR expression and 1 case shows moderate expression. Out of 8 cases with low Gleason score, 7 cases exhibits strong expression. It indicates that there is no association between Gleason score and AMACR expression.

Statistical analysis was done using pearson correlation test and pearson chi square test. From which coefficient correlation and p value was measured to find out whether there is any correlation between gleason grading of prostatic adenocarcinoma cases and AMACR expression. It showed coefficient correlation=0.009 and p value=0.966, which indicates that there is no correlation between them.

		coefficient correlation	**p value**
GLEASON SCORE	AMACR	0.009	0.966

DISCUSSION

Prostate cancer produces a major health problem, being the second most common cancer in men. So early recognition and treatment is necessary. Since the recognition of PSA as a screening tool, more and more number of core biopsies were done leading to early recognition and treatment of prostate cancer which resulted in reduced mortality. Even in prostatic core biopsies, pathologists encounter a small focus of atypical cells or benign mimickers of malignancy which make the diagnosis of prostatic carcinoma difficult. The mimickers of malignancy includes the conditions such as, atrophy, partial atrophy, post atrophic hyperplasia, BCH, clear cell cibriform hyperplasia, adenosis, nephrogenic adenoma, mesonephric hyperplasia and seminal vesicle.

In recent years, IHC has emerged as sensitive and specific diagnostic tool in diagnosing morphologically difficult cases, thereby increasing the diagnostic accuracy of prostatic cancer. The IHC markers commonly used are antibodies against basal cells such as p63, HMWCK (34 βE12) and the prostatic adenocarcinoma specific marker AMACR .

Basal cell markers which includes HMWCK (34 βE12), CK 5/6 and p63 are very helpful for demonstration of basal cells because their existence makes the diagnosis of invasive prostatic adenocarcinoma highly unlikely[96]. But there are many limitations with the usage of basal cell markers in the diagnosis of PC since some benign atypical conditions like

Atypical adenomatous hyperplasia (AAH), HGPIN, post atrophic hyperplasia (PAH) may also exhibit irregular or patchy staining, making the diagnosis difficult.

Studies done by Angela wu and Lakshmi P kunju[97], Giovanna et al[98] and Charles C Guo et al[99] reported aberrant positive p63 expresssion in cases of prostatic adenocarcinoma with atrophic features/basaloid features, atypical basal cell proliferation, basal cell carcinoma and intraductal carcinoma. So we should be very cautious in interpreting basal cell immunostains in diagnosing prostatic adenocarcinoma.

Eventhough basal cell markers are an extremely useful adjunct, it is important to use an additional cancer specific marker for adenocarcinoma, having a high sensitivity and specificity for confirmation of the diagnosis.

Multiple studies have now evaluated the utility of AMACR immunostain in the diagnosis of PC, making AMACR a useful immunohistochemical marker for prostate cancer. AMACR expression has also been noted in HGPIN, adenosis and even in benign prostatic glands which limits its usage as a cancer specific marker. So AMACR is more sensitive and specific when used in combination with basal cell markers.

In our present study we have used both p63 and AMACR antibodies and demonstrated the expression of both these markers in differentiating cancer mimickers and premalignant lesions from adenocarcinoma.

Grisanzio et al[100], who performed p63 staining in 130 cases of invasive prostate cancer and found p63 negativity in 126 (97%) cases. Four cases showed p63 positivity in < 1% of tumour cells.

Parsons et al[101], who studied p63 expression in a large series and described strong diffuse p63 protein expression in basal cells of normal and hyperplastic prostate glands, and patchy strong expression in proliferative atrophy and HGPIN.

Kalantari et al[88], studied p63 expression in 12 cases of adenosis , 16 cases of atrophy and 10 HGPIN cases.All cases showed p63 positivity. Also all the 38 adenocarcinoma cases evaluated in their study were p63 negative.

In Shah et al[96] study, 2 out of 27 partial atrophy cases showed p63 positivity. Also Wang et al study exhibited that 30% of partial atrophy cases were p63 positive.

In Vladimir et al study[102], they demonstrated p63 expression in 15 cases with severe morphological signs of chronic inflammation in prostate which showed positive basal cell staining.

In our study we had 24 prostatic adenocarcinoma cases,22 PIN cases and 8 cases of benign conditions which mimic adenocarcinoma. Out of 8 cancer mimickers, 2 cases are prostate atrophy, 2 cases are adenosis,2 cases are basal cell hyperplasia and 2 cases are chronic prostatitis. Out of 22 PIN cases, 19 cases are LGPIN and 3 cases are HGPIN.

Of these, 21 PIN cases (95%) and 8 cases(100%) of benign mimickers of adenocarcinoma showed moderate to strong degree of positive basal cell staining for p63. All 24 cases of adenocarcinoma (100%) showed negative staining for basal cells.

The association between p63 expression and noncancerous prostatic lesions is statistically significant as the p value is <0.0001. In this study, p63 has 100% sensitivity and 100% specificity in diagnosing cancer mimickers and adenocarcinoma cases. So it can be considered as a reliable basal cell markerin distinguishing prostatic adenocarcinoma from premalignant lesions and benign conditions which mimic carcinoma.

Zhou et al[103] demonstrated that, of 115 prostate biopsies diagnosed as atypical by an expert pathologist, 34 (30%) were changed to a final diagnosis of cancer based on a positive AMACR immunostain. In our study all 24 adenocarcinoma cases (100%) showed positive AMACR expression. The association between AMACR expression and adenocarcinoma cases is statistically significant as the p value is <0.0001. In our study, AMACR has 100% sensitivity and 88% specificity in diagnosing cancer mimickers and adenocarcinoma.

AMACR expression is also identified in 4-21% of benign prostatic glands[104,103] and up to 18-27% of cases of Adenosis. Yang et al[105], studied AMACR expression in 40 samples with AAH foci and found that 33 (83%) showed negative staining, focal stainig in 4 cases and diffuse

staining in 3 cases. In our study, both the 2 adenosis cases showed negative AMACR expression.

Studies done by Jiang et al[106], Luo et al[107] and Rubin et al[108], reported that positive AMACR staining was noted in all Adenocarcinoma and HGPIN cases.

Hosler and Epstein[73], in their study of Basal cell hyperplasia cases alone, reported that the immunohistochemical staining was useful for the identification of the nature of basal cell proliferation. They found 100% (7/7) positivity for p63 and complete negative expression for AMACR in all those cases of basal cell hyperplasia.

Fatma El-Zahraa Salah El-DeenYassin et al[109]who studied on BCH and HGPIN cases alone, noted the expression of p63 and AMACR in 9 basal cell hyperplasia and 16 HGPIN cases. Of these, all basal cell hyperplasia (100%) cases showed p63+ and AMACR-. Also all 16 HGPIN cases (100%) showed positive p63 expression, of these 3/16 (19%) cases with continuous pattern of staining and 13/16 cases(81%) with fragmented pattern of staining.

In our present study, we had 2 basal cell hyperplasia and 3 HGPIN cases. Out of the 3 HGPIN cases, 2 cases showed positive p63 expression(1 with continuous staining pattern and other with patchy staining pattern) and 1 case showed negative p63 expression Out of 2 BCH cases, both showed p63 positivity and 1 out of 2 cases showed AMACR positivity.

In contrast to previous studies, all the 3 HGPIN cases showed negative AMACR expression and 1 out of 2 BCH cases showed positive AMACR expression. So the HGPIN case which showed p63-/AMACR- and the BCH case which showed p63+/AMACR+ needs to be evaluated further and followed up to rule out malignancy.

Vincent molinie et al[110] in their study,found that all atrophic and benign lesions showed 10–100% persistent basal cell staining with p63 and all prostatic carcinomas were negative for p63.AMACR expression was noted in 2% of normal glands (4/260) with a focal weak staining . AMACR overexpression was noted in 97% of cancer cases with a heterogeneous staining pattern from weak, moderate and strong intensity, independently of the Gleason score (*P*=0.29). In our study, AMACR expression was negative in normal benign glands.

Also Zhong jiang et al[106], who compared Gleason grading with AMACR expression in 137 prostatic adenocarcinoma cases and noted strong positive expression of AMACR regardless of varying Gleason grade with 100% sensitivity.

In our present study also, it showed that intensity of AMACR expression did not correlate with the Gleason score(p=0.966 and coefficient correlation=0.009).

LIMITATIONS OF THE STUDY

1. Since our study is a retrospective study PSA values could not be collected for more number of cases. PSA value was available only in 17/54 cases. Hence the correlation of PSA values with the diagnosis of prostatic lesions couldn’t be done.
2. In our institution there are less number of cancer mimicker and HGPIN cases available for comparison of expression of these markers

SUMMARY

This study includes a total of 54 cases with prostatic lesions diagnosed from TURP specimens and prostatic needle biopsies. Of these 24 cases were adenocarcinoma, 19 cases were LGPIN, 3 cases were HGPIN and remaining 8 cases were benign mimickers of adenocarcinoma (2 atrophy, 2 adenosis, 2 chronic prostatitis and 2 BCH cases). We have done IHC staining for all the cases using p63 and AMACR antibodies to differentiate prostate adenocarcinoma from benign prostatic lesions which mimic carcinoma. Also to find out the expression of these markers in benign glands and premalignant lesions.

Out of 24 adenocarcinoma cases all showed p63-/AMACR+. Negative staining for p63 indicates that complete absence of basal cells in adenocarcinoma. Of 8 benign mimickers of adenocarcinoma cases,7 cases showed p63+/AMACR- and 1 Basal cell hyperplasia case showed p63+/AMACR+. All the 19 LGPIN cases showed p63+/AMACR-. Out of 3 HGPIN cases,2 cases showed p63+/AMACR- and 1 case showed p63-/AMACR-.

As in previous studies, p63 was positive in all BPH with LGPIN cases, 8 cancer mimickers and 2 HGPIN cases. p63 was negative in all adenocarcinoma cases. AMACR was positive in all adenocarcinoma cases and negative in LGPIN cases and 7 cancer mimickers in our study.

In contrast to previous studies, 1 BCH case was AMACR positive and all 3 HGPIN cases were AMACR negative in our study.

In this study, both sensitivity and specificity of p63 was 100% in cancer mimickers and adenocarcinomas. Sensitivity and specificity of AMACR in cancer mimickers and adenocarcinoma was 100% and 88% respectively. The association between p63 expression and noncancerous prostatic lesions is statistically significant as the p value is <0.0001 and also there is a statistically significant association present between AMACR expression and adenocarcinoma cases as the p value is <0.0001.

CONCLUSION

1. p63 is a 100% sensitive and 100% specific basal cell marker and can be used in morphologically difficult cases which mimic adenocarcinoma. AMACR has 100% sensitivity but 88% specificity in diagnosing the prostatic lesions which mimic adenocarcinoma. So When we use both these markers in combination the diagnostic accuracy can be improved.
2. There is no significant correlation between Gleason grading and AMACR expression in adenocarcinoma cases (p value =0.966).
3. Positive p63 expression and negative AMACR expression was noted in all benign glands. All LGPIN cases showed p63 positive and AMACR negative. Out of 3 HGPIN cases, 2/3 cases are p63 positive . So it can be inferred that p63 is a reliable basal cell marker in diagnosing benign and premalignant lesions and ruling out carcinoma.
4. We also conclude that, AMACR expression was negative in all 3 HGPIN cases in contrast to other studies. All adenocarcinoma cases showed AMACR+/p63-. So AMACR marker may be used in differentiating HGPIN and adenocarcinoma cases in addition to basal cell markers like p63.We also suggest that further study of AMACR expression with more number of HGPIN cases is necessary.

COLOUR PLATES

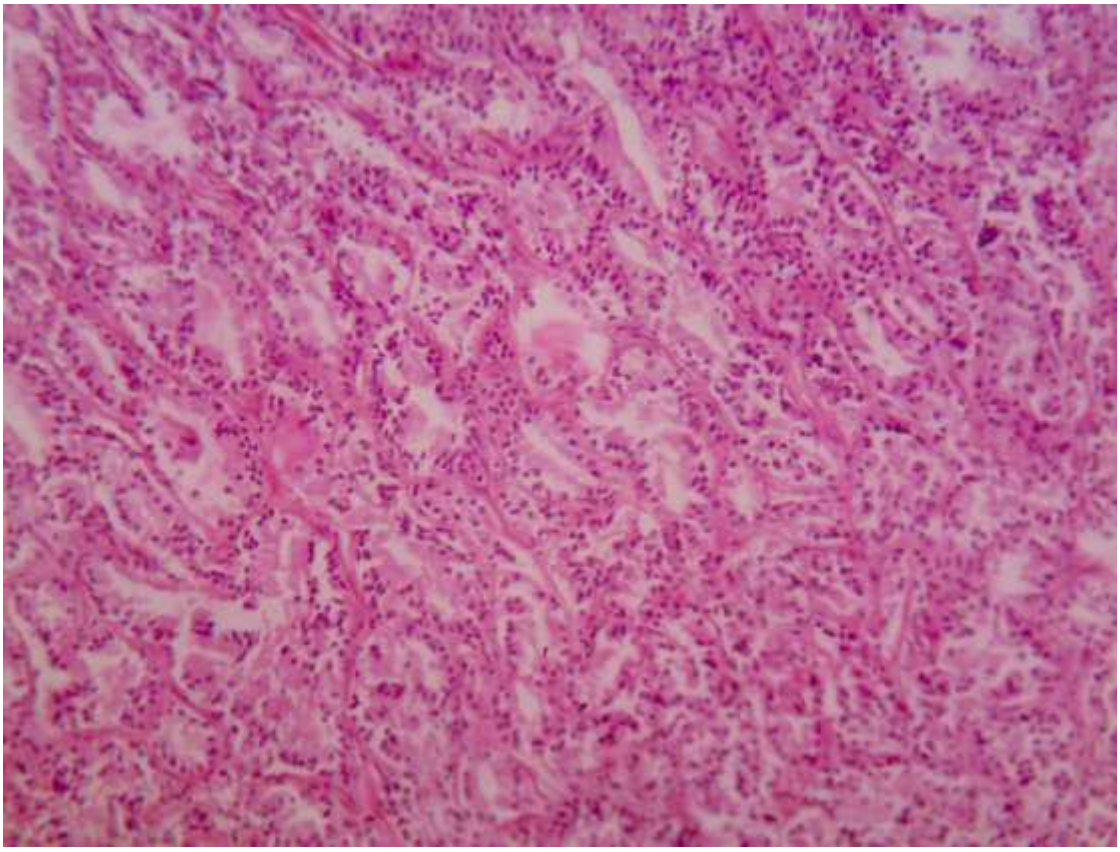


FIGURE 1:A CASE OF PROSTATIC ADENOCARCINOMA – H AND E IMAGE

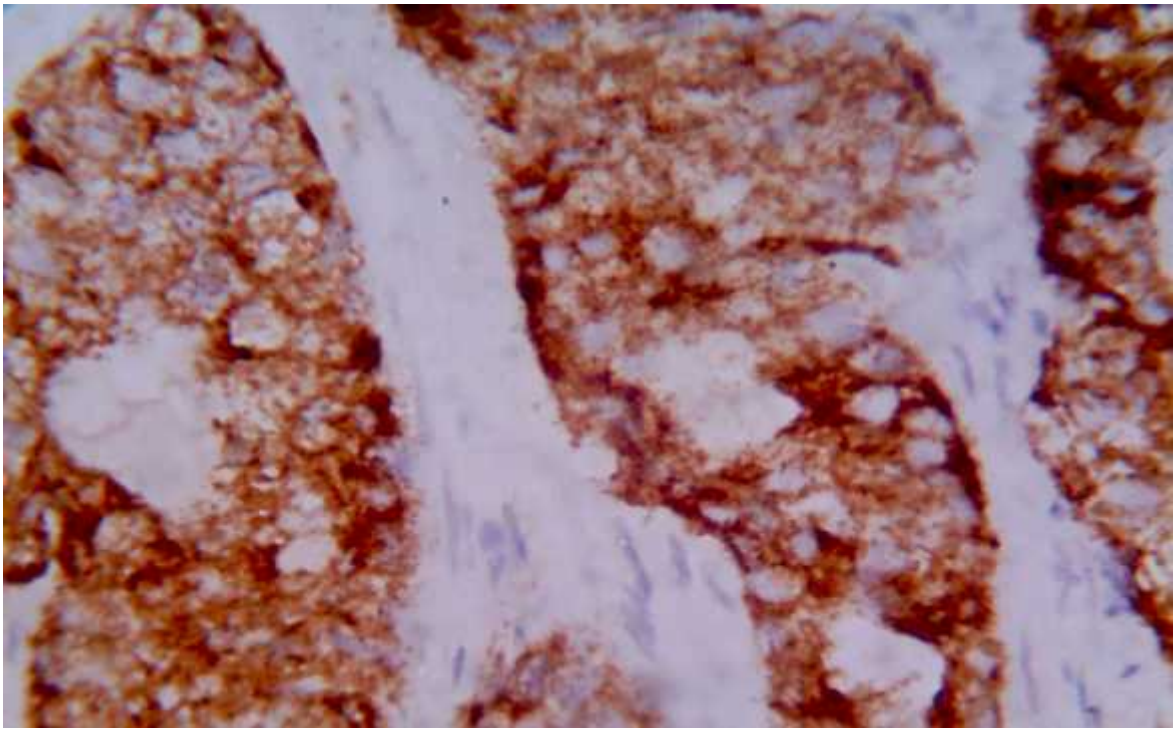


FIGURE :2-STRONG POSITIVE AMACR EXPRESSION IN ADENOCARCINOMA

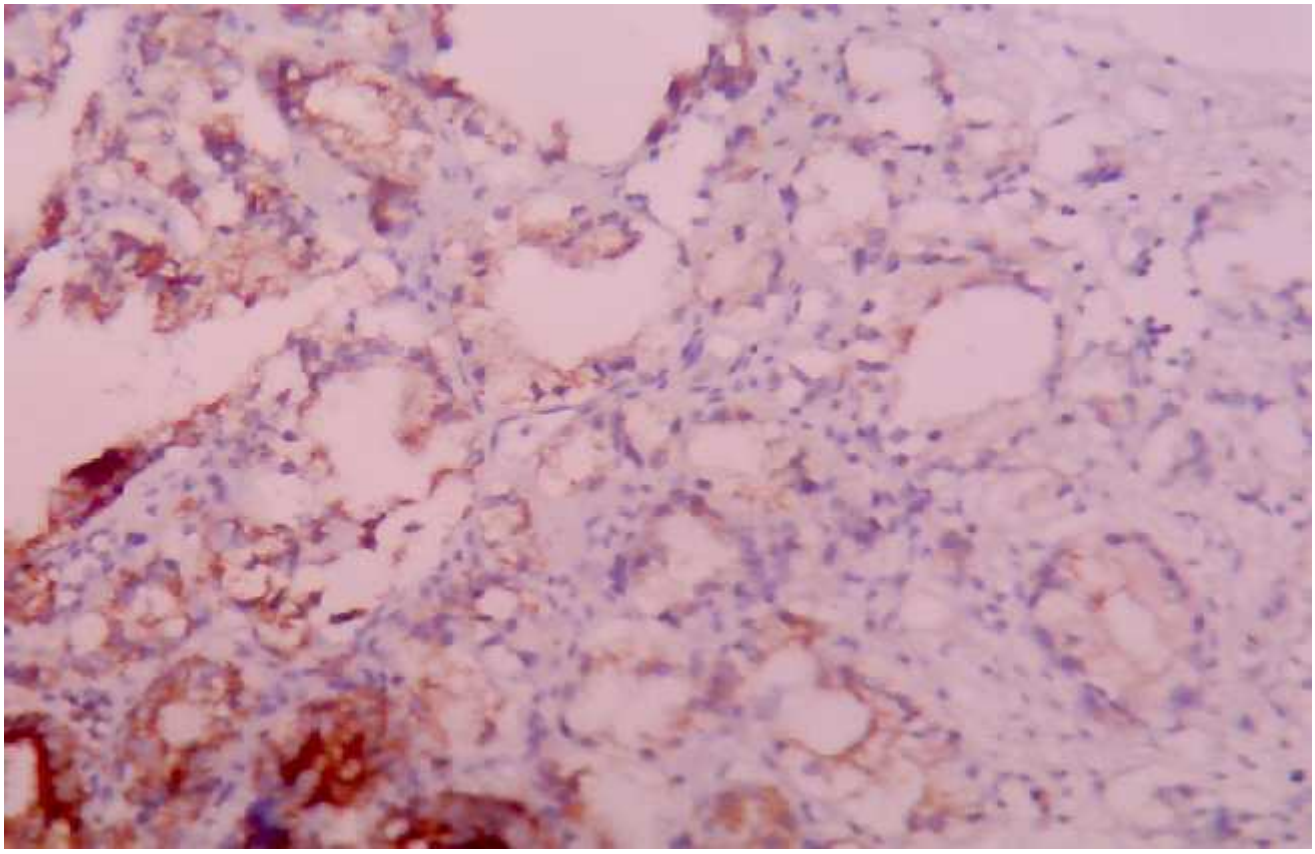


FIGURE 3: MODERATE POSITIVITY FOR AMACR IN ADENOCARCINOMA

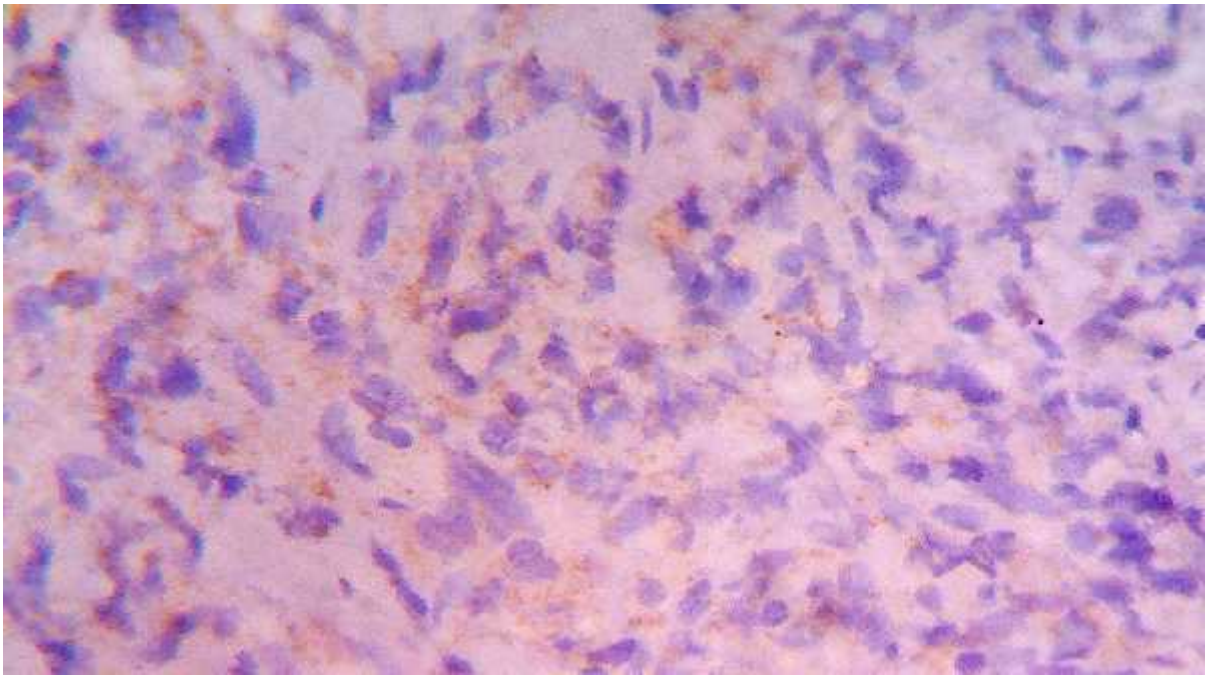


FIGURE 4:MILD POSITIVITY FOR AMACR EXPRESSION IN ADENOCARCINOMA

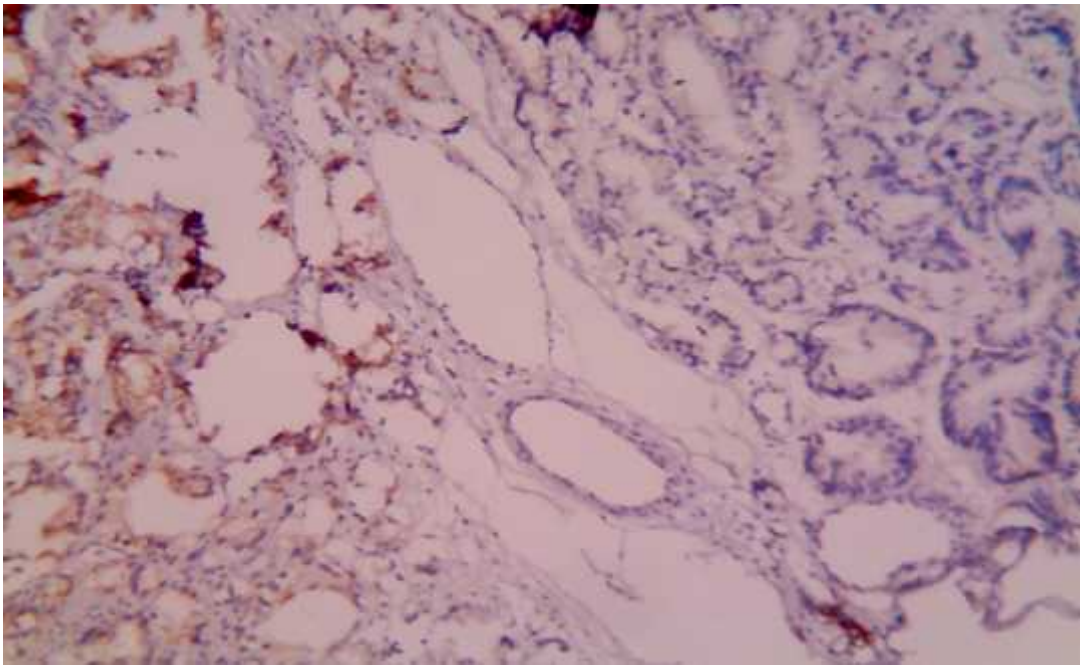


FIGURE 5: POSITIVE AMACR EXPRESSION IN ADENOCARCINOMA WITH ADJACENT BENIGN GLANDS SHOWING NEGATIVE AMACR STAINING.

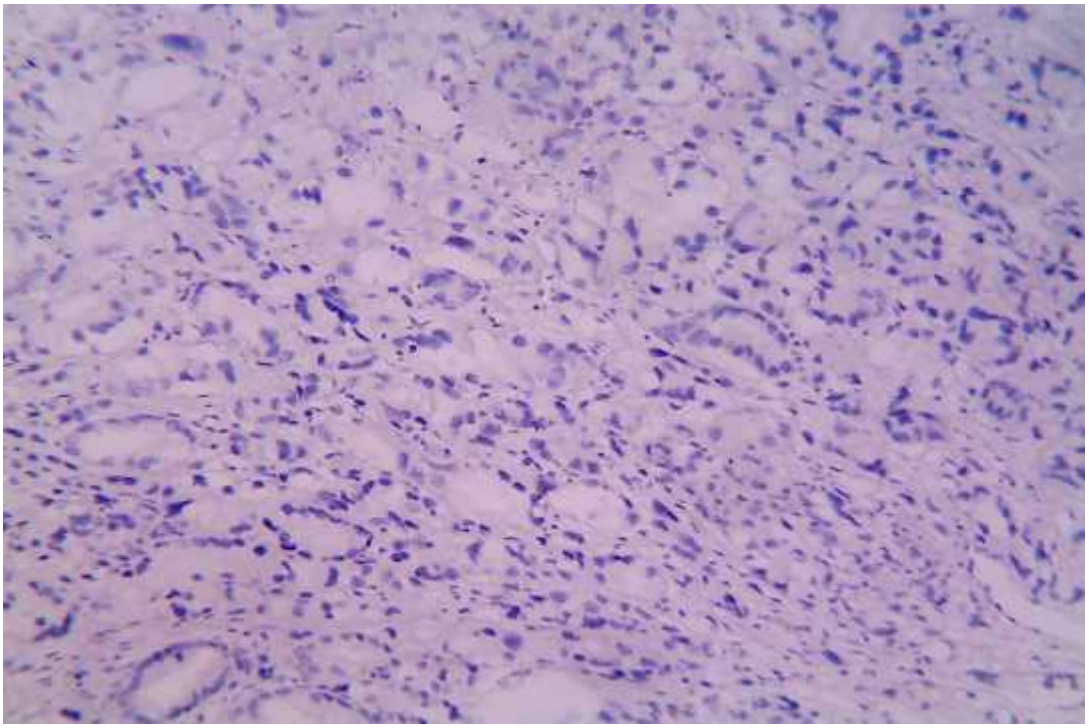


FIGURE 6: -NEGATIVE P63 EXPRESSION IN TUMOUR CELLS OF ADENOCARCINOMA

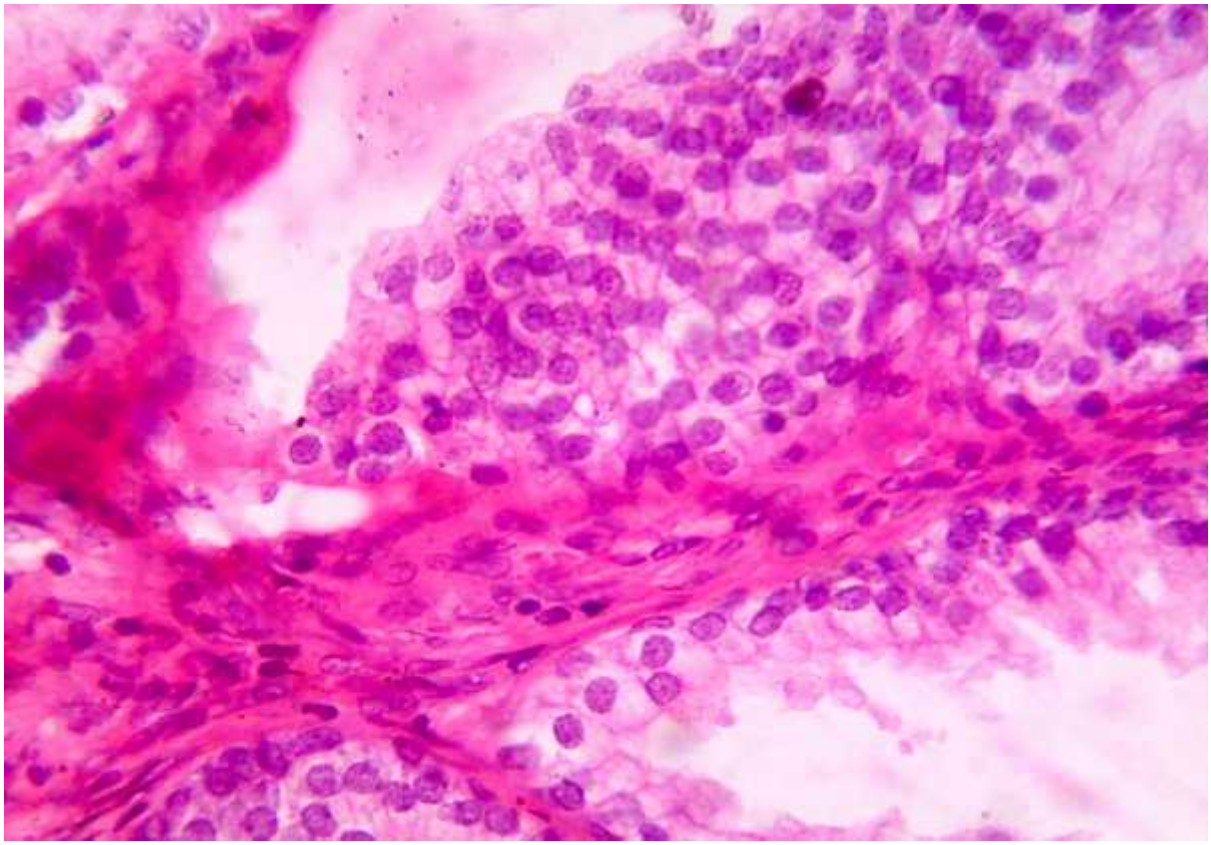


FIGURE 7: A CASE OF HIGH GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA H & E IMAGE

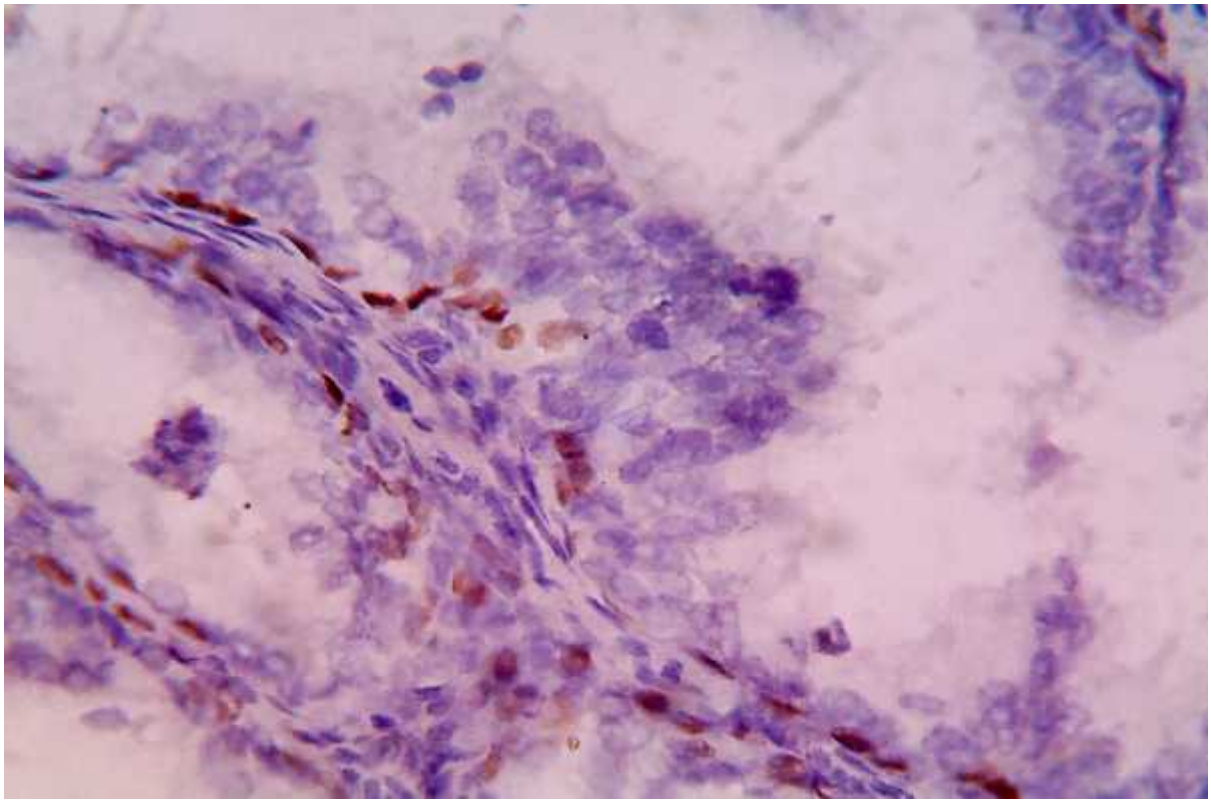


FIGURE 8: FOCAL STRONG POSITIVE P63 EXPRESSION IN HGPIN

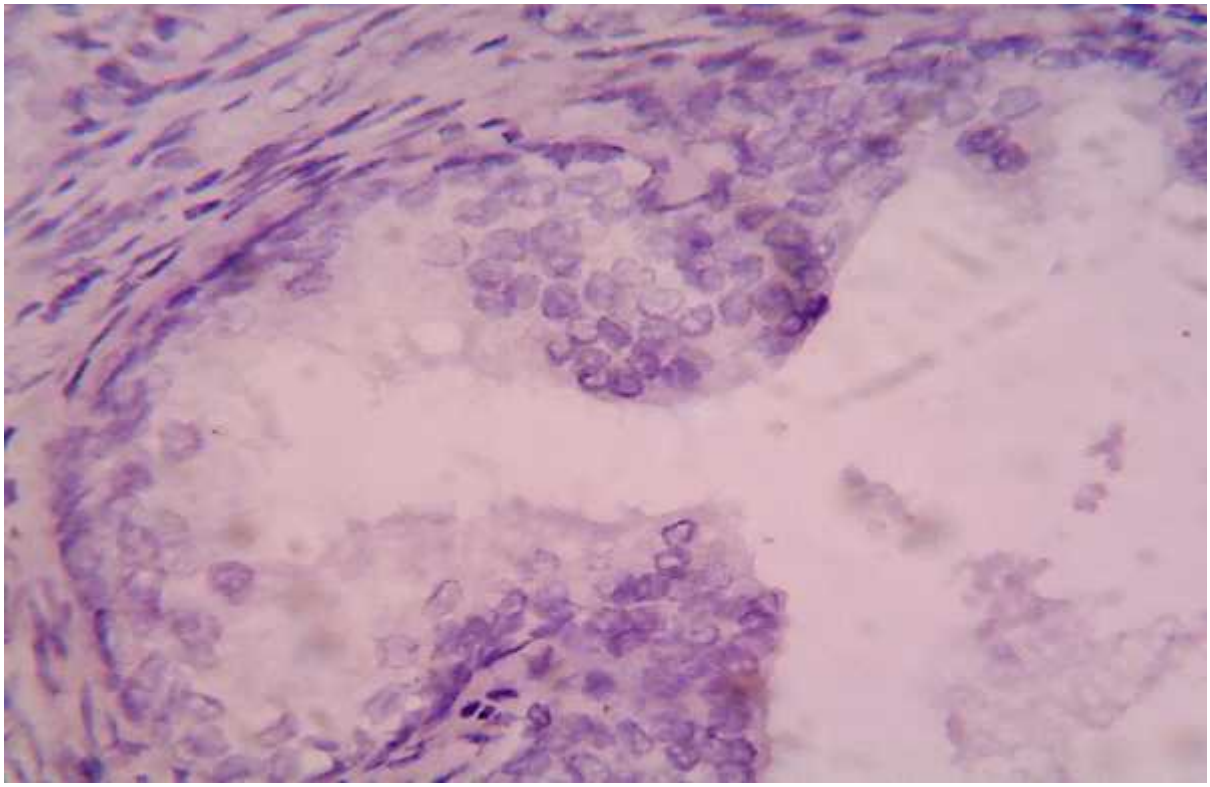


FIGURE 9: NEGATIVE AMACR EXPRESSION IN HGPIN

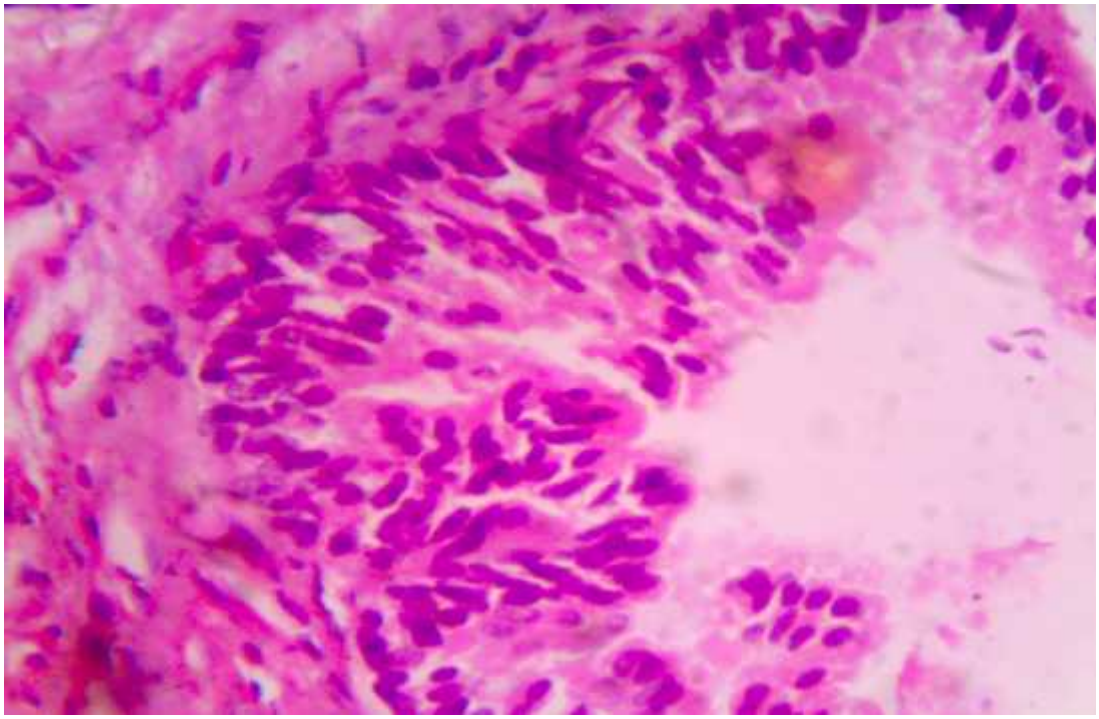


FIGURE 10: A CASE OF LOW GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA H& E IMAGE

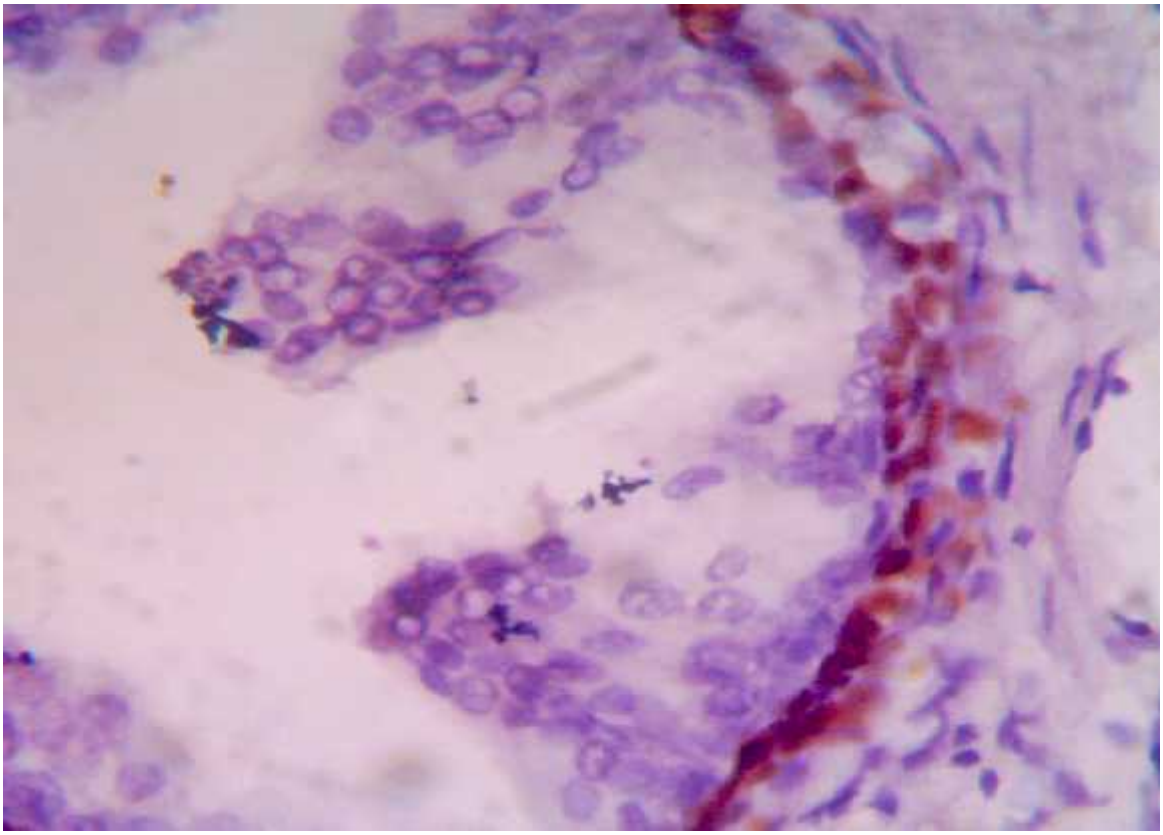


FIGURE 11:POSITIVE P63 EXPRESSION IN LGPIN FOCI

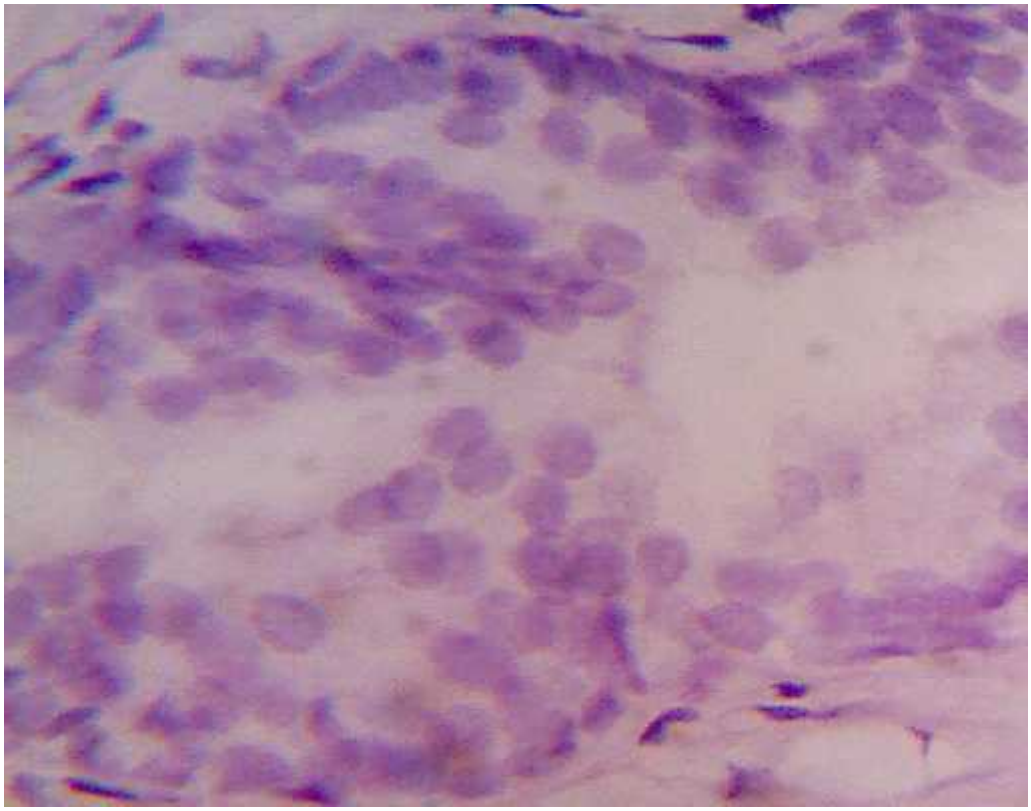


FIGURE 12-NEGATIVE AMACR EXPRESSION IN LGPIN

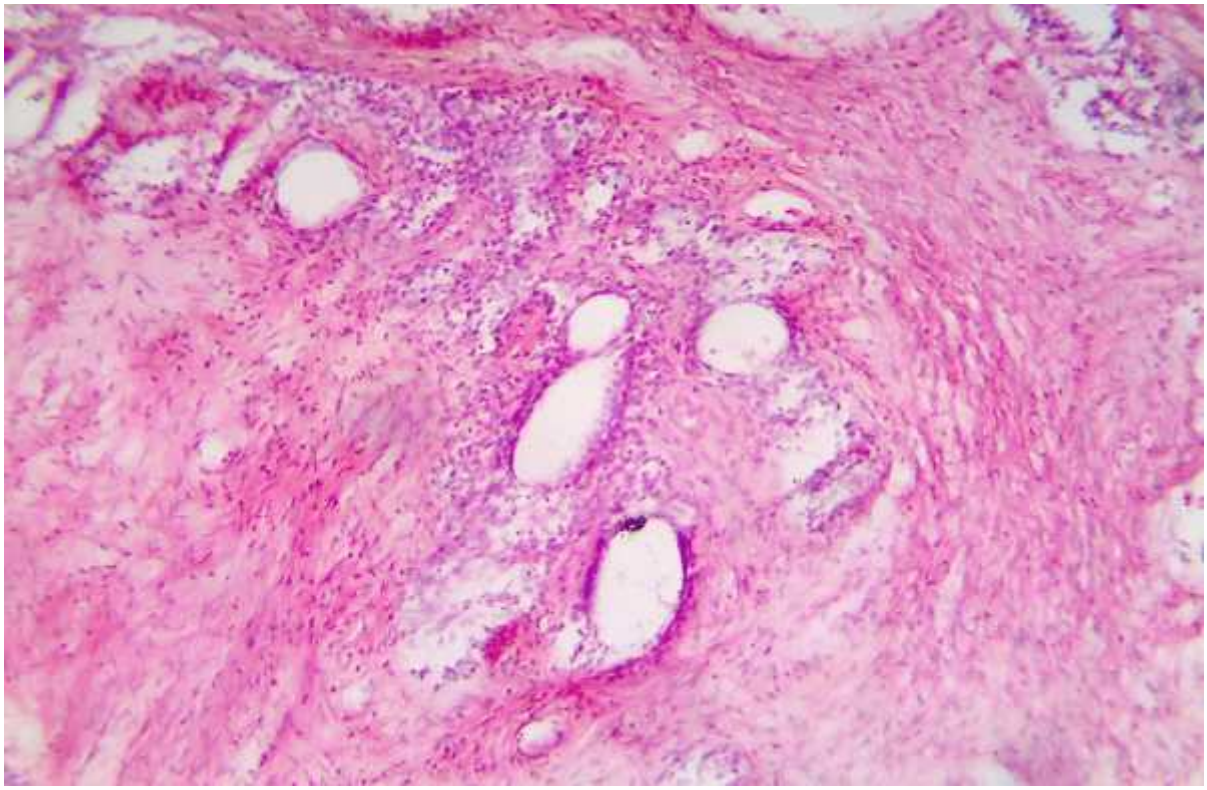


FIGURE 13: A CASE OF PROSTATE ATROPHY H& E LOW POWER IMAGE

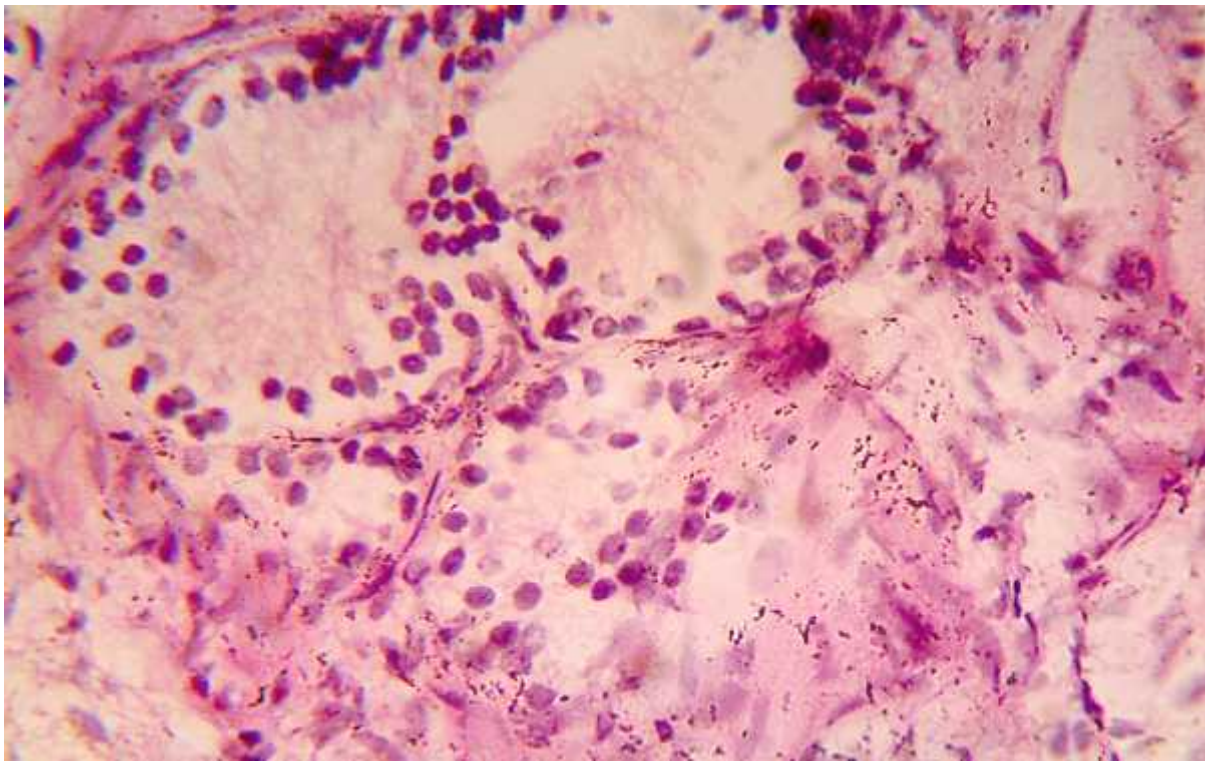


FIGURE 14: A CASE OF PROSTATE ATROPHY H& E HIGH POWER IMAGE

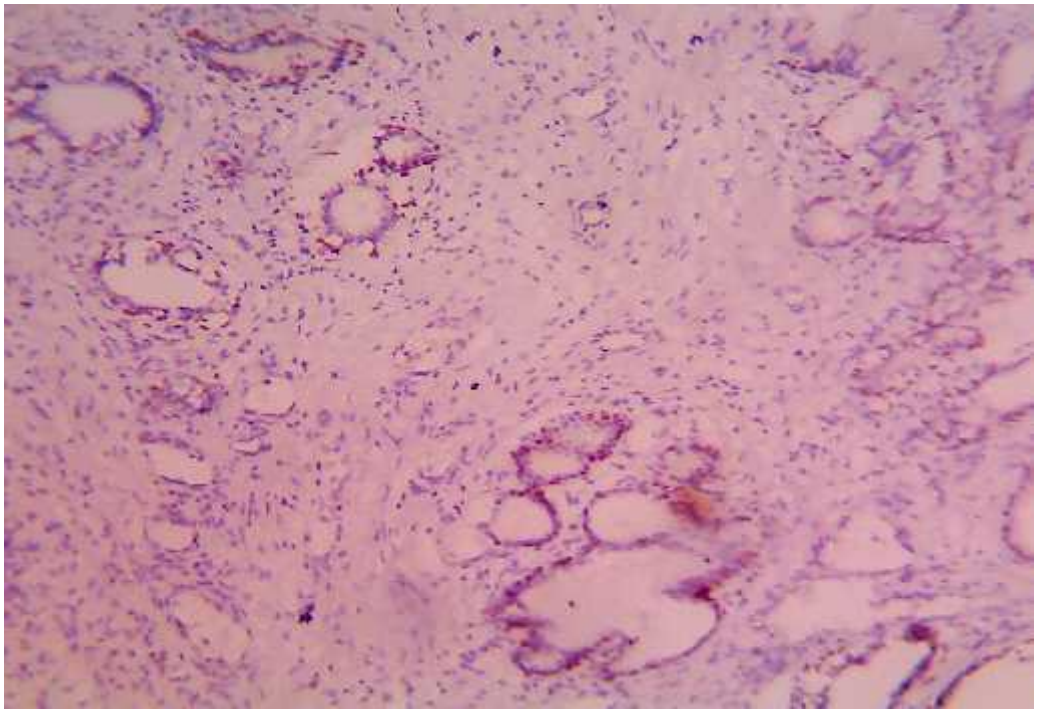


FIGURE:15 POSITIVE P63 EXPRESSION IN PROSTATE ATROPHY LOW POWER IMAGE

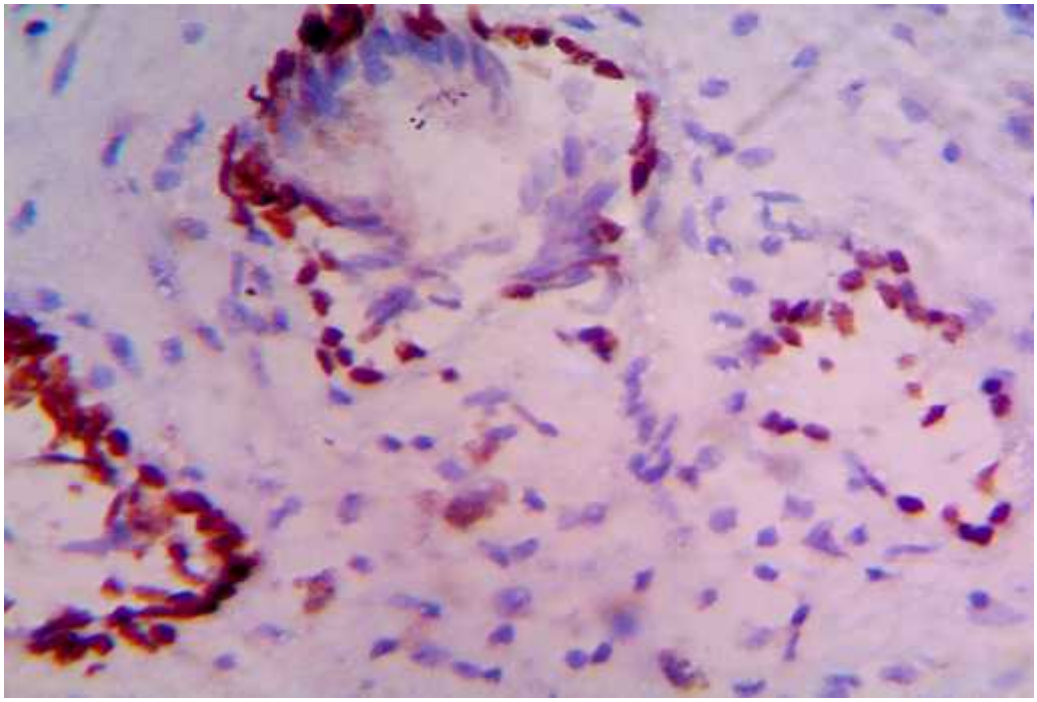


FIGURE 16: POSITIVE P63 EXPRESSION IN PROSTATE ATROPHY HIGH POWER IMAGE

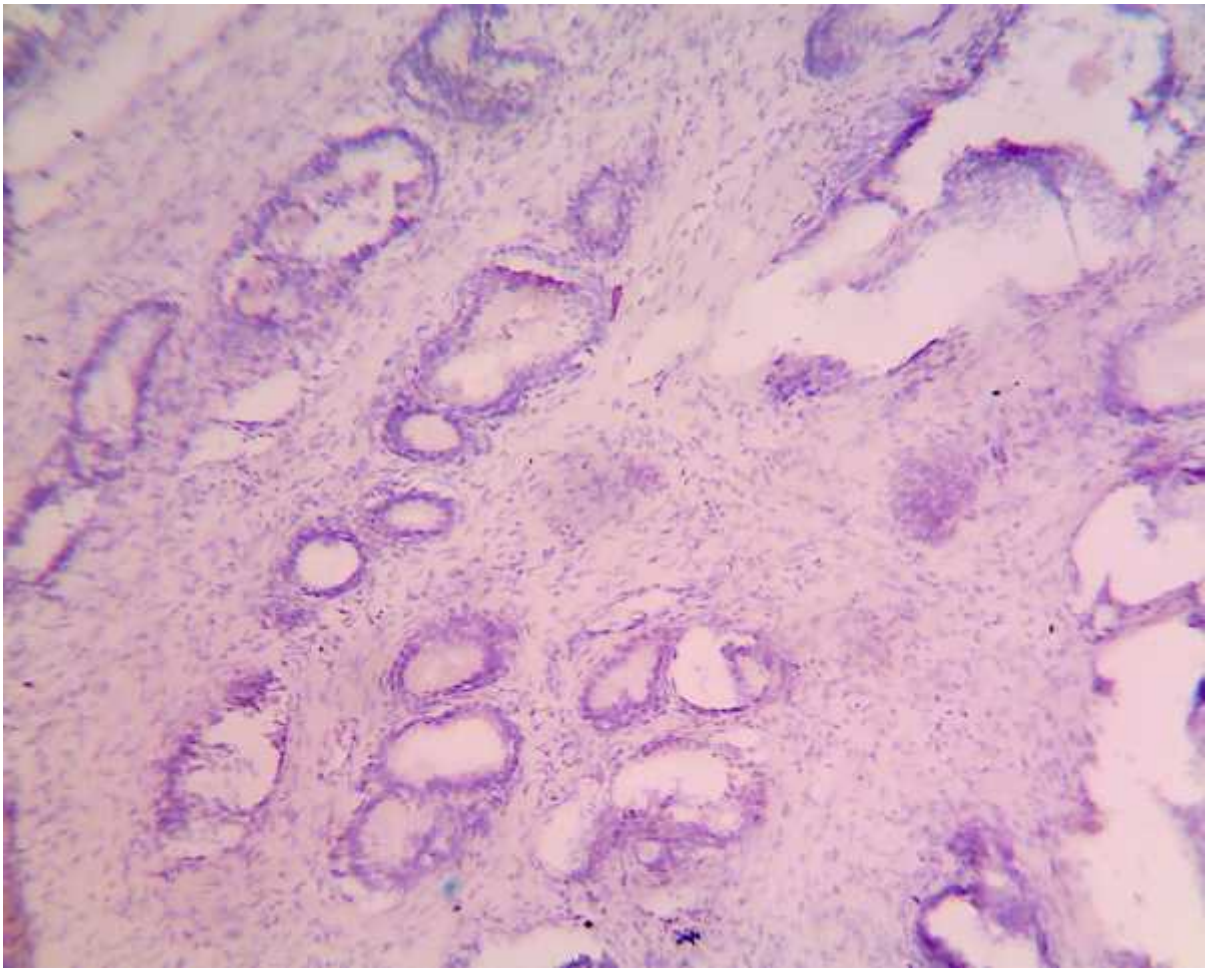


FIGURE 17: NEGATIVE AMACR EXPRESSION IN PROSTATE ATROPHY

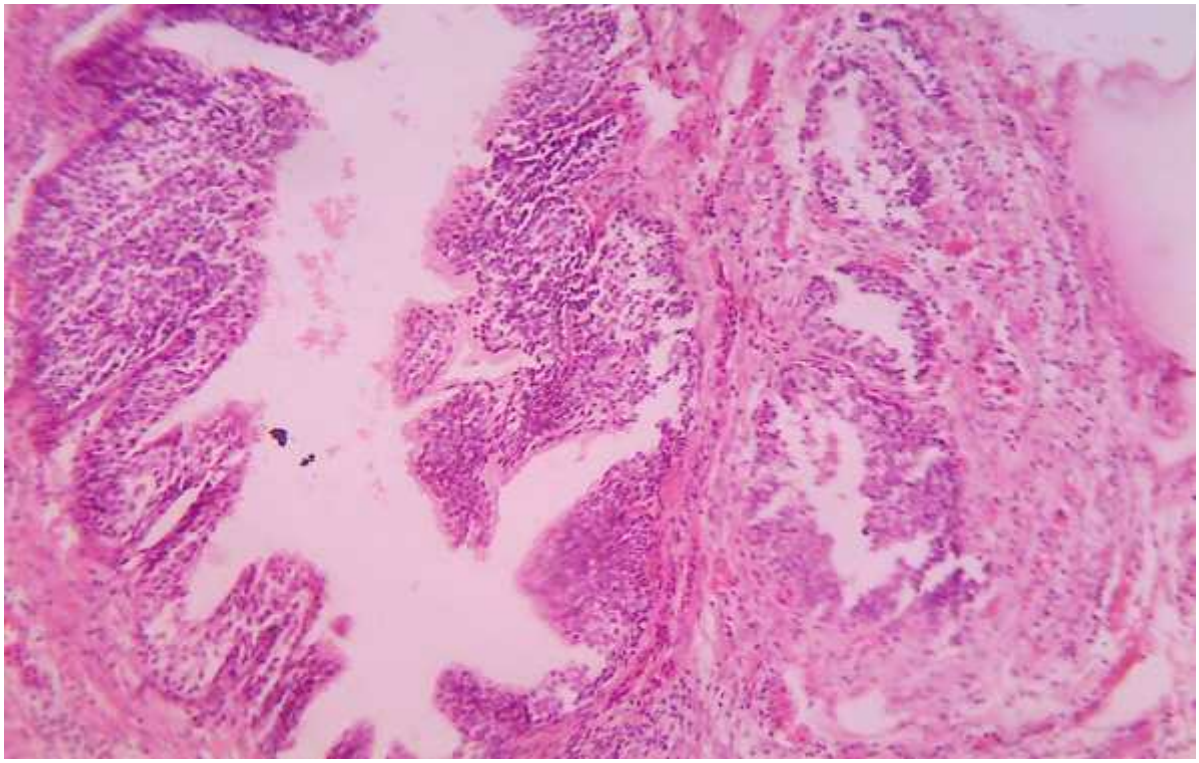


FIGURE :18 A CASE OF BASAL CELL HYPERPLASIA –H& E IMAGE

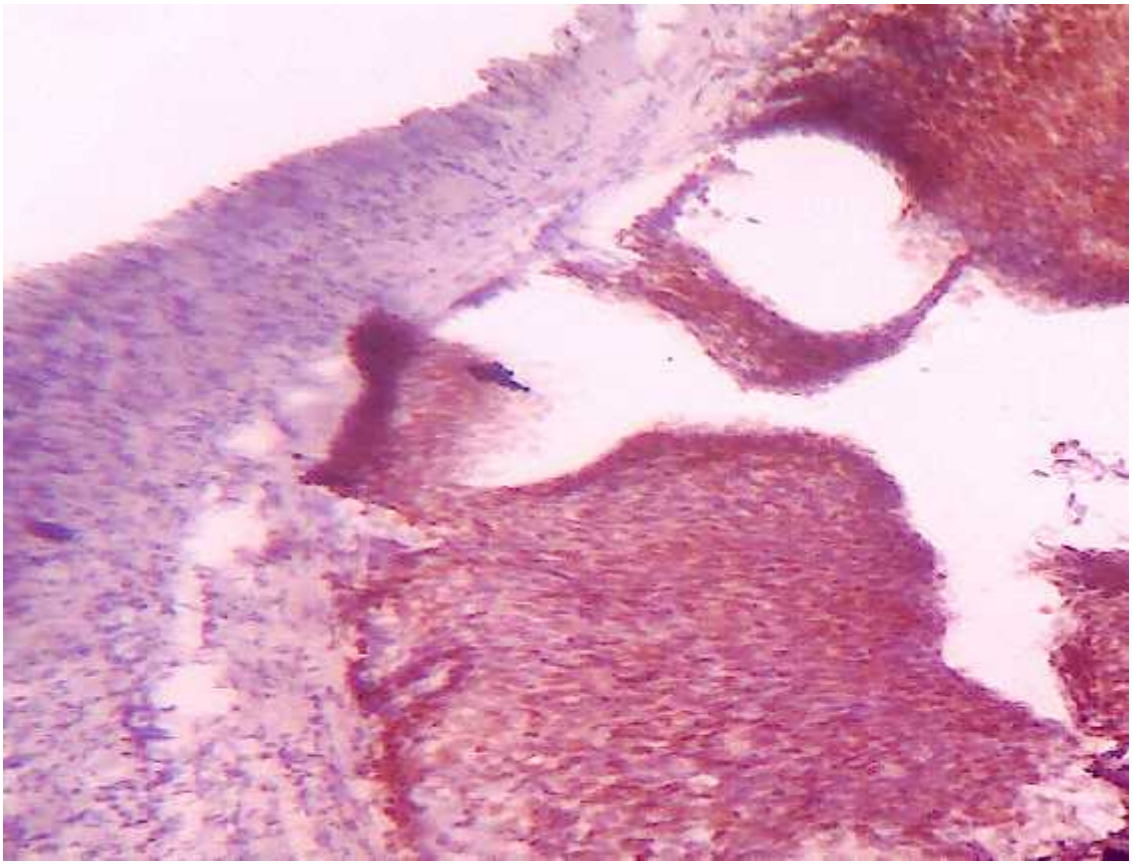


FIGURE 19: POSITIVE P63 EXPRESSION IN BASAL CELL HYPERPLASIA

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S.no	PATH NO	AGE IN YEARS	IP NO	SPECIMEN RECEIVED	USG FINDINGS	PSA VALUE	HPE	GLEASON SCORE	IHC RESULT-%EXPRESSION OF p63	P63 EXPRESSION	IHC RESULT-EXPRESSION OF AMACR	%positivity ofAMACR Expression
1	76/13	69	735	TURP	Enlarged prostate		HGPIN		55% BASAL CELLS POSITIVE & PIN FOCI POSITIVE	MODERATE	PIN FOCI NEGATIVE	
2	184/13	78	3501	TURP	Enlarged prostate		LGPIN		60% BASAL CELLS POSITIVE & PIN FOCI POSITIVE	MODERATE	PIN FOCI NEGATIVE	
3	209/13	62	3709	TURP	Enlarged prostate		LGPIN		>75%CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
4	611C/13	70	12603	TURP	Nodularprostate		LGPIN		>75% CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
5	735/13	67	17027	TURP	Nodularprostate		LGPIN		>75% CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
6	928/13	65	22828	TURP	Nodularprostate		LGPIN		>75%CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
7	1222/17	65	26842	TURP	Nodularprostate		LGPIN		>75%CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
8	2554/13	55	53110	TURP	Nodularprostate		LGPIN		>75% CELLS POSITIVE &PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
9	2579/13	70	52303	TURP	Enlarged prostate		LGPIN		85% CELLS POSITIVE& PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
10	2848/13	47	59321	TURP	Enlarged prostate		LGPIN		>75% CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
11	3023/13	78	60464	TURP	Enlarged prostate		LGPIN		50% CELLS POSITIVE & PIN FOCI POSITIVE	MODERATE	PIN FOCI NEGATIVE	
12	1208/14	50	21923	TURP	Nodularprostate		LGPIN		70% CELLS POSITIVE & PIN FOCI POSITIVE	MODERATE	PIN FOCI NEGATIVE	
13	1574/14	72	23946	TURP	Prostatomegaly		ADENOCARCINOMA	3+2=5	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	70%
14	1209/14	66	21923	TURP	Enlarged prostate		BASAL CELL HYPERPLASIA		60% BASAL CELLS POSITIVE	MODERATE	NEGATIVE	
15	1774/14	70	29868	TURP	Nodularprostate		LGPIN		>75% BASAL CELLS POSITIVE &PIN FOCI POSITIVE	STRONG	PIN FOCI NEGATIVE	
16	1923/14	55	32226	TURP	Enlarged prostate		LGPIN		<5% BASAL CELLS & PIN FOCI POSITIVE	MODERATE	PIN FOCI NEGATIVE	
17	2273/14	67	3835	TURP	Enlarged prostate		HGPIN		BASALCELLS POSITIVE IN PIN FOCI	MODERATE	PIN FOCI NEGATIVE	
18	1037/14	70	15820	TURP	Grade 2 prostatomegaly		ADENOCARCINOMA	4+3=7	TUMOUR CELLS-NEGATIVE	NEGATIVE	2+ POSITIVE	15%

19	1417/14	67	23928	TURP	Prostatomegaly		ADENOCARCINOMA	4+5=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	80%
20	1616/14	85	25264	TURP	Prostatomegaly		ADENOCARCINOMA	5+4=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	65%
21	2182/14	80	34722	TURP	Grade 4 prostatomegaly		ADENOCARCINOMA	4+3=7	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	90%
22	3875/14	70	68114	TURP	Prostatomegaly	7.6	ADENOCARCINOMA	3+2=5	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	60%
23	563/15	84	4717	TURP	Enlarged prostate		LGPIN		70% CELLS POSITIVE & PIN FOCI POSITIVE	MODERATE	NEGATIVE	
24	971/15	70	16715	TURP	Enlarged prostate		LGPIN		>75% CELLS POSITIVE &PIN FOCI POSITIVE	STRONG	NEGATIVE	
25	1534/15	67	26764	TURP	Enlarged prostate		LGPIN		>75% CELLS POSITIVE &PIN FOCI POSITIVE	STRONG	NEGATIVE	
26	1697/15	70	31386	TURP	Enlarged prostate		LGPIN		>75% CELLS POSITIVE & PIN FOCI POSITIVE	STRONG	NEGATIVE	
27	1700/15	60	30902	TURP	enlarged prostate		LGPIN		20% CELLS POSITIVE & PIN FOCI POSITIVE	MODERATE	NEGATIVE	
28	1049/15	68	17263	TURP	Grade 3 Prostatomegaly	9.4	ADENOCARCINOMA	2+3=5	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	85%
29	1163/15	61	20033	TURP	Prostatomegaly	8.6	ADENOCARCINOMA	2+3=5	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	100%
30	2257/15	54	42023	TURP	Grade 4 prostatomegaly	13.1	ADENOCARCINOMA	5+4=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	75%
31	2375/16	67	40994	TURP	Enlarged prostate		HGPIN		BASAL CELLS POSITIVE & PIN FOCI NEGATIVE	NEGATIVE	NEGATIVE	
32	2377/16	67	38428	TURP	Enlarged prostate		LGPIN		>75% CELLS POSITIVE &PIN FOCI POSITIVE	STRONG	NEGATIVE	
33	1354/16	80	24279	PROSTATE BIOPSY	Prostatomegaly	>100	ADENOCARCINOMA WITH PERINEURAL INVASION	5+5=10	TUMOUR CELLS-NEGATIVE	NEGATIVE	2+ POSITIVE	20%
34	1983/16	65	37321	PROSTATE BIOPSY	Prostatomegaly	26.4	ADENOCARCINOMA WITH PERINEURAL INVASION	4+4=8	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	90%
35	2083/16	75	38395	TRUCUT BIOPSY	Prostatomegaly	130	ADENOCARCINOMA	3+3=6	TUMOUR CELLS-NEGATIVE	NEGATIVE	1+ POSITIVE	5%
36	598/17	69	6420	TURP	Grade2 prostatomegaly	9	ADENOCARCINOMA	5+4=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	100%
37	2729/16	59	49164	PROSTATE BIOPSY	Grade3 prostatomegaly	8.1	SUGGESTIVE OF MALIGNANCY	2+3=5	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	60%
38	176/17	62	1823	PROSTATE BIOPSY	Prostatomegaly	49	ADENOCARCINOMA	5+4=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	65%

39	2411/17	55	44748	TURP	Prostatomegaly	12.6	ADENOCARCINOMA	5+5=10	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	95%
40	3977/16	60	75005	TURP	Enlarged prostate		ATROPHY		40 % BASAL CELLS POSITIVE	MODERATE	NEGATIVE	
41	4024/16	76	69396	TURP	Enlarged prostate		ATROPHY		45 % BASAL CELLS POSITIVE	MODERATE	NEGATIVE	
42	3288/16	57	45678	TURP	Prostatomegaly		ADENOSIS		>75% CELLS POSITIVE	STRONG	NEGATIVE	
43	3292/16	75	59672	TURP	Prostatomegaly		ADENOSIS		>75% CELLS POSITIVE	STRONG	NEGATIVE	
44	2744/13	55	57631	TURP	Enlarged prostate		BASAL CELL HYPERPLASIA		>75% BASAL CELLS POSITIVE	STRONG	>50% CELLS- 3+POSITIVE	
45	3290/16	75	58133	TURP	Enlarged prostate		CHRONIC PROSTATITIS		60% BASAL CELLS POSITIVE	MODERATE	NEGATIVE	
46	3289/16	65	46674	TURP	Enlarged prostate		CHRONIC PROSTATITIS		65% BASAL CELLS POSITIVE	MODERATE	NEGATIVE	
47	2714/17	74	47277	BIOPSY	Grade1 prostatomegaly	52.6	SUGGESTIVE Of PROSTATIC	3+2=5	TUMOUR CELLS NEGATIVE	NEGATIVE	3+ POSITIVE	80%
48	2715/17	60	49564	BIOPSY	Nodular prostate	8.7	SUGGESTIVEOF PROSTATIC ADENOCARCINOMA	3+3=6	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+ POSITIVE	100%
49	2531/17	62	46530	TURP	Grade 3 prostatomegaly	7.4	ADENOCARCINOMA	4+5=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	80%
50	2630/17	69	46525	TURP	Enlarged prostate with cystitis	9.1	ADENOCARCINOMA	5+4=9	TUMOUR CELLS-NEGATIVE	NEGATIVE	3+POSITIVE	90%
51	2632/17	74	47961	BIOPSY	Prostatomegaly	8.2	ADENOCARCINOMA	4+3=7	TUMOUR CELLS NEGATIVE	NEGATIVE	3+POSITIVE	85%
52	2492/16	65	40647	TURP	Prostatomegaly	13.1	ADENOCARCINOMA	4+4=8	TUMOUR CELLS NEGATIVE	NEGATIVE	3+POSITIVE	70%
53	3266/17	68	48624	TURP	Prostatomegaly		ADENOCARCINOMA	3+5=8	TUMOUR CELLS NEGATIVE	NEGATIVE	3+ POSITIVE	75%
54	3277/17	70	48923	BIOPSY	Prostatomegaly		ADENOCARCINOMA	5+3=8	TUMOUR CELLS NEGATIVE	NEGATIVE	2+ POSITIVE	45%