THE EXPRESSION AND DIAGNOSTIC UTILITY OF AMACR AND 34βE12 IN PROSTATIC LESIONS

Dissertation submitted in

Partial fulfilment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

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DECLARATION

I hereby declare that the dissertation entitled **THE EXPRESSION AND DIAGNOSTIC UTILITY OF AMACR AND 34β E 12 IN PROSTATIC LESIONS** was done by me in the Department of Pathology at Coimbatore Medical College and Hospital, Coimbatore during the period from August 2011 to July 2012 under the guidance and supervision of **Dr.A.Dhanalakshmi, M.D.,** Associate Professor, Department of Pathology, Coimbatore Medical College, Coimbatore. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree in Pathology. I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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GUIDE

Dr.A.Dhanalakshmi, M.D.,
Associate professor
Department of Pathology,
Coimbatore Medical College

Dr.M.Murthy,M.D.,
Professor and HOD
Department of Pathology,
Coimbatore Medical College

Dr.R.Vimala,M.D.,
Dean
Coimbatore Medical College

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : DR. L. ARTHI

Course : M.D. PATHOLOGY

Period of Study : AUGUST 2011 – JULY 2012

College : COIMBATORE MEDICAL COLLEGE

Dissertation Topic : EXPRESSION AND DIAGNOSTIC UTILITY OF 34 βE12 AND AMACR IN PROSTATIC LESIONS

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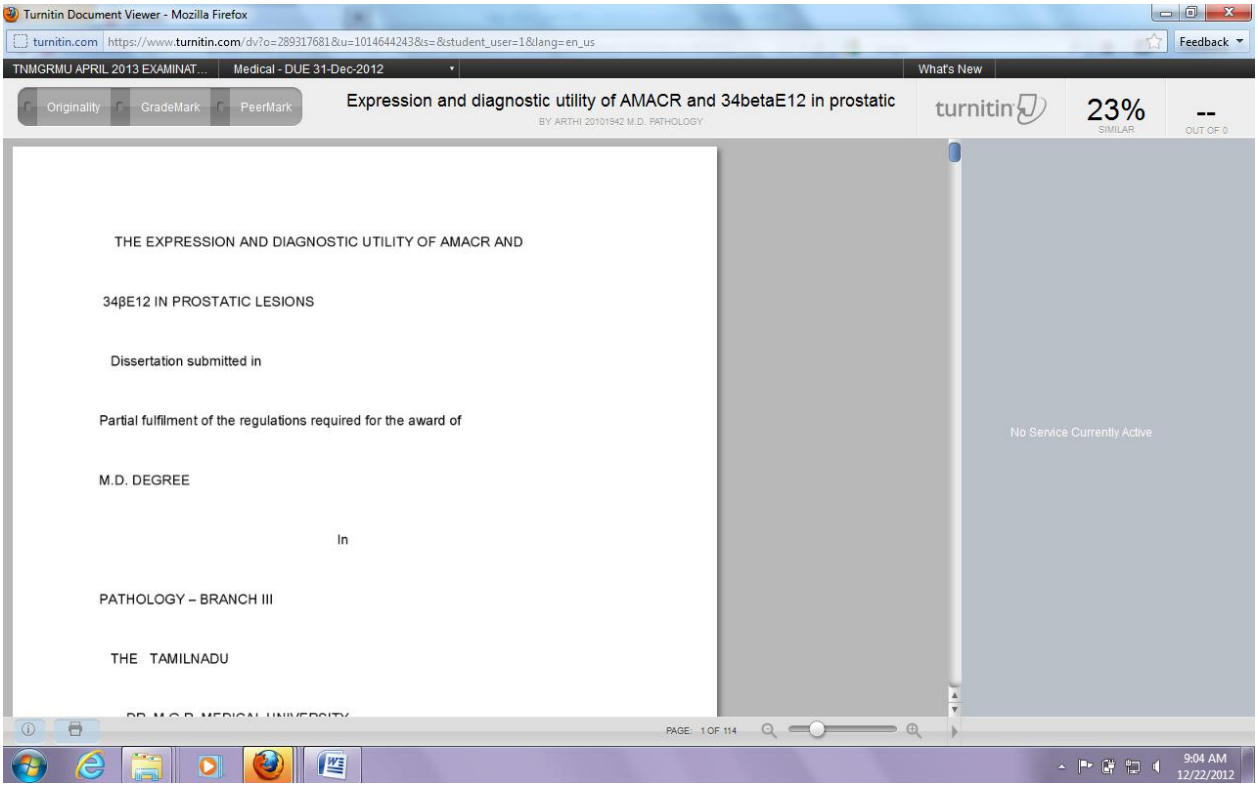

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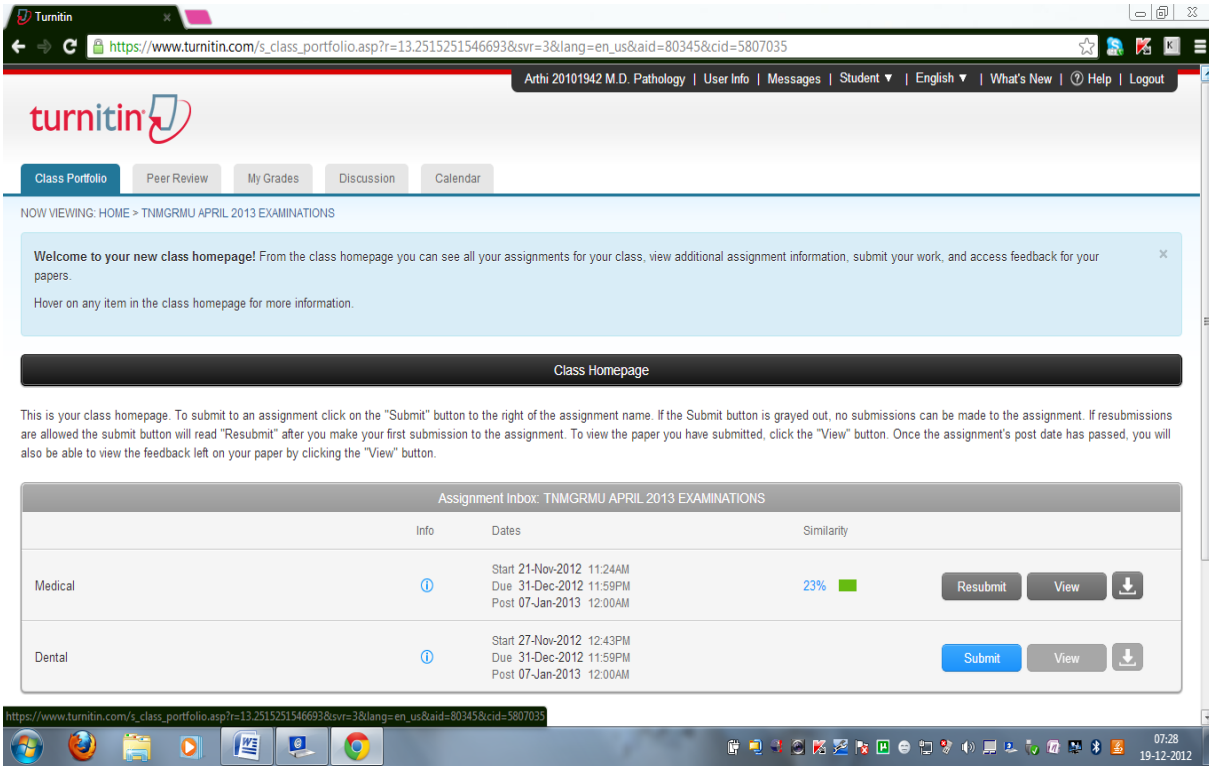
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LIST OF ABBREVATIONS

WHO	–	World Health Organization
BPH	–	Benign Prostatic Hyperplasia
PIN	–	Prostatic Intra Epithelial Neoplasia
ASAP	–	Atypical Small Acinar Proliferation
PC	–	Prostatic Carcinoma
PSA	–	Prostate Specific Antigen
PAP	–	Prostatic Alkaline Phosphatase
AMACR	–	α Methyl Acyl CoA Racemase
HMWCK	–	High Molecular Weight Cytokeratin
HPE	–	Histopathological Examination
IHC	–	Immunohistochemistry
DRE	–	Digital Rectal Examination
H&E	–	Hematoxylin And Eosin Staining

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INTRODUCTION

Across the world, Carcinoma of the Prostate is one of the most common malignancies, the accurate diagnosis of which is of major concern. In the list of malignancies excluding skin cancers, it comes second.[36]

Its incidence has steadily risen with time. This is attributed to the increased life span and also to the westernisation of lifestyle typified by diet with high calories and inadequate exercise to the body[14].

Serum PSA is increasingly being used as a screening tool. Consequent to it, prostate needle biopsies are increasingly performed in men. Increased prostate-specific antigen levels increases needle biopsies, for the exclusion of prostate cancer.

Prostatic needle biopsy is the preferred method. It has fewer side effects, and helps with accurate information regarding degree of tumor extension. The grade of tumor is also diagnosed with precision.

But, a needle biopsy presents problems. Only a small tissue amount is provided for microscopic examination. It is a difficult task to accurately diagnose small foci of prostate cancer for pathologists and to distinguish cancer from its benign mimickers. Small malignant infiltrating glands

graded as 6 on the Gleason score pose the volume of difficulty in prostatic specimens[30].

Only a few glands may be malignant, and they can be easily overlooked. To diagnose prostate cancer, no specific single histologic feature is sufficiently available. The combination of architectural and cytologic change gives the diagnostic clue.[30]

There are numerous benign mimickers posing as prostate cancer. These include benign conditions including atrophy, basal cell hyperplasia, small crowded glands and inflammatory atypia.[30]

Wrong diagnosis leads to serious issues, like radiation induced adverse effects, prostatectomies done unnecessarily because of falsely positive diagnosis. Also, falsely negative results cause delay in early effective treatment. Hence, definitive diagnosis with the available specimen is essential for the benefit of patients.[30]

Basal cells are noted in Benign glands. Prostate cancers do not contain basal cells. This helps in the diagnosis of specimens. Here comes the vital role of Immunohistochemistry. This is used by pathologists to diagnose suspicious lesions in small foci accurately. 34βE12 is a marker which is a high-molecular-weight cytokeratin, which takes positivity in benign glands. p63 is a newer basal cell marker.

The diagnosis of prostate adenocarcinoma is supported by the basal cells' absence. However high-molecular-weight cytokeratin and p63 are negative markers for prostatic carcinoma.

AMACR (α-Methyl Acyl CoA Racemase) is used in the diagnosis of prostate cancers as a positive marker with high sensitivity (76 – 100%) and high specificity(75 – 95%)[36]

So, the aim of this study is to study expression and use of immunohistochemical markers in various prostate biopsies. The role of HMWCK (34βE12) and AMACR (P504S) in diagnosis of prostate specimens is studied

AIM OF THE STUDY

To study the Expression and Diagnostic utility of Immunohistochemical markers AMACR and 34βE12 in various Prostatic lesions.

OBJECTIVES OF THE STUDY

1. To find out the incidence of various prostatic lesions in men
2. To study the clinical presentation of prostatic lesions
3. To study the sensitivity and specificity of AMACR and 34βE12
4. To study the expression of AMACR and 34βE12 in various prostatic lesions
5. To study the use of AMACR and 34βE12 in detecting limited samples of prostate .

REVIEW OF LITERATURE

Cancers of the Prostate account for the highest incidence of malignancies in men and is the second most common cause of morbidity. PSA used as a screening tool has resulted in Needle biopsies of the Prostate increasingly being performed. It has also increased the cases of difficult biopsies.

ANATOMY OF PROSTATE

Prostate is a functional coundit which allows urine to pass from urinary bladder to the urethra. It adds nutritional secretions to the sperm to form semen during ejaculation.[57]

Prostate is a tubulo alveolar gland located in true pelvis. Normal prostate in adults measures 4 x 3 x 2cms. Anatomically it is divided into glandular and nonglandular components.

Glandular components include Peripheral zone, Central zone, Transition zone and Periurethral gland zone.

Non glandular components include Anterior fibromuscular stroma, preprostatic sphincter, striated sphincter.

The normal adult prostate weighs approximately 30g and is funnel shaped. The prostate gland consists of concentric inner zone and outer

zone. Clinically detectable carcinomas affect the outer zone and BPH affects the inner aspect of the gland.[57]

It receives arterial supply from inferior vesical and middle rectal arteries, branches of internal iliac arteries. Prostatic venous plexus drains into internal iliac vein. Primary lymphatics drain into regional lymphnodes.

HISTOLOGY

The epithelial cells of prostate are Transitional, Secretory, Basal cell, and Neuroendocrine cell.

TYPES OF SPECIMEN

Needle biopsies[58]

TRUS guided core biopsies (Trans Rectal UltraSound guided) for diagnosis of prostate cancer is the Gold standard method now.

The standard protocol says that lesions identified on ultrasound or digital rectal examination have to be correlated with systematic biopsies. The bilateral apex, mid and base regions are included in the sextant protocol.

The center of each prostate's half is aimed at in Sextant biopsies. It is ensured that it is of equal distance, both from the lateral edge and the

midline. The dorsolateral region is the most common site of prostate cancer.

Many modifications of the said biopsy protocol have been suggested. Studies conducted recently say that protocols containing ten to thirteen systematic biopsies are superior with detection of prostatic cancers in about 35% of cases. This is better than the existent sextant protocol done traditionally.

Handling of needle biopsies[58].

The identification of the different glandular areas in biopsies of the Prostate is essential. The location of the biopsy site is to be known because, between base and apex, there is difference in the standard histology.

The clinician considers the extent and location when selecting options of treatment.

Trans Urethral Resection of Prostate(TURP)[58]

TURP detected Cancers are frequently transition zone tumours. when large, they may originate from the peripheral zone. More than 100g tissue may be present in a TURP sample. It is frequently necessary, for histological examination, to select a limited tissue amount.

Pathologic features

Gross examination of TURP specimens is of little significance, because benign processes can mimic prostate carcinoma.

Radical prostatectomies

Grossly identifiable prostate carcinoma is typically of higher grade and stage and larger diameter. In contrast to the adjacent normal prostate tissue, which appears tan and spongy, grossly evident prostate carcinoma is solid and firm. It ranges between white grey and yellow orange in color.

Prostate carcinomas discovered by PSA screening are less visible grossly, these cancers are often small(<5mm) and of lower grade and stage.

Patterns of spread and metastasis[58,59]:

Local extraprostatic extension typically occurs anteriorly for transition zone cancer ,posteriorly and posterolaterally for peripheral zone cancer. Prostate carcinoma can also spread superiorly into the bladder neck. Rarely, it can penetrate Deninvillier's fascia posteriorly to involve the rectum. Metastatic prostate carcinoma most commonly causes node enlargement regionally and affects axial skeleton bones and pelvis.

Gleason Grading System

Gleason grading system, designed by Dr. Donald Gleason, is the predominant grading system for prostate carcinoma. Architecture of the glands forms the basis; evaluation of nuclear atypia is not done. With decreasing glandular differentiation, 5 histologic patterns are defined. To get the Gleason score the first and second common patterns (in order of prevalence) are added. If a prostate carcinoma only has one pattern, doubling of the pattern is done to get the Gleason score. Along with the Gleason score, reporting of primary and secondary patterns is to be done.

Recently, several modifications have been made to the original Gleason grading scheme in an effort to adapt this grading system to present-day practice in a similar way. The modified Gleason grading system is given below. The significant changes include a stricter definition of Gleason pattern 3 cribriform glands, and grading ill-defined glands with poorly formed glandular lumina as pattern 4.

Gleason's microscopic grading system of prostatic carcinoma[60]

Grade 1 Separate, single , uniform glands in closely packed masses with

usually rounded, definite, edge limiting the areas of tumor

Grade 2 Nodular, separate, single, slightly less uniform glands, with less sharp edge(loosely packed)

Grade 3a Separate, single, much more variable glands, usually irregularly separated, may be closely packed but poorly defined, ragged edge

Grade 3b Like 3a, but tiny cell clusters or very small glands

Grade 3c Smoothly and sharply circumscribed masses of loose cribriform or papillary tumor.

Grade 4a Raggedly infiltrating, raggedly outlined glandular tumor in a fused manner.

Grade 4b Like 4a,with pale large (hypernephroid) cells

Grade 5a Rounded, sharply circumscribed masses usually with central necrosis; of almost solid cribriform tumor.

Grade 5b Ragged masses of anaplastic carcinoma with only vacuoles or enough gland formation for identification as adenocarcinoma

Gleason Pattern 1.[58]

This pattern is composed of a well-circumscribed nodule of tightly packed, uniform but separate glands. There is no or minimal infiltration

into adjacent tissue. The glands are similar in size or of intermediate size and shape. It is a very rare pattern. It is usually present in transition zone of cancers of prostate. The Gleason grading system with new modifications states that Gleason score of 2(1 + 1) is a grade that should be reported very rarely. It is commonly, only a minor component of carcinoma specimen.

Gleason Pattern 2.

There is a less well-circumscribed nodule of medium-sized glands, with some degree of variation in size and shape and looser arrangement. Gleason pattern 2 carcinoma is commonly seen in the transition zone. There can be minimal invasion of carcinomatous glands into adjacent tissue. Cytoplasm, in the grading by Gleason system, is not evaluated. But in Gleason patterns 1 and 2, the glands are pale-clear and abundant.

Gleason Pattern 3

This is the pattern with the highest frequency. The carcinomatous glands commonly infiltrate between the surrounding benign glands. They are commonly angular and vary in shape and size. Typical patterns are seen with small glands. When they are large, they have a cribriform or papillary configuration. The Gleason pattern 3 cribriform glands have smooth, round contours, in contrast to the large, irregular, pattern 4 of

Gleason, cribriform glands.

Gleason Pattern 4

In this pattern, the glands are poorly formed, cribriform or fused. Fused glands comprise a group of glands which are not separated by any stroma. Cribriform glands in pattern 4 are large and have irregular contour and jagged edges. The intraluminal cellular proliferation spans the entire diameter of the lumen. Poorly formed glands still have glandular configuration, but they have ill-formed glandular lumina. The hypernephromatoid pattern is an uncommon variant composed of fused glands showing very pale or clear cytoplasm.

Gleason Pattern 5

Cancer cells lack glandular differentiation. They manifest as strands, solid sheets, or single cells infiltrating the stroma. Comedo necrosis may be seen.

Tertiary pattern

Tertiary Gleason pattern refers to a minor pattern occupying less than 5% of the tumor volume. In radical prostatectomy, when high-grade tertiary pattern is seen, it affects the prognosis worsely. A bad prognosis is seen in a tertiary pattern five (Gleason score 4 + 3 = 7)

prostate carcinoma. In comparison, where a tertiary high-grade component is not seen, the same lesion has a relatively better prognosis. But, the prognosis isn't as bad as that of a 4 + 5 = 9 carcinoma. In prostate needle biopsies that harbor three patterns when the worst pattern is the least common, the highest pattern should be incorporated as the secondary pattern.

Grading of morphological variants

Morphologic variants of prostate carcinoma are uncommon and often are mixed with ordinary prostate carcinoma. Grading such variants should be based on the underlying cancer glandular architecture. In general, ductal adenocarcinoma and mucinous adenocarcinoma behave more aggressively, comparable to Gleason score 8 acinar cancers. Signet-ring cell and sarcomatoid variants are even more aggressive, comparable to Gleason score 9 or 10. On the other hand, squamous cell cancers, cancers of the urothelium, small cell cancers, and basaloid/adenoid cystic carcinoma are not assigned a Gleason grade.

Prostate carcinoma treated with hormonal ablation or radiation can appear artefactually to of higher Gleason grade.Therefore, Gleason grade should not be assigned to such cases. If no effect of the therapy is evident, a Gleason grade can be assigned.

Prostate carcinoma displays a remarkable intratumoral grade heterogeneity; therefore, the biopsy Gleason grade may in some cases represent undergrading or overgrading compared with the radical prostatectomy. Nevertheless, the concordance between needle biopsy and prostatectomy Gleason scores is mostly within 1 Gleason score.

Multiple studies have demonstrated that the Gleason grade is currently a very powerful prognostic indicator for cancers of the prostate. In radical prostatectomies, there is a good correlation with all the significant pathologic criteria, and with prognosis secondary to radical prostatectomy or radiation therapy. The distinction between Gleason scores 6 and 7 is difficult as well as important.Gleason 7 prostate carcinoma behaves significantly worse than Gleason 6 cancer but better than Gleason score 8 to 10 cancer.

Prognosis

The following Gleason scores combinations fall into similar prognosis groups:

Gleason score 2 to 4 - well-differentiated

Gleason score 5 to 6 - moderately differentiated

Gleason score 7 - moderately to poorly differentiated

Gleason score 8 to 10 - poorly differentiated.

The use of many names to predict progression of disease (after radiotherapy and surgery) and pathologic stage stress the significance of Gleason grade. These nomograms, including Partin tables and Kattan nomograms, use preoperative biopsy Gleason score, tumor extent, clinical stage, and serum PSA to predict the risk of invasion of seminal vesicle, adjacent extension, and nodal metastasis and probability of disease recurrence after treatment.

Prostate cancer diagnosis is usually made using histological, traditional parameters, not with any single diagnostic feature. They include nuclear features, tissue architecture and other features. In needle biopsies, tissue diagnosis of prostatic carcinoma is difficult. This is because of either the many benign mimickers of malignancy or a small focus of cancer.

Microscopic Findings

Prostate cancer has a collection of architectural, cytoplasmic, nuclear features[36].

Prostate Carcinoma – Pathologic Features

Gross Findings

- Firm, solid, white grey to yellow orange in contrast to tan, Spongy benign prostatic tissue

- PSA-detected cancer often not grossly visible

MICROSCOPIC FINDINGS OF PROSTATIC CARCINOMA

- **Architecture features**:

  Haphazard glandular arrangement; infiltrative growth; less differentiated glands with cribriform, fused glands, cords, sheets, or single tumor cell. Typically small glands with straight luminal border

- **Cytologic features**:

  Pale to amphophilic cytoplasm; no lipofuscin pigment

- **Nuclear features**:

  Enlargement, hyperchromasia, variably prominent nucleoli.

- **Cancer-specific features:**

  Mucinous fibroplasias (collagenous mironodules); glomeruloid formation; perineural invasion.

Histologic Variants of Prostate Carcinoma

- Ductal adenocarcinoma
- Atrophic carcinoma
- Pseudohyperplastic carcinoma
- Foamy gland carcinoma
- Mucinous carcinoma
- Small cell carcinoma
- Signet-ring carcinoma
- Squamous cell cancer
- Sarcomatoid carcinoma
- Urothelial carcinoma
- Basaloid carcinoma

Differential Diagnosis

- Normal prostatic/nonprostatic tissue (verumontanum glands, Cowper's glands, paraganglia, seminal vesicle/ejaculatory duct, mesonephric remnants)

- Benign conditions (atrophy, partial atrophy, postatrophic hyperplasia, urothelial/squamous metaplasia, basal cell hyperplasia, adenosis, sclerosing adenosis, inflammation, nonspecific granulomatous prostatitis, BPH)

- HGPIN

- Treatment effect (radiation atypia)

The differential diagnosis of prostate carcinoma is complex. In many instances, the differential is with normal prostatic and nonprostatic structures, including seminal vesicles/ejaculatory duct epithelium, Cowper's gland, paraganglia, and mesonephric duct remnants. A wide variety of benign pathologic processes, such as inflammation, atrophy (simple atrophy, partial atrophy, and postatrophic hyperplasia), metaplasia (urothelial, squamous, and mucinous), basal cell hyperplasia, BPH, and radiation and hormonal treatment effects, can simulate prostate carcinoma to varying degrees. The prostate gland can rarely be involved by primary urothelial, small cell, mucinous, and signet-ring cell carcinoma. However, such a diagnosis should be made only after a metastasis from other sites is diligently excluded.

- On the other hand, prostate carcinoma can also mimic benign

conditions. For example, a well-differentiated Gleason score 2 to 4 prostate carcinoma should always be differentiated from adenosis. Cribriform prostate carcinoma should be distinguished from benign cribriform hyperplasia or cribriform HGPIN. Atrophic and foamy prostatic carcinomas may be confused with benign atrophy and Xanthoma, respectively. Pseudohyperplastic prostate carcinoma shares some architectural features with BPH, although the former invariably has significant nuclear atypia. Careful evaluation of the architectural and cytologic features and prudent use of AMACR and basal cell markers will lead to a correct diagnosis.

- A**typical small acinar proliferation (ASAP)**[1,35]

Sometimes a glandular focus or gland raises suspicion of prostate carcinoma, yet a definitive cancer diagnosis cannot be established due to the lack of sufficient architectural and cytologic atypia. The terms “atypical small acinar proliferation (ASAP)” and “focal atypical glands” have been used. Unlike HGPIN or prostate carcinoma, ASAP is a diagnostic term rather than a defined disease entity. It encompasses such lesions as HGPIN, reactive atypia, benign mimickers of prostate carcinoma and cases of focal cancer. ASAP found in needle biopsy denotes a high risk (~ 50%) of detecting prostate carcinoma in subsequent biopsies.

The following are the benign mimickers of prostate,

BENIGN MIMICKERS OF PROSTATE [2,9,36]

1. Adenosis,
2. Atrophy,
3. Partial atrophy,
4. Clear cell hyperplasia,
5. Basal cell hyperplasia,
6. Post atrophic hyperplasia,
7. Mesonephric hyperplasia,
8. Nephrogenic adenoma,
9. Seminal vesicle & Cowpers glands.

The following are the features help to differentiate prostate cancer from its benign mimickers.

Architecture

Gland-forming prostate carcinomas are more crowded than benign glands and typically exhibit a haphazard growth pattern, with malignant glands separated irregularly by bundles of smooth muscle and

perpendicular orientation to each other. They also display "infiltrative growth pattern," with malignant glands situated between or flanking benign glands. When prostate carcinoma becomes less differentiated, it loses glandular differentiation and forms cribriform structures, fused glands, poorly delineated glands, solid sheets or cords, or even single tumor cells.

Cytoplasm.

In contrast to benign glands with irregular and undulating luminal borders, prostate carcinoma glands are smaller and have straight luminal borders. They may have arnphophilic, or darker, cytoplasm that is evident even at low magnification. However, low-grade prostate carcinoma often has pale-clear cytoplasm, indistinct from benign glands. Prostate carcinoma typically lacks lipofuscin pigment.

Nuclei

Typically, prostate cancer shows nuclear features which is distinct from its surrounding benign glands. Some prostate cancers have hyperchromatic and enlarged nuclei and do not show prominent nucleoli. Apoptotic bodies and Mitoses are more frequent in prostate cancer, but they are infrequent in benign glands..

Intraluminal content.

Crystalloids - dense eosinophilic, crystal-like structures found within the glandular lumina - are found more commonly in carcinoma. However, they are also frequently found in adenosis, a benign condition that mimics low- grade prostate carcinoma. Intraluminal pink, acellular, dense secretions mucin are additional findings seen preferentially in prostate carcinoma. In contrast, corpora arnylacea are commoner in glands of benign nature and are not commonly seen in prostate cancer.

Stroma.

Ordinary prostate carcinoma does not elicit a desmoplastic response or stromal inflammatory reaction. Ductal adenocarcinoma of prostatic origin, however, may induce such stromal reactions with fibrosis containing hemosiderin- laden macrophages.

Cancer-specific features.

There are 3 histologic features diagnostic of prostate cancer, because they are not seen in benign glands. Mucinous fibroplasia, or collagenous micronodules are seen within or adjacent to cancer glands. It is frequently surrounded by a crescentic space, resembling glomerulus of kidney . The pathognomonic feature of prostate cancer is Perineural invasion with completely or near-completely encircling the cancer glands. The circumferential expansion of benign glands about a

nerve has not been mentioned.

Prostatic biopsies sometimes show small foci of proliferative atypical acini. They show some features diagnostic of adenocarcinoma, but not all. A variety of terms like suspicious, suspicious - but not diagnostic of malignancy, atypical focus and atypical small acinar proliferation (ASAP) have been used to describe them. ASAP is the most accepted term of these[36].

ASAP include lesions such as BCH, atrophy, HGPIN, atypical adenomatous hyperplasia, reactive atypia. The list also includes cases which in retrospect display minute carcinoma but contain inadequate architectural or cytological atypia for concrete diagnosis of carcinoma. The chance of occurence of prostate carcinoma on subsequent biopsy in people with ASAP diagnosis on initial biopsy varies between 21% and 49%[36].

Among the prostate cancer - benign mimickers, partial atrophy and atrophy are often wrongly diagnosed as prostate cancer.[37,45] Maintenance of lobular architecture , absence of nucleoli, uniformity of cytology, and presence of a layer of basal cells are some of the criteria against the diagnosis of cancer.

Prostatic partial atrophy:

It is the commonest benign mimicker of prostatic carcinoma. It has a diffuse growth pattern with features of glandular crowding and is frequently lobular to disorganized. Glands often have undulating luminal surfaces with papillary infoldings. Cytoplasm is pale-staining. Nuclei are more spaced than in typical atrophy. In areas, nuclei reach the full height of the cells and are usually benign-appearing. On occasion, prominent nucleoli may be present, although they are typically not as large as seen in prostatic adenocarcinoma. It is often difficult to identify basal cells in partial atrophy on H&E-stained sections. Therefore, it is commonly confused with prostatic adenocarcinoma.

Partial atrophy[36]:

The pattern is lobular to disorganized with glandular crowding . The cells are paler and angulated. Nuclei are sometimes large with prominent nucleoli. Pseudo nerve invasion and absent/ patchy basal cells make it to mimic adenocarcinoma.

Adenosis

This contain severely crowded small glands, with more benign glands admixed with them. Glands show pale to clear cytoplasm. The

nuclei do not have a very prominent nucleoli. There is a continuous or fragmented basal cell layer.

Basal cell hyperplasia :

This mimicks prostate adenocarcinoma commonly in needle biopsies. It is commonly visualised in the transition zone. It shows nodular expansion of round uniform glands seen with a clear cytoplasm. Seen are basal cells in multiple layers. With scant cytoplasm, they are dark; and show oval or round hyperchromatic spindled nuclei. They are usually negative for racemase and with basal cell markers, stain positively[36].

Nephrogenic adenoma: [53]

It can uncommonly affect the prostatic urethra and is a metaplastic, benign response to injury by the urothelium. Extension into the underlying fibromuscular prostatic stroma by small tubules of nephrogenic adenoma can cause a wrong diagnosis of prostate cancer of low grade.

Cowpers glands:

It can look similar to either foamy gland carcinoma or low grade adenocarcinoma. A bland cytological picture is seen in either.

Seminal vesicle :

Sometimes, seminal vesicles seen on biopsy specimens can mimic prostate cancer. It is seen with the seminal vesicle's complex papillary architecture visualised at the tissue core's edge with surrounding small glandular clusters. A leading point is lipofusin granules in abundance in the seminal vesicle's cytoplasm.

PIN: Prostatic Intraepithelial Neoplasia[32]:

PIN is the abnormal proliferation of foci of premalignancy, with carcinoma in situ and cellular dysplasia; there is no stromal invasion within the ductules, large acini and prostatic ducts.

The prostate's peripheral zone is the region where most of prostate cancers occur. It is also the commonest site for PIN. PIN is seen commonly in the peripheral zone, displaying multicentricity.

The commonest preinvasive stage of prostate cancer is Prostatic Intraepithelial Neoplasia. PIN has a significantly high predictive value as an indicator for prostate cancer. The diagnosis needs a repeat biopsy to rule out subsequent or concurrent invasive cancer.

There is no significant elevation of serum PSA - prostate-specific antigen in PIN. Neither is there any in their derivatives. Present imaging

methods such as ultrasound cannot detect PIN. Biopsy is the only available detection method. Many PIN patients, within 10 years, will go on to develop cancer. Androgen deprivation therapy has, in chemoprevention, a significant role. It reduces the extent and prevalence of PIN.

Grading[26,56]:

Previously graded from 1 to 3, Prostatic intraepithelial neoplasia currently recommends 2 grades of PIN (low & high grade). Low-grade PIN was Grade 1, high-grade PIN included grades 2 and 3. Presently, without qualification, 'PIN' means only high-grade PIN. The important distinguishing feature between high and low grade PIN is, rather than architecture, the nuclear appearance.

The earliest accepted stage in carcinogenesis is High-grade prostatic intraepithelial neoplasia (PIN). It has most of the biochemical, phenotypic and genetic changes of cancer. PIN doesn't invade the acini's basement membrane.

4 main patterns in high-grade PIN exist:

1. Tufting

2. Cribiform

3. Flat /Atrophic.

4. Micropapillary

Among this, tufting pattern is the most common pattern[36], it presents in about 97% of cases. According to a report, a higher coexistent cancer risk is noted with the cribriform pattern. High-grade PIN has no clinically known significant differences between architectural patterns.

Other unusual patterns:

These include Foamy gland pattern, small-cell neuroendocrine pattern and signet ring cell pattern.

The earliest evidence of cancer is early invasion of stroma. It occurs with high-grade PIN at sites of basal cell disruption and acinar outpouching in acini. Nearly 2% PIN show such microinvasion and is seen with equal frequency in all architectural patterns.

PIN Differential diagnosis

PIN's differential diagnosis includes

1. Lobular atrophy

2. Post atrophic hyperplasia

3. Atypical basal cell hyperplasia

4. Cribriform hyperplasia

5. Metaplastic changes

The most common benign lesions mimicking HGPIN are basal cell hyperplasia and cribriform hyperplasia.

PIN was often diagnosed as adenocarcinoma as showed in a study from the Mayo Clinic files of transurethral resections from 1960 to 1970.

Likewise, clusters of PIN are wrongly diagnosed as cancer in prostate fine-needle aspiration.

In diagnostic pathology, the detection of limited prostate cancer is a difficult challenge. Differences between atypical glands and benign glands in cytoplasmic features, nuclear features and intraluminal contents are to be known. This assists in diagnosing on needle biopsy, small foci of atypical glands..

In the diagnosis of limited adenocarcinoma, Immunohistochemistry in prostate needle biopsies plays a vital role. High molecular weight cytokeratin and p63 are basal cell markers which stain positively in basal cells. They are not seen in carcinoma of the prostate. But there are problems in specificity and sensitivity in immunohistochemistry studies.

Features favouring the diagnosis of limited prostate adenocarcinoma[28,43]

Diagnostic of cancer

Mucinous fibroplasia

Glomerulations

Perineural invasion

Favoring cancer

Nuclear Hyperchromasia

Prominent nucleoli

Nuclear Enlargement

Cytoplasmic Amphophilia

Straight, even luminal borders

Mitotic figures

Basophilic mucinous secretions

Intraluminal Pink dense secretions

Crystalloids

Features against the diagnosis of limited adenocarcinoma

Inflammation

Adjacent PIN

Small glands merging with benign glands

Tangential section or outpouching of PIN

Atrophic features

CARCINOMAS MIMICKING BENIGN GLANDS[22,32]

Between 5% and 10% of prostate cancers are histologic variants. They are consistently seen related with acinar prostate cancer. The histological variants, several times differ from cancer in clinical, immunophenotypic, ultra structural and genetic features. They also differ in their therapeutic approach and prognosis.

Some carcinomas resemble benign prostate glands in their architectural pattern similar to benign mimickers of prostate cancer. They may not be recognized as carcinomas.They are :

1. Foamy gland cancers
2. Peudohyperplastic prostatic cancers
3. Atropic prostatic cancers

1.Foamy gland cancer

Cancer cells have abundant foamy or "xanthoma"-like cytoplasm. Even though the cytoplasm has a xanthomatous appearance, it contains empty vacuoles rather than lipid. There is a very low nuclear/cytoplasmic ratio. In foamy gland prostate cancer cells nuclei are typically hyperchromatic and small.. The diagnosis of foamy gland prostate carcinoma is based on its architectural pattern of infiltrative and/or crowded glands, abundant foamy cytoplasm, and frequent pink, dense, acellular intraluminal secretions. Even with its bland cytology, most of the cases have Gleason score 6 or greater. It is consequently, reported as a intermediate-grade carcinoma.

The diagnosis is aided by its architectural pattern of infiltrative glands, foamy, abundant cytoplasm, and frequently present acellular pink secretion. Prominent nucleoli and nuclear enlargement, which are the common features of adenocarcinoma, are often not seen. This makes this lesion difficult to diagnose as carcinoma.

Nuclei occupy only 10% of the cell height in foamy gland carcinoma because of the copious cytoplasm. Typically, the nuclei in foamy gland carcinoma are round, small, and densely hyperchromatic. They are often rounder than the nuclei in benign prostatic secretory cells.

2. Atrophic prostate cancers

They are uncommon and may be seen as a benign lesion on needle biopsy. The cancer glands may be confused with benign atrophy because they have scant cytoplasm. The diagnosis is based on a number of features. They are these carcinomas displays an infiltrative growth pattern, with atrophic glands with mixture of larger benign glands. But, benign atrophy usually has a lobulated pattern. Atrophic prostate carcinoma has significant cytologic atypia, nuclear pleomorphism and prominent nucleoli.At last, atrophic prostate carcinoma is frequently intermixed with non atrophic ordinary prostate carcinoma.

3.Pseudohyperplastic prostate cancer

Resembling benign prostatic glands, pseudohyperplastic prostate carcinoma glands are large with branching and papillary infoldings[19,20]. However, the malignant glands are much more closely packed than benign glands, and they display malignant nuclear features typical of prostate carcinoma. The diagnosis of pseudohyper-plastic prostate carcinoma on needle biopsies is often difficult and requires immunohistochemistry. This confirms that basal cells are absent. Pseudohyperplastic prostate carcinoma, inspite of its benign appearance, can be seen with typical intermediate-grade cancer.

Aggressive behavior can be noted.

When composed of severely dilated glands with abundant cytoplasm, a pseudohyperplastic adenocarcinoma variant may be difficult to diagnose as malignant. It can be identified by numerous large glands with abundant cytoplasm, with straight even luminal borders that are almost back-to-back. Cytologic atypia in some of these specimens also distinguishes them.

Atrophic adenocarcinoma of the prostate and Pseudohyperplastic adenocarcinoma are less commonly (62 – 77%) positive for AMACR.[22]

Mucinous (Colloid) Carcinoma.

Colloid carcinoma is defined as a cancer in which 25 % or more of the tumor consists of abundant extracellular mucin. Prostate carcinoma with less than 25 % mucinous component should be classified as having mucinous features. Prostate cancer with intraluminal mucin without extracellular mucin is not considered as mucinous prostate carcinoma. The average age for colloid prostate carcinoma is similar to that for the ordinary prostate carcinoma, even though the clinical staging at presentation is frequently advanced, or metastatic disease. Microscopically, tumor cells float in lakes of extracellular mucin that are sharply demarcated from the

stroma.Tumor cells are arranged in cribriform pattern, cords, strands, acini, or tubules. Cytologically, they appear bland with infrequent prominent nucleoli.

Signet-Ring-Like Carcinoma[35].

Defined as 25 % or more of tumor mass consisting of signet-ring-appearing cells, this histologic variant is a rare entity with anaggressive clinical course. Microscopically, the signet ring-like tumor cells display displacement of nuclei and clear cytoplasmic vacuolar indentation. In majority of cases, these vacuoles contain lipid rather than mucin, as with true signet cells. The cancer cells grow as single cells, in small clusters and in sheets. They are invariably mixed with ordinary acinar prostate carcinoma components. PSAP and PSA Immunostains are positive in most cases. Stains for CK7, CK20, and HMWCK are negative in all cases. Before establishing a diagnosis of prostatic signet-ring carcinoma, a metastasis from other anatomic sites, including stomach, lung, colon, and pancreaticobiliary system, must be excluded. On the other hand, a prostatic signet- ring carcinoma should be considered when one encounters a signet-ring-cell carcinoma of unknown primary, especially if mucin stains are negative.

Sarcomatoid Carcinoma (Carcinosarcoma).

Sarcomatoid prostate carcinoma shows both malignant spindle cell and epithelial elements. It may be a de novo diagnosis, or patients may have a past history of prostate carcinoma post hormonal or radiation ablation treatment or both. Serum Prostate Specific Antigen is often normal in many cases, despite the frequent presence of nodal and distant metastases. The 5-year survival rate is less than 40%. Histologically, sarcomatoid prostate carcinoma is biphasic, with variable Gleason patterns seen in the gland component and a sarcomatoid component often exhibiting nondescript malignant spindle cell proliferation. Specific mesenchymal differentiation can also be present, including osteosarcoma, chondrosarcoma, and rhabdomyosarcoma. Immunohistochemically, the epithelial elements are positive for PSA and/or pancytokeratins, whereas the sarcomatoid elements react with corresponding mesenchymal differentiation markers and express cytokeratins in a variable manner.

The reasons why the small focus in needle biopsies may not be conclusive of cancer are:

1. Decreased number of minimally atypical glands

2. High-grade PIN

3. Difficuty in ruling out adenosis

4. Difficulty in differentiating atrophic cancers from atrophy

5. Inflammation associated causing reactive atypia

6. Distortion of tissue by crush artifact

Prostate-specific Antigen (PSA)

It is an important screening method for the detection of Prostatic cancers. Normal PSA levels are 2 -4 ng/ml. It increases with age. The following table shows the age specific reference values.

IMMUNOHISTOCHEMISTRY

Prostate-specific Antigen (PSA) [58,59]

It is detected in secretory cells of benign prostate glands in all anatomic zones, but not in seminal vesicle/ejaculatory duct epithelium, basal cells, or prostatic urothelial cells. Most prostate carcinomas also express PAS, although there is considerable intratumoral and intertumoral heterogeneity, and the expression is decreased in a minority of high-grade prostate carcinoma. After androgen deprivation and radiotherapy, some cancers can lose PSA expression. PSA immunoreactivity can be detected to variable degrees in some nonprostatic tissues and tumors, including urethral and periurethral glands, cystitis cystic and glandularis, urachal remnants,

bladder adenocarcinoma, and extramammary Paget's disease of the penis.

Cytokeratins[58]

Basal and benign secretory prostatic cells are immunoreactive for antibodies to low-molecular-weight and broad-spectrum cytokeratins (CKs). Negative staining for both CK7 and CK20, which is typical of prostate cancer, is useful to differentiate prostate carcinoma from urothelial carcinoma, which is typically positive for both markers.

Basal Cell Marker[36,54]

HMWCK is expressed not by secretary cells, only by prostate basal cells. It is identified by 34βE12 antibody clone. The clone identifies CK1, CK5, CK10, and CK14, or the antibody cocktail that recognizes CK5 and CK6. Basal cell layer is invariably not seen in Prostate carcinoma. Thus it is negative for HMWCK. A diagnosis of prostate cancer is supported by the absence of a basal lining. It is shown by lack of immunostain for HMWCK

However, prostate carcinoma can occasionally contain sparse tumor cells positive for 34βE12. They may not be in a basal cell distribution, especially after radiation or hormonal therapy. Spread of

prostate cancer intraductally or benign entrapped glands may also be mistaken as residual cells in prostate carcinoma. Conversely, some benign conditions, including partial atrophy and adenosis (atypical adenomatous hyperplasia) may at times have an absent or discontinuous basal lining of cells.

p63[59]

p63 is a nuclear protein expressed in pseudostratified epithelia in their basal cells. It has similar diagnostic utility and pitfalls as HMWCK. Only very rarely, it shows variability in staining. This is particularly seen in TURP specimens affected by cautery artifact. Thus it is easily interpreted due to its sharp and strong nuclear staining.

α-Methylacyl-Coenzyme A Racemase.[36,54,59]

AMACR is an enzyme involved in the intermediates of bile acid metabolism and metabolism of fatty acids (branched-chain). It is overexpressed in the most of prostate cancers. Because of its intratumoral heterogenous patterns of expression, in only eighty percent of cancers, AMACR is positive. Numerous prostate carcinoma histologic variants, such as atrophic, foamy gland, and psedohyperplastic prostate carcinoma, exhibit decreased expression of

AMACR. It is not completely specific for prostate carcinoma. This is due to the reason that it is present in HGPIN (90%), partially atrophic glands, adenosis (17.5%), and at times, morphologically benign glands. AMACR is used as a confirmatory staining for prostate cancer, in combination with basal cell markers and H&E histology.

Dual chromogen[36]

AMACR and basal cell markers can be combined together in a single immunostaining reaction. Such "cocktail" staining may be useful when carcinoma is present only in one tissue section for the work – up of a small focus.

Presently, in diagnostically challenging prostate cases, Alpha-Methylacyl- CoA- Racemase (AMACR) and basal cell markers are being used in addition to morphology. This has caused a rise in accuracy of diagnosis of prostate cancer across the world.

Basal cell markers:

HMWCK (34βE12) and P63 and CK 5/6 are critical for demonstration of basal cells in benign glands. When they are seen, a invasive prostatic cancer diagnosis is very less likely.

34 β E12

This is a high molecular weight cytokertin immunochemical marker.It binds to high molecular weight cytokeratin,intermediate filament, not in luminal cells of prostate but in the basal cells' cytoplasm.

Interpretation[36]

It is interpreted as positive / negative and continous / discontinous

Limitations :[36]

For the diagnosis of prostate cancer, many limitations are noted in using basal cell markers. Stressing on absence of basal cell staining, a negative finding, to decide on a positive diagnosis of cancer is the most important. Also, some of benign prostatic glands (5% - 23%), some specimens of atrophy(23%), up to half of specimens of adenosis, 66% specimens of mesonephric hyperplasia, 44%-75% samples of nephrogenic adenoma may lack basal cell staining. This is the reason why negative basal cell marker immunostaining in singularity cannot conclusively pinpoint carcinoma.

Ejaculatory duct epithelium and seminal vesicle are invariably positive for basal cell markers. But the status of Cowper's glands is contradictory.

Hence, one must be cautious while reporting negative basal immunostains. They support the diagnosis of prostatic carcinoma along with the presence of appropriate H&E picture. The decisive one is that some high grade prostatic carcinomas are positive for basal cell markers.. They are commonly easily detected based on H&E picture.

In addition, some cases of invasive acinar adenocarcinomas, in 1% of cases, harbor basal cells. A few of these could be flat HGPIN or cancer outpouchings of HGPIN glands. So, even though being very useful, basal cell markers are to be interpreted carefully in the diagnosis of cancer. The definitive criterion is that a detectable positive basal cell layer is absent. Also, false-negative staining of basal cells can occur in prolonged formalin fixation.

CK 5/6

CK 5/6 is a mesothelial cell marker. It is expressed normally by complex epithelium and also in mammary carcinomas, as well as bile tract, pancreatic and malignant mesothelioma. This antibody does not react with HPIN or prostatic tumoral cells but with prostatic basal cells.

p63 antibody

It is an antibody to basal cells of prostate. It has a critical role in development of the prostate. It is expressed in normal prostatic glands by

basal cells. Most cases (89–94%) of prostate cancer do not express p63. Immunostaining with p63 is very useful in suspicious prostate cases.

AMACR[36,58]

Thus, a specific and sensitive positive immunohistochemical marker is necessary. This increases the accuracy in pathological diagnosis of prostate malignancies. Also known as p504 S or racemase, AMACR, is an enzyme identified recently by microarray and cDNA subtraction technology. It is invariably up regulated in prostate cancer, being a specific and sensitive IHC tool.

It is overexpressed in prostate cancer with marked differential staining between malignant and benign glands. It is highly sensitive and is seen in 75-95% in prostatic carcinomas.[45]

The AMACR gene product, in prostate cancer, was identified to be over expressed. This was identified with a small number of prostate adenocarcinoma samples in conjunction with high-throughput microarray analysis by complementary DNA library subtraction. It is a protein whose activity is increased in prostatic adenocarcinoma. Its gene is located on 5p13, and its product resides in peroxisomes and mitochondria. The protein has an important role in the β oxidation of bile acid intermediates and branched-chain fatty acids. Since beef and dairy products are the

major sources of branched -chain fatty acids, their intake has been linked with an increased risk of prostate cancer. AMACR overexpression and diet have, in the natural history of prostate cancer, complementary roles.[25]

It has been recognized that AMACR is also expressed in the precursor lesion to prostate cancer, HPIN, and even in low grade PIN[34,38]. At the protein level, AMACR overexpression is tightly linked to prostate cancer. It occurs in almost all stages and grades and also in untreated and hormone-refractory patients. DNA microarray analyses have also found significant overexpression of AMACR in prostate carcinoma.

Interpretation[36,39]

Positive staining of AMACR refers to diffuse dark or granular, luminal or cytoplasmic, but circumferential staining. From 0+ to 3+, the percentage positivity is graded as below:-

0% cells (negative, 0+)

1-10% cells (mild, 1+)

11-50% cells (moderate, 2+)

> 51% cells (strong, 3+)

There should be no more than weak staining or noncircumferential partial staining in the surrounding benign prostatic glands .

Negative staining refers to focal or no staining, fine weak noncircumferential staining.

Expression of AMACR in prostatic cancer is upregulated. About 75-95% of prostate cancers in immunohistochemistry are positive for AMACR. Therefore, AMACR is used as a positive prostate cancer marker in combination with negative basal cell markers (p63 or high-molecular-weight CK). This helps in the diagnosis of suspicious prostate needle biopsies.

A significant advantage of AMACR immunostain exists. A diagnosis of malignancy is based on a positive indicator; not based on loss of signal.

Limitations:[14,36]

A few morphological variants of prostatic cancer are a serious diagnostic problem. In these cases, immunohistochemistry is specifically needed to clinch the diagnosis of carcinoma. They have been seen to express less AMACR reaction when contrasted with the more conventional cases. AMACR expression is found in 62-68% of foamy

gland carcinomas and ~ 70-77% of pseudohyperpalstic cancers. Added to prostate cancer, in 90% cases of HGPIN, AMACR positivity is seen. This proves that HGPIN should be excluded with care by the use of basal cell markers and morphology, before AMACR positivity is used to report the diagnosis of cancer. In HGPIN, AMACR positivity varies between strong and weak. AMACR expression is also identified in 18-58% cases of nephrogenic adenoma, in 4%-21% benign prostatic glands and in 18-27% cases of adenosis.[39]

So, even though AMACR is an immunohistochemical marker of use in prostate carcinoma, it has significant drawbacks. It is so stressed that AMACR is to be interpreted in the suitable morphological scenario and with combination of basal cell markers.

The commonest reason for error in diagnosis in TURP and needle biopsies is that the malignant foci are very limited (3-10%). The important reasons for the difficulty in detecting limited prostatic cancer are listed below.

Most importantly, for histopathological examination, there may only be a few acini seen in the limited number of carcinomatous glands. Also, for the detection of prostate carcinoma a single feature sufficient and specific is not available. It is based on a combination of cytological and architectural features and extracellular material such as crystalloids

or secretions tinged blue. Many of these microscopic diagnostic conditions may be seen in benign conditions of the prostate sometimes. Added to that, the consequences may be very serious such as radiation exposure, unnecessary prostatectomy or delay in effective treatment when associated with a false negative or positive diagnosis. Various cases with change of diagnosis to malignant/premalignant from benign are underdiagnosed because of the presence of limited adenocarcinoma[36].

Also, causes of error are inflammation, missing out on HGPIN (not judiciously looking at high power), and diagnostic mistakes with benign mimics of carcinoma.[40]

In nonspecific staining of carcinomatous cells by basal cell markers, a few methods of antigen retrieval are implicated. Nonspecific tumor cell immunoreactivity is noted in the hot plate antigen retrieval method. But, for the overall staining, it is better than others. The microwave retrieval and pepsin predigestion methods did not cause this condition, however some benign basal acinar cells did not stain with these methods.

Uncommonly, HMWCK is expressed in high grade prostate carcinomas. But it is not a diagnostic problem usually, since malignant cells are positive for AMACR.

Vincent et[28] al in his study stated that p504s was a highly sensitive and specific marker for prostate cancer. AMACR is extensively upregulated at the transcript and protein levels in HPIN and adenocarcinoma.

Xu et al[10], in conjunction with microarray high-throughput screening, using cDNA library subtraction, discovered 3 proteins - P504S, P503S, and P510S, specific for carcinoma to differentiate between malignant and benign prostate tissue.

Xu et al[10] also stated that a 382-amino-acid protein is P504S. It is actually AMACR - human α-methylacyl coenzyme A racemase. AMACR plays a role in the β-oxidation of fatty acid derivatives and branched-chain fatty acids. P504S mRNA - messenger RNA is overexpressed in approximately 30% (microarray screening) to 60% (quantitative polymerase chain reaction analysis) of prostate cancers. It is undetectable or low in normal prostate tissues.

In 2001, Jiang et al[5] stated that P504S (AMACR) was, for prostate tumors, a new immunohistochemical marker. P504S/AMACR is a marker with high sensitivity for prostate carcinoma. In 92% of cases, a diffuse staining pattern (>75% cancer positive) was visualised without regard to Gleason score. P504S/AMACR in high-grade prostatic intraepithelial neoplasia (PIN) was also strongly positive.

The study also reported that of P504S/AMACR and high-molecular- weight cytokeratin expression was mutually exclusive. AMACR/ P504S is a marker for prostate cancer with high specificity. Benign tissue samples of prostate (88%), including benign prostate tissue surrounding carcinomas were completely negative for P504S/AMACR in contrast to cancers. Therefore, it was showed that, the AMACR/ P504S staining pattern must be an adjunct to distinct benign and malignant glands; and it is to be applied in conjunction with the histological criteria.

Luo et al,[12] in his study assessed the association of the 2 antibodies.p63 as a negative marker and p504s as a positive marker were studied. This significantly helps the diagnosis of prostate carcinoma. It causes an increase in diagnostic precision, a decrease in the false negatives risk, and increased specificity and sensitivity in detecting prostate cancers.

In 2002, Rubin et al[53] confirmed increased AMACR expression in prostate carcinoma with polyclonal antibody to AMACR and cDNA microarrays. Rubin et al reported that in three of four DNA microarray analyses (128 samples) independently and microarray tissue specimens, including 17 metastatic prostate cancers, significant AMACR overexpression in prostate carcinoma was note.

Hameed et al[33] stated in his study that Basal cell markers like antibodies directed against cytokeratin 5 and 6 or p63 and 34BetaE12 antibody help to demonstrate basal cells. Their presence is against a diagnosis of invasive prostatic cancer. Although, many benign mimickers of PC, including nephrogenic adenoma, atypical adenomatous hyperplasia (AAH), atrophy and mesonephric hyperplasia, with these markers, can stain negatively, a negative basal cell marker immunostain cannot singularly rule out a diagnosis of benignancy. Despite the fact that there are instances in literature of high grade PC that, with a few of the basal cell markers, stain focally; these are usually easily detected based on microscopic appearances. They are less likely to be confused with such benign mimickers.

(AMACR) - Alpha-methylacyl-coenzyme-A racemase is a sensitive marker of Prostate cancer (except for a few rare variants: foamy gland, atrophic, and pseudohyperplastic variants). It’s detection in atypical prostatic lesions by immunohistochemistry is very helpful in confirming a diagnosis of prostate cancer. AMACR expression may also be seen in prostatic atrophy, high grade prostatic intraepithelial neoplasia (PIN), benign prostatic glands and AAH. Therefore, a report of Prostate Cancer must not be based singularly on a positive immunostain of

AMACR. This is more important in conditions where the luminal staining is noncircumferential and/or weak.

Luo et al [12] discovered that out of all histologically normal prostatic epithelium, < 4% showed positive staining for AMACR; while in prostate cancers, > 95% stained positively. They also showed 81% and 93% positivity of AMACR in thirty two metastatic prostate cancers from non–hormone-refractory disease and fourteen hormone-refractory metastatic prostate cancers, respectively. They stated finally that AMACR is a positive immunohistochemical marker that adds advantage to concretely diagnose prostate cancer, along with the traditional basal cell stains.

Beach et al[29] in his study from 405 prostatic specimens, showed that P504S monoclonal antibody was positive in 376 prostate needle biopsy specimen. Also reported that in biopsy specimens,82% of 186 cases showed positivity for AMACR immunostaining , but foci of benign prostate epithelium showed only 21% positivity.As well as they show faint,focal and non circumferential staining. The most specific staining pattern of AMACR is diffuse and circumferential cytoplasmic staining in prostate carcinoma and no staining in benign prostate tissue. Positive staining was not found in the transitional metaplasia, specific small gland

proliferation of postatrophic hyperplasiaas well as in basal cell hyperplasia.

Leav et[20] al in his study showed that AMACR (P504S) expression in prostate carcinoma is common in the transition zone. All 25 cases in the study with Gleason grade 1 carcinoma were positive for AMACR.But compare to high grade carcinoma staining was less intense in grade 1.

Magi-Galluzzi et al[25] studied numerous cases (209 cases), all are needle biopsy with minimal foci (<5% of a core) of prostate cancer. 88% were positive for AMACR in the small foci of prostate cancer. They also studied that the among the different groups the sensitivity varied.80 % to 87% for cases from outside institutions and100% for the in-house cases .They were chosen to include the differences in processing and fixation in various pathology laboratories. Eventhough it is essential to recognize AMACR negative staining in some minimal cancers, they came to conclusion that from the needle biopsy specimen positive staining of AMACR may increase the range of confidence in establishing a definitive diagnosis of malignancy.

(1) If small, focal atypical glands stain with basal cell markers but not with AMACR/P504S, the diagnosis is benign.

(2) When atypical glands are positive for 34βE12/p63 and AMACR/P504S, malignancy can be ruled out. The differential diagnoses include high-grade PIN, adenosis, and even some benign glands based on the findings on H&E staining.

(3) If small atypical glands, excluding high-grade PIN and nephrogenic adenoma, are negative for basal cell markers but positive for AMACR/P504S, a malignant diagnosis is established.

(4) In the scenario that small atypical glands are negative for 34βE12/p63 and AMACR/P504S, the diagnosis might be malignant or benign.

In their study, the likelihood of negative staining of both 34βE12/p63 and AMACR/P504S in small focal carcinoma in needle biopsy specimens is rare (<6%).

Magi-Galluzzi et al[25] reported a variable sensitivity (80%-100%) of AMACR for the diagnosis of minimal prostatic cancer. They emphasize that it is important to recognize that some small focal cancers might be negative for AMACR/P504S.[44,45]

In a recent study, Jiang et al[5] examined, on prostate needle biopsy specimens, 41 foci of “atypical cases” with a AMACR / P504S combination and 34βE12 stains. The study described that when the

antibodies combination was used, more than half the suspicious atypical foci were diagnosed definitively.

Oppenheimer et al[30] showed patchy basal cells in 12 cases stained for high-molecular-weight cytokeratin (HMWCK). Hence, partial atrophy can sometimes cause diagnostic challenges in prostate needle core biopsy specimens.

A recent report by Herawi et al[25] identified 567 atypical but benign foci in specimens from 345 patients received in consultation. The authors found that partial atrophy was the most common mimicker of adenocarcinoma (203 of 587 cases [34.6%]

Jiang, Zhong et al [5,30]in his study, described cases with a small focus of prostate cancer (73) measuring ≤1 mm and benign prostatic cases (69), totalling 142 needle biopsies. They were studied by using immunohistochemistry for (34βE12) - high molecular weight cytokeratin and P504S. Out of 73 cases, 69 (94.5%) of carcinoma showed P504S immunoreactivity. It was not seen in any benign prostates (none out of 69) or benign glands adjacent carcinomatous glands. In all 73 cases, immunostaining with 34βE12 demonstrated that in the focus of carcinoma, basal cells were absent. Its significant diagnostic value in pathologic practice was confirmed by the high sensitivity and specificity of P504S in the diagnosis of minimal prostate cancer. Utilising a

combination of 34βE12 and P504S on needle biopsy helps the detection of limited prostate cancer.

Vogel and Gown[50] described the utility of 34βE12, a anti-high molecular weight cytokeratin monoclonal antibody. This was applied to mark prostate's basal cells. They were described to be characteristic of benign glands which retain the layer of basal cells. In a bigger series, Epstein and Wojno to diagnose adenocarcinoma used 34βE12 in suspicious glands detected in prostate needle biopsy series.

Jiang et al[5] stated that HMWCK (34bE12) and AMACR immunohistochemistry, in the study of 41 atypical small acinar proliferation (ASAP) foci caused a 76% agreement rate between the three pathologists involved.

Zhou et al [13] demonstrated that based on a positive AMACR immunostain, out of 115 biopsies of prostate detected by an expert pathologist, as atypical, 34 (30%) were labelled a final diagnosis of cancer.

Browne et al [21] described that the utility of a combination of both AMACR and basal cell antibody immunostain resolved the diagnosis in 86/123 (70%) of suspicious prostate biopsies.

Sanderson et al [22] used AMACR/ p63 combination to redesignate 2 of 7 (29%) atypical needle biopsies as prostate cancer.

Kunju et al [14] were able to resolve 27 of 29 (93%) atypical biopsies due to immunostaining with basal cell marker and AMACR.

Tara Jane Browne[21] showed that in microscopically difficult cases, utilising combination of stains can be a helpful approach since it increases the chance that a conclusive diagnosis can be arrived at while decreasing the possibility of an inconclusive diagnosis. But, a disadvantage of the said method is the loss of tissue in such small lesions, hinting that combining BCC and AMACR on a single slide will be better than using either separately.

MATERIALS & METHODS

Study Design :

Prospective study

Study Period :

From August 2011- July 2012

Study Place :

Coimbatore Medical College Hospital, Coimbatore

Sample size :

A total number of 37 cases

From case records, brief clinical data were collected, which included age, presenting complaints, digital rectal examination (DRE) findings, serum PSA levels and clinical diagnosis.

The following inclusion and exclusion criteria were adopted.

Inclusion criteria

1. All prostatic specimens- needle biopsies, TURP- transurethral resection of prostate and radical prostatectomy specimens.
2. Patients in all age groups

Exclusion criteria

1. Ill fixed samples
2. Inadequate samples

A proforma was used to document demographic data, age, dietary habit, clinical presentation and previous history as given in Annexure – 1.

The study was conducted in the same hospital.

Methods:

Among the total cases received in the department of pathology of our hospital during study period, 37 cases were taken into study as per inclusion criteria and 3 cases were eliminated from the study because of insufficient material and as the biopsy was non representative. 37 cases were finally evaluated further. Included were 29 needle biopsies and 8 TURP specimens.

The received samples were then fixed in 4% formalin, embedded in paraffin and stained with H&E.

After eosin and hematoxylin staining all slides were reviewed by pathologists and assigned to the following groups - Benign prostatic

hyperplasia (10), Basal cell hyperplasia (1), PIN (5), malignant (20) and suspicious (1).

There are 37 cases.They consisted of 29 needle biopsies and 8 TURP specimens. The age group were differed between 48 and 85 years.

The value of Serum Prostate specific antigens was available for 3 cases and ranged between 7 and 100.

PROCEDURE OF IMMUNOHISTOCHEMISTRY

The blocks from control and selected cases were cut and mounted on poly l- lysine coated glass slides .Blocking of Endogenous peroxidase activity was done by 0.3% hydrogen peroxide in methanol, freshly prepared, for twenty minutes. Then, epitope retrieval by heat was performed by using buffer of Tris EDTA at pH 9. Immunohistochemistry was done by utilising a monoclonal anti-HMWCK antibody (clone no 34βE12 of 1:50 dilution) and a rabbit monoclonal anti-AMACR antibody (p504 S, clone no 13H4 of 1:50 dilution).

IMMUNOHISTOCHEMISTRY

METHOD:

Two-step indirect technique.

PRINCIPLES OF THE PROCEDURE:

Using a two-stage process, antigens in cells and tissues were detected. The first was the binding to specific epitopes of the primary antibody. Second was a calorimetric reaction to detect the binding. Sections of tissue were fixed and attached to slides. The paraffin-embedded sections were then dewaxed. Antigen retrieval procedure was done. This consisted of the heating in microwave of formalin-fixed tissue in an aqueous solution. It recovered full antigenicity with a most of the antibodies. These also included cases that were formerly unreactive with formalin-fixed tissue. Subsequently, the tissue sections were treated with Peroxide-Block and Power-Block for blocking endogenous peroxidise nd non-specific protein-protein interactions, respectively.

REAGENTS USED

1) Peroxide Block: 3%hydrogen peroxide in water.Power Block Reagent: A highly effective universal protein blocking reagent. Contains casein and propriety additives in PBS with 15mM sodium azide.
2) Chromogen: DAB-3,3’-diaminobenzidine.
3) Liquid DAB Substrate: Comprises Tris buffer containing the peroxide and stabilizers.
4) Super Enhancer Reagent.

5) Poly-HRP Reagent.

6) Counter stain: Mayer's Hematoxylin.

7) Buffer solutions:

TRIS BUFFER: (ph -7.6)

TRIS Buffer salt : 0.605 gm

Sodium chloride : 8 gm

Distilled water : 1000 ml

1N Hydrochloric acid : 3 ml

CITRATE BUFFER: (ph-6.0)

Trisodium citrate : 2.94 gm

Distilled water : 1000 ml

1 N Hydrochloric acid : 5 ml

TRIS EDTA: (ph-9.0)

TRIS Buffer salt : 6.05 gm

Disodium EDTA : 0.744 gm

Distilled water : 1000 ml

PROCEDURE:

1) Sections were deparaffinised in xylene for 30 minutes.
2) Washed in absolute alcohol for 5 minutes with 2 changes.
3) Slides were then washed for 10 minutes in tap water

4) Rinsed for 5 minutes in distilled water.
5) Antigen retrieval was done by placing the slides with appropriate buffer solution in microwave : Medium-10 minutes: High-10 minutes.
6) They were then cooled to room temperature and rinsed in distilled water.
7) Washed in TBS buffer for 5 minutes with 2 changes.
8) Treated with Peroxide Block for 10 minutes.
9) Washed in TBS buffer for 5 minutes with 2 changes.
10) Treated with Power Block for 10 minutes.
11) Slides were drained and covered with primary antibody (supplied from DAKOCYTOMATION) for 2 hours.
12) Washed in TBS buffer for 5 minutes with 2 changes.
13) Slides were covered with Super Enhancer for 30 minutes.
14) Washed in TBS buffer for 5 minutes with 2 changes.
15) Poly HRP reagent was applied and left for 30 minutes.
16) Washed in TBS buffer for 5 minutes with 2 changes.
17) Treated with DAB Chromogen with Substrate buffer for 5 to 8 minutes.
18) Washed in TBS for 5 minutes with 2 changes.

19) They were then washed for 5 minutes in tap water.

20) They were counterstained for 1 minute with Mayer's Hematoxylin.

21) Washed for 5 minutes in tap water.

22) Slides were air dried and mounted with DPX.

Tumor cells were scored positive if there was golden brown cytoplasmic, nuclear or membrane staining in the neoplastic cells. Negative diagnosis was made when no golden brown staining was noted.

Interpretationof Immunohistochemistry:

Criteria for positive/ negative staining

AMACR[36]

Positive staining refers to granular or dark diffuse, luminal or cytoplasmic staining. The percentage positivity was graded between 0+ and 3+ as below:-

- negative (0+, 0%cells)
- mild (1+,5-10% cells)
- moderate (2+,11-50% cells)
- strong (3+,51% cells)

Negative staining refers to focal or no staining, fine or weak and partial or noncircumferential staining.

34βE12[36]

The basal cell marker of benign prostatic glands, High molecular Weight Cytokeratin, was interpreted as positive/negative cytoplasmic or membrane staining and discontinuous/continuous staining.

OBSERVATION AND RESULTS

A total of 37 cases were selected as per Inclusion and Exclusion criteria. Among the 37 cases, 2 cases were negative for both AMACR and 34βE12. It could be because of improper fixation - overfixation or underfixation.

Considering the clinical details and morphology, in the present study of Immunohistochemistry with AMACR and HMWCK, 35 cases were chosen for evaluation.

Prostate carcinoma :

17 out of 19 cases categorised as prostatic carcinoma showed moderate to strong positive cytoplasmic staining of AMACR in malignant areas, but not in any benign glands adjacent to that. 2 out of 19 cases of prostatic carcinoma showed negative staining with AMACR.

Immunostaining with 34βE12 confirmed that basal cells were absent in the cancer focus in all 19 cases of prostatic carcinoma.

Prostatic Intraepithelial Neoplasia

3 out of 5 cases categorised as prostatic intraepithelial neoplasia showed focal, weak and granular cytoplasmic positivity with AMACR. Added to it, staining with 34βE12 highlighted the basal cell layer in 3 out

of 5 cases. Compared to prostate cancers, a weaker intensity of AMACR expression was noted in high grade PIN cases.

Atypical focus suspicious of malignancy

1 case categorised as ASAP , showed positive staining in luminal cells by AMACR and negative staining with 34βE12 in basal cells. Thus it was diagnosed as positive for malignancy.

Benign prostatic Hyperplasia

Among 9 cases categorised as Benign prostatic Hyperplasia ,8 cases showed positivity for HMWCK in benign glands and 1 out of 9 showed negativity for HMWCK.

All the 9 cases of benign prostatic hyperplasia showed negativity for AMACR.

INCIDENCE OF PROSTATIC LESIONS

Totally 50 specimens of prostate were received in the Department of Pathology during the study period.

TABLE 1: INCIDENCE OF VARIOUS PROSTATIC LESIONS

PROSTATIC LESIONS	No of cases	PERCENTAGE
BPH	24	48%
Basal cell hyperplasia	1	2%
Atypical foci	1	2%
PIN	5	10%
Prostatic cancer	19	38%
Total	50	100%

The incidence of Benign Prostatic Hyperplasia was highest in the study with 24 cases contributing to 48%, followed by 19 cases of Prostatic carcinoma comprising 38% of cases. Prostatic Intraepithelial Neoplasis comprised 10% of the cases. Basal cell hyperplasia and Atypical foci were with 1 case each.

CHART I : INCIDENCE OF VARIOUS PROSTATIC LESIONS

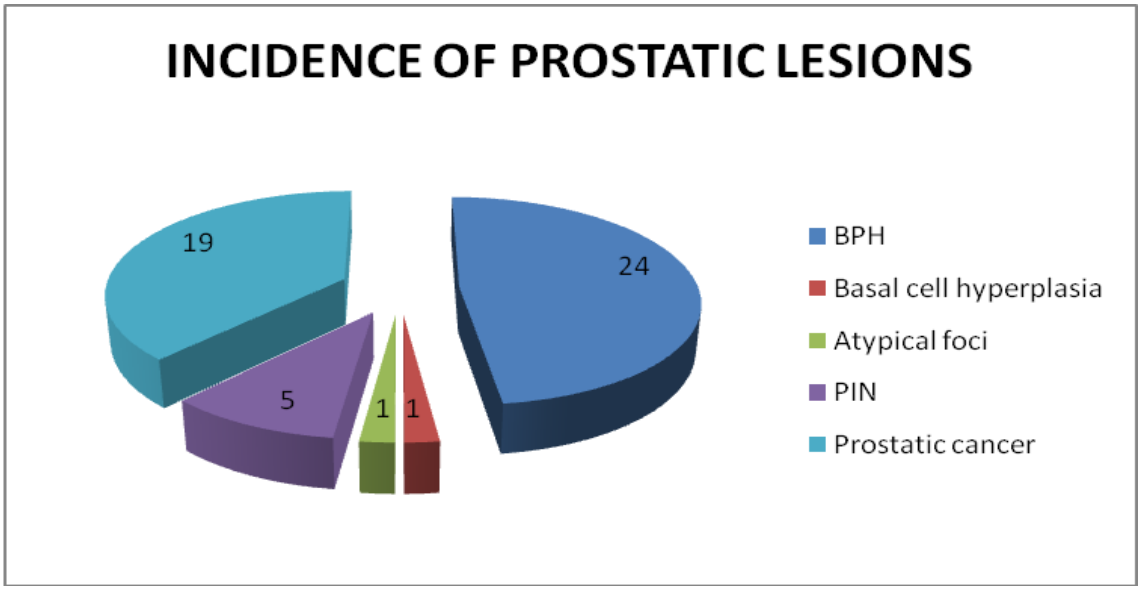


TABLE 2: AGE DISTRIBUTION OF PROSTATIC CARCINOMAS

AGE (years)	NO.OF CASES	PERCENTAGE
60-65	3	15.78%
66-70	5	26.31%
71-75	9	47.36%
75-80	2	10.52%
Total	19	100%

In the present study, the incidence of Prostatic carcinoma was highest in the age group of 71 to 75 years, comprising about 48% of the cases followed by 66 to 70 year category with 5 cases (26 %),60-65 years(16%),75-80(11%).

CHART II: AGE DISTRIBUTION OF PROSTATIC CARCINOMAS

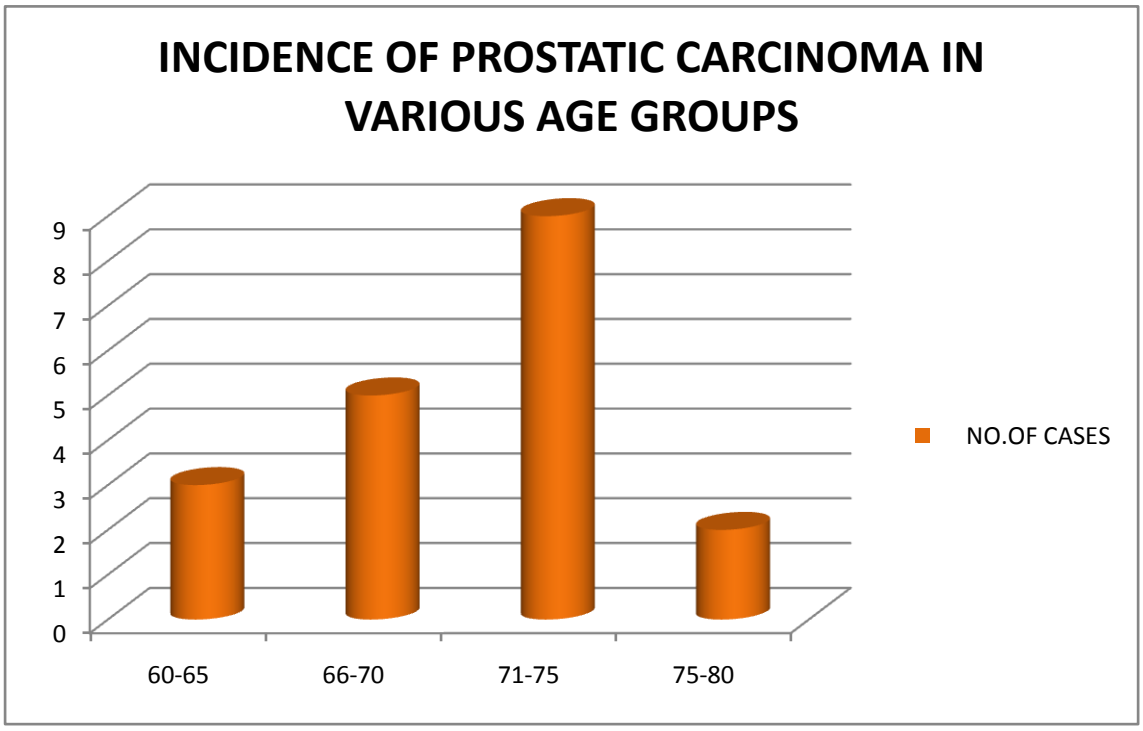


TABLE 3: DISTRIBUTION OF PROSTATIC CARCINOMA WITH REFERENCE TO GLEASON'S SCORE

Gleason score	No of cases	Percentage
6	5	26.31 %
7	6	31.57%
8	4	21.05%
9	3	15.78%
10	1	5.26%
Total	19	100%

Out of the 19 cases of prostatic carcinoma, 6 cases (31.5%) were of grade 7 and 5 cases (26 %) were of Grade 6. Grade 8, 9 and 10 had 4(21%), 3(16%) and 1(5%) cases respectively.

CHART III: DISTRIBUTION OF PROSTATIC CARCINOMA WITH REFERENCE TO GLEASON'S SCORE

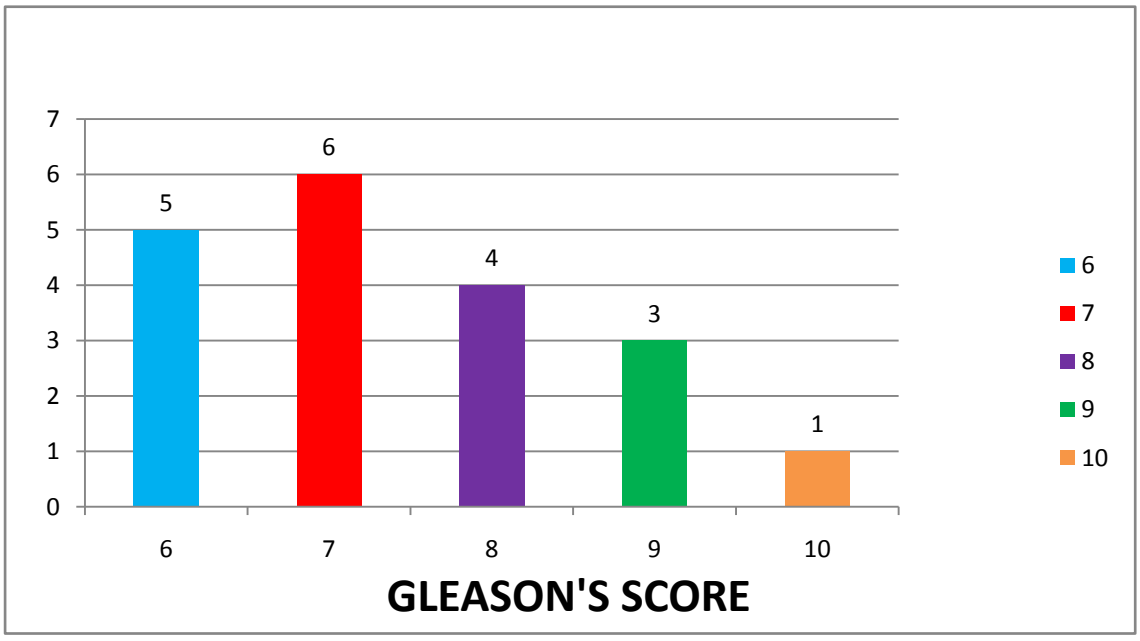


TABLE 4 - EXPRESSION OF AMACR

	No of cases	AMACR positive	AMACR Negative
Prostatic Carcinoma	19	17	2
PIN	5	3	2
ASAP	1	1	0
BCH	1	0	1
BPH	9	0	9

BPH - Benign Prostatic Hyperplasia

BCH - Basal Cell Hyperplasia

PIN - Prostatic Intra epithelial Neoplasia

ASAP - Atypical Small Acinar Proliferation

CHART IV: EXPRESSION OF AMACR

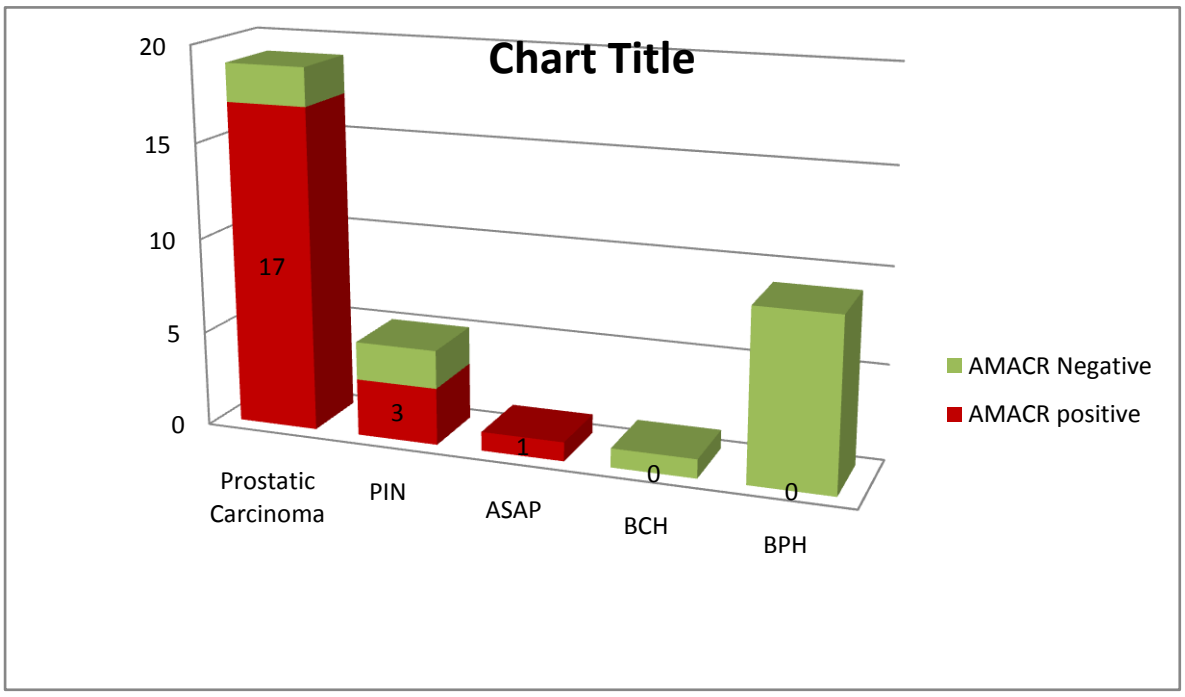


TABLE 5 - EXPRESSION OF 34BE12

	No of cases	Positive	Negative
Prostatic Carcinoma	19	0	19
PIN	5	+/-3	2
ASAP	1	0	1
BCH	1	1	0
BPH	9	8	1

+- indicates focal and discontinuous positivity

BPH - Benign Prostatic Hyperplasia

BCH - Basal Cell Hyperplasia

PIN - Prostatic Intra epithelial Neoplasia

ASAP - Atypical Small Acinar Proliferation

CHART V - EXPRESSION OF 34βE12

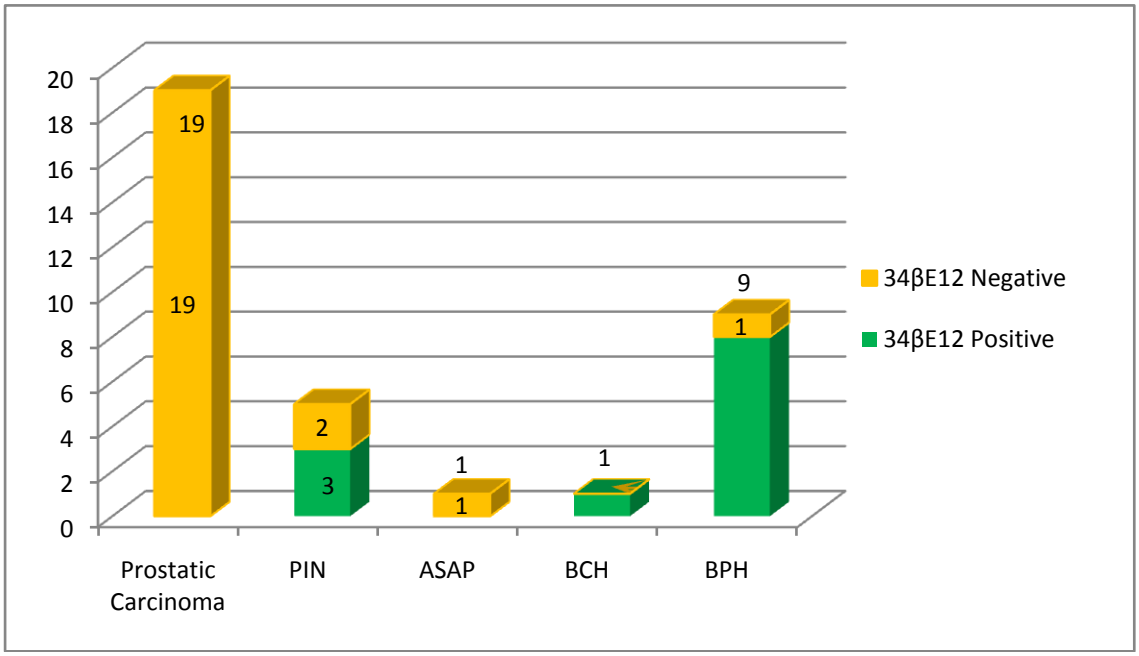


TABLE 6: AMACR GRADING IN PROSTATIC CARCINOMA

Grading	No of cases	Percentage (%)
0(Negative)	2	10.5%
1(Weak)	1	5.25%
2(Moderate)	2	10.5%
3(Strong)	14	73.6%
Total	19	100%

Grade 3 positivity of AMACR was observed in 14 cases of Prostatic carcinoma ,Grade 2 in 2 cases ,Grade 1 in 1 case.

CHART VI: AMACR GRADING IN PROSTATIC CARCINOMA

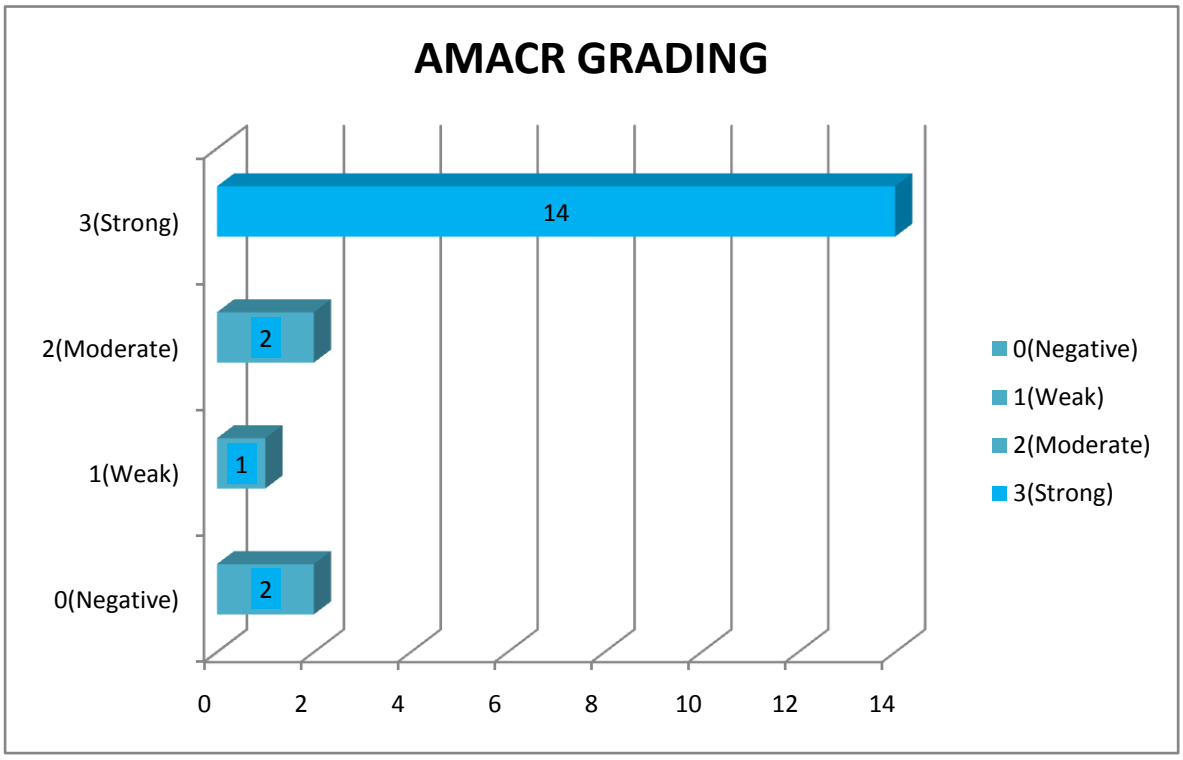


Table 7 - AMACR EXPRESSION

	Prostate carcinoma	BPH
Positive	17	0
Negative	2	9

Detection of prostatic carcinoma by AMACR

Sensitivity : 89.47%

Specificity : 100%

CHART VII: AMACR EXPRESSION

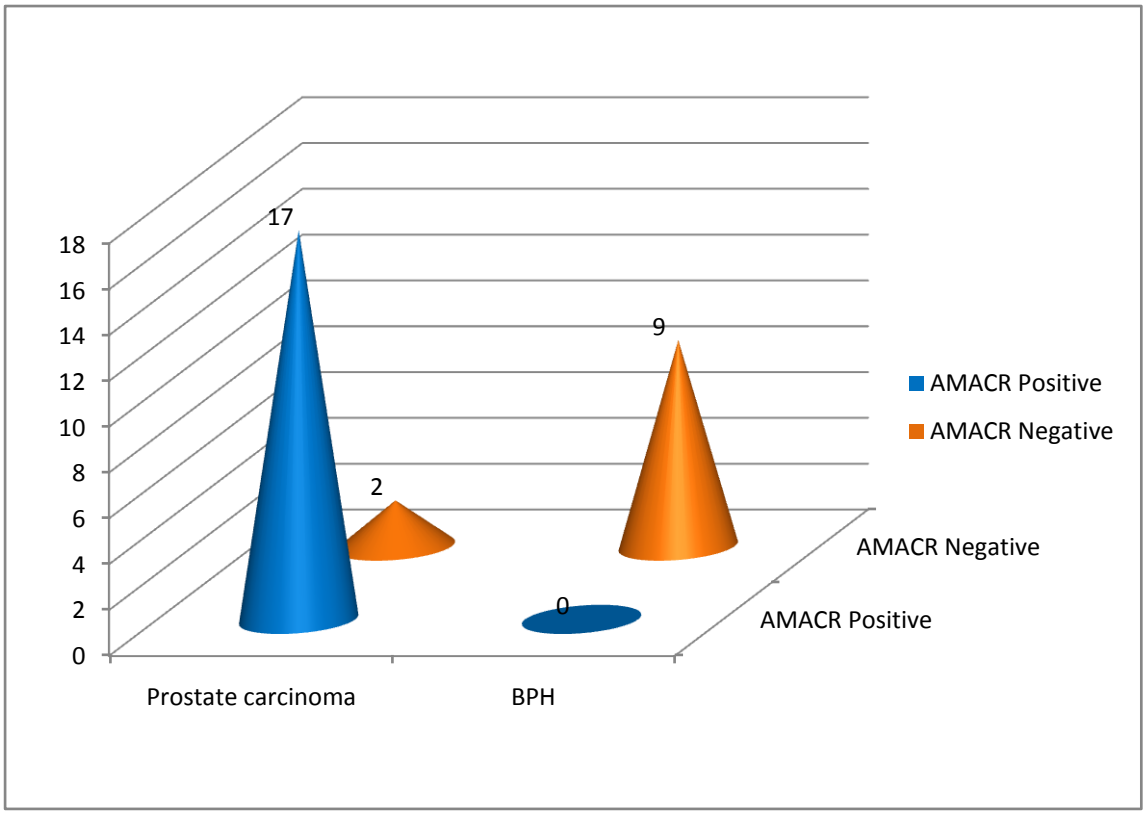


TABLE 8 :34βE12 EXPRESSION

	BPH	Prostate carcinoma
Positive	8	0
Negative	1	19

Detection of prostatic carcinoma by 34βE12

Sensitivity : 100%

Specificity : 88.88%

CHART VIII: 34βE12 EXPRESSION

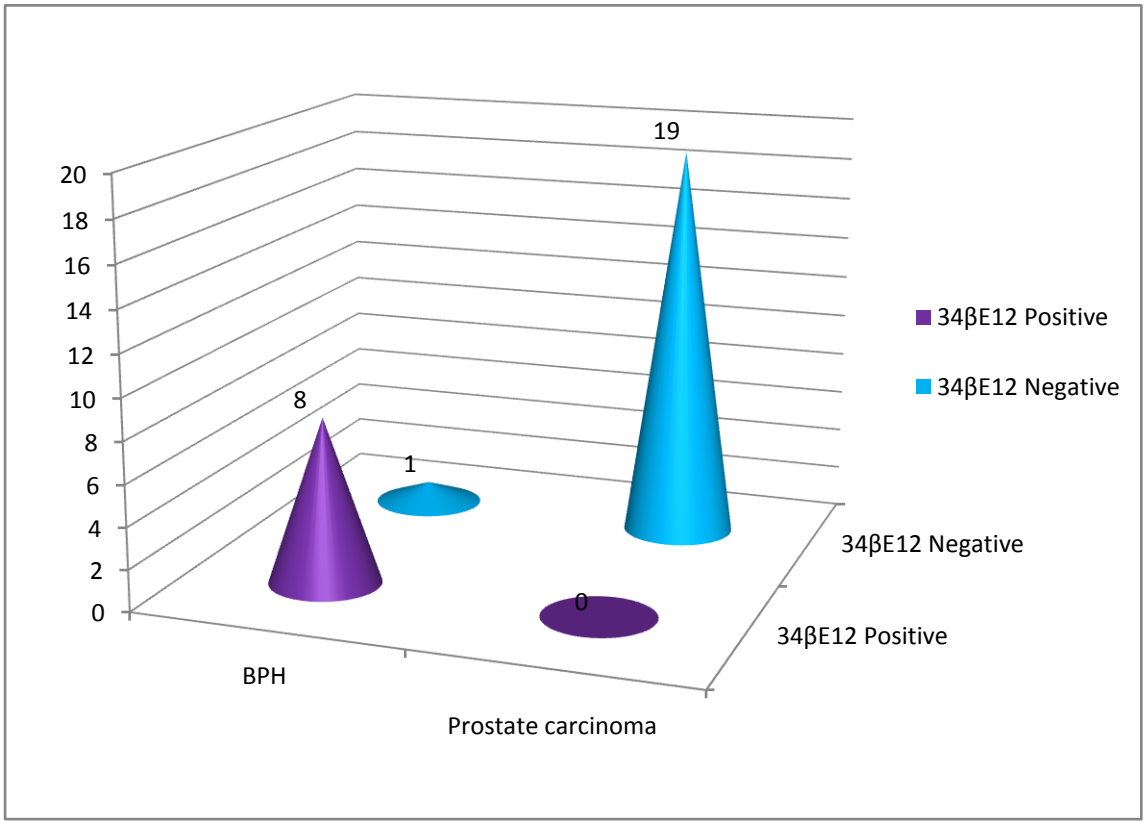


TABLE 9:COMPARISON OF AMACR AND 34BE12 IN DETECTING PROSTATE CARCINOMAS

	Sensitivity	Specificity
AMACR	89.47%	100%
34βE12	100%	88.88%

CHART IX: COMPARISON OF AMACR AND 34BE12 IN DETECTING PROSTATE CARCINOMAS

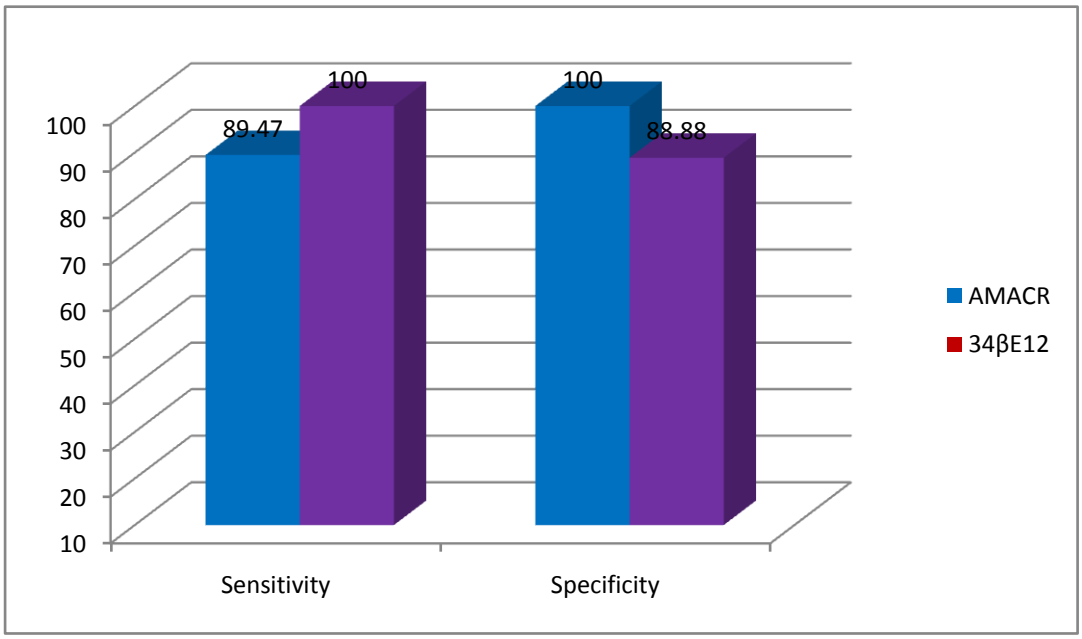


TABLE 10: PERCENTAGE POSITIVITY OF AMACR & 34βE12

	No of cases	AMACR Positive	34βE12 Positive
PC	19	17/19 (89.47%)	0/19(0%)
Benign	9	0/9 (0%)	8/9(88.8%)

TABLE 11:COMPARISON OF AMACR INDICES IN VARIOUS STUDIES

	Total no of PC cases	AMACR positive	Sensitivity
Sung et al	49	35	71%
Zhong jiang (1) et al	73	69	95%
Victor et al	113	108	96%
Kumerasan et al	25	23	92%
Present study	19	17	90%

CHART :X COMPARISON OF AMACR INDICES IN VARIOUS STUDIES

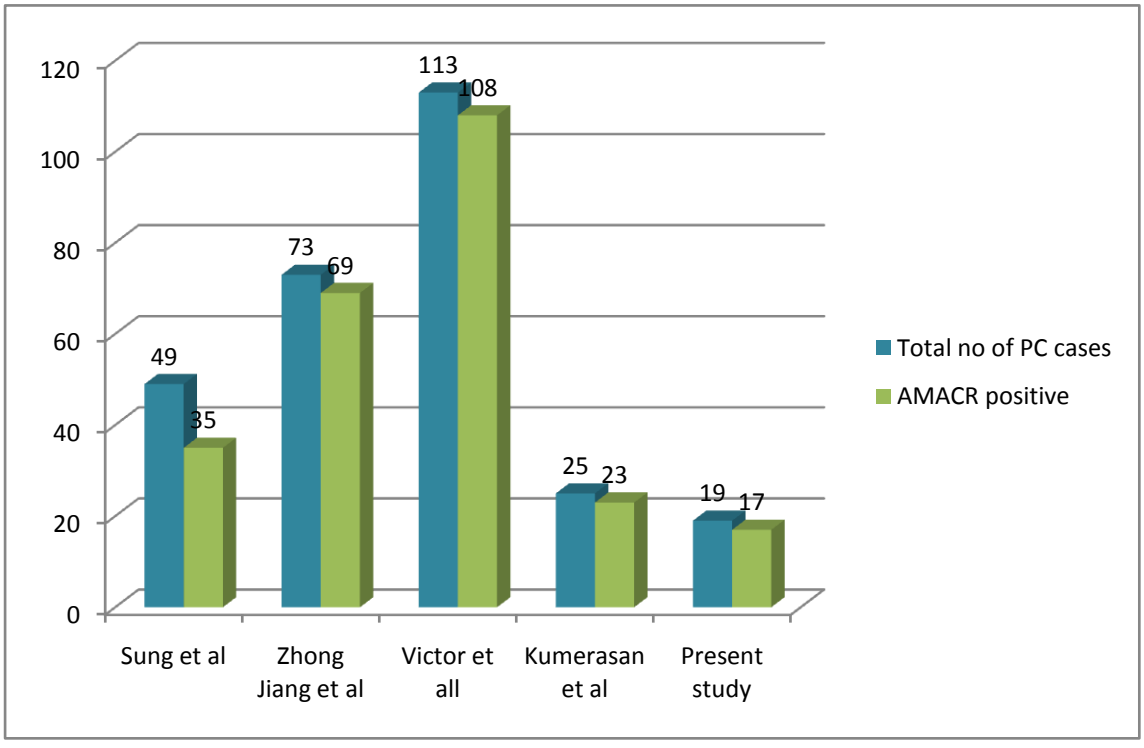
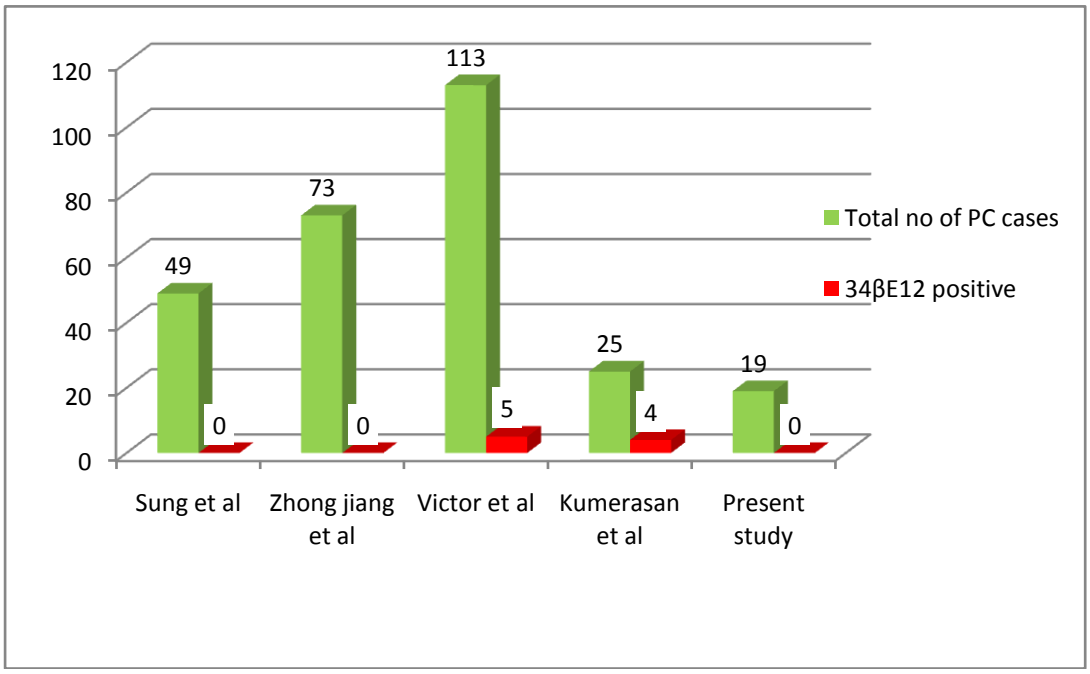


TABLE 12: COMPARISON OF 34BE12 INDICES IN VARIOUS STUDIES

	Total no of PC cases	34βE12 positive	Specificity
Sung et all	49	0	100%
Victor et all	113	5	93%
Zhong jiang	82	0	100%
Kumerasan et al	25	4	84%
Present study	19	0	100%

CHART XI COMPARISON OF 34BE12 INDICES IN VARIOUS STUDIES



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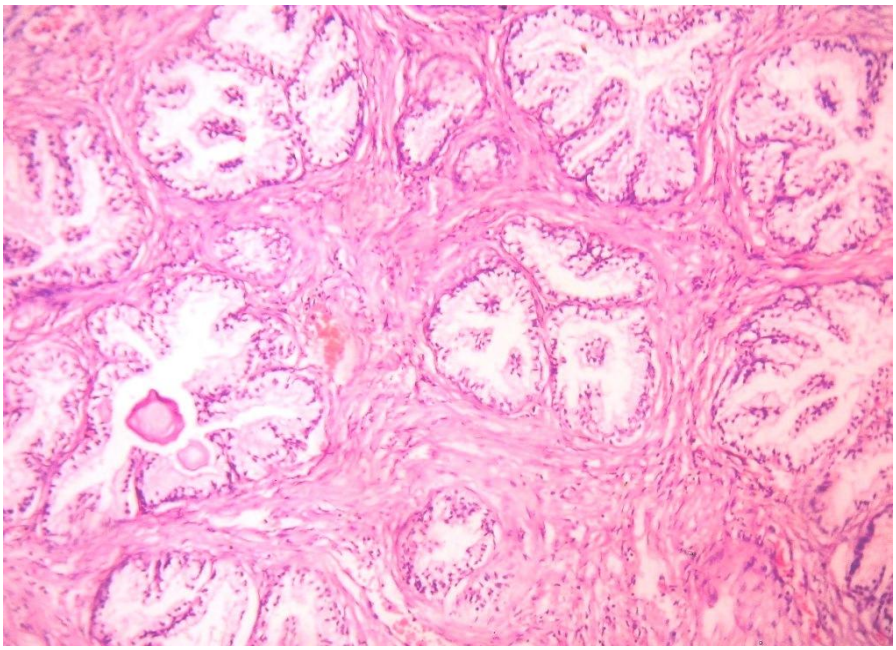


Fig.1.H&E shows Benign prostatic Glands with secretions inside it with fibromuscular stroma .10X

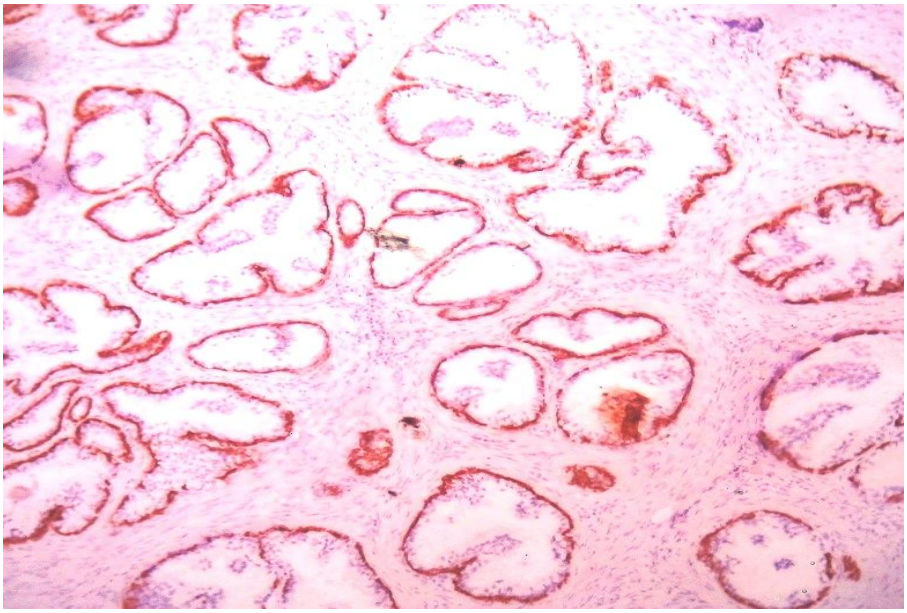


Fig.2. Continous 34βE12 positivity of basal cell layer in Benign prostatic Glands.10X

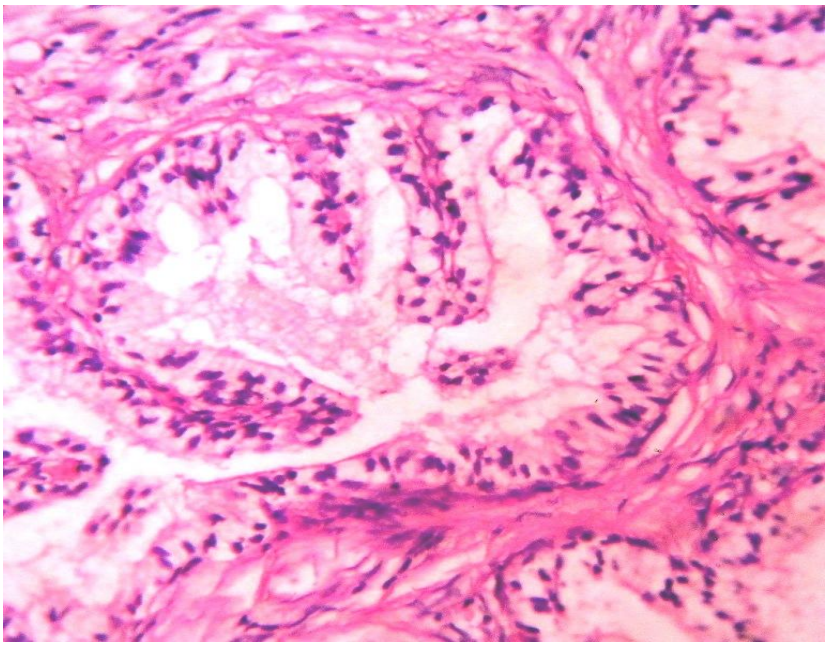


Fig.3.H&E shows magnified view of Benign prostatic Glands

40X

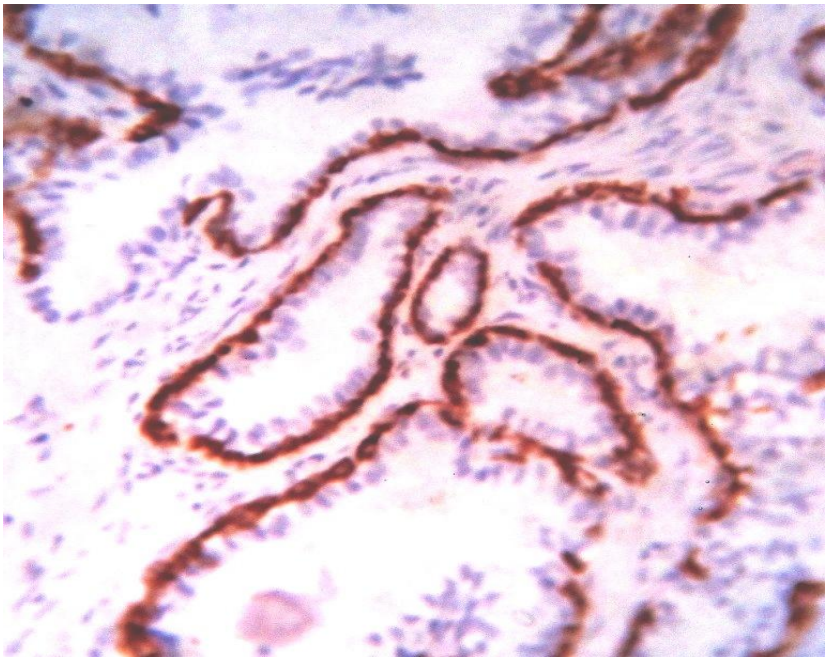


Fig.4. Continous 34βE12 positivity of basal cell layer in Benign prostatic Glands.40X

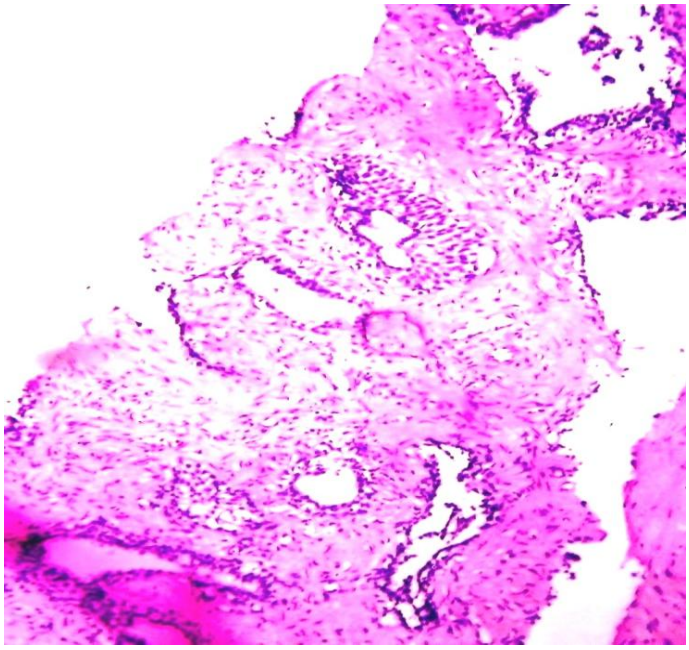


Fig.5.H&E shows benign prostatic glands in needle biopsy. 10X

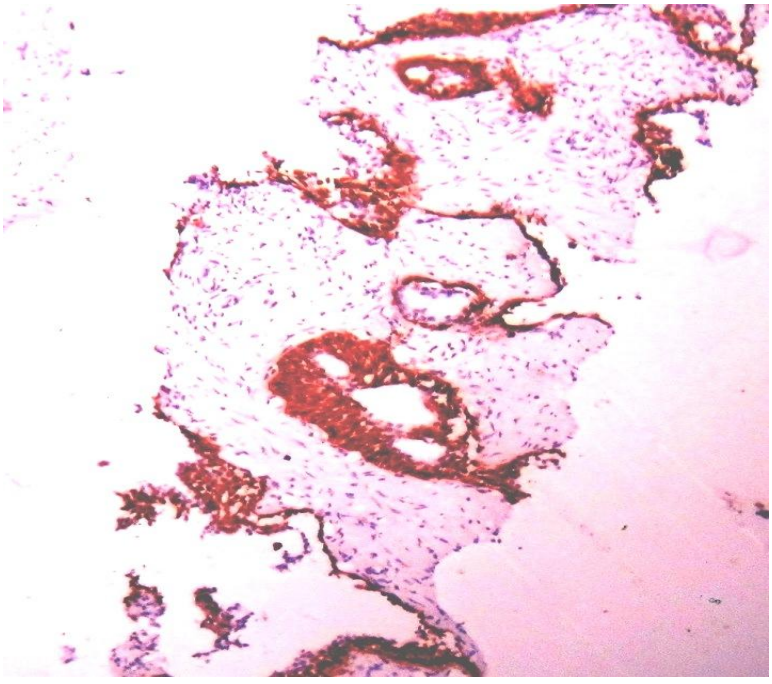


Fig.6.34βE12 shows continous positivity of basal cell layer in benign prostatic glands. 10X

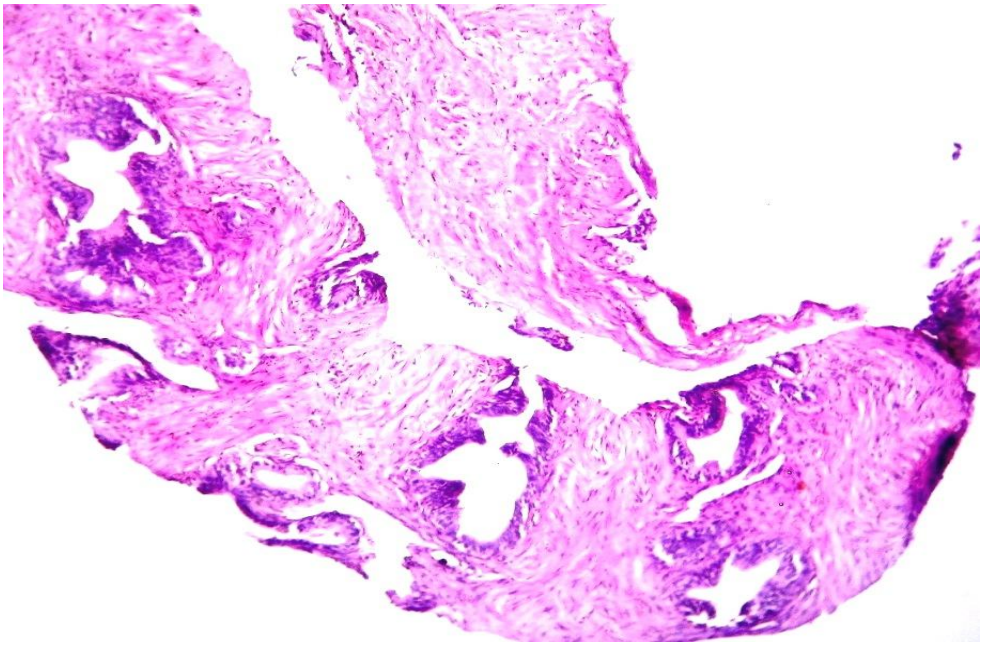


Fig.7.H&E shows Benign Prostatic Glands in needle biopsy.10X

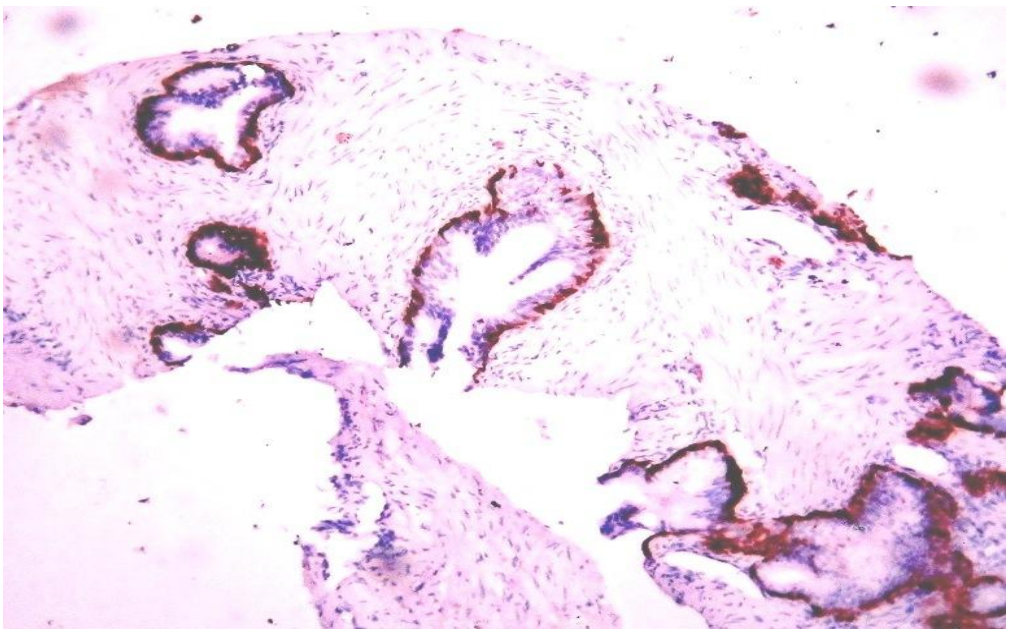


Fig.8.Continous 34βE12 positivity of basal cells in benign prostatic glands.10X

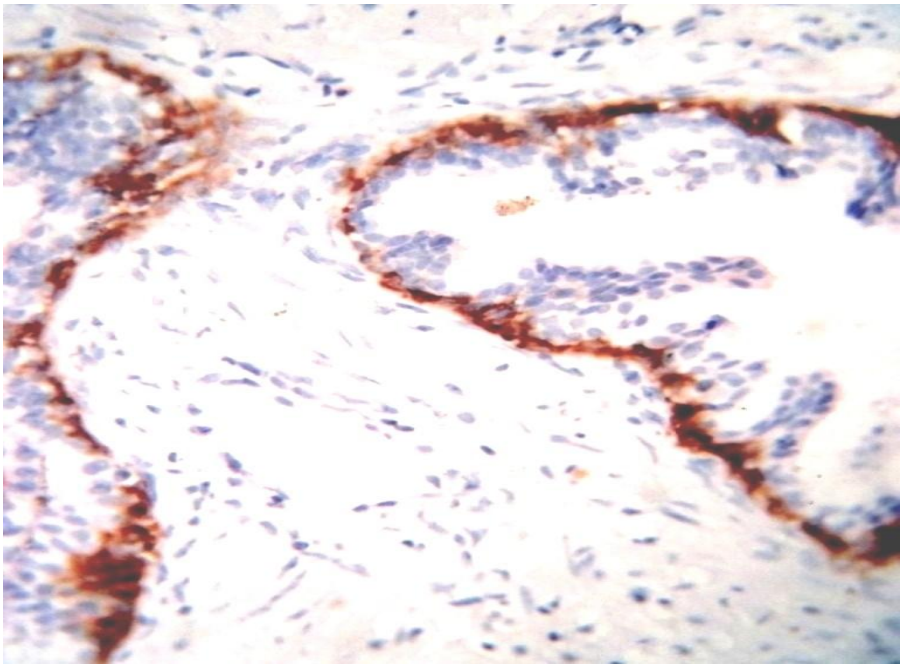


Fig.9.Continous 34βE12 positivity of basal layer in benign prostatic glands.40X

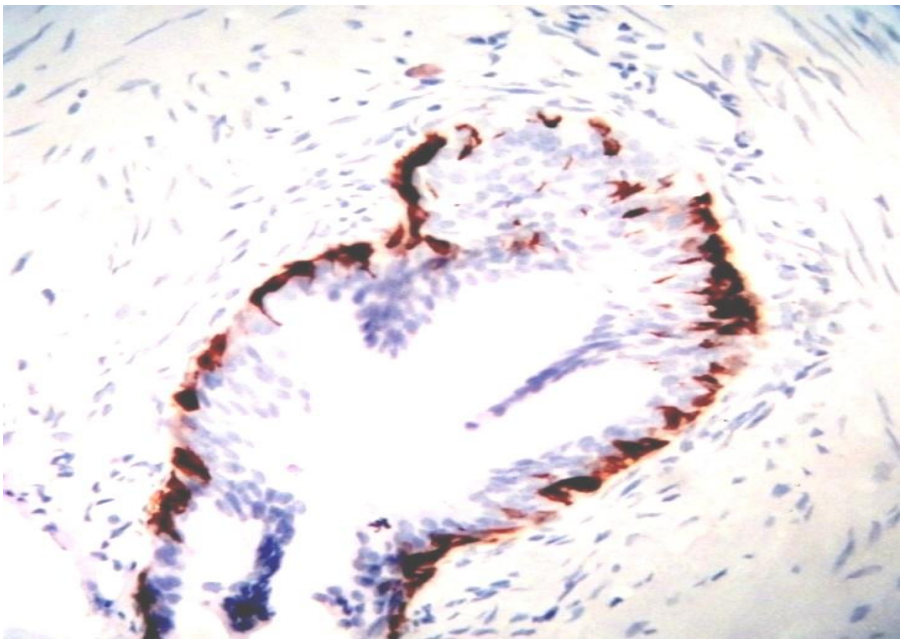


Fig.10.Continous 34βE12 positivity of basal layer in benign prostatic glands.40X

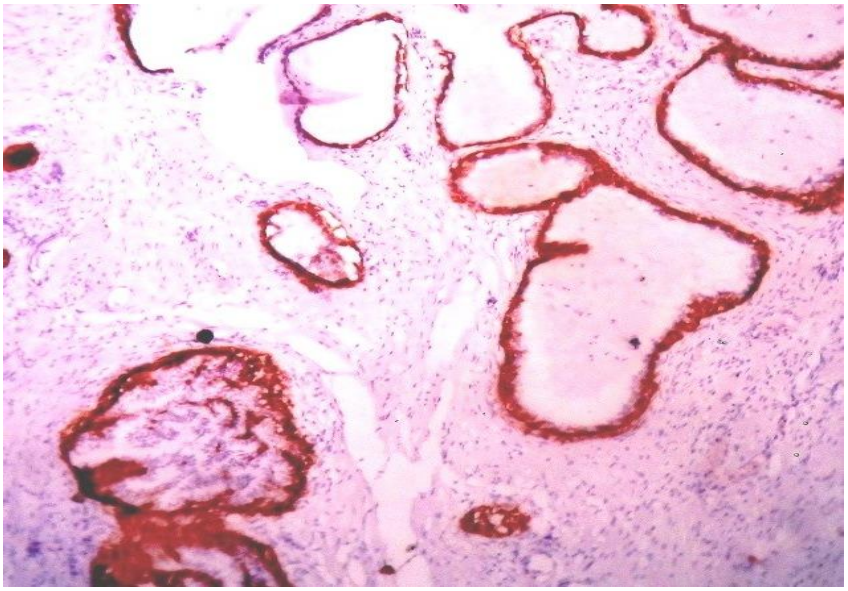


Fig.11.Continous 34βE12 positivity of basal layer in benign prostatic glands.10X

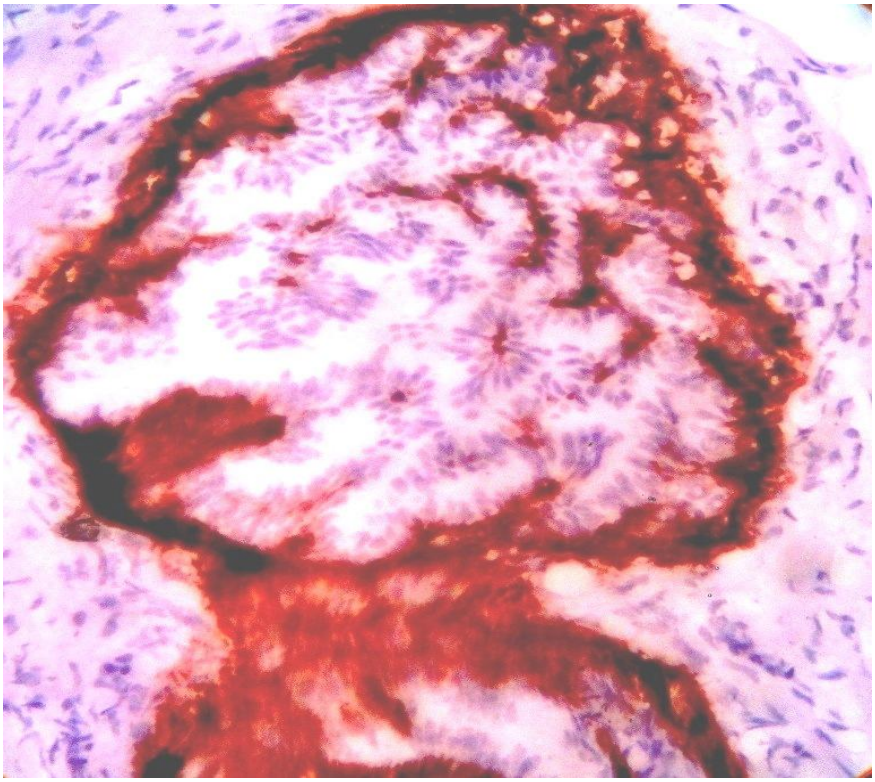


Fig.12.High power view of Continous 34βE12 positivity of basal layer in benign prostatic glands.40X

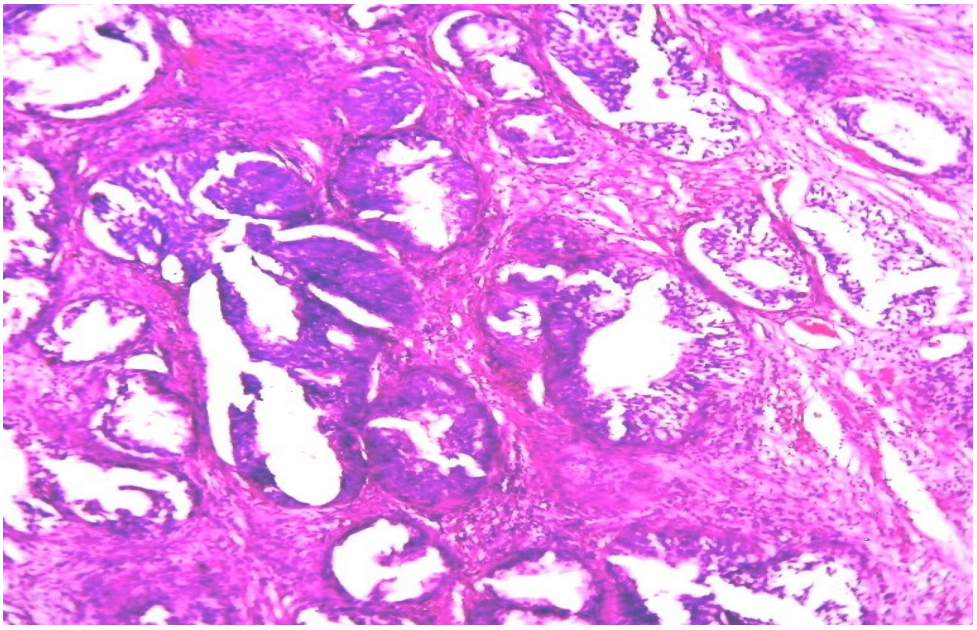


Fig.13.H&E of Basal cell Hyperplasia shows multilayering of basal cells .10X

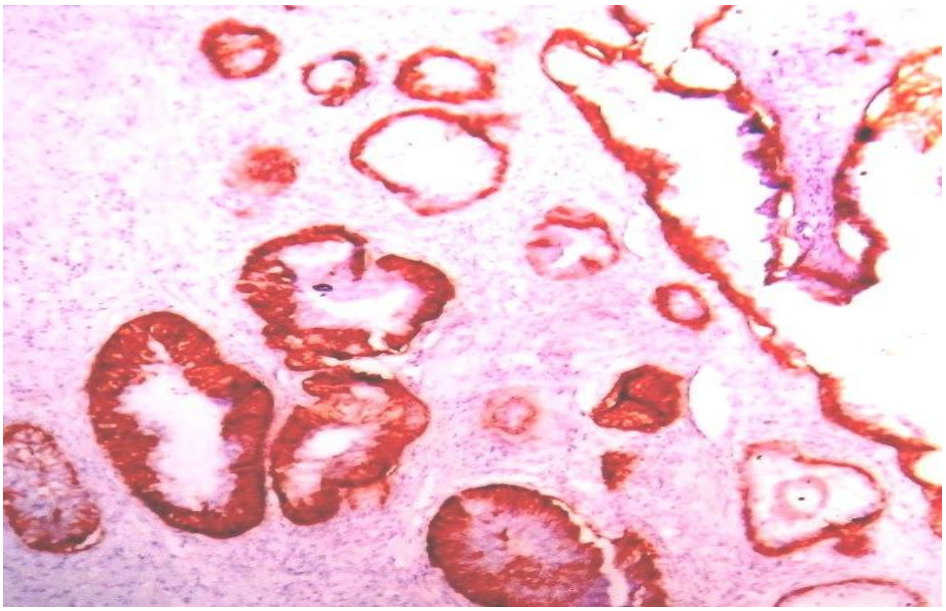


Fig.14.Continous positivity of 34βE12 in Basal cell Hyperplasia shows multilayering of basal cells .10X

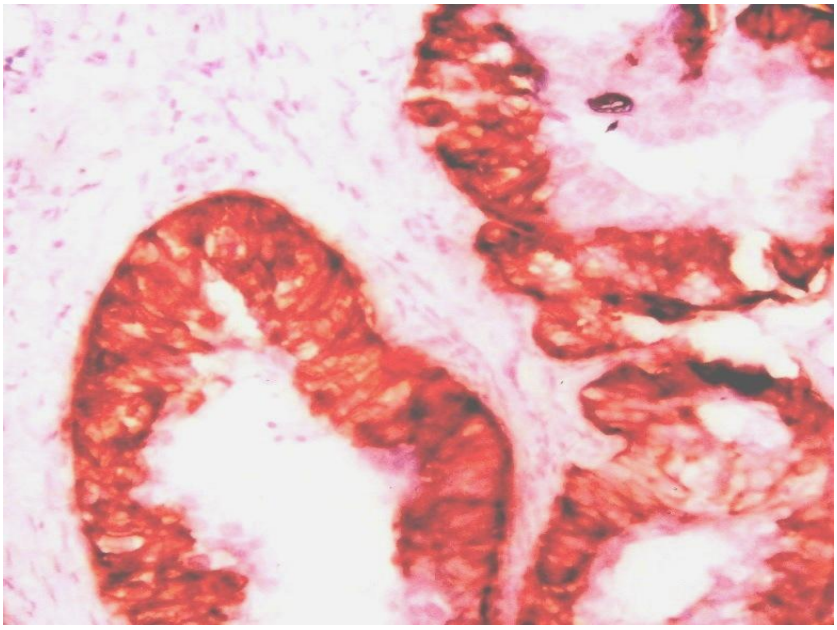


Fig.15.Continous positivity of 34βE12 in Basal cell Hyperplasia shows multilayering of basal cells .40X

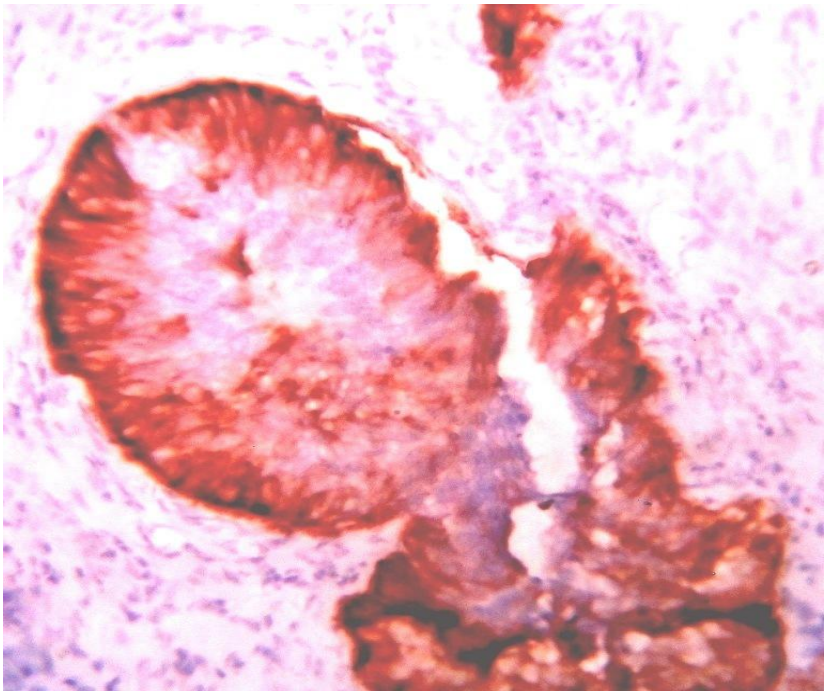


Fig.16.Continous positivity of 34βE12 in Basal cell Hyperplasia shows multilayering of basal cells .40X

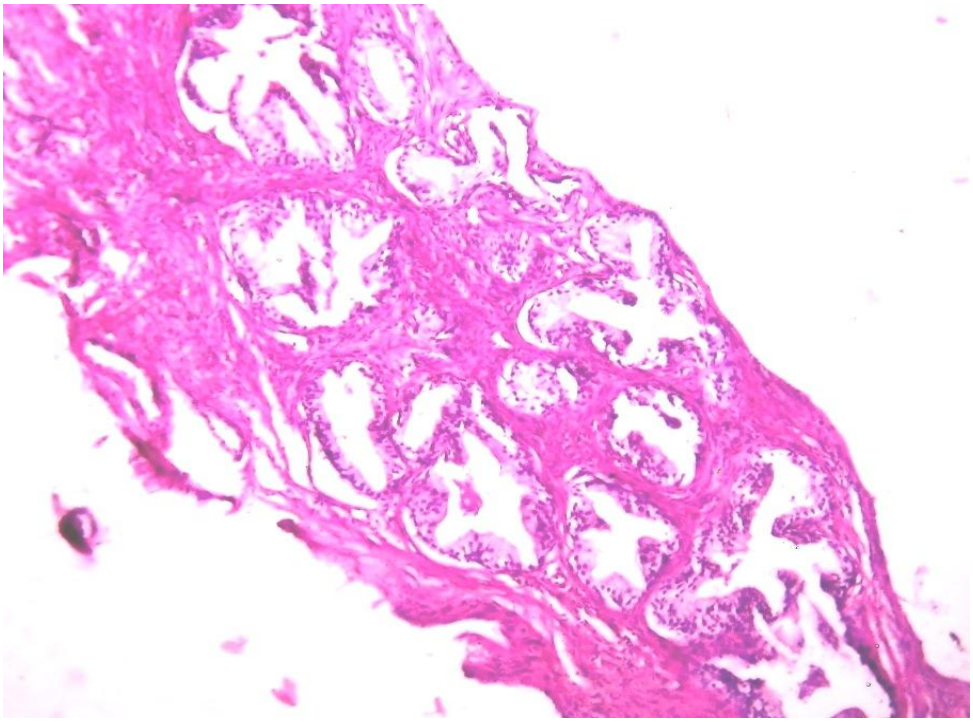


Fig .17.H&E shows Low grade Prostatic Intraepithelial Neoplasia.10X

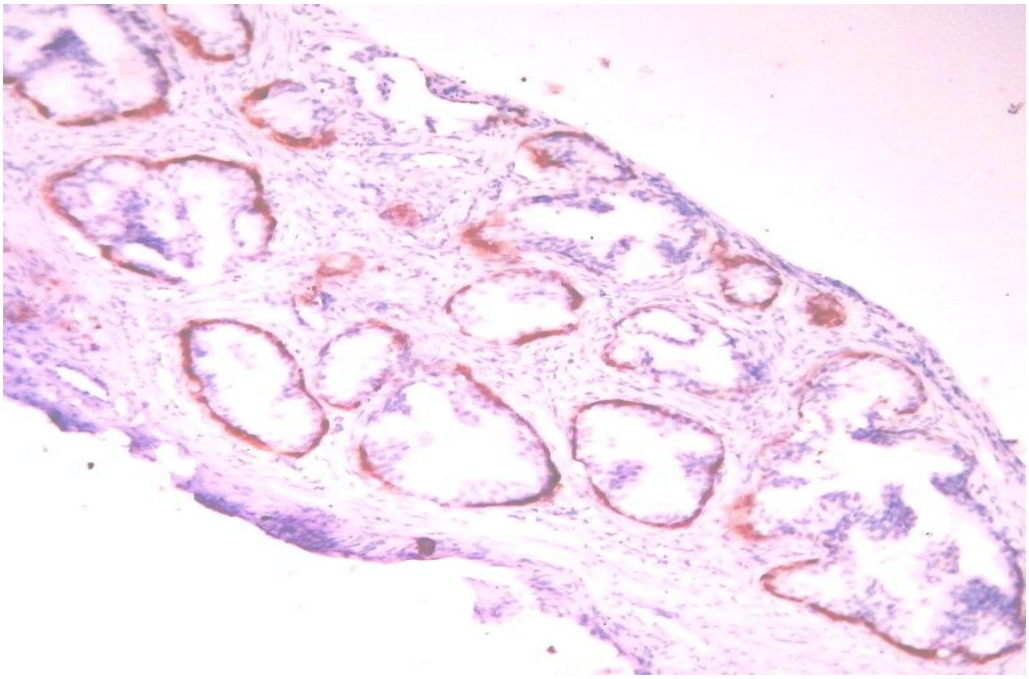


Fig .18.Discontinous 34βE12 positivity of basal cell layer in Low grade Prostatic Intraepithelial Neoplasia.10X

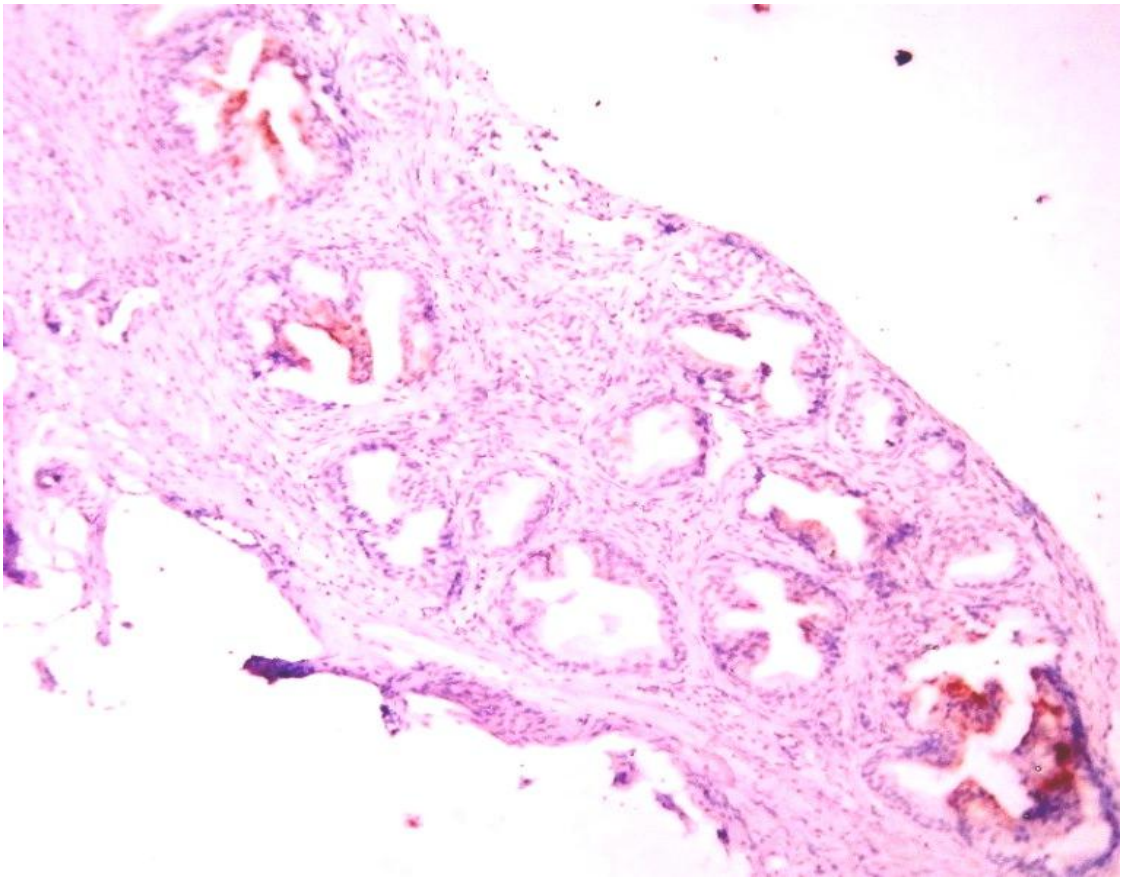


Fig .19.Weak & Non-circumferential positivity of AMACR in luminal epithelial cells in Low grade Prostatic Intraepithelial Neoplasia.10X

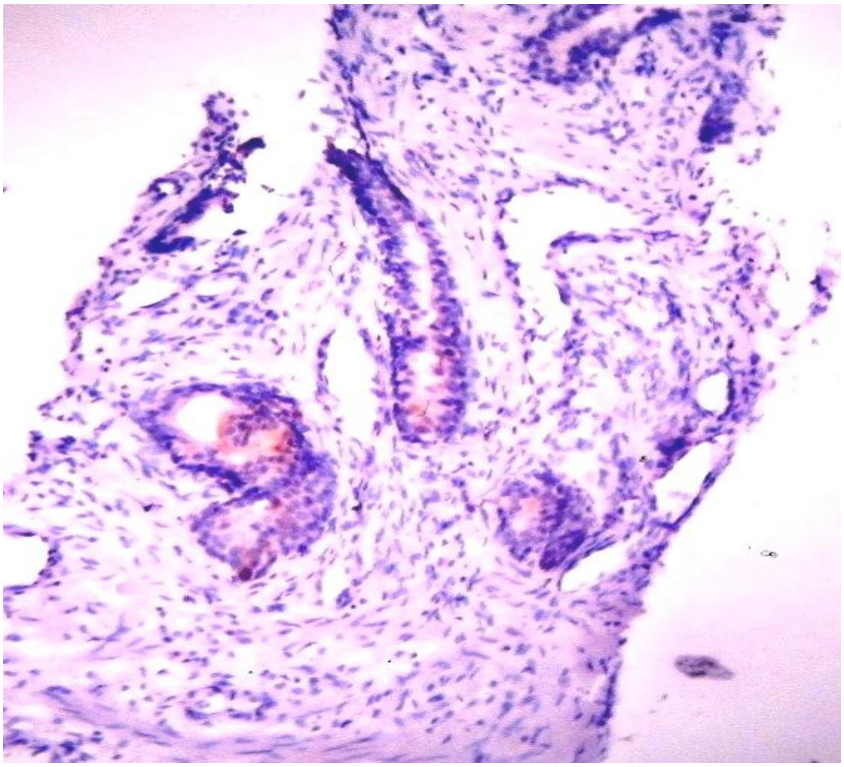


Fig .20.Weak & Non-circumferential positivity of AMACR in luminal epithelial cells in Prostatic Intraepithelial Neoplasia.10X

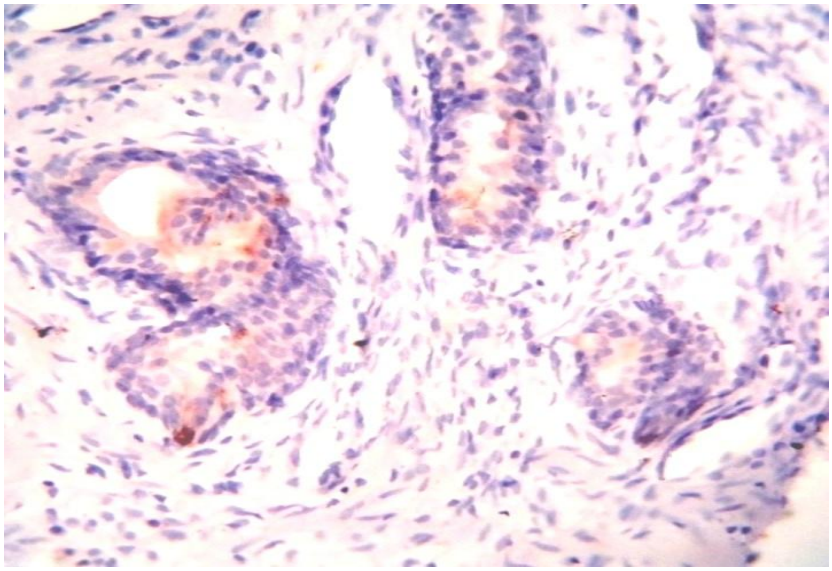


Fig .21.Weak & Non-circumferential positivity of AMACR in luminal epithelial cells in Prostatic Intraepithelial Neoplasia.40X

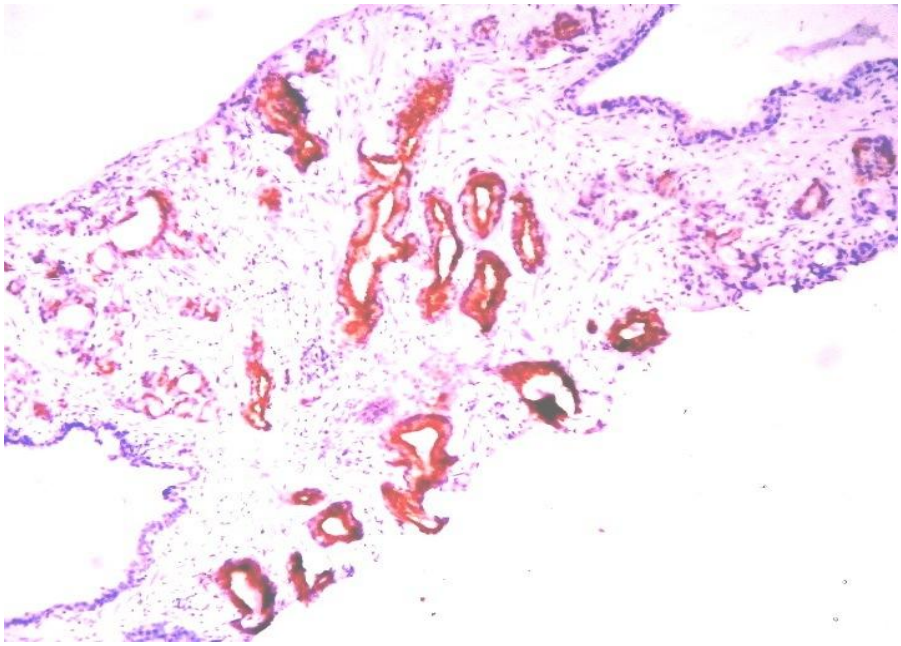


Fig .22.Continous, strong positivity of AMACR in luminal epithelial cells in Prostatic carcinoma.10X

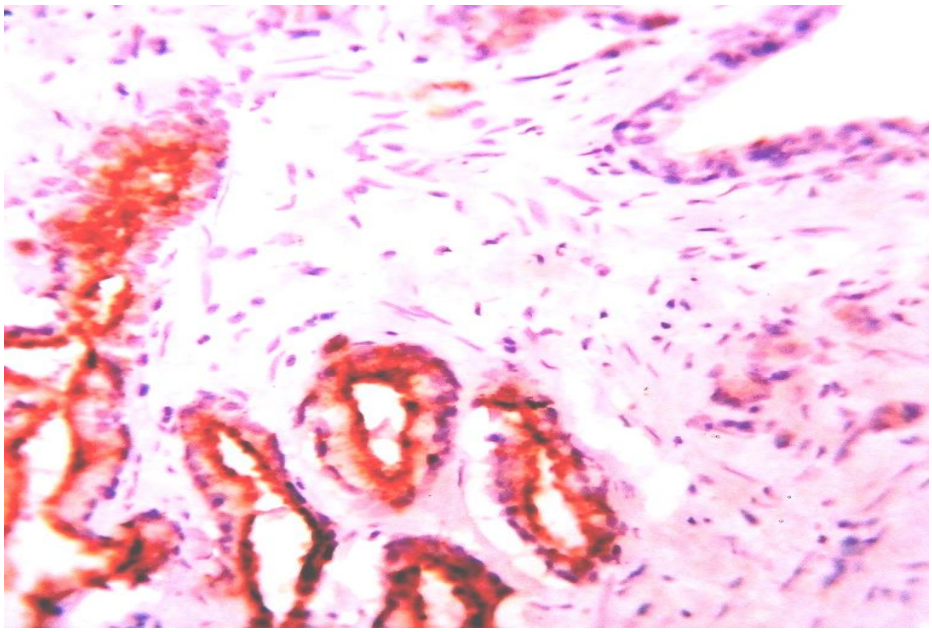


Fig .23.Continous, Granular &Strong positivity of AMACR in luminal epithelial cells in Prostatic carcinoma.40X

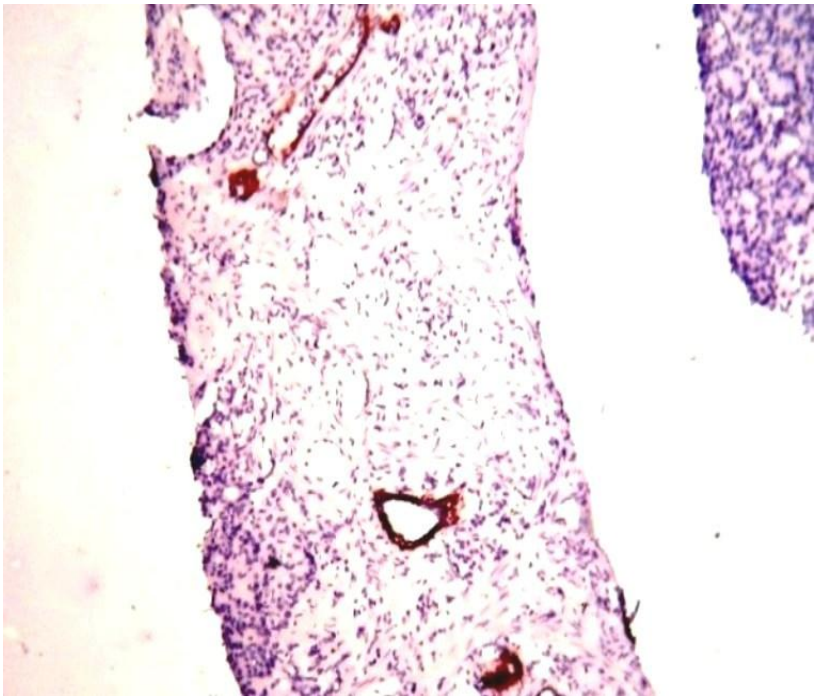


Fig .24.Continous 34βE12 positivity of basal cell layer and adjacent malignant foci shows negativity of 34βE12 in Prostatic carcinoma.10X

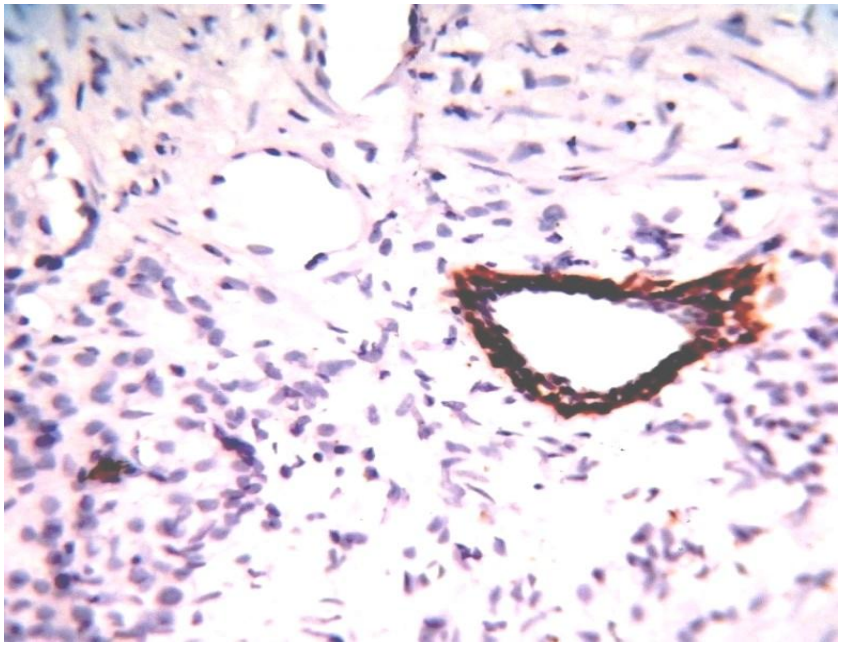


Fig.25.Continous 34βE12 positivity of basal cell layer and adjacent malignant foci shows negativity of 34βE12 in Prostatic carcinoma.40X

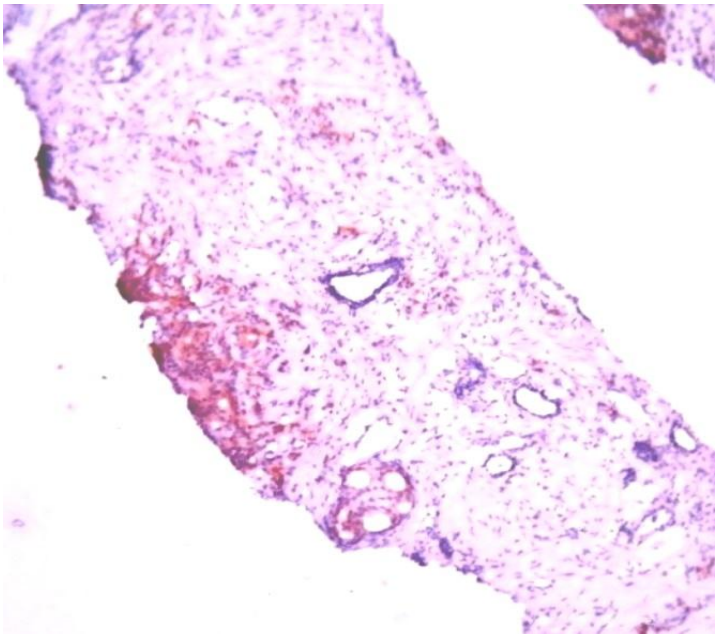


Fig .26. Granular &Strong cytoplasmic positivity of AMACR in malignant epithelial cells & its absence in adjacent benign glands in Prostatic carcinoma.10X

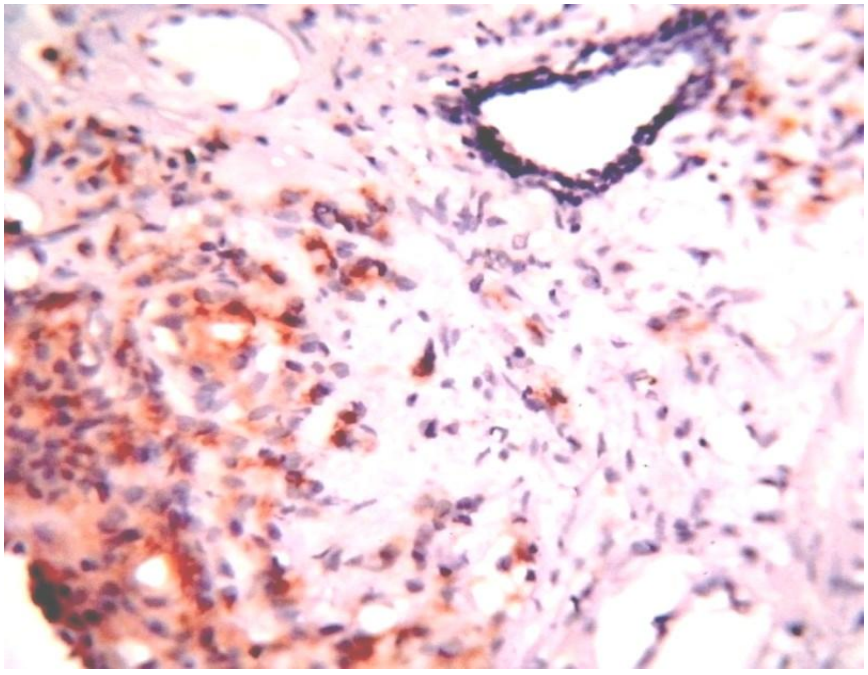


Fig .27. Granular &Strong cytoplasmic positivity of AMACR in malignant epithelial cells & its absence in adjacent benign glands in Prostatic carcinoma.10X

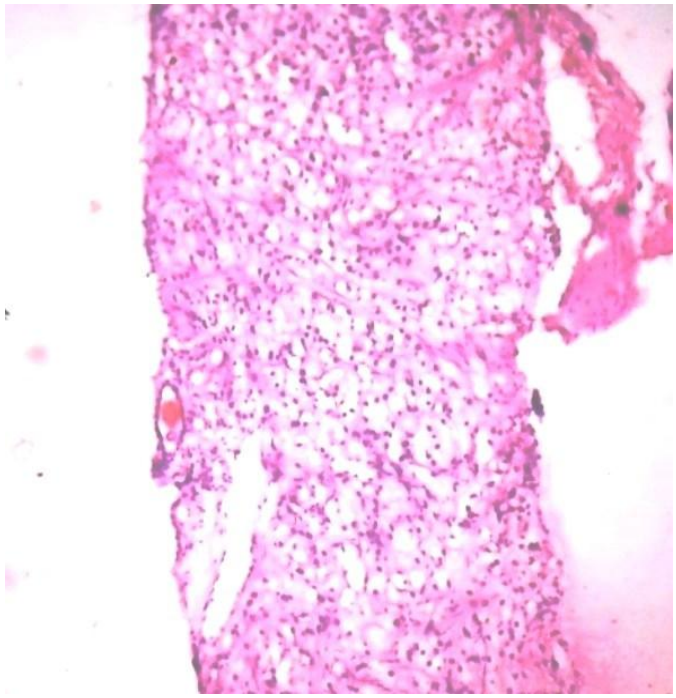


Fig.28. H&E shows Signet ring carcinoma of Prostate.10X

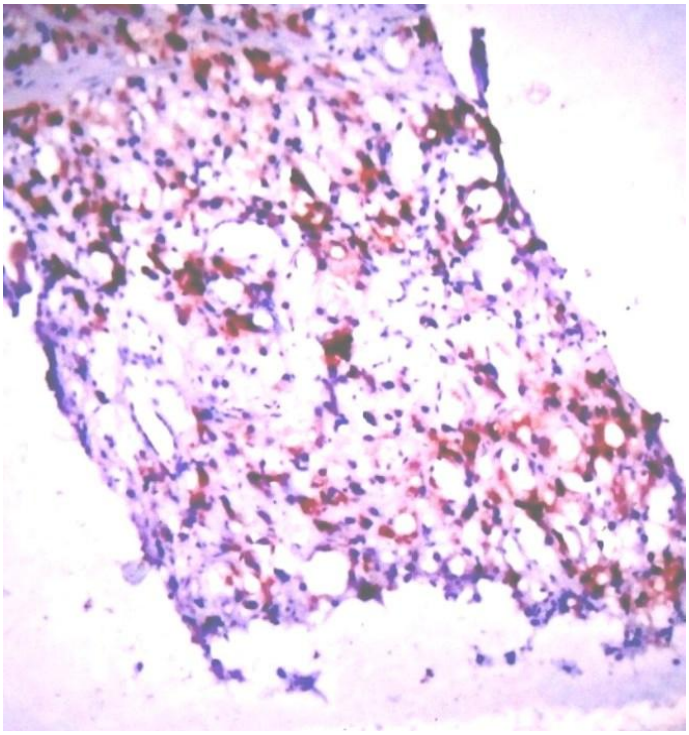


Fig.29.AMACR shows strong and granular cytoplasmic positivity in Signet ring carcinoma of Prostate.10X

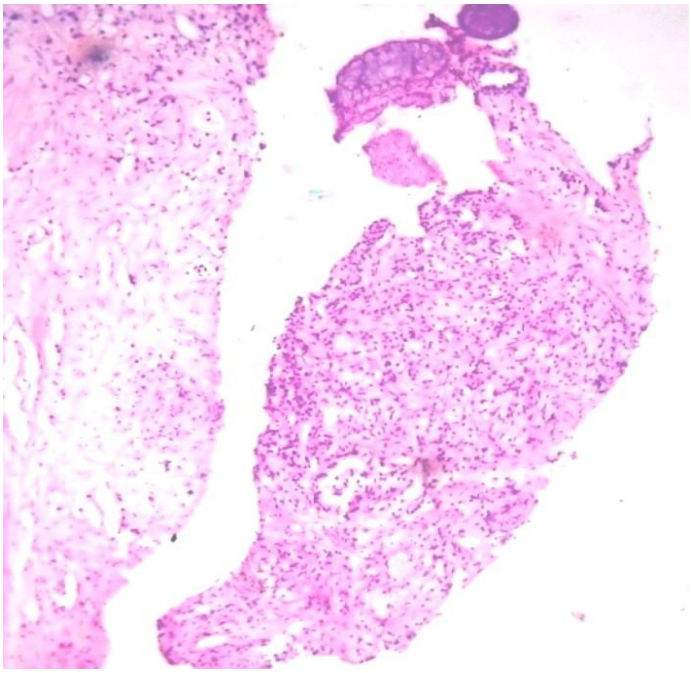


Fig.30. H&E shows High grade Carcinoma of Prostate in needle biopsy.10X

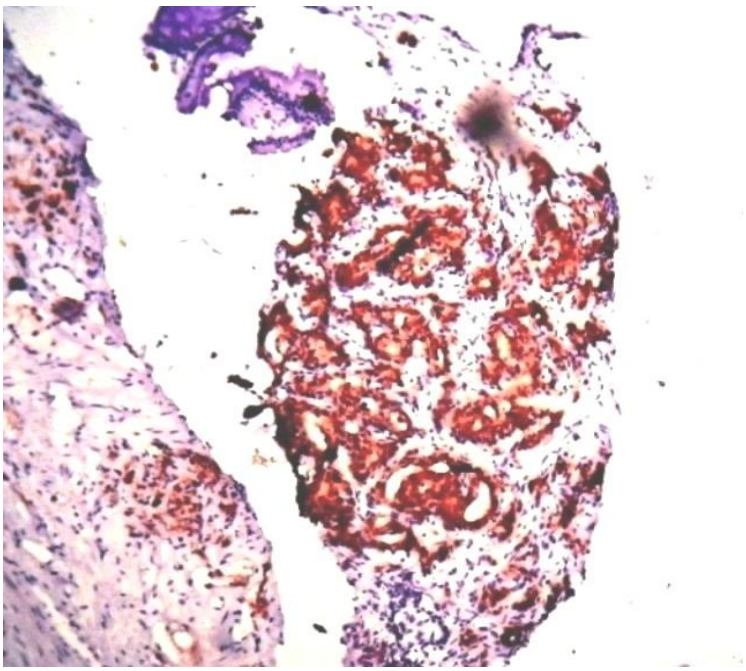


Fig.31.AMACR shows diffuse ,strong and granular positivity of malignant foci in Carcinoma of Prostate.10X

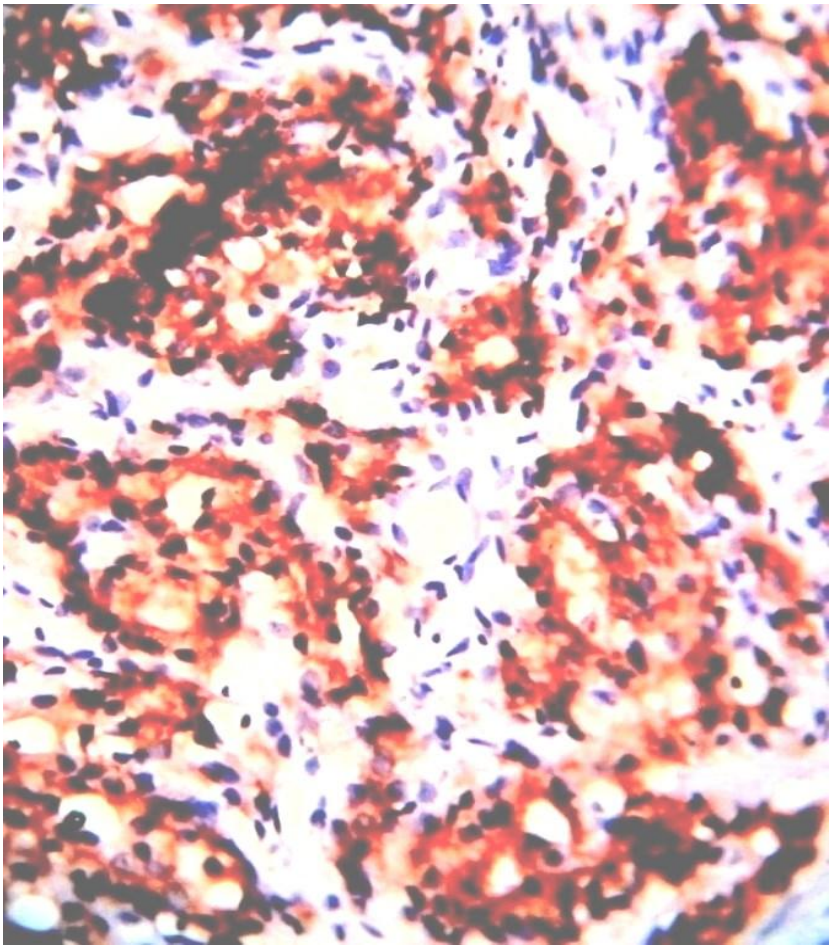


Fig.32.AMACR shows diffuse,strong and granular positivity

Carcinoma of Prostate in needle biopsy.40X

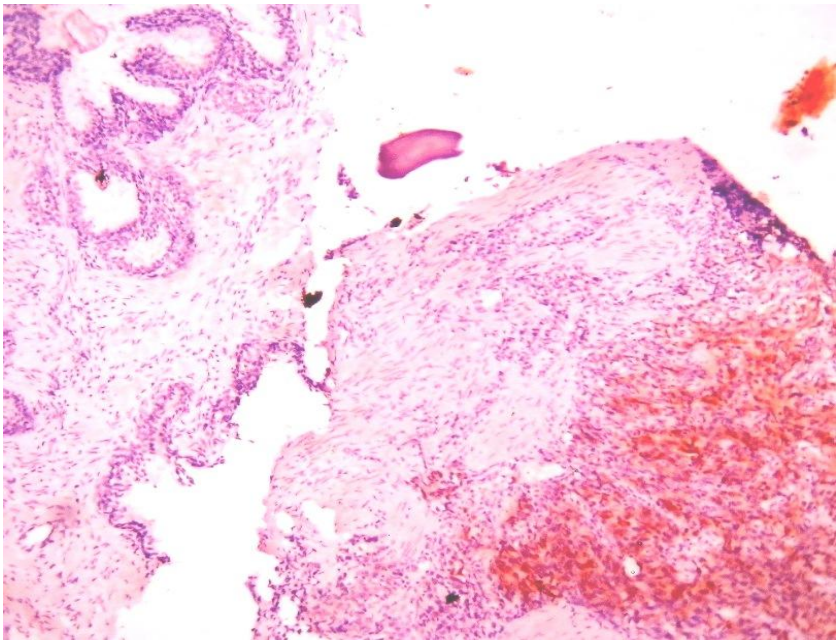


Fig .33. Granular &Strong cytoplasmic positivity of AMACR in malignant epithelial cells & its absence in adjacent benign glands in Prostatic carcinoma.10X

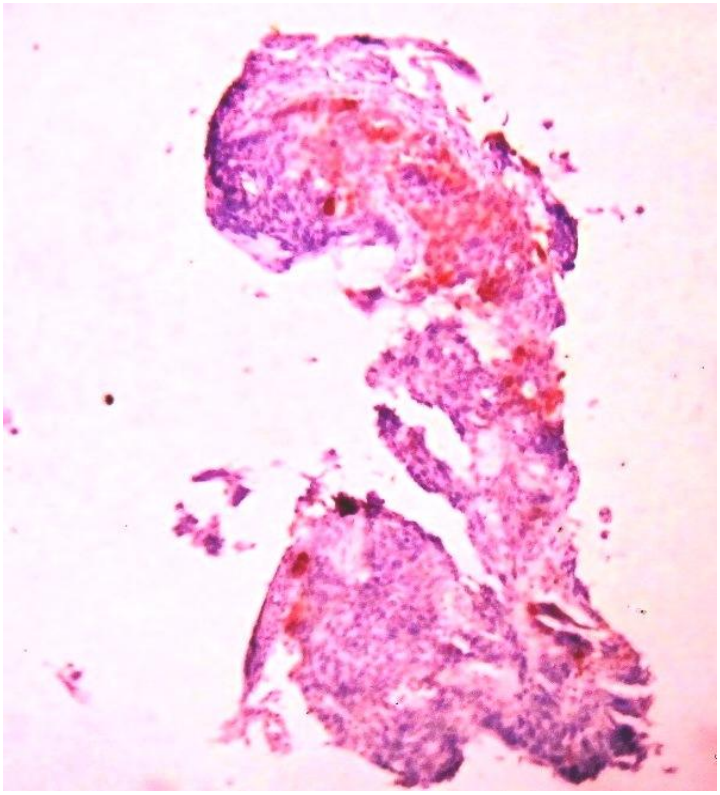


Fig.34.AMACR shows strong and granular positivity Carcinomaof Prostate in needle biopsy.10X

DISCUSSION

Some cases of needle biopsies are difficult in instances where only a few cancerous glands (minute carcinomas, small focus carcinomas) or benign mimics of cancer are there.

Considering the histologic features, based on standard histological staining, an initial diagnosis of “atypical small acinar proliferation” may be done. The diagnosis of such inconclusive cases affects 1.5–9% of prostate biopsies.

In this present study, in the 19 cases of prostatic carcinoma, AMACR positivity was detected in 17 cases, showing positive cytoplasmic granular staining. All 19 cases showed negative basal staining with 34βE12 .

The sensitivity of AMACR was 17/19 (90%) and the specificity of 34βE12 was 0/19 (100%).

Among the 10 benign cases including 1 case of basal cell hyperplasia, all the 9 cases stained positive with 34βE12 with a continous cytoplasmic positivity in basal cells. None of the above 10 cases were positive for AMACR staining.

Detection of prostatic carcinoma by 34βE12 had a Sensitivity of 100% and Specificity of 88.88%.

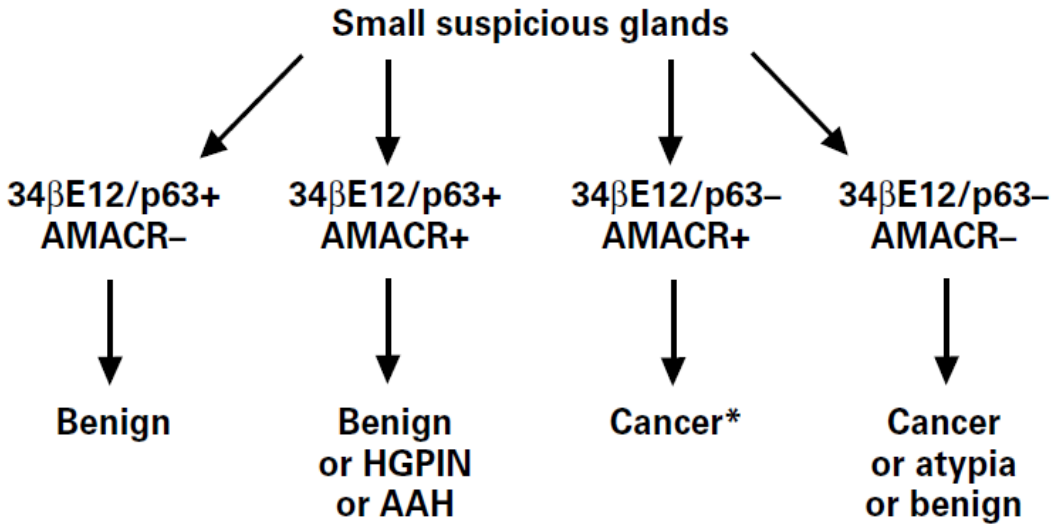
The accuracy of AMACR in detecting prostatic carcinoma was 90 % (17 out 19 of cases)

The accuracy of AMACR in detecting benign prostatic lesions was 100 % (9 out 9 of cases)

The accuracy of 34βE12 in detecting prostatic carcinoma was 100 % (19 out 19 of cases)

The accuracy of 34βE12 in detecting benign prostatic lesions was 88.88 % (8 out 9 of cases)

ALGORITHM FOR THE IMMUNOHISTOCHEMICAL MARKERS



The observation and results of previously conducted study relevant to his study are discussed below.

Vincent et al study[28] stated that prostatic cells in high-grade PIN, expressed with an intracytoplasmic granular pattern, p504s in 20–80 % of cases.

We also observed that this expression is generally weaker and more focal than the positivity of the tumoral prostatic glands.

Vincent study [28] results confirms that p504s is absent in cases of transitional metaplasia, atrophy, basal cell hyperplasia and postatrophic hyperplasia.

In this study we also observed the absence of expression of p504s in cases of basal cell hyperplasia.

Vincent's study[28] indices in terms of specificity and sensitivity (95 and 98%) are in concordance with the numbers described in literature. The combination of p504s and 34βE12 helps the confirmation of neoplastic transformation in the prostate gland; this has been stated by several groups as a helpful marker of tumoral prostatic cells in a diagnostic scenario. There is an increased specificity of up to 100% and sensitivity of up to 97%. This applies both in standard and tissue array biopsies. The results in this study are concordant with our study.

Luo et al,[12] stated in a simple assay that the two antibodies 34βE12 and p504s, when associated or combined together, one (34βE12) as a negative marker and the other(p504s) as a positive marker, significantly helps the detection of carcinomatous prostate cells. This results in improved specificity and sensitivity, a rise in diagnostic precision, and a fall in the risk of false negatives. The results in this study are concordant with our study.

Vincent's study[28] states that out of the "atypical small acinar proliferation" group, 89.4% can be detected using a combination of the two antibodies. It decreased the percentage of additional biopsies and inconclusive interpretations.

Zhiang Jiang[30] in his study, reported that immunoreactivity with p504s was found out of 73 cases in 69 (94.5%) of carcinoma. It was not seen in any benign gland surrounding malignant glands or case of benign prostate (0 of 69) or. The 34βE12 immunostaining, in all 73 cases, confirmed that basal cells were absent in the carcinoma focus. The results in this study are concordant with our study.

Luo et al[12] stated that < 4% of histologically normal prostate epithelium was positive for AMACR, while > 95% of prostate carcinoma stained positively. The results in this study are concordant with our study.

Victor et al[27] showed that AMACR was positive in 88% cases of small foci of prostatic carcinoma. They found that among the different groups AMACR sensitivity varies: 80% to 87% for cases from outside institutions and 100% for the in-house cases. P504S immunostaining was found in (94.5%) 69 of 73 cases of prostatic cancer but not in any benign glands adjacent to malignant glands and any benign prostates (0 of 69). In all 73 cases,the basal cells show absent 34βE12 immunostaining confirmed the focus of carcinoma. The results in this study are concordant with our study.

Beach et al [29] studied that (82%) that is153 of 186 biopsy specimens with prostate cancer shows positivity for AMACR.But only

21% of the benign prostatic epitheliai cells showed faint, focal,and noncircumferential staining .

Rubin et al [53] showed that among 94 specimens of prostate needle biopsy, demonstrated 100% specificity and 97% sensitivity of AMACR in prostate carcinoma detection.

Sung MTJiang[43] et al studied that basal cell markers (p63 or 34betaE12) were totally absent in all malignant acini. In (29%) 14 of 49 cases of prostatic carcinoma cells failed to demonstrate AMACR expression . In the remaining cases of 35 (71%), positive immunostaining with variable intensities and percentages of cells of AMACR was seen. In benign gland,positive staining for AMACRs was not seen in any case. In all benign acini cases, basal cells were strongly stained by p63 with a mean positive percentage of 96%. The results in this study are concordant with our study.

Victor et all[27] in his study showed AMACR specificity ranges from 79% to 100% and the sensitivity varies from from 82% to 100%

Rubin et al[53]in his study showed that in 94 specimens of prostate needle biopsy ,AMACR has 100% specificity and 97% sensitivity in the detection of prostate carcinoma

The results and indices in this study are in conformity with the previously conducted studies.

Thus it goes on to show that immunohistochemistry has a vital role in detection of morphologically difficult prostatic lesions.

CONCLUSION

In the present study the incidence of prostatic carcinoma was common in the age group of 71-75 years.

Incidence of prostatic carcinoma was 38%, prostatic intraepithelial neoplasia was 10%, benign prostatic hyperplasia was 48%

AMACR grading:

74% cases showed Grade 3 positivity

10% cases showed Grade 2 positivity

5% cases showed Grade 1 positivity

10% cases showed Grade 0 positivity

The sensitivity of AMACR in detection of prostate carcinoma was 90% and specificity was 100%.

The sensitivity of 34βE12 detection of prostate carcinoma was 100% and specificity was 89%.

Newer antibodies against prostatic tumor cells (p504s) and prostatic basal cells (34βE12,p63,CK 5/6) have proven to beneficious. The results showed that, for ambiguous lesions such as atypical small acinar proliferation, small foci of prostatic carcinoma not diagnosed ,but

suspected to be malignant can be benefited by the use of these markers. Immunohistostaining with 34βE12 and p504s has improved diagnostic uitility in microscopically difficult cases. It helps to avoid newer and subsequent prostatic biopsies ,which are costlier and causing morbidity in the patients. The application of these newer antibodies individually is less relavant than the combined use of these antibodies. Compared to a new series of biopsies, the cost of immunohistochemical techniques remains lower .

So, the conclusion is that in conjuntion with the clinical scenario and morphology, a combination of prostatic epithelial marker AMACR and basal cell marker HMWCK is of better value in diagnosing the prostate carcinoma cases and other morphologically difficult lesions. Hence, the accuracy of diagnosis in prostate cancer is significantly increased. However, it should be kept in mind about the limitations of both the markers.

In summary, from several institutions, various type of studies have demonstrated that AMACR/ P504S is an important positive epithelial cell marker for prostate cancer. A sensitivity ranging from 82% to 95% and a specificity ranging from 79% to 100% was achieved, regardless of tumor grade; as well as with different criteria for positive stains in benign and malignant glands[20,33,36,37,45,59].

Hence,AMACR has the potential to be a useful marker which can be used seperately for prostate carcinoma in clinical pathology practice. Similarly 34βE12 has a high specificity and sensitivity for identifying prostate lesions in biopsies.

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ANNEXURE 1

PROFROMA

Coimbatore medical college

Department of pathology

Coimbatore

Particulars of the patient:

Name: Age:

Ward: IP/OP No:

Address: Occupation:

Presenting complaints:

Dysuria

Burning micturition

Drippling of urine

Duration of presenting complaint:

Past history:

History of previous surgeries

History of chemotherapy/Radiotherapy

Family history:

Personal history

Diet

General examination

Nourishment : Built: Consious:

Pallor: Jaundice: Cyanosis: Clubbing:

PR: RR: BP: Febrile/afebrile:

Lymphadenopathy: Edema:

Digital Rectal Examination:

Clinical diagnosis:

Investigations:

Serum PSA level

USG Report

FINAL REPORT

- Specimen : Biopsy/TURP
- HPE Diagnosis : PC/ PIN/ ASAP / BPH/ BCH
- Gleason grading :
- AMACR : Positive/ Weak positive/Negative
- 34Beta E12 : Positive/ Weak positive/Negative
- Final Diagnosis : PC/ PIN/ ASAP/ BPH/ BCH

Sno	IP No	Pt Name	Age	HPE No	Specimen	HPE	Gleason grading	AMACR	34Beta E12	Final Diagnosis
1	119110	Saranraj	75	314/11	TURP	PC	5+4	negative	negative	PC
2	69111	Perumal	63	315/11	Biopsy	PC		focal positive	negative	PC
3	6349	Murugasamy	80	352/11	Biopsy	BPH		negative	negative	BPH
4	11397	Suyambu	69	486/11	Biopsy	PIN		negative	negative	PIN
5	11975	Arokiadoss	58	607/11	Biopsy	BPH		negative	positive	BPH
6	22581	Manickam	72	986/11	Biopsy	PC	4+3	negative	negative	PC
7	25040	Mani	55	1025/11	Biopsy	PC	4+3	positive	negative	PC
8	27827	Raju	67	1033/11	TURP	PC	4+4	positive	negative	PC
9	32900	Ramasamy	85	1229/11	TURP	BPH		negative	positive	BPH
10	310109	Samraj	75	1293/11	TURP	BPH		negative	positive	BPH
11	33753	Moosa	58	1296/11	TURP	BPH		negative	positive	BPH
12	32891	Karuppusamy	80	1312/11	Biopsy	PC	5+4	Focal positive	negative	PC
13	44229	Karuthachalam	36	1903/11	Biopsy	PC	5+4	positive	negative	PC
14	4821	Chinnasamy	60	2040/11	Biopsy	PIN		weak positive	positive	PIN
15	66193	Arumugam	62	2042/11	TURP	BPH		negative	positive	BPH
16	45496	Vadamalai	65	2209/11	Biopsy	PC	4+3	positive	negative	PC
17	20431	Muthusamy	55	2235/11	Biopsy	PC	4+3	positive	negative	PC
18	53840	Subramani	61	2305/11	Biopsy	PC	4+3	positive	negative	PC
19	52224	Krishnan	75	2363/11	TURP	BCH		negative	positive	BCH
20	55919	Sampath	50	2441/11	Biopsy	PC	4+3	positive	negative	PC
21	2072	Arumugam	62	2442/11	Biopsy	PC	4+4	positive	negative	PC
22	45496	Vadamalai	65	2515/11	Biopsy	PC	3+4	positive	negative	PC
23	42198	Sugaprama	78	2516/11	Biopsy	PC	4+5	positive	negative	PC
24	62043	Karuppusamy	70	2646/11	Biopsy	ASAP		positive	negative	ASAP
25	66418	Karuppusamy	66	2799/11	Biopsy	PIN		weak positive	dis.positive	PIN
26	70529	Natarajan	74	2972/11	Biopsy	BPH		negative	positive	BPH
27	2936	Ramalingam	62	137/12	Biopsy	BPH		negative	positive	BPH
28	17753	Mookannan	80	814/12	Biopsy	PIN		weak positive	positive	PIN
29	18458	Duraisamy	70	857/12	Biopsy	PIN		weak positive	positive	PIN
30	18495	Devaraj	64	858/12	Biopsy	PC	4+5	positive	negative	PC
31	20139	Kasinathan	55	964/12	Biopsy	PC	4+4	positive	negative	PC
32	22225	Myilsamy	65	965/12	Biopsy	PC	3+4	positive	negative	PC
33	24813	Moideen	69	1109/12	Biopsy	PC	4+5	positive	negative	PC
34	41264	Munusamy	68	1986/12	Biopsy	PC	4+4	positive	negative	PC
35	13452	Kaliappan	59	2313/11	TURP	BPH		negative	positive	BPH

KEY TO MASTER CHART

BPH	–	Benign Prostatic Hyperplasia
PIN	–	Prostatic Intra Epithelial Neoplasia
ASAP	–	Atypical Small Acinar Proliferation
PC	–	Prostatic Carcinoma
AMACR	–	α-Methyl Acyl CoA Racemase
HMWCK	–	High Molecular Weight Cytokeratin
HPE	-	Histopathological Examination
IHC	–	Immunohistochemistry
H&E	-	Hematoxylin And Eosin Staining

ABSTRACT

Prostatic carcinoma accounts for the highest incidence of malignancies in men and it is the second most common cause of morbidity. Increased prostate-specific antigen levels increases needle biopsies, for the exclusion of prostate cancer. But, a needle biopsy presents problems, only a small amount of tissue is provided for microscopic examination. It is a difficult task to accurately diagnose small foci of prostate cancer for pathologists and to distinguish cancer from its benign mimickers. Hence, definitive diagnosis with the available specimen is essential for the benefit of patients. The diagnosis of prostate adenocarcinoma is supported by the absence of basal cells which is highlighted by Immunohistochemical markers high-molecular-weight cytokeratin and p63 but they are negative markers for prostatic carcinoma. Now, a newer marker, AMACR (α-Methyl Acyl CoA Racemase) is used in the diagnosis of prostate cancers as a positive marker with high sensitivity (76 – 100%) and high specificity (75 – 95%).

TITLE: THE EXPRESSION AND DIAGNOSTIC UTILITY OF AMACR AND 34β E 12 IN PROSTATIC LESIONS

AIM AND OBJECTIVE:

To study the Expression and Diagnostic utility of Immunohistochemical markers AMACR and 34βE12 in various Prostatic lesions.

MATERIALS & METHODS:

Among the total cases received in the Department of Pathology of our hospital during study period, 37 cases were taken into study. Included were 29 needle biopsies and 8 TURP specimens.

The received samples were then fixed in 4% formalin, embedded in paraffin and stained with H&E. After eosin and hematoxylin staining all slides were reviewed by pathologists and assigned to the following groups - Benign prostatic hyperplasia, Basal cell hyperplasia, PIN, malignant and suspicious.

OBSERVATION & RESULTS:

In this study, a total of 37 cases were evaluated. 17 cases prostatic carcinoma, 5 cases prostatic intraepithelial neoplasia, 10 benign prostatic hyperplasia were detected. The sensitivity of AMACR in detection of prostate carcinoma was 90% and specificity was 100%.The sensitivity of 34βE12 detection of prostate carcinoma was 100% and specificity was 89%.

CONCLUSION:

In conjunction with the clinical scenario and morphology, a combination of prostatic epithelial marker AMACR and basal cell marker HMWCK is of better value in diagnosing the prostate carcinoma cases and other morphologically difficult lesions.