CORRELATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF ALPHA-METHYL ACYL-COENYME ARACEMASE/P504S WITH GLEASON GRADE and serum PSA level in prostate carcinoma

BY

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Dissertation submitted to

BLDE (Deemed to be University) Vijayapur, Karnataka

In partial fulfillment of the requirements for the degree of

DOCTOROFMEDICINE IN

PATHOLOGY

Under the guidance of

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DEPARTMENTOF PATHOLOGY

BLDE (Deemed to be University)

SHRIB.M.PATILMEDICALCOLLEGE

HOSPITAL & RESEARCH CENTRE, VIJAYAPUR

KARNATAKA

2020

"CORRELATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF ALPHA-METHYL ACYL-COENZYME A RACEMASE/P504S WITH GLEASON GRADE AND SERUM PSA LEVEL IN PROSTATE CARCINOMA"

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ABSTRACT

INTRODUCTION:

Carcinoma of the prostate is the second most common cause of cancer. AMACR is a diagnostic marker for prostatic carcinoma. There is a significant lack of studies relating to the immunohistochemical expression of AMACR in prostatic cancer tissue and its prognosis.

OBJECTIVES:

To study the pattern of expression of AMACR in prostatic carcinoma on immunohistochemistry and its correlation with Gleason grade and serum PSA level of patients.

MATERIAL AND METHODS:

This was a retroprospective study of 45 cases of histopathologically diagnosed Primary Adenocarcinoma of prostate. Immunohistochemistry for AMACR was performed on tissue core biopsies, TURP chips and its expression was evaluated in relation to Gleason grade and PSA level.

RESULTS:

AMACR expression was noted in 42(93.33%) cases of malignant lesions of the prostate. Statistically significant relation was obtained between high AMACR expression and high Gleason grade. No significance was noted between AMACR expression and serum PSA level.

CONCLUSION:

This study adds to an understanding of efficacy of immunoexpression of AMACR in prostate carcinoma. Strong AMACR expression is a poor prognostic indicator as it is associated with high Gleason grade in prostatic carcinomas.

KEY WORDS: AMACR, Gleason grade, Prognosis.

LIST OF ABBREVATIONS USED

PC	-	Prostate carcinoma
BPH	-	Benign Hyperplasia of Prostate
AMACR	-	Alpha-Methylacyl-CoA Racemase
PSA	-	Prostate-specific specific antigen
PAP	-	Prostate Acid Phosphatase
LTUS	-	Lower Urinary Tract Symptoms
AR	-	Androgen Receptor
CRPC	-	Castrate-Resistant Prostate Carcinoma
ADT	-	Androgen Deprivation Therapy
PIN	-	Prostatic Intraepithelial Neoplasia
PNI	-	Perineural Invasion
PAA	-	Prostate Acinar Adenocarcinoma
TURP	-	Transurethral Resection of Prostate
IHC	-	Immunohistochemistry
KLK3	-	Kallikrein 3
KDa	-	KiloDalton
TRUS	-	Transrectal Ultrasonography
BCH	-	Basal Cell Hyperplasia
AAH	-	Atypical Adenomatous Hyperplasia
CCCH	-	Clear Cell Cribriform Hyperplasia
SA	-	Sclerosing Adenosis
HMWCK	-	High Molecular Weight Cytokeratin
CK	-	Cytokeratin
SMA	-	Smooth Muscle Actin

ISUP	-	International Society of Urological Pathology
GG	-	Gleason Grade
IDC	-	Intraductal Carcinoma of Prostate
GS	-	Gleason Score
PIRADS	-	Prostate Imaging Reporting and Data System
HGPIN	-	High-Grade Prostatic Intraepithelial Neoplasia
DAB	-	Diaminobenzidine

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INTRODUCTION

Carcinoma prostate is the second most common cause of cancer and the sixth leading factor in men's cancer-related deaths globally,[1] with an average age of 66 at diagnosis; prostate cancer mortality and incidence are connected with global ageing. It is predicted that there will be 2,293,818 new cases up until 2040, with an increase of 1.05% in mortality.[2]

There is a constant and rapidly increasing incidence of prostate cancer in India. In men under 40 years of age, prostate cancer is rare, and the risk increases exponentially after 50 years of age. 6 out of 10 prostate cancers are detected in men over the age of 60.[3]

Previously, the prevalence of prostate cancer in India was thought to be much lower than in Western countries, but there have been increasing migrations of the rural population to increased awareness, urban areas, lifestyle changes, and increased health care that have led to increase in prostate carcinoma prevalence in India. Ease of access to facilities has led to more cases of prostate cancer. The increase in cancer has made us realize that we are not far behind rates in Western countries. We are reporting new information, and we see a significant increase in cancer cases over the next few years.[1]

Most tumors remain asymptomatic and present with only mild urinary complaints like dysuria, hesitancy, increased frequency etc. It is diagnosed by digital rectal examination or trans rectal ultrasound.[4]

Prostate specific antigen (PSA) is the most used biomarker for prostate cancer diagnosis and prognosis assessment. However, since it is present in cells of benign and malignant prostatic epithelium, PSA is not a cancer-specific marker. In benign conditions, such as benign prostatic hyperplasia (BPH) and prostatitis, serum PSA

levels are also elevated. Consequently, to rule out or confirm the presence of carcinoma of the prostate, patients with an elevated serum PSA level must undergo a biopsy. Other biomarkers are expressed in prostate carcinoma, including "prostate acid phosphatase (PAP), prostate-specific membrane antigen, prostate inhibin peptide, PCA-1, PR92, prostate-associated glycoprotein complex, PD41, 12-lipoxygenase, p53, p27, hepsin, PIM-1 kinase, and EZH2."[5]

The localised disease frequently reacts to traditional therapies such as androgen ablation by castration and/or chemical inhibitor administration. However, researchers still face challenges with advanced conditions resistant to any curative therapies. Increasing attempts are being made to improve the likelihood of identifying positive and sensitive immune markers for prostate cancer diagnosis and treatment. Alpha-methyl-acyl-CoA racemase (AMACR) is a dietary "branched-chain fatty acid and C27 bile acid" intermediate enzyme that acts in peroxisomal beta-oxidation.[6]

In the development and progression of prostate cancer, the use of branched fatty acids and increased fatty acid production may play significant roles. In premalignant and malignant prostate lesions, AMACR is overexpressed relative to within the normal prostate. As prostate carcinoma goes to higher grades and stages, the level of AMACR expression is continuously elevated. Studies have shown that the chromosomal region 5p13 for AMACR is a candidate gene region, and AMACR gene polymorphism is common in prostatic carcinomas of families with inherited prostatic carcinomas. For optimal in vitro growth of prostate carcinoma cells, AMACR is essential, and this enzyme may be a complementary androgen ablation target in the treatment of prostate carcinoma.[6]

Many investigations have been conducted to determine importance of expression of AMACR stain as a diagnostic marker for prostatic cancer, especially for the differential diagnosis of benign prostatic mimics.[7]

To distinguish between low-risk non-aggressive indolent carcinomas and high-risk aggressive prostate carcinomas, several risk stratification systems have been devised, including the combination of clinical and pathological characteristics (such as Gleason score, serum PSA levels, ISUP grade of the tumor, as well as clinical and pathological staging). To differentiate between aggressive and non-aggressive carcinomas or to predict the course of the disease, these methods are still insufficient.[8] There is a much more significant lack of studies relating to the immunohistochemical diagnosis of AMACR in prostate carcinoma tissue and its prognosis.[7]

Hence the study was undertaken to test the significance of immunohistochemical AMACR expression in prostate carcinoma and correlate the AMAC expression with Gleason grade and serum PSA level.

Objectives of the study:

- To study the pattern of expression of AMACR in prostatic carcinoma on immunohistochemistry.
- To correlate the pattern of AMACR expression with Gleason grade and serum PSA level of patients.

REVIEW OF LITERATURE

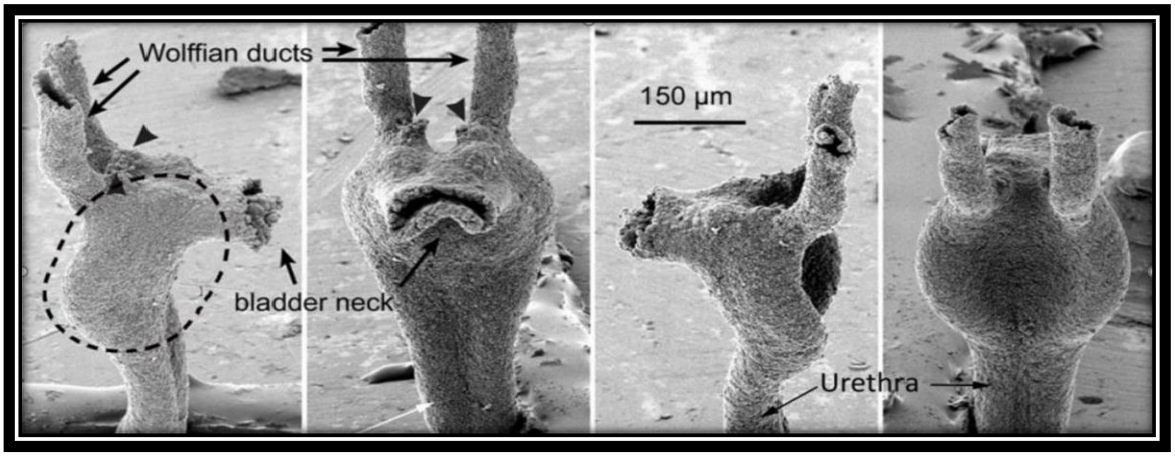
DEVELOPMENT OF PROSTATE:

The prostate develops from embryonic urogenital sinus (UGS) epithelial buds. Several stages of prostate development can be distinguished. (Figure 1):[9]

(a) pre-bud UGS, (b) urogenital sinus epithelium (UGE) produces firm prostatic epithelial buds, (c) bud branching and extension, (d) solid epithelial cords are canalisated, (e) differentiation of luminal and basal epithelial cells, and (f) secretory cytodifferentiation (Table 1)

TABLE 1: TIMELINE OF PROSTATIC DEVELOPMENT IN HUMANS SHOWS: [9]

Developmental event	Human Age
Pre-bud stage	8–9 wks
Initial budding	10–11 wks
Bud elongation & branching morphogenesis	>11 wks
Canalisation	>11wks



A B C D

Fig 1: Electron microscopy photograph showing 90° rotations of the tissue, starting with a lateral view (A) which indicates portion of the UGS from which prostatic buds will emerge. Arrowheads show entry of Mullerian ducts into the UGS epithelium. [9]

ANATOMY OF PROSTATE:[10]

The prostate is a male reproductive system auxiliary gland. The prostate is located behind the lower portion of the pubic arch in the lesser pelvis, beneath the urinary bladder's neck. It is situated next to the rectum's ampulla.

Along with the secretions of the bulbourethral glands and seminal vesicles, this gland's secretions contribute bulk to the seminal fluid. In consistency, it is firm.

Shape, size and weight:

Its base measures approximately 4 cm transversely, 3 cm vertically, and 2 cm anteriorly and posteriorly. It resembles an inverted cone. It weighs eight Gms.

The prostate gland has five surfaces, comprising a base, an apex, an anterior surface, a posterior surface, and inferolateral surface. The confluence of the prostatic and membranous portions of the posterior urethra is encircled by the apex, which is angled downward. The base is upward-pointing and physically connected to the bladder neck. The anterior surface is convex from side to side and narrow. It is separated by retropubic fat and is 2 cm behind the pubic symphysis. The anterior wall of the rectum is interrupted by the posterior surface, which is triangular and flat. The levator ani fascia, which is located above the urogenital diaphragm, is where the inferolateral surface connects to the anterior surface and rests.

Zones of the prostate:

The prostate has been divided into three zones: "The central zone (CZ), transition zone (TZ), and peripheral zone (PZ)" (Figure 2). The histology, anatomical landmarks, biological functions, and susceptibility to pathological diseases of these three zones, which have distinct embryologic origins, can serve as distinguishing characteristics. 70% of the glandular tissue is composed in the peripheral zone. It is located posteriorly and is the most frequent location from which prostate cancer

develops, and it primarily derives from the urogenital sinus. 25% of the glandular tissue is in the central zone. This zone is Wolfian in origin and is not affected by the disease. Benign prostatic hyperplasia (5%) originates from the periurethral transition zone in men.

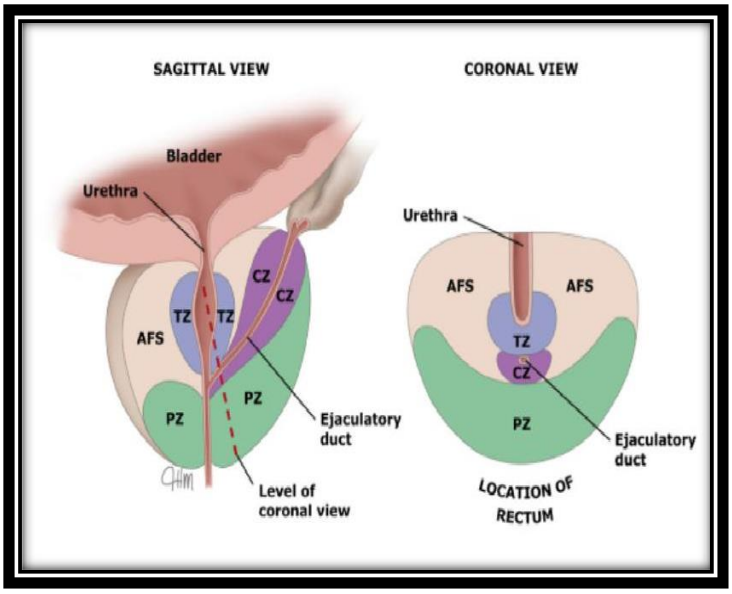


Fig 2: Zones of prostate

At microscopic level, the prostate gland is made up of tubuloacinar glands that are encased in the fibromuscular stroma. The duct has a complicated architectural layout made up of a glandular system and a tubule-like structure.[11] Acini and ducts are lined by luminal, basal, and neuroendocrine cells make up the glandular epithelium. Typically, the acini seem papillary to undulating.[12] In the central zone, this papillary shape is notably more apparent. The columnar luminal cells have round nuclei close to the base and pale eosinophilic cytoplasm. Specialized cells, known as luminal cells, produce a range of substances into the lumen that helps seminal fluid develop. [12]

A small number of glands have proteinaceous prostatic secretions in their lumens, but most glandular acini have luminal spherical prostatic concretions that resemble corpora amylacea.[13]

The stroma of the prostate is fibromuscular and contains fibroblasts, blood vessels, nerves, and a large number of smooth muscle cells. The prostate doesn't have any adipose tissue.[14]

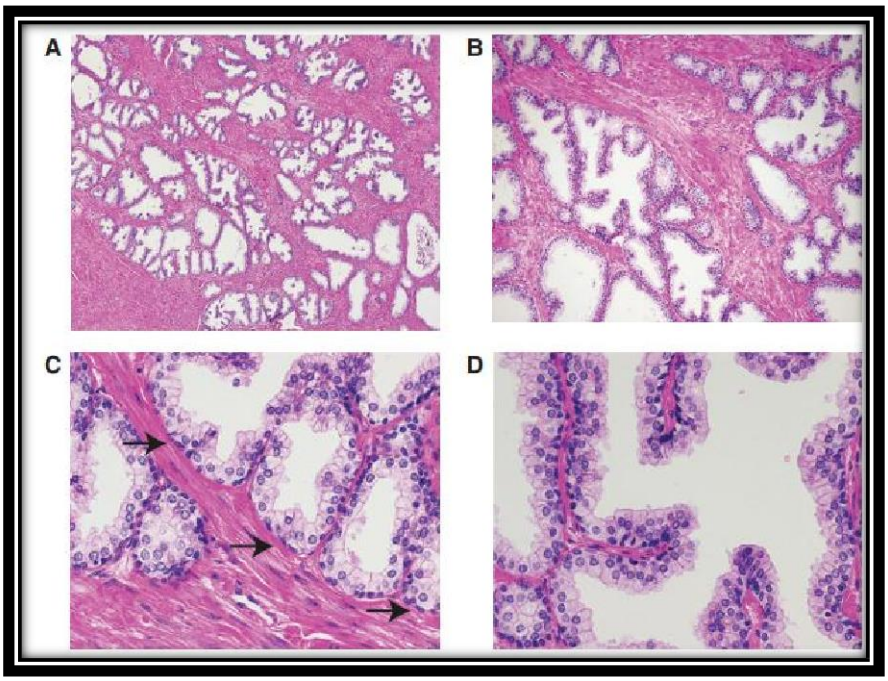


Fig 3: Human prostate.[14] (A) Low, (B) medium, and (C, D) high-power views of the peripheral zone of the human prostate. Note the loosely lobulated arrangement of acini (A, B). Basal cells are characterized by ovoid nuclei and lie near the basement membrane (C, arrows). The acini can have small papillae (D).

PSA

The secretory cells in the prostatic ducts and acini produce the serine protease known as prostate-specific antigen (PSA), which belongs to the kallikrein family. Semen liquefaction is caused by its secretion into the seminal fluid.[1] The most popular blood test for diagnosing prostate cancer or monitoring treatment effectiveness is

serum PSA. It does not, however, accurately reflect the molecular state or biological properties of the tumour cells. Men without prostate carcinoma typically have serum PSA measurements that are low.[15] 4 ng/ml is considered to be the typical value of serum PSA. Serum PSA levels >10ng/ml suggest a strong propensity for malignancy, while levels 4–10ng/ml indicate suspicion of malignancy. [14]

The PSA level's potential as a screening tool was realized following the publishing of papers on various series in which the need for a prostate biopsy was determined by PSA test results. With further practice, it became widely accepted that a PSA level of more than 4.0 ng per millilitre is indicative of carcinoma of the prostate.[16] Because PSA level is frequently increased in benign hyperplasia of prostate and prostatitis, and when the the gland is mechanically moved, it has limits in screening. [1]

PSA immunohistochemistry exhibits high positivity in luminal cells. Using immunohistochemistry for nuclear p63 (nuclear) and high-molecular cytokeratins (cytoplasmic), basal cells can be more easily distinguished. H&E sections cannot be used to accurately identify neuroendocrine cells, however, immunohistochemistry can be used to highlight these cells using neuroendocrine markers such as chromogranin and synaptophysin.[12]

PAP

Initially detected in high concentrations in seminal fluid, human Prostatic Acid Phosphatase (PAP) is a prostate epithelium-specific differentiation antigen. PAP protein can be found intracellularly as the cellular form (cPAP) in seminal fluid as secretory form (sPAP) in normal differentiated prostatic epithelia.[17] It is a secreted glycoprotein (100 kDa) enzyme that is produced by cells of the epithelial of the prostate gland. PAP protein quantities reported in prostate tissue are roughly 0.5 mg/g

total weight and 1 mg/ml in seminal fluid. The sex hormone testosterone, which is in charge of secondary sexual features, is connected to PAP expression. PAP may be discovered in higher concentrations in males with prostate cancer. PAP was shown to be highly expressed in prostate cancer with a high Gleason score.[18]

CARCINOMA OF PROSTATE

Prostate carcinoma is the second most common cause of cancer in male.[1] Prostate cancer accounted for about 1,276,106 new cases and nearly 3.5 lakhs deaths which is around 3.8% of deaths in men with cancer during the year 2018.[19]

Black males have a prostate cancer incidence that is almost 60% greater than that of White men, although native Chinese and Japanese communities a low death and incidence rates.[20] Prostate cancer in African American males is typically discovered at an earlier age and in more severe stages.[21]

The lowest incidences are in Asia, several countries in the Middle East, and Africa. Both genetic and environmental influences have been implicated in these incidence differences. An environmental component (possibly related to diet) is confirmed by the fact that low-risk Asian men who move to a high-risk geographical area (such as the USA) have a marked increase in prostate cancer incidence.[22]

Prostate cancer has a high prevalence at autopsy, being found in the completely embedded prostate glands of 1 5-20% of men in Asia and 30-40% of men in western countries.[22] The incidence of latent prostate cancer discovered at autopsies varies significantly by region, much like with clinically recognised prostate cancer.[22,23] In western men, incident prostate cancer is discovered in 50% of specimens from cystoprostatectomy that have all of the prostate glands embedded.[24, 25] In contrast, the average prevalence in men from Asian countries is around 10%.[26, 27]

In India, according to "Population Based Cancer Registries (PBCRs) for the cities of Bangalore, Barshi, Bhopal, Chennai, Delhi, Mumbai, Kamrup, Ahmedabad, Kolkata, Kollam, Nagpur, Pune, Trivandram, and Wardha" between 2008-2011, prostate cancer was among the top ten most common cancers in these cities.[28]

For four "Population Based Cancer Registries (PBCRs), namely Delhi, Kolkata, Nagpur, and Thiruvananthpuram," prostate cancer is the second most prevalent type of cancer. In several states, including "Gujrat (Ahmedabad and Wardha PBRCs) and Madhya Pradesh," Prostatic adenocarcinoma incidence is comparatively low (Bhopal PBCR). However, India's north-eastern area has the lowest prevalence of prostate cancer. [28]

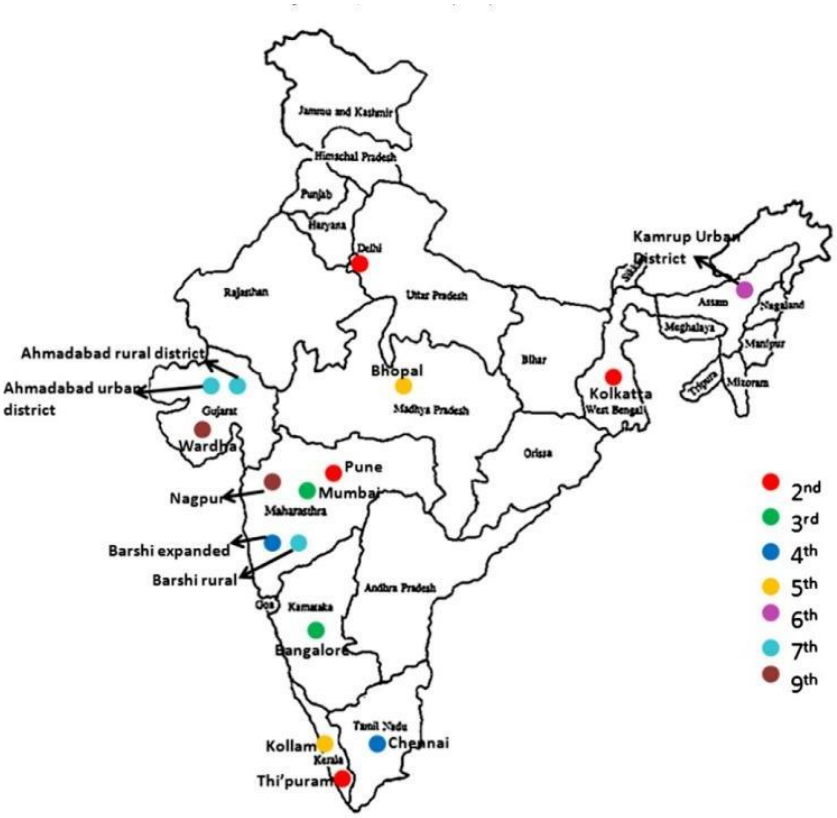


Fig 4: A map of India showing the rank of prostate cancer among top ten leading sites of all cancers, for different population based cancer registries of India.[28]

Prostate cancer detection rates are highly correlated with patient age, with most cases being found in men over 60. Only 1% of prostate cancers in men under 50 years old are clinically detected. In contrast, prostate cancer is evident at autopsy in 30% of American males between the ages of 30-50 years.[29]

Up to 30-50% of men over the age of 50 report with moderate to severe lower urinary tract symptoms (LUTS), which include frequency, nocturia, hesitation, weak stream, inadequate emptying, and straining.[30] LUTS sufferers frequently worry that their symptoms point to prostate cancer. As part of a normal prostate screening, many men with LUTS are checked for prostate cancer using PSA testing and a digital rectal examination.[31] Several credible studies have shown that men with LUTS are at no greater risk of prostate cancer than asymptomatic men of the same age.[32, 33] Despite this evidence, screening of symptomatic men is widespread.[31] Patients frequently believe that these symptoms are unimportant, general, or comparable to symptoms of other less serious diseases. Since prostate cancer is far more treatable in its early stages, it is crucial to start prostate cancer screening behaviours as soon as possible. [31]

Etiology and Pathogenesis

There is speculation that a number of variables, such as age, family history, hormone levels, race and environmental effects, may be involved in the development of the prostate cancer. The hypothesis that environmental variables are at work is supported by the increased incidence of this disease after migrating from a low-incidence to a high-occurrence area. There are numerous potential environmental influences, but none have been established as causal.[34]

For instance, it has been suggested that increasing consumption of lipids or carcinogens found in charred red meats is to blame. The dietary components

lycopenes (found in tomatoes), vitamin D and soy products are among those thought to prevent or delay the onset of prostate cancer. [34]

India is a diverse country. From one region to the next, there are differences in the religions, customs, environments, literacy rates, and eating patterns of the people. The incidence of prostate cancer might vary significantly across the nation depending on these variables. [35]

"There are several risk factors implicated in the causation of prostate cancer, namely, positive family history,[36] history of diabetes mellitus,[37] height, weight and obesity,[38] smoking habit, physical activity,[39] body mass index (BMI)."[40]

The Indian population is heavily reliant on agriculture and related businesses. As a result, these individuals may be directly or indirectly exposed to particular types of pesticides (at work or in the environment). Most of the time, there are only inadequate safety precautions used when using and handling these cancer-causing substances. This could result in the broad dispersion of these dangerous and cancer-causing substances, endangering people.[35]

Pesticides with estrogenic qualities, primarily organochlorine pesticides (OCPs), are sometimes referred to as xenoestrogenic pesticides. These pesticides may enhance the risk of prostate cancer incidence in the population exposed to these carcinogenic chemicals because prostate cancer is an estrogen-dependent malignancy.[34]

In prostate cancer, androgens are crucial. Similar to their normal counterparts, prostate cancer cells depend on androgens for growth and survival. Androgens bind to the androgen receptor (AR) and promote growth and survival by production of the gene. [34]

Due to the presence of luminal cell markers in prostate carcinoma and the absence of basal cells, luminal cells are thought to be the origin of prostate cancer.[41]

A polymorphic sequence made up of repeats of the codon CAG is present in the X-linked AR gene, which is interesting in light of racial disparities in prostate cancer risk (which codes for glutamine). The therapeutic value of castration or antiandrogen therapy, which typically promote recovery of the disease, can be used to demonstrate the significance of androgens in supporting the proliferation and survival of malignant cells of prostate carcinoma.[34]

"Somatic mutations are less common in early-stage, treatment-naive cancers but are frequently seen in castrate-resistant prostate carcinoma (CRPC) patients because AR mutations suggest a selective response to Androgen deprivation therapy (ADT)".[41]

Although there has been the identification of at least one missense mutation that may be able to predict prostate carcinoma with the early start, germline mutations linked to cancer risk are uncommon in all phases of prostate carcinoma progression.[42] In prostate cancer cell lines and tumour tissues, active AR splice variants (SVs) resulting from gene splicing or genomic rearrangement were found.[43,44] The majority of mutations in the AR are gain-of-function alterations that can be localised to the ligand-binding domain and cause androgen hypersensitivity or decreased ligand selectivity.[41]

These include modifications that result in enhanced PI3K/AKT signalling pathway activation (such as the deletion of the PTEN tumour suppressor gene), which is most frequently seen in cancers that have developed resistance to antiandrogen therapy.[45]

Men who have one first-degree relative who has prostate cancer are twice as likely to have it as those who don't, and those who have two first-degree relatives are five times as likely. Prostate cancer also has a tendency to strike men younger when there is a strong family history of the condition. A germline mutation in HOXB13, a homeobox gene that codes for a transcription factor that controls prostatic development, also confers a significantly elevated risk in the small fraction of families that carry it. Both of these mutations raise the risk of prostate cancer in men by 20-fold. However, changes in other loci that only slightly raise the risk of cancer account for the great majority of familial prostate malignancies.[34]

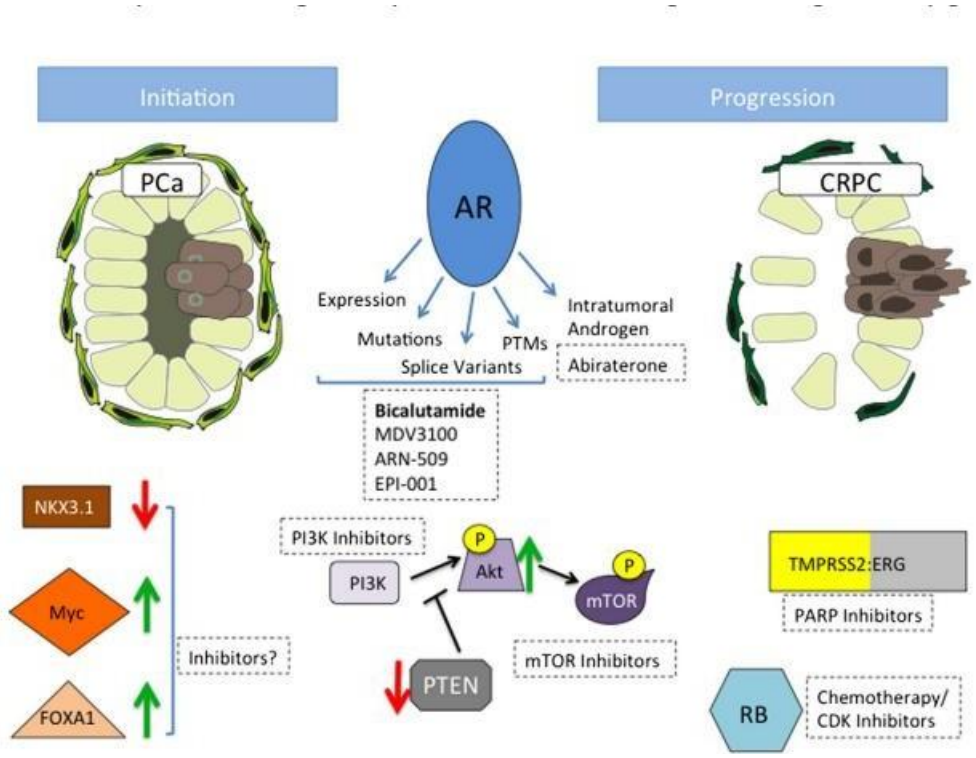


Fig 5: "Molecules implicated in prostate carcinoma initiation and progression." [41]

Deregulation of several signalling pathways plays a role in the initiation of prostate carcinoma and progression to castrate-resistant prostate carcinoma (CRPC). Early stage prostate carcinoma exhibits altered expression of NKX3.1, FOXA1, and

Myc, which contributes to the development of the illness. However, there aren't any efficient ways to combat these aberrations at the moment. Additional compounds that can be pharmacologically inhibited are disturbed during the shift to CRPC.[41]

Loss of PTEN expression increases PI3K/AKT/mTOR signalling, which can be inhibited with targeted inhibitors, while TMPRSS2:ERG fusion expression promotes invasive phenotypes and can be inhibited by PARP1 inhibitors, RB status (loss of expression or function) can stratify patients for responsiveness to chemotherapy or CDK inhibitors, and these are all factors that can influence patient response. [41]

"AR signalling is crucial to prostate carcinoma development and CRPC progression. Bicalutamide, an AR antagonist, is used to treat invasive prostate carcinoma before CRPC, although new inhibitors like MDV3100, ARN-509, and EPI-001 are currently undergoing clinical studies and could be even more successful. Increased expression, gain-of-function mutations, constitutively active splice variants, posttranslational modifications (PTMs), and intratumoral androgen production are some of the mechanisms used to restore AR activity in response to antagonists. Abiraterone is approved for the treatment of CRPC and inhibits autocrine androgen production"[41]

The fact that prostate carcinoma is efficiently detected by a total absence of basal cells, even though prostate epithelial stem cells have been theorised to dwell in the basal layer, has long been a mystery in relation to prostate cancer.[46]

Prostatic intraepithelial neoplasia (PIN), prostatic cancer in situ, invasive cancer, and metastatic cancer are the typical phases that prostate adenocarcinoma typically progresses through.[46]

The impact of microenvironment of tissue, which significantly affects how tumour cells behave and their ability to spread, is a key factor in the progression of carcinoma. It is generally determined that that in vivo interactions between cells and between cells and the matrix have a significant impact on how various cells react to various stimuli.[47]

These interactions are crucial for cell function in healthy tissue and are crucial for the theory of a "stem cell niche." In order to support progenitor cells' undifferentiated phenotype, a cell habitat called a niche offers essential signals of maintenance for stem cell. These pertinent signals include those from the Hedgehog, Wnt, or Notch pathways, which are all crucial for early oncogenesis and the regulation of cell differentiation and proliferation.[46]

Because it is inextricably connected to the development of neovasculature and controls the generation of proangiogenic elements, hypoxia is a crucial component of the niche.[47] The vessels that have been created are premature, tortuous, possibly dilated, devoid of innervation, and intravascular shunts, blind ends, and prone to excessive branching.[48]

Numerous genes are known to express in hypoxic environments; out of them, the hypoxia-inducible factor-1 (HIF-1) regulate the majority of the genes. Under hypoxic conditions (3–5% O2), the expression of the gene may be changed toward an immature phenotype, encouraging de-differentiation of prostate tumour cells into more “stem-like” ones.[49]

OCT-4, a transcription factor that binds to octamers, is more abundantly expressed in hypoxic cells as a result of interactions between HIF-1 and HIF-2. OCT-4 is a gene that is involved in embryonic stem-ness. OCT-4 is a direct target of HIF-2α, and upregulation of it may aid in the growth and maintenance of stem cells of

cancer.[50] Xenotropic murine leukemia virus-related virus (XMRV) is a new human gammaretrovirus identified in prostate cancer tissue from patients homozygous for a reduced-activity variant of the antiviral enzyme RNase L.[51] The catalytic activity of the R462Q RNase L variation is three times lower than that of the wild-type enzyme, and those who carry the R462Q mutation (QQ) have a two-fold higher chance of developing prostate cancer.[52]

With these concepts, the prostate oncogenesis hypothesis—which states that PIN is preceded by inflammatory atrophy and that cells of prostatic epithelium have a higher level of Ki-67-marked proliferation—has been strengthened, as shown in the flow chart.[46]

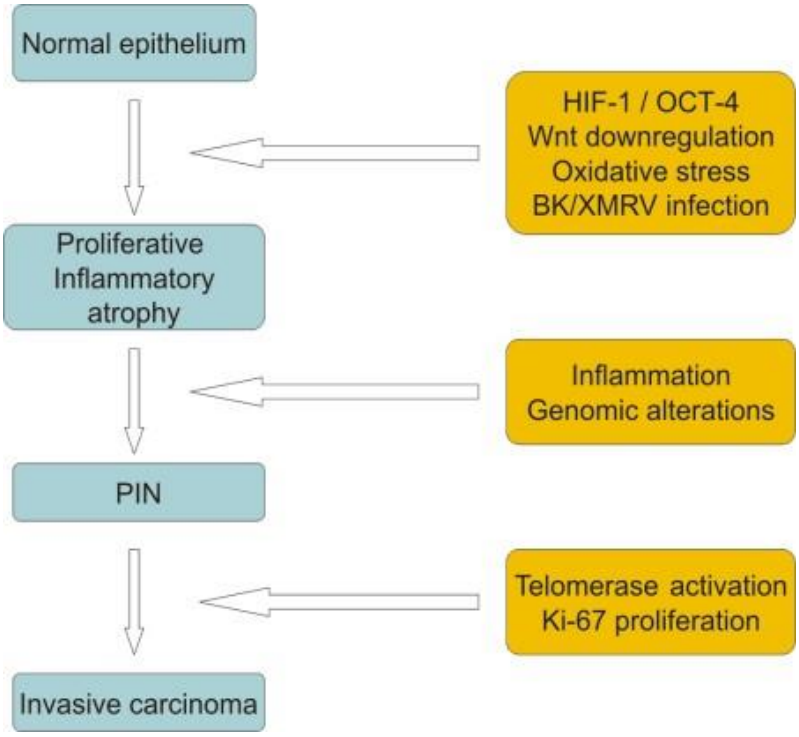


Fig 6: A model for prostate oncogenesis. The normal epithelium will have an increased proliferative capacity and eventually give rise to prostate intraepithelial neoplasia when exposed to oxidative stress, Wnt down-regulation, or human gamma retrovirus Xenotrophic MuLV-related virus infection (PIN). The progression from PIN to invasive, metastatic, and treatment-resistant cancer is determined by the strong proliferative potential and telomerase activation.[46]

Methods of tissue diagnosis

1. **Fine-needle aspiration:** Before the modern core needle technique was created, fine-needle aspiration cytology of the prostate was rather frequently used to diagnose prostate cancer.[53] One significant drawback is that immunohistochemistry cannot be used to establish basal cell absence, Gleason grading cannot be used, and tumor area cannot be determined. Consequently, it is no longer advised to use this method.[1]

2. **Needle core biopsy:** For diagnosis, at least 10-12 systematic prostate 18-gauge core biopsies should be performed, guided by trans rectal ultrasound, with extra (targeted) cores taken from a suspect lesion recognised by digital rectal examination or imaging.[1] As long as precautions are taken to straighten and flatten the differentially inked cores during the pre-embedding stage of processing, 1-2 (maximum 3) differentially inked cores may be embedded in a single cassette.[54,55]

3. **Transurethral resection:** When medical therapy or alternative treatments are ineffective for a patient, transurethral resections are still done.[1] Transurethral resection specimens weighing less than 12 g should be submitted in full, usually in six to eight cassettes.[56] Eight cassettes must be submitted in order to identify nearly all stage T1 b tumors and 90% of stage T1 tumours.[57,58]

   A base weight of 12 g plus one cassette for every extra 5 g of tissue should be submitted for specimens that weigh more than 12 g.[56]

4. **Open (simple) prostatectomy/ enucleation:** Urologists may perform open prostatectomy for benign prostatic hyperplasia in patients with large prostates.[1]

5. **Radical prostatectomy:** One of the only effective therapies for clinically

localised prostate cancer is radical prostatectomy, which can be carried out openly, laparoscopically, or robotically.[1] Slices of the prostate's body should be made at intervals of 3 to 4 mm in a transverse plane that is parallel to the rectal surface.[59,60,61] You can utilize whole mounts or ordinary blocks, and they both supply the same information.[62] A crucial slice should contain the soft tissue surrounding the connection with the prostate's base.[63]

MORPHOLOGY OF PROSTATIC ADENOCARCINOMA

Without further explanation, the phrases "prostate cancer" or "prostate adenocarcinoma" refer to the common or acinar type of prostate cancer. Prostate cancer typically develops in the posterior region of the peripheral zone of the gland, where it may be felt on rectal examination, in around 70% of cases.[34]

Architecturally, the finding of crowded small glands is suspicious but not diagnostic of carcinoma. Small atypical glands on either side of a benign gland or unusual glands in a row that is the breadth of core are more distinctive characteristics of cancer.[1]

The majority of lesions are adenocarcinomas, which result in distinct, easily detectable gland patterns. The cuboidal or low columnar epithelium lines the glands, which are smaller than benign glands. Prostate cancer glands differ from benign glands in that they are more crowded and typically lack branching and papillary infolding.[34]

There are four main cytoarchitectural patterns. These patterns include cribriform, diffuse individual cell infiltration, medium-sized glands, and tiny glands.[14]

Due to their closely spaced distribution, irregular form, smooth inner surface, and sparse intervening stroma, medium-sized gland carcinomas can be identified on low-power examination.[14]

Although prominent nucleoli are the most widely recognized cytological characteristic of prostate adenocarcinoma, this characteristic should not be the only one used to make the cancer diagnosis. On needle biopsy, prominent nucleoli are not always present in cases of cancer of the prostate and can be seen in a variety of cancer mimickers.[64,65]

A lack of conspicuous nucleoli may indicate a sampling issue, such as when thick sections conceal nuclear detail or when no biopsies are taken from tumour tissue in areas with prominent nucleoli or are overstained. [64,66]

These nucleoli are numerous and are marginated.[65] Mitoses are also important, however they are infrequently observed in well-differentiated tumours made up of tiny or medium-sized glands.[14]

The cytoplasm of prostate cancer may be amphophilic compared to that of the nearby benign glands, which is pale to clear.[1]

The majority of prostate cancers don't have a noticeable desmoplastic or inflammatory response. However, stromal response is frequently linked to high-grade cancer if it is present. Although more frequently observed around prostate cancer glands, retraction artefact is not totally specific for cancer.[67]

There are three characteristics that have not yet been found in benign glands but which, by themselves, are diagnostic of cancer: glomerulations, mucinous fibroplasia (collagenous micronodules), and perineural invasion.[1]

In a prostate needle biopsy, evaluating an unusual focus should be done carefully. When analysing needle biopsies, one should create a mental balance sheet with features supporting the detection of carcinoma on one side of the column and features opposing the detection of cancer on the other. At the end of a case evaluation, all of the criteria should, ideally, be put on one side of the column or the other in order

to establish a clear diagnosis. Instead of depending solely on one criterion, the diagnosis of cancer should be made using a variety of characteristics.[68]

Although significant in the identification of cancer on needle biopsy, prominent nucleoli shouldn't be the only factor considered when making the diagnosis. Relying solely on prominent nucleoli for prostate cancer detection could result in both an under- and over diagnosis of the disease. It is important to consider the architectural pattern, additional case characteristics, and the significance of conspicuous nucleoli. Although they are not always visible in adenocarcinomas of the prostate, mitotic figures help distinguish between malignant and benign glands.[68]

After radical prostatectomy, perineural invasion (PNI) on prostate tissues is independently linked with poor pathologic characteristics, lower disease-free survival, and overall worse survival. When advising prostate cancer patients about their treatment options, PNI should probably be brought up as a potential indicator of worse oncologic outcomes. The most popular method of prostate cancer risk stratification divides patients into low-risk, intermediate-risk, and high-risk groups according to their risk of the illness returning after therapy using pre-treatment PSA, biopsy Gleason score, and clinical stage. When choosing a course of treatment, PNI could be a useful characteristic to take into account. In men with low-risk or low-volume intermediate-risk illness, PNI on the prostate specimen may suggest the necessity for definitive treatment rather than continuing with active surveillance. The perineural space may offer a conduit of lesser resistance to tumour propagation, which could explain why the oncologic results found with PNI are poorer. Additionally, it has been discovered that tumour cells in the perineural area exhibit enhanced proliferation and decreased apoptosis. Numerous research have been found to support the conclusion that PNI increases the chance of biochemical recurrence after

prostatectomy, according to a comprehensive review of the subject. Furthermore, clinical results for PNI patients receiving external-beam radiation could be poorer.[69]

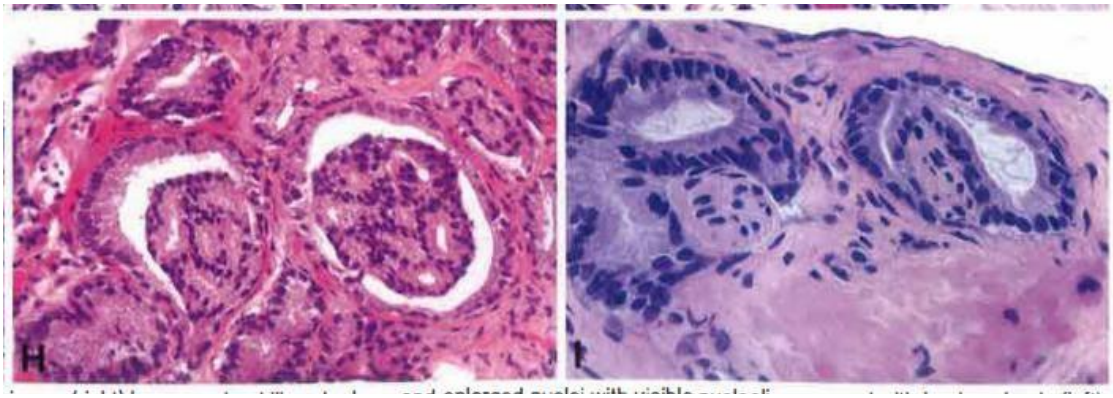


Fig 7: H. Malignant gland showing glomerulations. **I**. Perineural invasion with malignant glands that have amphophilic cytoplasm and blue mucinous secretions partially encircling nerves.[1]

The typical histological and cytological features of prostatic acinar adenocarcinoma (PPA) can usually be used to make the diagnosis. However, there are times when the diagnosis is difficult because many prostatic or nonprostatic benign or malignant lesions and normal tissue structures can resemble prostatic acinar adenocarcinoma, particularly in a small piece of tissue from a biopsy taken or transurethral resection of the prostate that was guided by transrectal ultrasound (TRUS). This could result in an incorrect diagnosis and ineffective treatment.[70]

Among the mimickers of Prostate Adenocarcinoma, Prostatic /Lobular atrophy is the most common lesion among the elderly population. The atrophic glands typically form many lobules that are divided by fibrotic stroma. The cells have scant cytoplasm and small, hardly detectable nucleoli in their hyperchromatic nuclei. It is possible for the basal layer to be discontinuous, which is sometimes mistaken for PAA.[70]

Under the light microscope, atypical basal cell hyperplasia or basal cell hyperplasia (BCH) with large nucleoli may be mistaken for high-grade prostatic intraepithelial neoplasia or cancer. BCH often coexists with BPH and displays similarly shaped lobules with smooth edges. Traditional BCH can take the form of solid basaloid nests or glands with multiple layers of basal cells. The nuclear characteristics of the hyperplastic basal cells are bland, devoid of nucleoli, and pleomorphic.[71] However, some of its uncommon variants, such florid BCH and atypical BCH, could resemble PAA. According to its morphology, florid BCH is a nodule-forming widespread proliferation of basal cells that involves more than 100 closely packed tiny acini (Per slice). The growth of basal cells with pronounced nucleoli is atypical BCH. There are extremely few mitoses, intracytoplasmic hyaline globules, intraluminal secretions, nuclear atypia, and intraluminal secretions in these two subtypes.[71,72]

BPH's morphological variation, clear cell cribriform hyperplasia (CCCH), has been recognised. Cribriform hyperplasia is characterized by a crowded proliferation of complex glands without cytologic atypia. The cribriform glands often feature homogeneous rounded lumina and clear cytoplasm. The cuboidal to low columnar secretory-type cells that make up the core cribriform regions have uniformly rounded nuclei and transparent cytoplasm. They lack nucleolar enlargement and nuclear atypia. Around the periphery, basal cells are visibly present. [73] The presence of a prominent row of basal cells at the edge of the lesion, which is highlighted by the 34E12 keratin stain, is a crucial indicator in the differential diagnosis of malignancy.[14]

Atypical adenomatous hyperplasia (AAH), also known as adenosis, is a benign lesion characterised by the growth of tiny acini. AAH has been detected in up to 33% of radical prostatectomy specimens and in 1.5-19.6% of transurethral restates particularly in a prostate biopsy under TRUS guidance, AAH is frequently mistaken

for prostatic acinar adenocarcinoma (PPA). AAH is a lobular-appearing lesion that is well circumscribed and comprised of small, rounded, and closely spaced glands. Some of them combine to form more substantial and complex acini. It may have crystalloids, packed and disorganized glands, a border that is expansile or hardly infiltrative, and medium-sized nucleoli that resemble low-grade prostatic acinar adenocarcinoma. In contrast to the aforementioned characteristics, AAH lacks macro nucleoli (>3 micron), mucin, and straight luminal margins, which are typically associated with prostatic acinar adenocarcinoma.[70]

Prostate sclerosing adenosis (SA) and breast sclerosing adenosis are quite similar. It is a benign lesion with the growth of both small acini and fibrous stroma. The lesion appears nodular with a distinct boundary but lacks a capsule.[74] The hyperplastic glands contain both amphophilic basal cells and transparent secretory cells. Additionally, a layer of dense, eosinophilic material that resembles a basement membrane surrounds some glands. The cells may have intraluminal acid mucin and prominent nucleoli. Sclerosing adenosis and tiny acinar adenocarcinoma should be separated from one another. The presence of smooth muscle actin (SMA), S-100 protein, and HMWCK (e.g., 34E12), CK5/6, and p63 in the basal cells during IHC labelling indicates that those cells have undergone myoepithelial differentiation. [75,76]

A few other uncommon mimickers of prostate Carcinoma include florid hyperplasia of mesonephric remnants, Cholesterol-laden macrophages, Signet-ring-like changes, melanosis and extramedullary haematopoiesis. During a needle biopsy, rectal tissue may also be found in the prostate; when these glands are deformed, they may likewise resemble prostatic cancer.[14]

Normal structures and several benign tumours might also resemble PAA. Therefore, it is crucial for the pathologist to be aware of and recognise these

microscopic traits. However, when there is a small suspicious area in prostatic specimens, it can be difficult for pathologists to make a proper diagnosis. Both PAA and its harmless imitators have extremely different morphologies. However, characteristics such as perineural invasion and the presence of micronodules unequivocally establish the PAA diagnosis. A complicated pattern, nuclear pleomorphism, hyperchromatic nuclei with large nucleoli, a lack of mitotic figures, and glandular lumen with eosinophilic secretions, in addition to the features mentioned above, favour the diagnosis of PAA. Ancillary techniques, including IHC staining and careful analysis of clinical history and investigations, if done, can be useful in making correct diagnoses leading to appropriate treatment.[74]

GRADING SYSTEMS FOR PROSTATE CARCINOMA

The prostate cancer grading system currently used worldwide was developed in 1966-1974 by Dr Donald Gleason.[1]

For grading prostate cancer histopathologically, many different schemes have been developed. The primary points of contention have been whether prostate cancer should be graded according to its least differentiated or dominant pattern, and whether nuclear atypia and glandular differentiation should both be taken into account when assigning grades.[77]

TABLE 2:"NUMEROUS GRADING SCHEMES FOR PROSTATIC ADENOCARCINOMA USING ROUTINE MICROSCOPY:"

Study	Year	Descriptions
Jewett *et al.*[78]	1968	"Grades I-III, three grades of well, moderately and poorly differentiated cancer."
Mobley & Frank[79]	1968	"Grades I-III, using gland-forming ability and nuclear features."
Utz & Farrow[80]	1969	"Grades I-IV, based on architecture, cytology, and mitotic figures."
Hohbach & Dhom [81]	1972, 1977	"Four groups based on histologic pattern, in order from best to worst prognosis: well differentiated, poorly differentiated, cribriform, solid-anaplastic"
Gaeta *et al.*[82]	1980	"Grades I-IV, defined by gland formation and organization, amount of stroma, nuclear atypia, and mitotic count"
Uchida *et al.* [83]	1988	"Japanese General Rules of Prostatic Cancer: well, moderately, and poorly differentiated"

The Gleason grading system (1966) is one of the few number of systems that employs a low-power architectural approach. At present time, the Gleason Grading system is the most commonly used grading system followed worldwide.[84]

Gleason's first study was done only on morphology only, not immunohistochemistry. Some of the patterns 1 and 2, originally described in Gleason's 1974 paper, which accounted for one-third of cancers, were actually recently described as mimics of cancer, such as adenosis and partial atrophy. The probability is high.[85,86]

Gleason advised reporting the primary (most frequent) and secondary (second-most frequent) patterns, however, the report omitted to indicate a modest component of higher-grade malignancy, if it was present. As a result, the problems with higher grade tertiary patterns on radical prostatectomies and biopsies were not addressed. Gleason did not offer advice on how to report tumour nodules of various grades in radical prostatectomies or numerous cores affected by malignancy from various sites. With the development of prostate-specific antigen testing, modern technique generally samples at least 12 cores, with "1, 2, or 3 cores per container, whereas in Gleason's day only a few large-gauge needles were put into palpable tumours."[87]

Last but not least, new prostatic carcinoma patterns that needed to be added to the Gleason grading system have been described in more recent years.[88,89,90]

First, Gleason scores 2 through 5 were discontinued, and some patterns that Gleason described as a score of 6 are now rated as 7, resulting in a better prognosis for current Gleason score 6 tumours than for historical score 6 cancers.[91]

Second, despite being on a scale of 2 to 10, the lowest score currently issued is 6, in actuality. As a result, patients make the rational but false assumption that the cancer detected by biopsy is in the middle of the grade scale. This increases their dread of receiving a cancer diagnosis and makes them believe that certain treatment is always required. [91]

Third, despite the fact that 3+ 4 = 7 against 4+ 3 = 7 and 8 versus 9 to 10 have significantly varied prognoses, merging Gleason scores into a 3-tier classification (6, 7, 8-10) is most usually employed for prognostic and therapeutic purposes. [91]

New Grading System:

Two unique aspects of the Gleason grading system are that it is based solely on the architectural pattern of the tumour and that the grade is defined as the sum of the two most common grade patterns (the Gleason score) rather than assigning the worst grade as the grade of the carcinoma.[1]

The primary (predominant) and secondary (second most prevalent) architectural patterns are assigned a number from 1 (the most differentiated) to 5 (the least differentiated). If a tumour has only one histological pattern, then the primary and secondary patterns are assigned the same number.[1]

For example, A tumour that is predominantly Gleason pattern 3 with a lesser amount of Gleason pattern 4 has a Gleason score of 7 (3 + 4). The Gleason score is calculated by twice the tumor's single pattern if there is just one pattern, e.g. Gleason score 4+4=8. Gleason pattern 1 is uncommon, hence Gleason scores 2 and 3 are only given in extreme cases.[1]

Gleason pattern 1 is made up of a highly well-circumscribed nodule with numerous discrete, densely packed glands that don't invade nearby benign prostatic tissue. Pattern 1 is quite uncommon.[77]

Gleason pattern 2 consists of smooth-ended circular or oval glands. Compared to those of Gleason pattern 1, the glands are less homogeneous in size and shape and are more loosely distributed. Neoplastic glands may just slightly invade the surrounding healthy prostatic tissue.[77]

Gleason pattern 3 is the most prevalent; compared to patterns 1 and 2, more glands are infiltrative, and their distance from one another varies. The spaces between nearby non-neoplastic glands are frequently invaded by malignant glands. The pattern 3 glands have variable size and shape and are frequently angular. Pattern 3 typically has small glands, although there may also be huge, atypical glands. Each gland is surrounded by stroma and their lumen is open. Rare is cribriform pattern 3. [77]

In Gleason pattern 4, the glands may be poorly defined, cribriform, or seem merged. A collection of glands that are no longer entirely divided by stroma make up fused glands. Normal polarity has lost some of it.[77]

In Gleason pattern 5, there is an almost total elimination of glandular lumina, with sporadic lumina still visible. The epithelium invades the stroma in the form of solid sheets, solid strands, or solitary cells. Possible comedo necrosis is present.[1]

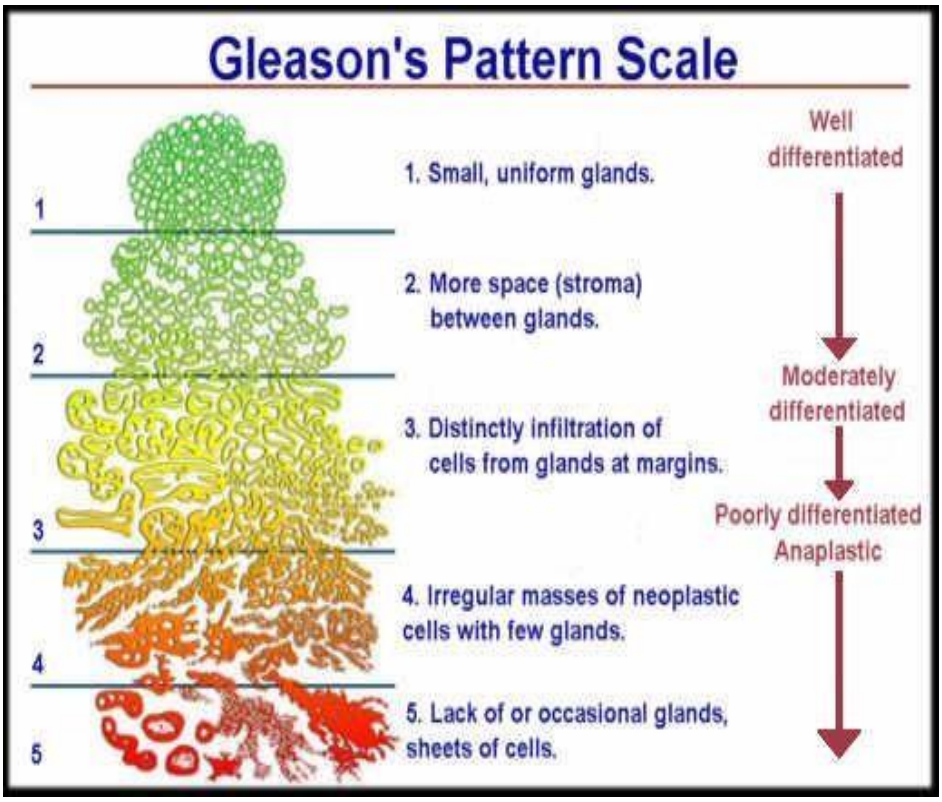


Figure 8: Modified Gleason grading schematic diagram

Since the 2005 consensus meeting, the International Society of Urological Pathology

(ISUP) has advocated for the GS's introduction of tertiary higher grade patterns for biopsies, regardless of degree. A needle biopsy that has a 60% Gleason pattern 4, 36% Gleason pattern 3, and 4% Gleason pattern 5 would be recorded as GS 4+5=9. (GG5). In light of the possibility that with only a very modest second higher grade cancer component, the primary and secondary grades can be similar, it has recently been recommended that the term "minor" rather than "tertiary" is preferred.[92]

"Below is the Summary of the Modification made By ISUP in the year 2019" (ISUP Consensus2019)[92]

- Report the percentage of samples with Gleason pattern 4 for all GS 7 (ISUP GG 2 and 3)
- Include the presence of tertiary/minor Gleason patterns 4 and 5 in the GS for radical prostatectomies if they account for more than 5% of the tumor volume.
- Describe the existence of tertiary/minor Gleason patterns 4 and 5 in radical prostatectomies.
- Do not grade Intraductal prostate cancer (IDC) in the absence of invasive malignancy.
- When invasive cancer is present, include the grade of IDC in the GS.
- Comment on the existence and importance of IDC in biopsies and specimens from radical prostatectomy.
- Comment on the existence and importance of invasive cribriform carcinoma in biopsies and tissue from radical prostatectomy.
- Report in systematic biopsies a separate GS (ISUP GG) for each individual biopsy site

- Report in multiparametric- Magnetic Resonance Imaging-targeted biopsies a global (aggregate) GS (ISUP GG) for each suspicious MRI lesion
- Report specific benign histologic findings in suspicious (PIRADS 4-5) MRI-targeted biopsies without cancer

TABLE 3: MODIFIED GLEASON GRADING ON H & E SLIDE:[1]

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

IMMUNOHISTOCHEMISRY

GENERAL PRINCIPLE:

Frequently employed in using anatomic surgical pathology to classify and identify cells, immunohistochemistry (IHC) uses antibodies directed against specific antigens in particular tissues and cells to make it easier to identify the cell type and organ of origin. Despite the fact that it was initially created on frozen sections and that tissue embedded in plastic can also be used, because it may be easily adapted for storage, the method is frequently performed on formalin-fixed paraffin-embedded (FFPE) tissue.[93]

The evaluation of prognostic and predictive biomarkers in various cancers, those of the lung, hematolymphoid, breast, central nervous systems and gastrointestinal tract, and, has lately increased the use of IHC.[93]

As an overview, the IHC's sequential steps are as follows: antigen retrieval (AR), the addition of primary antibody, putting a secondary antibody that binds the primary antibody on a target, and adding a detecting agent to help the primary antibody find its location. [93]

The application of a particular primary antibody (usually made by immunising a mouse or rabbit with a peptide/antigen of interest) is the first and most important step in IHC after antigen retrieval. This is followed by vigorous washing to eliminate excess levels of primary antibody.[94] Depending on the target antigen and antibody, there are a variety of antigen retrieval techniques, although the majority often involve physically or chemically removing protein cross-links created by fixation with substances like formalin. The most widely used technique at the moment is a process known as heat-induced antigen retrieval (HIAR), which frequently makes use of microwave ovens alongside pressure cookers, autoclaves, and water baths.[95]

The primary antibody, which may be monoclonal or polyclonal, is titrated to achieve the highest primary antibody dilution possible while still improving the contrast between positively stained tissue and background staining that is not specific.[96,97] In general, polyclonal antibodies, which can bind a variety of epitopes, are more sensitive than monoclonal antibodies, which can only bind a single epitope.[97]

A secondary antibody that is aimed towards the species' immunoglobulin in which the main antibody was made must be labelled in order to see the interaction of the antigen and antibody under light microscopy.[93] The primary antibody is quickly labelled and applied to the tissue using the direct method, although this approach is less frequently utilized because it does not amplify signals, necessitating larger antibody concentrations and the labelling of every primary antibody. In the indirect method, the secondary antibody is labelled, enabling the amplification of the signal and use with a variety of primary antibodies. There are many labels that can be employed, including fluorescent compounds and enzymes that produce coloured products after being incubated with chromogenic substrates like diaminobenzidine (DAB), such as horseradish peroxidase or alkaline phosphatase.[97]

Due to the binding of endogenous biotin, the avidin-biotin-peroxidase approach suffers from strong background staining and is currently mainly disregarded. Many peroxidase molecules and secondary antibodies are used in polymer-based techniques; they are coupled to a dextran polymer backbone and enable enhanced sensitivity.[97]

Endogenous peroxidase activity may cause background staining, which is more problematic in tissues with rich hematopoietic elements like bone marrow, and nonspecific antibody binding, which is more common in polyclonal antibodies. Using

the same species' normal serum as the secondary antibody during preincubation or it is possible to lessen nonspecific antibody binding by using an accessible universal blocking agent.[97]

ROLE OF IMMUNOHISTOCHEMISTRY IN PROSTATE LESIONS

Immunohistochemistry is probably used most frequently to identify basal cells, which are absent with very few exceptions in prostate cancer. Although typically benign glands are positive for basal cells with IHC, adenosis, partial atrophy, and high-grade prostatic intraepithelial neoplasia (HGPIN) can have very patchy or absence of basal cells in a focus on needle biopsy.[98]

Immunostains for epithelial cells (low-molecular-weight cytokeratins and PSA), among the basal cell marker high-molecular-weight cytokeratins (HMWK) and p63, and the cancer-associated proteins AMACR and ERG can help to identify histologically inconspicuous cancer cells. Although AMACR expression is downregulated in some cases after androgen deprivation therapy, resulting in only 45-71% of cases being AMACR-positive.[1]

Numerous research contrasting the two have found p63 to be marginally superior to HMWCK.[99] HMWCK and p63 can be used as a double cocktail to reduce staining variability while increasing the sensitivity of basal cell detection.[100]

Prostate cancer dramatically upregulates AMACR. This protein has been targeted by antibodies (P504S). The majority of the carcinoma of prostate are IHC-positive for AMACR, with sensitivity ranging from 82% to 100% in different studies.[98]

ERG is the most recent marker to be suggested as a tool for the diagnosis of restricted prostate carcinoma. About 40% to 50% of prostate carcinomas include androgen-regulated transmembrane protease serine 2 (TMPRSS2) and ERG gene

fusions. With the exception of the fact that 16% to 20% of HGPIN also exhibits the gene fusion, this gene fusion is very specific for prostate cancer. There are monoclonal anti-ERG antibodies for the TMPRSS2-ERG gene fusion that correlate well with cancers that are fusion-positive. Evidence suggests that postatrophic hyperplasia, partial atrophy, and adenosis are all negative for ERG antibodies.[98]

A combination of immunohistochemical markers should be employed to diagnose benign and malignant prostatic lesions because there are many questions surrounding their diagnosis.

ROLE OF AMCAR IN PROSTATIC LESIONS:

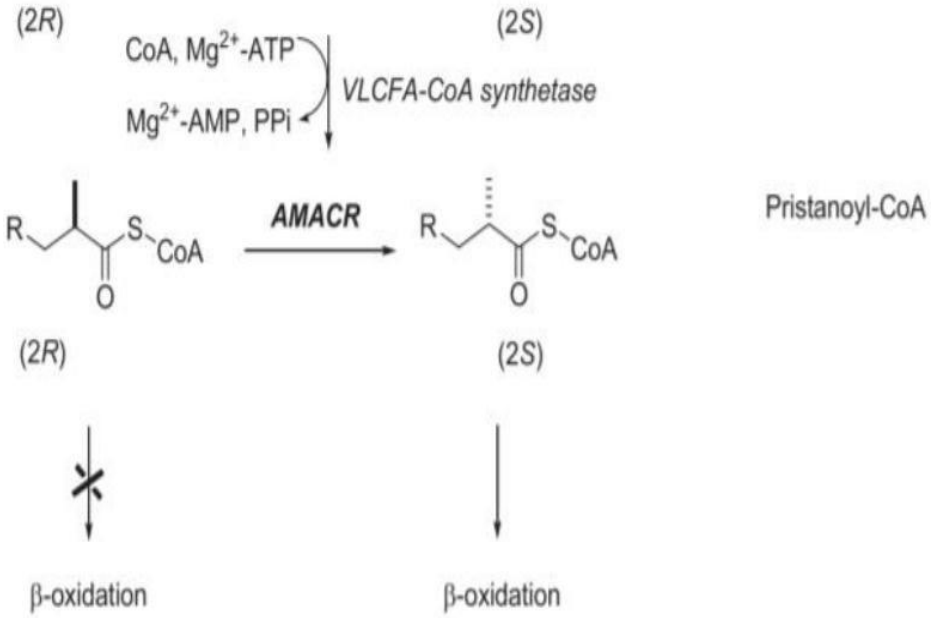
In 1995, scientists researching lipid metabolism first isolated and examined this enzyme.

A 382 amino acid protein was found to be overexpressed in prostate cancer cells as compared to benign or normal prostate epithelial cells with the gene for AMACR found on 5p13. An important part of the peroxisomal –oxidation of dietary branched-chain fatty acids and C27-bile acid intermediates is played by the well-studied enzyme AMACR. The (R)-alpha-methyl-branched-chain fatty acyl-CoA esters are catalysed into their (S)- stereoisomers. During their subsequent peroxisomal-oxidation, only the (S)-stereoisomers can act as substrates for branched-chain acyl-CoA oxidase. Two aspects of this pathway may have particular relevance for prostate carcinogenesis: (a) the main sources of branched-chain fatty acids in humans (milk, beef, and dairy products; have been implicated as dietary risk factors for prostate cancer; and (b) peroxisomal β-oxidation generates hydrogen peroxide, a potential source of procarcinogenic oxidative damage.[101]

Two genes, P503S and P504S, were shown to be increased in both healthy and malignant prostatic tissue utilising cDNA extraction combined with high throughput microarray screening. Additional research demonstrated that P504S was overexpressed only in prostatic cancer and not in healthy prostatic tissue. Bioinformatics and colony hybridization was used to fully code P504S, and the result was determined to be human Alpha Methyl Acyl CoA Racemase.[102]

Adult-onset sensory-motor neuropathy has been linked to mutations in the AMACR gene, which are linked to decreased enzyme activity. This is likely to be a result of persistent increases in the plasma concentrations of branched-chain fatty acids such as pristinic acid. Zheng and colleagues discovered that in families with hereditary prostate cancer, specific sequence mutations in the AMACR gene highly co-segregate with the development of prostate cancer.[103]

FIGURE 9: MECHANISM OF ACTION OF AMACR:[103,104]



AMACR EXPRESSION IN NORMAL TISSUES:

- AMACR is normally expressed in cells of salivary gland like acinar and ductal cells, Duct epithelial cells of the breast, Hepatocytes, proximal convoluted tubule epithelium, glands of Colon.

In malignant tissues, it is expressed in carcinoma of lung, breast, carcinoma of stomach, renal cell carcinoma and adenocarcinoma of Colon.

Various combinations combining antibodies for AMACR and basal cell-specific markers have been studied. One combination includes antibodies to AMACR and p63, both of which are labelled with a brown chromogen.[98]

In order to preserve tissue for IHC, a triple-stain combination that uses a brown chromogen for both HMWCK and p63 and a red chromogen for AMACR has been found to be superior to basal cell markers alone. [98]

A study done by Evans stated criteria for immunohistochemical staining with Alpha Methylacyl CoA racemase (AMACR/P504S) that would support a detection of cancer based on needle biopsies of prostate that on low power examination, the strong staining is readily visible, which can be granular, circumferential, luminal (apical) to diffuse cytoplasmic staining of acini with malignant features on H&E sections and negative or weak staining termed as "non-circumferential staining" refers to nearby benign glands.[103]

Identifying the incidence of high-grade prostatic intraepithelial neoplasia and other conditions that resemble prostate cancer and show positive AMACR/P504S positivity in prostatic needle biopsies, which is critical for interpreting this marker.[103]

MATERIALS AND METHODS

SOURCE OF DATA

- Specimens of the prostate such as prostatic needle biopsy, chips of transurethral resection of the prostate and radical prostatectomy received in the Histopathology Section, Department of Pathology, BLDE (Deemed to be University), Shri B.M Patil Medical College, Hospital and Research Centre, Vijayapura were included.
- **Study period**: January 2020 to August 2022 (1.5 years- Prospective study, 1 year- Retrospective study).

Inclusion criteria:

- Histologically diagnosed cases of primary prostate carcinoma were included in the study.

Exclusion criteria:

- Mimickers of prostatic adenocarcinoma, like prostatic atrophy, basal cell hyperplasia, atypical adenomatous hyperplasia, and clear cell cribriform hyperplasia were excluded from the study.

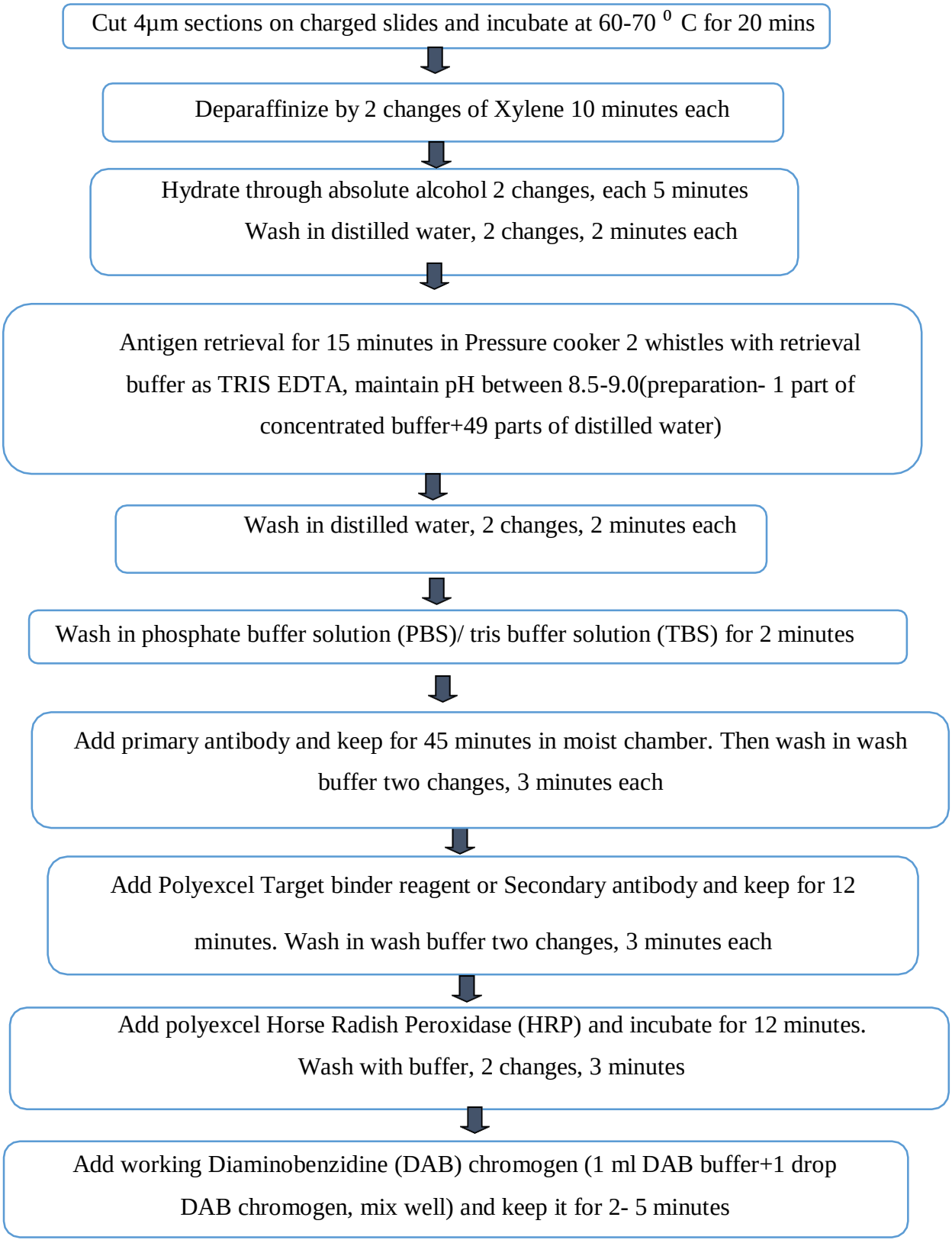
METHODS OF COLLECTION OF DATA.

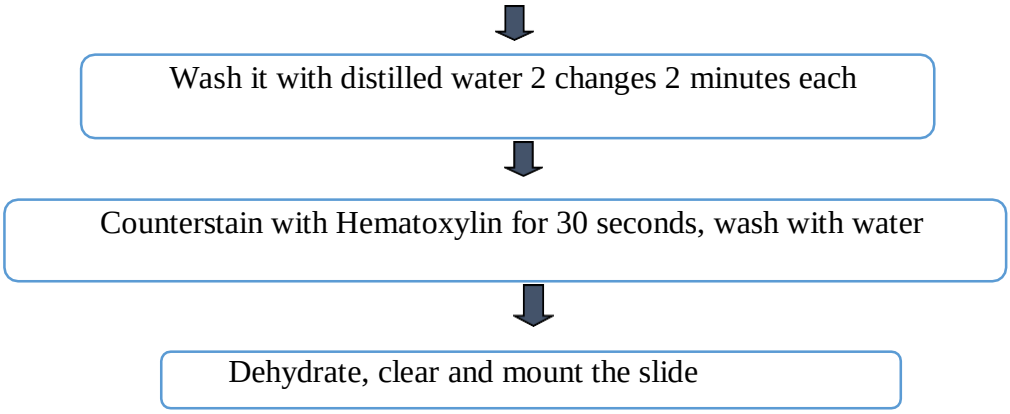
Specimens of the prostate, such as prostatic needle biopsy and chips of transurethral resection of the prostate, were received in the Histopathology Section, Department of Pathology, BLDE (Deemed to be University), Shri B.M Patil Medical College, Hospital and Research Centre, Vijayapura. Information regarding Clinical details, PSA levels and findings on imaging if any, was obtained.

The tissue was preserved in 10 % buffered formalin and processed routinely. Two four-micron-thick sections were prepared from each tissue block. One section was stained with Haematoxylin and Eosin (H & E) for morphologic diagnosis and

Gleason's score and grade, and another section was mounted on polylysine-coated slides, which was used for AMACR immunohistochemical staining.

Fig 10: IMMUNOHISTOCHEMICAL STAINING PROTOCOL [55]





MODIFIED GLEASON GRADING ON H & E SLIDE:[1]

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5	4+5, 5+4, or 5+5	9 or 10	"Lack of gland formation (or with necrosis) with or without poorly formed /fused /cribriform glands."

- Prostate cancer cases were graded histologically according to the Gleason grading system based on total score:[104]

TABLE 4: GRADING OF PROSTATE CARCINOMA BASED ON GLEASON SCORE:[104]

Grading	Score
Well Differentiated carcinoma	2–4
Moderately Differentiated carcinoma	5–7
Poorly Differentiated carcinoma	8–10

IHC INTERPRETATION OF AMACR:

- Positive AMACR staining was confined to dark diffuse or granular, cytoplasmic or luminal areas. However, circumferential staining was observed. [104]
- The AMACR positive percentage was graded from 0+ to 3+ as follows:[104]

TABLE 5: THE PERCENTAGE POSITIVITY OF AMACR EXPRESSION

Percentage of tumor cells with AMACR Positivity	Score
0 %	0+, Negative
1-10 %	1+, Mild
11-50 %	2+, Moderate
>50 %	3+, Strong

A correlation of immunohistochemical expression AMACR with Gleason grade and serum PSA level was done.

STATISTICAL ANALYSIS

All attributes were described in detail. The summary statistics of mean±standard deviation (SD) were employed for continuous variables. In the data summaries and diagrammatic display of categorical data, numbers and percentages were used. The Chi-square (2) test was used to determine the association between two categorical variables. The formula for the chi-square statistic used in the chi-square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript "c" is the degrees of freedom. "O" is the observed value, and E is the expected value. C= (number of rows-1)*(number of columns-1)

The difference in the means of analysis variables between the two independent groups was tested by unpaired t-test.

The t statistic to test whether the means are different can be calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

s_1^2 = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

s_2^2 = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise, it was considered as not statistically significant. Data were analysed using SPSS software v.23 (IBM Statistics, Chicago, USA)and Microsoft office.

RESULTS

The total number of cases included in the present study were 45 histologically diagnosed cases of primary adenocarcinoma of the prostate.

TABLE 6: AGE WISE DISTRIBUTION OF CASES:

Age (years)	Number of cases	Percentage
50-60	4	8.9
60-70	11	24.4
70-80	16	35.6
80-90	14	31.1
Total	45	100.0

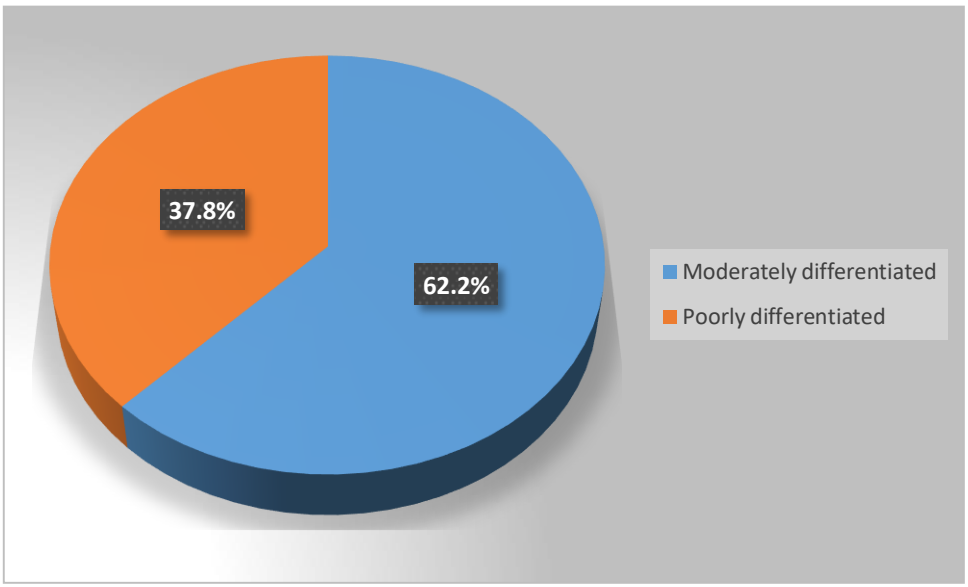
In the present study, cases of prostate cancer were noted with a variable age group of 50-90 years; among them, most of the patients, i.e. 16 (35.6%), were in the age range of 70-80 years, followed by 14 (31.1%) patients in the age group of 80-90 years. There were 11 cases belonging to the age group of 60-70 years, amounting to 24.4%, and 4 (8.9%) patients belonging to the age group of 50-60 years (Table 6).

TABLE 7: TYPES OF PROSTATE CARCINOMA SPECIMENS RECEIVED IN THE STUDY:

Type of specimen	Number of cases	Percentage
TURP chips	39	86.7%
Trucut biopsy	6	13.3%
Total	45	100%

Out of 45 cases, 39 (86.7%) were TURP chips and 6 (13.3%) cases were of Trucut biopsy (Table 7).

FIGURE 11: HISTOLOGICAL GRADING OF ADENOCARCINOMA OF PROSTATE:

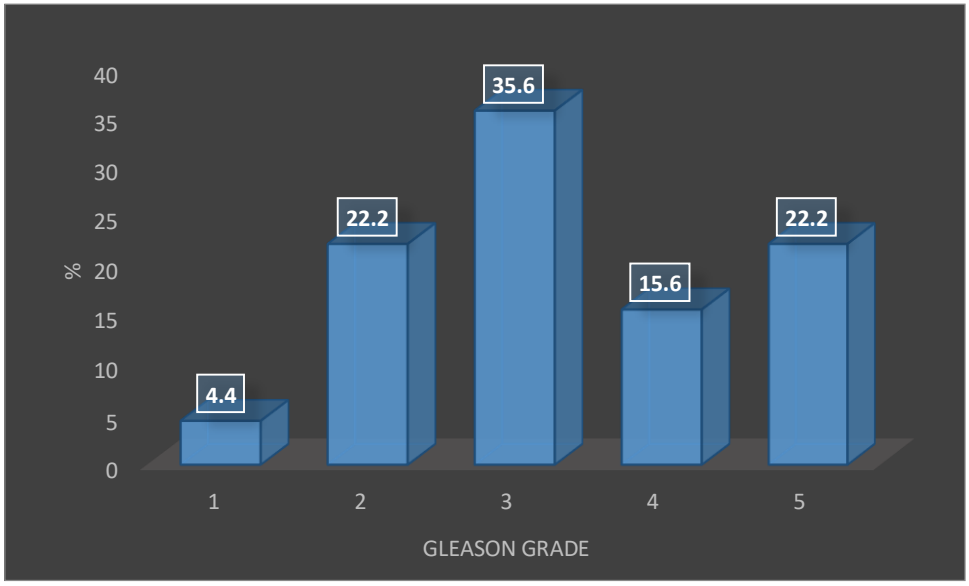


In this study, the majority of cases, i.e. 28 (62.2%), were of moderately differentiated adenocarcinoma, and 17 cases belonged to poorly differentiated adenocarcinoma amounting to 37.8% (Figure 11).

TABLE 8: DISTRIBUTION OF GLEASON GRADE GROUP IN PROSTATE CARCINOMA CASES

GLEASON GRADE	Number of cases	Percentage
1	2	4.4
2	10	22.2
3	16	35.6
4	7	15.6
5	10	22.2
Total	45	100.0

FIGURE 12: DISTRIBUTION OF GLEASON GRADE GROUP IN PROSTATE CARCINOMA CASES



Out of 45 cases of Prostate carcinoma, 16 cases were seen belonging to Gleason Grade group 3, accounting for 35.6,% followed by 10 patients belonging to Gleason Grade group 5, amounting to 22.2%. 10 cases were seen belonging to Gleason Grade group 2 constituting 22.2%. Only 7 out of 40 patients belonged to Gleason Grade group 4 constituting about 15.6% (Table 8, Figure 12).

TABLE 9: DISTRIBUTION OF CASES ACCORDING TO GLEASON SCORE

GLEASON SCORE	Number of cases	Percentage
2+3	1	2.2
3+3	1	2.2
3+4	10	22.2
4+3	16	35.6
3+5	3	6.7
4+4	3	6.7
4+5	5	11.1
5+3	1	2.2
5+4	4	8.9
5+5	1	2.2
Total	45	100.0

Out of 45 cases of adenocarcinoma of the Prostate, the majority of cases, i.e. 16, had a Gleason score of 4+3, accounting for 35.6%, followed by 10 cases with Gleason score of 3+4, constituting for 22.2%. 5 cases had a Gleason score of 4+5, amounting for 11.1%, and 4 cases had a Gleason score of 5+4, amounting for 8.9%followed by 3 cases with a Gleason score 3+5 and 4+4 amounting for 6.7% each. There were single cases with a Gleason score of 2+3, Gleason score of 3+3, a Gleason score of 5+3, and Gleason score of 5+5 constituting 2.2% each. (Table 9)

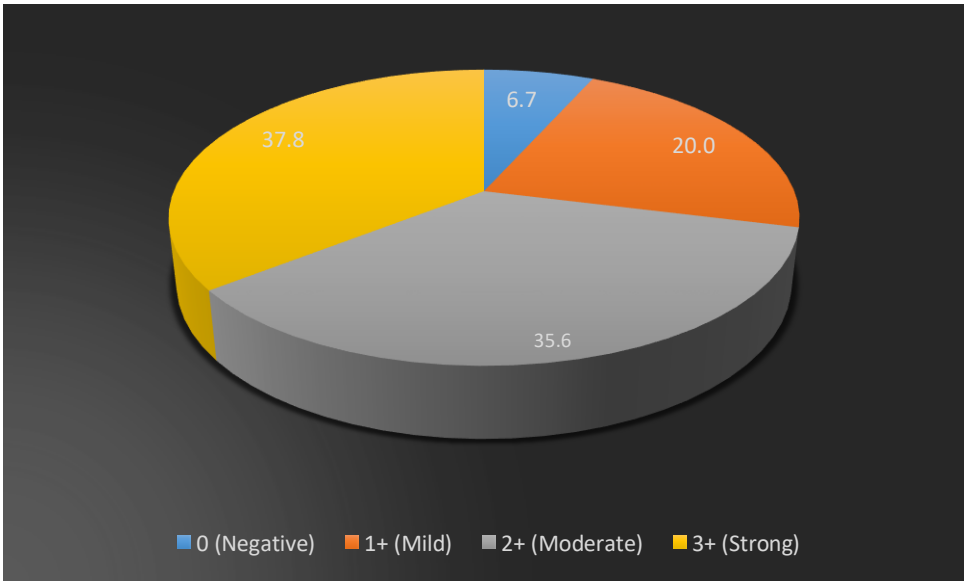
Serum PSA level was available in all the patients, ranging from 1.90 to 156 ng/ml, with a median level of 100 ng/ml.

TABLE 10: DISTRIBUTION OF CASES ACCORDING TO SERUM PSA LEVEL

Serum PSA level	Number of cases	Percentage
>10 ng/ml	38	84.44
<10 ng/ml	7	15.55
Total	45	100

We have categorised the cases having PSA levels <10 ng/ml and >10 ng/ml. Out of 45 cases, 38 patients had serum PSA levels >10ng/ml and 7 cases had serum PSA levels <10 ng/ml. (Table 10)

FIGURE 13: DISTRIBUTION OF CASES ACCORDING TO AMACR EXPRESSION:



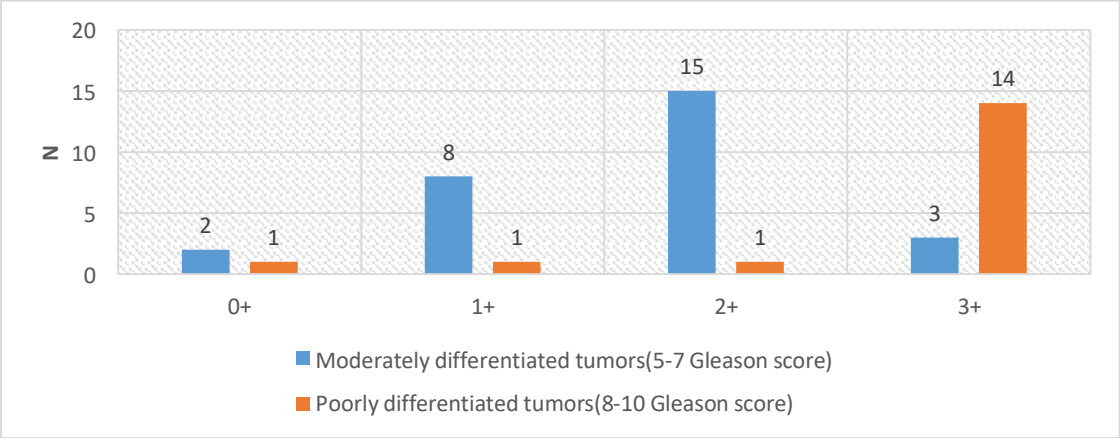
Among 45 cases of prostate adenocarcinoma, AMACR expression was noted in 42(93.3%) cases.

Out of total 42 cases, mild (1+) expression of AMACR was seen in 9 (22.2%) cases, moderate (2+) expression of AMACR was seen in 16 (35.6%) cases, strong (3+) expression of AMACR was seen in 17 (35.6%) cases. (Figure 13)

TABLE 11: FREQUENCY OF THE AMACR EXPRESSION IN RELATION TO TUMOR DIFFERENTIATION AND GLEASON SCORE (N=45):

AMACR Expression	Prostatic carcinoma		Total	p value
	Moderately differentiated carcinoma(5-7 Gleason score)	Poorly differentiated carcinoma (8-10 Gleason score)		
0+	2 (7.1%)	1 (5.9%)	3 (13%)	0.0001
1+	8 (28.6%)	1 (5.9%)	9 (34.5%)	
2+	15 (53.6%)	1 (5.9%)	16 (59.5%)	
3+	3 (10.7%)	14 (82.4%)	17 (93.1%)	
Total	28 (62.22%)	17 (37.77%)	45 (100.0%)	

FIGURE 14: FREQUENCY OF THE AMACR EXPRESSION IN RELATION TO TUMOR DIFFERENTIATION AND HISTOLOGICAL GRADE (N=45):

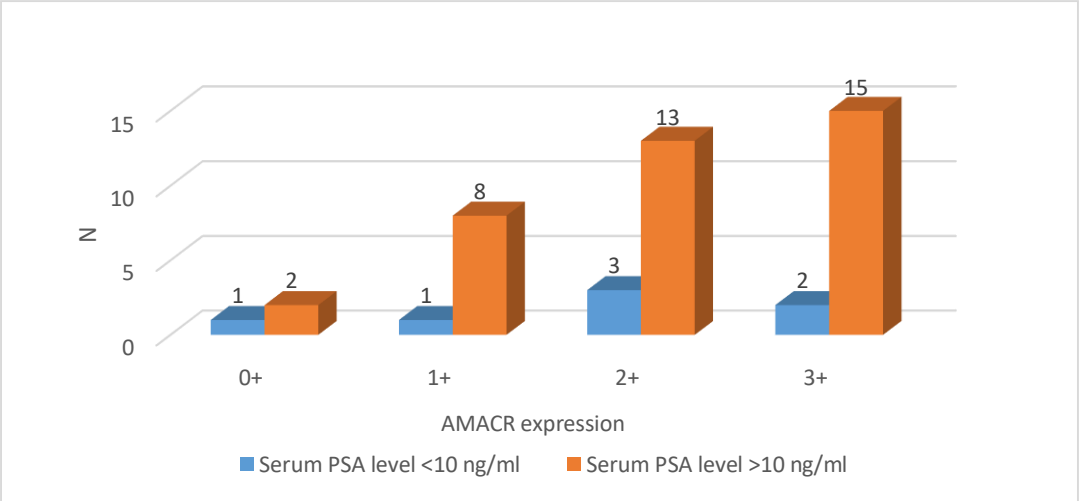


AMACR expression was significantly increased with increase in grade of prostate cancer. Out of 28 (62.2%) cases of moderately differentiated carcinoma, 15 cases (53.6%) cases showed 2+ positivity, while 3 cases (10.7%) showed 3+ positivity, and 8 (28.6%) cases had 1+ positivity and 2 cases did not show expression. Out of 17 (37.77%) cases of poorly differentiated carcinoma, 14 cases (82.4%) showed 3+ positivity, 1 (5.9%) case showed 2+ positivity, 1 (5.9%) case showed 1+ positivity and 1 (5.9%) case was negative for AMACR expression. The p-value for the correlation between AMACR expression and Gleason grade was 0.0001 suggesting a significant correlation between AMACR expression and Gleason's grade of carcinoma of the prostate. (Table 11, Figure 14)

TABLE 12: FREQUENCY OF THE PSA LEVEL IN RELATION TO AMACR EXPRESSION (N=45):

AMACR expression	Serum PSA level		Total	p value
	<10 ng/ml	>10 ng/ml		
0+	1 (14.3%)	2 (5.3%)	3 (6.7%)	0.761
1+	1 (14.3%)	8 (21.1%)	9 (20.0%)	
2+	3 (42.9%)	13 (34.2%)	16 (35.6%)	
3+	2 (28.6%)	15 (39.5%)	17 (37.8%)	
Total	7 (15.55%)	38 (84.44%)	45 (100.0%)	

FIGURE 15: FREQUENCY OF THE PSA LEVEL IN RELATION TO AMACR EXPRESSION (N=45):



Serum PSA level was available in all cases of prostate carcinoma. It was ranging from 1.90 to 156 ng/ml with a median level of 100 ng/ml. Cases were divided into 2 groups, serum PSA level <10 ng/ml and >10 ng/ml. There were 7 (15.55%) cases with serum PSA level <10 ng/ml and 38 (84.44%) cases with serum PSA level >10 ng/ml. Among the 7 (15.55%) cases with serum PSA level <10 ng/ml, 3 cases (42.9%) cases showed 2+ positivity, while 2 cases (28.6%) showed 3+ positivity, 1 (14.3%) case showed 1+ positivity and 1 (14.3%) case had negative staining. Out of 38 (84.44%) cases with serum PSA level >10 ng/ml, 15 cases(39.5%) showed 3+ positivity, 13 (34.2%) cases showed 2+ positivity, 8 (21.1%) cases showed 1+ positivity and 2 (5.3%) cases showed negative staining. The p-value for correlation between AMACR expression and serum PSA level was 0.761 suggesting no significant correlation between AMACR expression and serum PSA level of carcinoma of the prostate. (Table 12, Figure 15)

MICROPHOTOGRAPHS

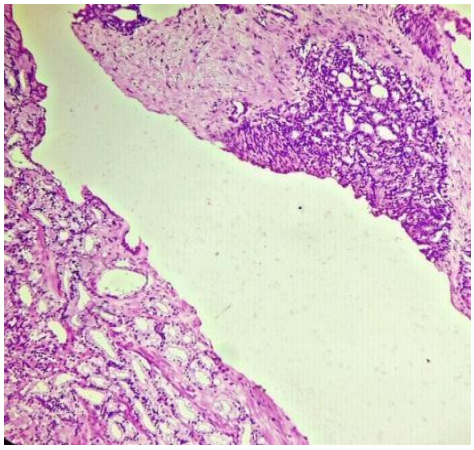


Figure 16: Microphotograph of Prostate Adenocarcinoma showing well-formed glands with lesser component of poorly formed glands (H&E,100X) (Gleason score=3+4)

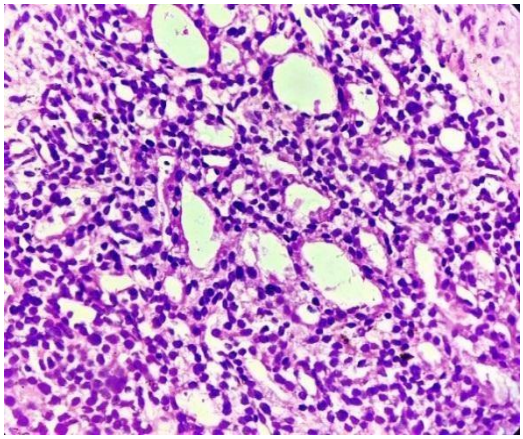


Figure 17: Microphotograph of Prostate Adenocarcinoma showing poorly formed glands (H&E, 400X).

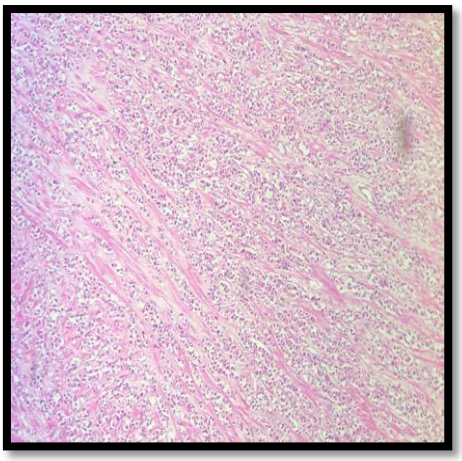


Figure 18: Microphotograph of Prostate Adenocarcinoma showing tumor tissue arranged in infiltrating cords (H&E, 100X)

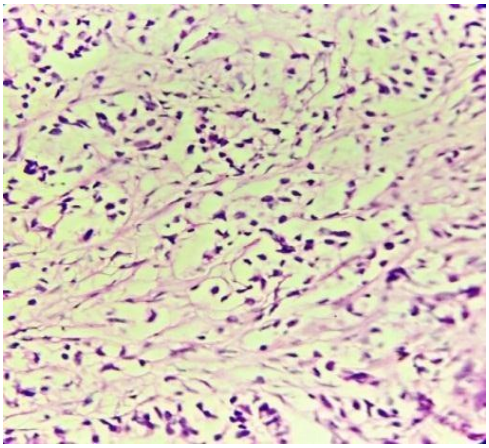


Figure 19: Microphotograph of Prostate Adenocarcinoma showing infiltrating cords of tumor cells (H&E, 400X)

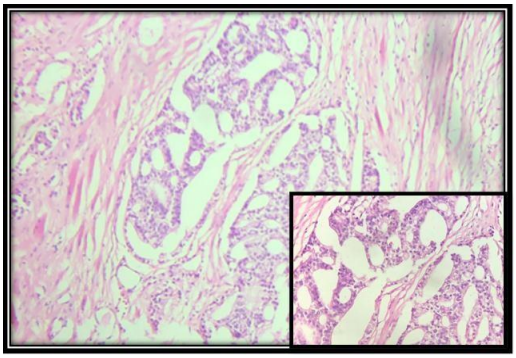


Figure 20: Microphotograph showing H&E Stain showing tumor arranged in cribriform pattern(100X) and image at the bottom right corner showing closer view of tumor cells (400X) in prostate adenocarcinoma. Gleason score=5+5

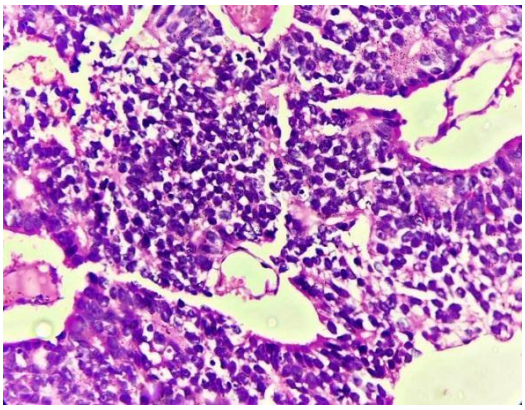


Figure 21: Microphotograph of Prostate Adenocarcinoma showing tumor cells with enlarged hyperchromatic nuclei (H&E, 400X)

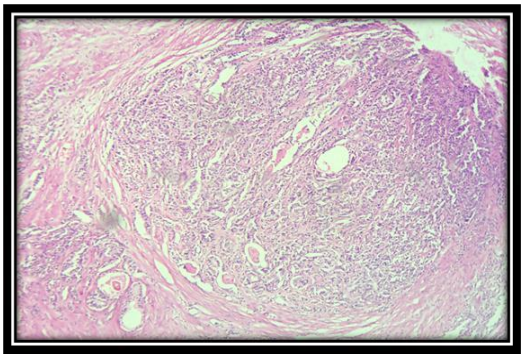


Figure 22: Microphotograph showing tumor in lobules in prostatic adenocarcinoma (H&E, 100X) (Gleason score=4+5)

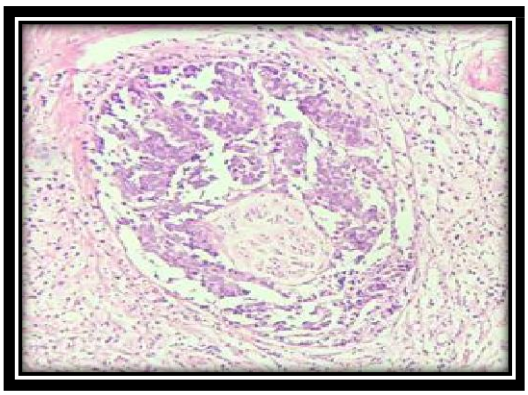


Figure 23: Microphotograph showing perineural invasion. (H&E, 200X)

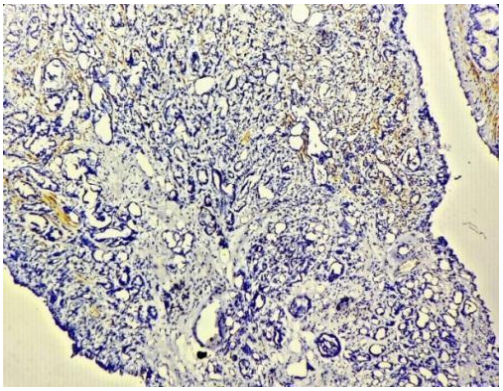


Figure 24: Microphotograph showing negative (0+) AMACR expression (100x).

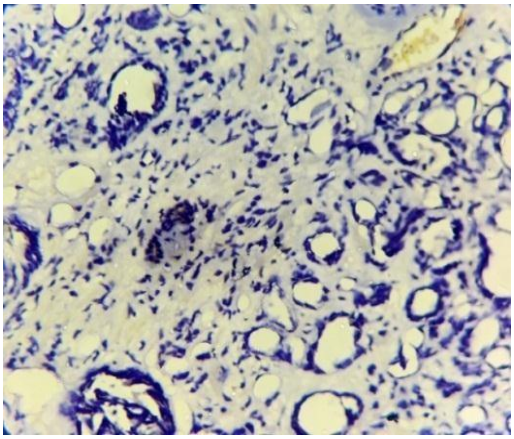


Figure 25: Microphotograph showing negative (0+) AMACR expression (400X).

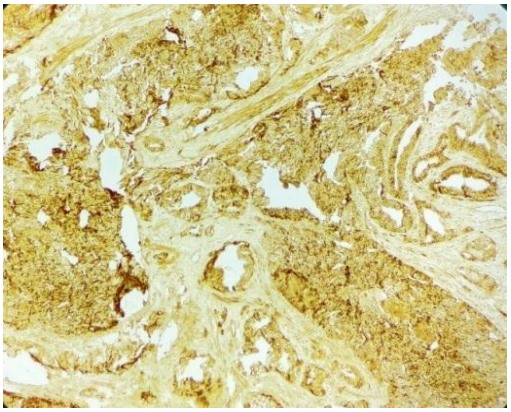


Figure 26: Microphotograph showing mild (1+) AMACR expression (100X).

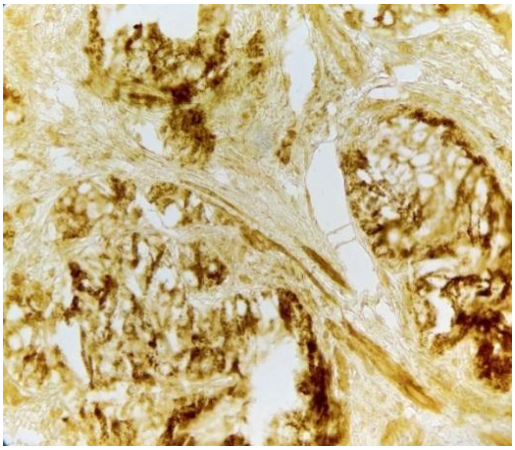


Figure 27: Microphotograph showing mild (1+) AMACR expression (400X).

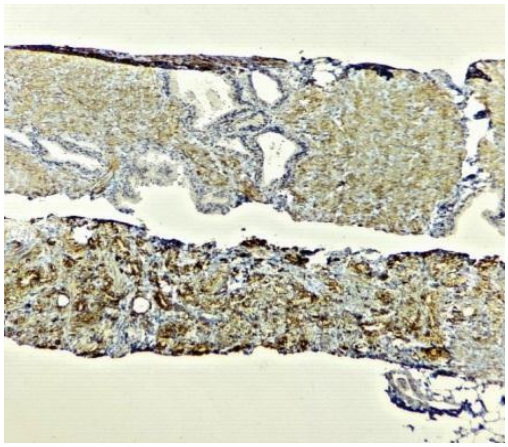


Figure 28: Microphotograph showing moderate (2+) AMACR expression (100X).

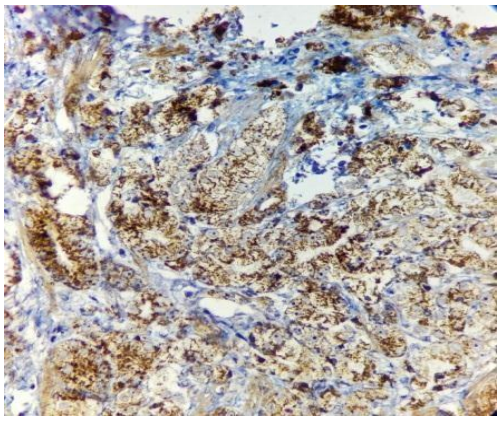


Figure 29: Microphotograph showing moderate (2+) AMACR expression (400X).

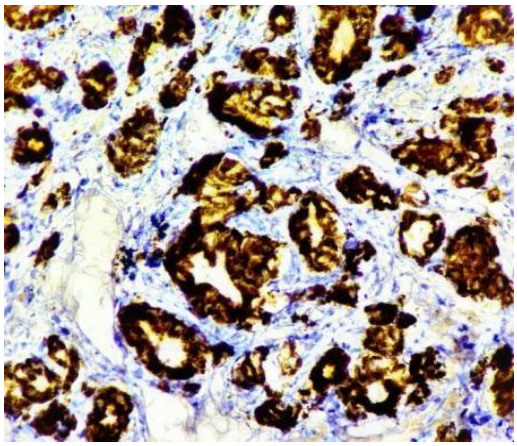


Figure 30: Microphotograph showing strong (3+) AMACR expression (400X).

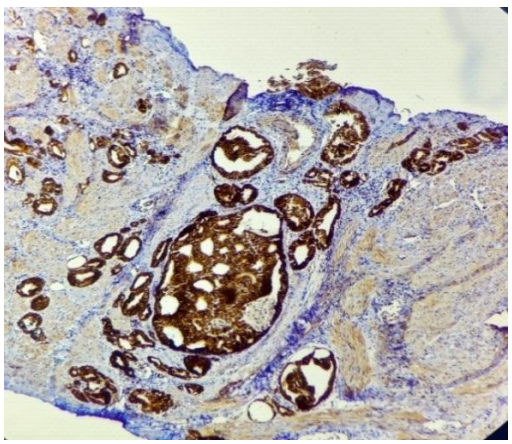


Figure 31: Microphotograph showing strong (3+) AMACR expression (100X).

DISCUSSION

Carcinoma of the prostate is the second most common cause of cancer, with an estimated 1.1 million new cases in 2012, and the sixth leading cause of cancer-related deaths in men worldwide, with an estimated 3,07,000 prostate cancer deaths in 2012.[1,105]

Prostate cancer accounted for about 1,276,106 new cases and 358,989 deaths which are around 3.8% of deaths in men with cancer during the year 2018.[19]

The figures, however, differ globally. Carcinoma of prostate incidence has been estimated to range throughout Asian nations, from 3.0/100,000 in Iran to a high of 20.3/100,000 in the Philippines. Age-standardized incidence rates (ASIRs) of prostate cancer have steadily risen in Asian nations during the past few decades. Although cancer rates in India are lower than those in Western nations, it has been observed that as life expectancies rise and lifestyles change, cancer rates—particularly prostate cancer—in this nation are rising.[35]

One of the most common issues in prostate pathology is the under diagnosis of restricted prostate cancer on needle biopsy. Due to different architectural patterns and the existence of numerous mimics of prostate adenocarcinoma, the identification of prostate carcinoma has become difficult for pathologists; however, one of the gold standards for the diagnosis of prostatic carcinoma is light microscopic observations. However, there can be a significant variation in the morphology of basal cells, including secretory cells that are cut tangentially, stromal fibroblasts, tumour cells that exhibit distortion and crushing artefacts in a small focus of cancer that resembles basal cells, and malignant tumour cells. The use of antibodies that identify the basal cells of the prostate may help to make the diagnosis when a lot of atypical glands are present for investigation.[68]

Recent research has identified the cytoplasmic protein AMACR, also known as P504S, as a tumour marker for a number of malignancies, but its function in prostatic carcinogenesis is yet unknown. Prostate cancer considerably upregulates AMACR expression, according to recent studies. According to IHC, this marker has been found to be positive in a considerable percentage of high-grade prostatic intraepithelial neoplasia (PIN), certain foci of adenosis, and some completely benign glands. However, the majority of prostate malignancies (80–100%) are positive for AMACR.[106]

According to various studies, AMACR has a specificity of 79-100% and a sensitivity of 80-100%.[107]

Prostate cancer has been linked to high rates of AMACR overexpression, and AMACR staining, when paired with basal cell markers, can be used confidently in the differential diagnosis of problematic prostatic biopsies.[7]

Currently, AMACR is more frequently used in an antibody cocktail with basal cell markers to diagnose suspicious foci in prostate specimens.[108]

In the present study, the age group of presentation of prostate carcinoma ranged from 50-90 years; among them, most of the patients, i.e. 16 patients (35.6%), were in the age group of 70-80 years, followed by 14 (31.1%) patients in the age group of 80-90 years. The youngest patient was 50 years old, while the eldest patient was 90 years old.

The age distribution of cases in the present study was similar to the study conducted by Norton *et al.*[107] in which the maximum number of malignant cases was in the age group of 70-79 years.

These results were also comparable with the study done by Garg *et al.*[109] and Jain *et al.*[104], where adenocarcinoma was most commonly found in the 7-8 decade.

TABLE 13: TYPES OF PROSTATIC SPECIMENS OBTAINED IN VARIOUS STUDIES:

PROCEDURE	Present study	Jain *et al.*[104]	Wadgaonkar *et al.*[110]	Khatib *et al.*[111]
TURP chips	39(86.7%)	15(23.1%)	69(86.5%)	72 (81.1%)
Needle Biopsies	06(13.3%)	78(64.5%)	06(7.5%)	10(11.36%)
Prostatectomy Specimens	00	15(12.4%)	05(6.2%)	06(6.81%)

In our study, prostatic chips from the TURP procedure comprised the majority of samples received, i.e. 39 followed by 6 needle core biopsies which were comparable to the study conducted by Wadgaonkar *et al.*[110] and Khatib W *et al.*[111] On the contrary, Jain *et al.*[104] received more needle biopsy as compared to TURP specimen. (Table 13)

According to a study done by Chandanwade SP *et al.*, TURP is the commonest procedure performed for prostatic lesions when compared with en block removal of the prostate in India.[112]

In the present study, Gleason score 7 was the most common pattern, with a total of 26 cases (57.8%) showing Gleason score of 7. Results were similar to a study done by El-Kasem *et al.*[7] On the contrary, in a study by Jain *et al.*,[104] Gleason score of 6 was the most common pattern.

TABLE 14: COMPARISON OF HISTOLOGICAL GRADES OF CARCINOMAS IN VARIOUS STUDIES:

Gleason grade	Present study	Jain *et al.*[104]	Norton et al[107]	El-Kasem *et al.*[7]
Well-differentiated	00	5.4%	00	00
Moderately differentiated	62.2%(28 cases)	67.56%	42.25%	59.1%
Poorly differentiated	37.8%(17 cases)	27%	57.7%	40.9%

In our study, majority of cases, that is, 28 cases (62.2%), were moderately differentiated carcinomas (Gleason score 5–7) and 17 cases (37.8%) (Gleason score 8–10) were poorly differentiated carcinomas. The results were in a similar line with the observations of Jain *et al.*,[104] El-Kasem *et al.*[7] and Gleason,[113] They discovered that the majority of prostate cancers in their study were of intermediate grade. A study done by Norton *et al.*[107] observed majority of cases being of poorly differentiated adenocarcinoma followed by moderately differentiated carcinoma, which is not in a similar line with the present study. (Table 14)

TABLE 15: COMPARISON OF AMACR EXPRESSION IN VARIOUS STUDIES:

AMACR expression	Present study N= 45	Jain *et al.*[104] N= 37	Norton *et al.*[107] N= 71	Molinié *et al.*[114] N= 260
0 (Negative)	6.7% (3 cases)	10.8%	1.23%	3%
1+ (Mild)	22.2% (9cases)	13.5%	2.81%	30%
2+ (Moderate)	35.6% (16 cases)	27.0%	35.7%	31`%
3+ (Strong)	35.6% (17 cases)	48.6%	60.6%	36%
Total	100% (45 cases)	100%	100%	100%

Among 45 cases of prostate adenocarcinoma, expression of AMACR was noted in 42(93.3%) cases. Out of the total of 42 cases, the majority of the cases showed both moderate (2+), i.e. 35.6% cases and strong (3+), i.e. 35.6% AMACR expression.

In the Study done by Jain *et al.*,[104] Norton *et al.*,[107] and Molinié *et al.*[114] got the majority of the cases had strong (3+) positivity, which is similar to the present study. (Table 15)

The present study showed expression of AMACR was significantly up-regulated in poorly differentiated carcinoma of the prostate with high Gleason score and grade. We reported high statistical significance between AMACR expression and Gleason grade in the adenocarcinoma of the prostate.

Out of 17 cases (37.77%) of poorly differentiated carcinoma, 14 cases (82.4%) showed strong (3+) AMACR positivity, and out of 28 cases (62.22%) of moderately differentiated carcinoma, only 3 cases (10.7%) showed strong (3+) AMACR positivity.

El-Kasem *et al.*[7] observed a highly statistically significant relationship between AMACR and Gleason's score, with 70.7% of cases with Gleason's score ≥7 having a strong AMACR expression [p =0.004], which is consistent with the current study.

Lee *et al.* have tested the relation between AMACR expression in nasopharyngeal carcinoma and reported that high expression of AMACR significantly correlated with higher primary tumor status and independently predicted worse disease-specific survival (DSS), distant metastasis-free survival (DMFS) and local recurrence-free survival (LRFS).[115]

Rubin M *et al.* investigated 204 patients of prostate carcinoma for potential expression of AMACR as a biomarker for aggressive prostate cancer. They discovered that reduced AMACR tissue expression was linked to a poorer prognosis for prostate cancer. Among those with both low AMACR expression and high Gleason score, the risk of prostate cancer death was 18-fold higher (P = 0.006).[116]

Barry *et al.* concluded that Low AMACR expression in primary tumor specimens was not independently associated with the development of metastatic and lethal prostate cancer after treatment over a 20-year follow-up period, after adjustment for important clinical covariates at diagnosis, yet they discovered that lower AMACR intensity was linked to greater prostate-specific antigen levels and more advanced clinical stages (p=0.003 and p=0.06, respectively) at diagnosis.[117]

Because there is no evidence-based scoring system and no agreement on a cut-off point to divide cases as low and high expressions, these inconsistent results could be related to different scoring systems utilised by each study to measure AMACR expression.

Studies done by Jain *et al.*,[104] Norton *et al.*, [107] Molinié *et al.*, [114] Rubin *et al.*,[118] and Ozgur *et al.*[119] revealed that there was no statistically significant relationship between the positivity of AMACR and Gleason's grade, which is not in concordance with the present study.

In our study, serum PSA level were available in all the patients and the values ranged from 1.90 ng/ml to 156 ng/ml, with a median level of 100 ng/ml.

We have categorised the cases having PSA levels <10 ng/ml and >10 ng/ml. Out of 45 cases, 38 cases had serum PSA levels>10% ng/ml, and 7 cases had serum PSA levels <10 ng/ml. There was no statistically significant association between AMACR expression and serum PSA level of prostate carcinoma. (P = 0.761)

A study done by El-Kasem *et al.*[7] revealed the median level of PSA in cases with strong AMACR expression was 141.8 ng/ml, compared to 58.9 ng/ml in cases with mild AMACR expression [p=0.08]. This result is in similar line with the present study.

These results were also comparable with the study done by Jain *et al.*,[104] Luo *et al.*[101]and Ozgur *et al.*,[119], that showed no correlation between AMACR expression and serum PSA level of prostate carcinoma.

On the contrary, Rubin *et al.*[118] showed a significant correlation between AMACR expression and serum PSA level of prostate carcinoma, with a p-value <0.001.

A study done by Norton *et al.*[107] stated that the correlation between PSA levels and AMACR expression was discovered to be substantial, with increased AMACR expression corresponding with higher PSA levels (P=0.02), which is not in a similar line with the present study.

Once expressed, the AMACR protein remains raised as the prostatic carcinoma develops to higher grades and stages and even metastasizes. Prostate carcinomas and the presumed precursor lesion (high-grade prostatic intraepithelial neoplasia) consistently scored significantly higher than matched normal prostate epithelium, according to a thorough immunohistochemical analysis of samples from 168 primary prostate cancer cases using both standard slides and tissue microarrays.[101]

SUMMARY

- A hospital-based retroprospective cross-sectional study was conducted. The study included specimens of prostate carcinoma received in the Department of Pathology from 1st Janauary, 2020 to 31st August, 2022(2.5-year study).
- Total of 45 prostatic specimens in the form of TURP/Biopsy were studied. The diagnosis of all cases included in this study was based on routine microscopic examination on H&E stain.
- Gleason grading was done according to the H&E stained section and serum PSA level of the patients obtained.
- Among the 45 cases of prostate carcinomas included in the study, majority of the cases were between the age group of 70-90yrs. The youngest patient was 50yrs, and the oldest was 90yrs.
- Expression of IHC marker, AMACR was noted in 42 cases out of 45 prostatic carcinomas, and the AMACR expression was correlated with Gleason grade and serum PSA level.
- AMACR expression was significantly up-regulated in poorly differentiated carcinomas of the prostate.
- Out of 17 cases (37.77%) of poorly differentiated carcinomas, 14 patients (82.4%) showed strong (3+) AMACR positivity and out of 28 cases (62.22%) of moderately differentiated carcinomas, only 3 cases (10.7%) showed strong (3+) AMACR positivity.
- A statistically significant correlation was observed between AMACR expression and Gleason's grade of prostate carcinoma (P = 0.000).
- 84.4% cases had serum PSA level >10 ng/ml, and out of them, the majority of the cases showed 3+ AMACR positivity. A statistically significant correlation

was not observed between AMACR expression and serum PSA level of prostate carcinoma (P = 0.761).

CONCLUSION

- The present study was carried out on immunohistochemical expression of AMACR in prostate carcinoma. The expression of AMACR was correlated with Gleason grade and serum PSA level. We found a significant correlation of AMACR expression with Gleason grade, but there was no correlation noted with serum PSA level.
- This study adds to our understanding of the efficacy of the immunohistochemical marker that is AMACR in prostatic carcinoma.
- Strong immunoexpression of AMACR is a poor prognostic indicator and is associated with high Gleason grade in prostate carcinoma.
- As the sample size was less, and there was discordance with few of the other studies, further extensive study is needed to standardize the immunoexpression of this marker.

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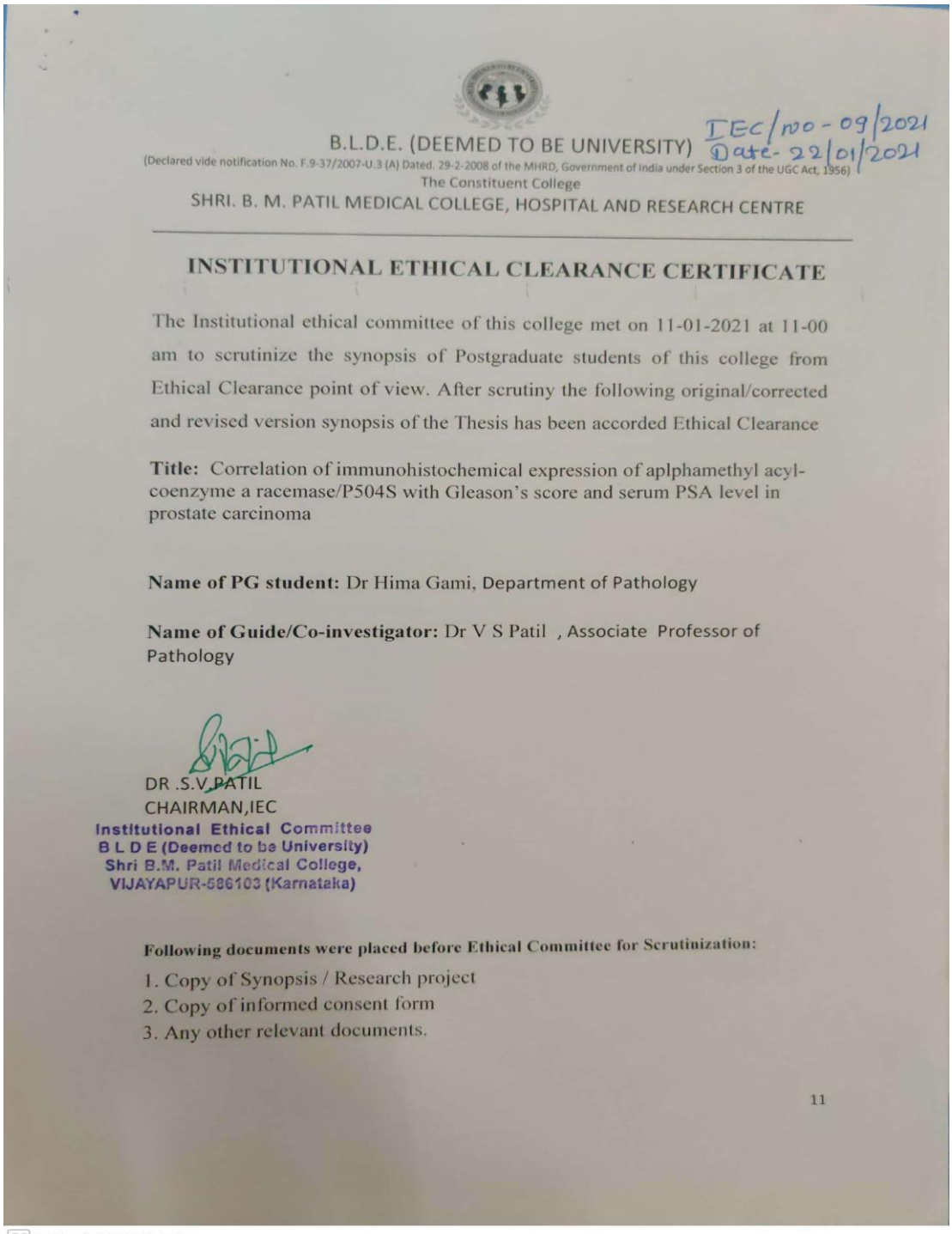
ANNEXURE-I



B.L.D.E. (DEEMED TO BE UNIVERSITY)

IEC/no-09/2021
Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Correlation of immunohistochemical expression of aplphamethyl acyl-coenzyme a racemase/P504S with Gleason's score and serum PSA level in prostate carcinoma

Name of PG student: Dr Hima Gami, Department of Pathology

Name of Guide/Co-investigator: Dr V S Patil , Associate Professor of Pathology

DR .S.V.PATIL
CHAIRMAN,IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

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ANNEXURE-II

B.L.D.E (DEEMED TO BE) UNIVERSITY,

SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURAA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, , S/O D/O W/O , aged years, ordinarily resident of _ do hereby state/declare that Dr of Hospital has examined me thoroughly on at (place) and it has been explained to me in my own language that I am suffering from disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research t i t l e d under the guidance of Dr requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept

secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor: Witness:

1.

2.

Date:

Place:

ANNEXURE-III

PROFORMA

NAME **:** OP/IP NO. **:**

AGE **:**

SEX **:** D.O.A**:**

RELIGION **:** D.O.D**:**

OCCUPATION **:**

RESIDENCE **:**

PRESENTING COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

TREATMENT HISTORY :

PER RECTUM :

USG (PROSTATIC FINDING) :

PSA LEVEL :

VITALS:

PR: RR:

BP: TEMPERATURE:

WEIGHT:

BIOPSY: NEEDLE BIOPSY-

TURP-

RADICAL PROSTATECTOMY-

HPR FINDING:

GLEASON SCORE-

GLEASON GRADE-

IHC: - AMACR expression: Negative (0+):

Mild (1+):

Moderate (2+):

Strong (3+):

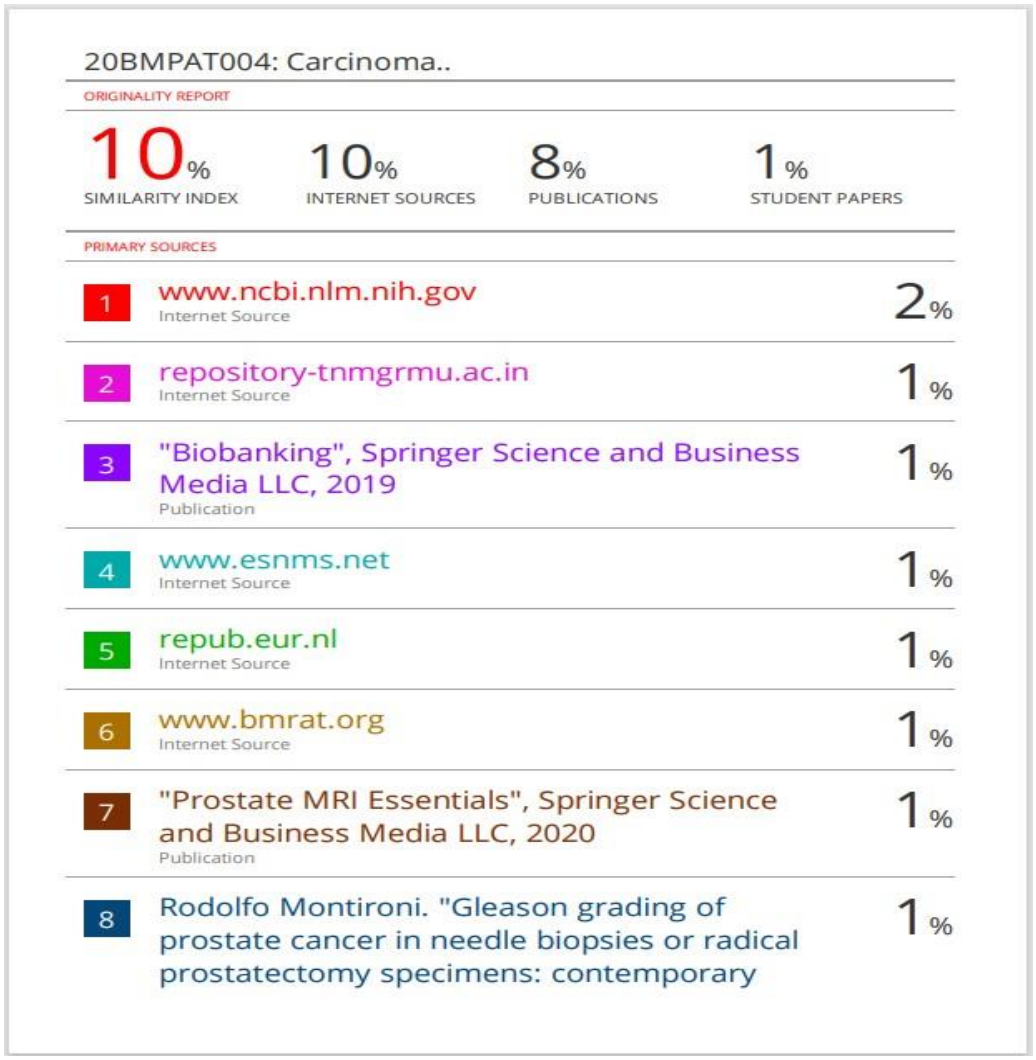
KEY TO MASTER CHART

1. Sl. No.	Serial Number
2. HPR no	Histopathology Reporting number
3. Yrs	Years
4. TURP	Transurethral Resection Of Prostate
5. PSA	Prostate Specific Antigen
6. ng/ml	Nanogram/millilitre
7. AMACR	Alpha-Methylacyl-CoA Racemase

MASTER CHART

Sr. No	HPR No.	Age	Specimen type	HPR Diagnosis	Gleason pattern	Gleason score	Gleason Grade	PSA (ng/ml)	AMACR positivity score
1	910/21 A	70 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	33	2
2	1250/21	80 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	72	2
3	863/21 A	61 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+5	9	5	100	3
4	1284/21 A	83 yr	Trucut Biopsy	Mod. Adenocarcinoma of Prostate	3+4	7	2	156	1
5	1991/21 B	50 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	3+5	8	4	120	3
6	1575/21	65 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	45.12	3
7	2018/21 A	60 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+5	9	5	50.9	3
8	1197/20 A	80 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	22	2
9	2080/20 B	56 yr	TURP Chips	Poorly differentiated Adenocarcinoma of prostate	5+4	9	5	100	3
10	2127/20 A	80 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	5+4	9	5	110	3
11	2197/21 A	70 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	39.1	3
12	2354/20 B	67 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	4.6	3
13	2416/20 E	70 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	7.2	0
14	2374/20 B	75 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	28	1
15	2450/20	70 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	45	2
16	2511/20	80 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	9.5	1
17	2734/20 A	87 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	110	2
18	2912/20 B	70 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+5	9	5	107	3
19	2922/20 B	75 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	3+5	8	4	112	3
20	3566/20 B	80 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	134	2
21	3848/20	68 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	5+4	9	5	100	3
22	3956/20 A	65 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	5+5	10	5	120	3
23	4219/20 A	70 yr	TURP Chips	poorlyAdenocarcinoma of Prostate	3+5	8	4	149	3
24	4282/20 H	70 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+4	8	4	30	3
25	2558/21	88 yr	Trucut Biopsy	Mod. Adenocarcinoma of Prostate	4+3	7	3	62	2
26	2796/21 B	71 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+4	8	4	32	3
27	2917/21 A	75 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	87	1
28	3305/21 A	80 yr	Trucut Biopsy	Mod. Adenocarcinoma of Prostate	2+3	5	1	121	2
29	3461/21 B	65 yr	Trucut Biopsy	Poorly Adenocarcinoma of Prostate	5+3	8	4	120	3
30	3992/21 D	55 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+4	8	4	77	1
31	4038/21 A	80 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	9.5	2
32	4531/21 A	71 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+3	6	1	11.5	1
33	5008/21	65 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	54	0
34	5153/21	70 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	28	2
35	5404/21 A	79 yr	Trucut Biopsy	Mod. Adenocarcinoma of Prostate	4+3	7	3	21.9	1

36	5567/21	85 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	36	2
37	5777/21 A	73 yr	Trucut Biopsy	Mod. Adenocarcinoma of Prostate	4+3	7	3	10	2
38	2294/22	65 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	98	2
39	2314/22 A	75 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	43	1
40	2416/22 A	50 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+5	9	5	110	0
41	3292/22A	85 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	4+5	9	5	117	2
42	3590/22 A	80 yr	TURP Chips	Poorly Adenocarcinoma of Prostate	5+4	9	5	1.9	3
43	4661/22 B	83 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	4+3	7	3	23	1
44	4554/22	65 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	31	2
45	4792/22	65 yr	TURP Chips	Mod. Adenocarcinoma of Prostate	3+4	7	2	6.3	2



20BMPAT004: Carcinoma..

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