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**STUDY OF IMMUNOHISTOCHEMICAL EXPRESSION OF Akt (PROTEIN  
KINASE B) IN PROSTATIC ADENOCARCINOMA -A DESCRIPTIVE  
STUDY AT TERTIARY CARE HOSPITAL, BELAGAVI**

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**By  
REG. NO.: BN0119010**

**Dissertation**

**Submitted to the  
KLE Academy of Higher Education and  
Research, Belagavi, Karnataka**

**In partial fulfilment  
of the requirements for the degree of**

**DOCTOR OF MEDICINE  
IN  
PATHOLOGY**

**JAWAHARLAL NEHRU MEDICAL COLLEGE,  
BELAGAVI, KARNATAKA**

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**K.L.E. ACADEMY OF HIGHER EDUCATION AND  
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## ACCEPTANCE LETTER

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## LIST OF ABBREVIATIONS USED

AMACR	Alpha-methylacyl-CoA racemase
BMI	Body mass index
BPH	Benign prostatic hyperplasia
DPX	Dibutylphthalate polystyrene xylene
DRE	Digital rectal examination
ETS	Erythroblast transformation specific
FDG	Fluorodeoxyglucose
GG	Grade group
GSTP-1	Glutathione S transferase P 1
H&E	Hematoxylin and eosin
IDC	Intraductal carcinoma
IHC	Immunohistochemistry
ISUP	International Society of Urological pathology
LHRH	Luteinizing hormone releasing hormone
mp-MRI	Multiparametric magnetic resonance imaging
MRD	Medical records department
p-Akt	phosphorylated-Akt
PBS	Phosphate buffered saline

PET	Positron emission tomography
PIN	Prostate intraepithelial neoplasia
PNI	Perineural invasion
PSA	Prostate specific antigen
PSAD	Prostate specific antigen density
RB	Retinoblastoma
TRUS	Transrectal ultrasound
WHO	World Health Organization

## **ABSTRACT**

**“STUDY OF IMMUNOHISTOCHEMICAL EXPRESSION OF Akt (PROTEIN KINASE B) IN PROSTATIC ADENOCARCINOMA – A DESCRIPTIVE STUDY AT TERTIARY CARE HOSPITAL, BELAGAVI.”**

**BACKGROUND:** In men, prostatic cancer is the third most common malignant tumour and the second leading cause of carcinoma death.

P-Akt is a phosphorylated protein kinase B, a central signalling molecule which participates in the genesis and progression of malignancies. Increased Akt expression has been associated with high Gleason’s scores.

Prostate specific antigen (PSA) is a biochemical tumour marker in prostatic adenocarcinoma and helps in diagnosing cancer and cancer recurrence. The Gleason’s score is used to evaluate the prognosis of prostatic cancer.

**OBJECTIVES:** To study immunohistochemical expression of p-Akt and the correlation of histological grading with PSA in prostate adenocarcinoma.

**METHODOLOGY:** 50 cases of Prostate adenocarcinoma were studied. Gleason’s score and Group grade was obtained. Immunohistochemical staining was done with p-Akt and the expression was studied. Correlation was done between the Gleason’s score and Grade group with P-Akt immunoexpression and also with PSA levels.

**RESULTS:** The commonest age group was in the 8<sup>th</sup> decade. The commonest Gleason’s pattern was 4 with score 7 being the commonest score. Grade group 4 and 5 were found in majority. The average PSA levels ranged from 121 to 1000 ng/ml. Intensity of immunoreaction of p-Akt and the immunoreactivity score showed a statistically significant correlation with Gleason’s score and Grade group. A

statistically significant correlation of PSA with Gleason's score and Grade group was also seen.

**CONCLUSION:** Intensity of immunoreaction of p-Akt, immunoreactivity score and PSA showed a statistically significant correlation with both Gleason's score and Grade group.

**KEY WORDS:** Gleason's score, Grade group, p-Akt, Prostate specific antigen.

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## **INTRODUCTION**

Prostatic carcinoma is one of the most common malignancies and it is the second common cause of cancer mortality in males.<sup>1</sup> It is an indolent disease but 25-30% of the cancers behave in an aggressive manner which results in poor prognosis and leads to death of the patients.

Biological activity of prostatic carcinoma is related to its histological appearance and this histological appearance is then graded as well differentiated, moderately differentiated and poorly differentiated. Gleason's grading system which is widely used correlates with the mortality and morbidity and also helps in predictions of metastasis.<sup>2</sup> It is used to evaluate the prognosis of prostatic cancer. The grading is done based on microscopic appearance. Carcinomas with a higher score are more aggressive and have worse prognosis. A new contemporary prostate cancer grading system has been introduced which is used in this study. It includes 5 grade groups.

Prostate-specific antigen (PSA) is also called gamma-seminoprotein or kallikrein-3.<sup>3</sup> PSA belongs to kallikrein related peptidases. Serum PSA level is a biochemical tumour marker in the diagnosis of prostatic adenocarcinoma. Levels of total PSA have been correlated with the Gleason grading and scoring and it helps in diagnosing cancer recurrence. From PSA levels, PSA density can be calculated which helps in predicting prostate carcinoma. It is found that the prostatic cancer which is associated with an increased level of serum PSA is more likely to be higher grade.

P-Akt is a phosphorylated protein kinase B which is a central signalling molecule which participates in the genesis and progression of malignancies.<sup>4</sup> On activation there is suppression of the apoptotic response. There are 3 isoforms of Akt which are Akt-1, Akt-2 and Akt-3. It is found that there is an increased Akt

expression in prostatic carcinomas of a higher grade. There are limited studies on p-Akt expression in prostatic carcinoma and more research is needed.

Literature search did not find any study doing a correlation of p-Akt immunoexpression with Grade groups, which was done in this study.

There is an urgency to better understand the biological and biochemical parameters of prostate cancer such that new prognostic markers can be established and cellular functions can be detected suitable for therapeutic interference. This study aims at studying the immunohistochemical expression of Akt in prostatic adenocarcinoma and determining correlation between histological grading of cancer prostate with PSA levels.

## **OBJECTIVES**

**Primary Objective-** To study the immunohistochemical expression of Akt in prostatic adenocarcinoma.

**Secondary Objective-** To study the correlation of histological grading of prostatic adenocarcinoma with PSA levels.

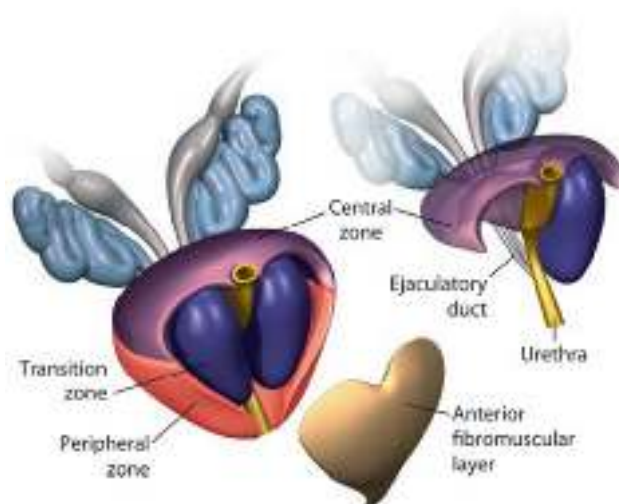
## REVIEW OF LITERATURE

### EMBRYOLOGY OF PROSTATE

Prostate gland is developed from buds arising from various parts of prostatic urethra. The secretory epithelium of the gland develops from buds arising from epithelium of prostatic urethra and also from the pelvic part of urogenital sinus. The inner glandular zone is formed by the buds from the mesoderm of the prostatic urethra and the outer glandular zone from the buds arising from the endoderm of the prostatic urethra. The peripheral mesenchyme forms the capsule of the gland along with its connective tissue and muscle. The outer glandular zone is often the site of carcinomatous changes later on in life with increasing age.<sup>5</sup> In females, paraurethral glands of Skene represent the prostatic gland.

### ANATOMY OF PROSTATE

Prostate is an accessory gland which is firm and pale and lies in the pelvic cavity. The dense fibromuscular stroma gives the gland the firm consistency. It resembles a horse-chestnut in shape and size.<sup>6</sup> The gland lies upon the rectum and hence is felt digitally when enlarged and lies postero-inferiorly to the pubic symphysis.



**Figure 1. Anatomy of Prostate<sup>7</sup>**

## **GROSS FEATURES**

It has a base, apex and four surfaces-anterior, posterior and two inferolateral.<sup>8</sup> The apex which is directed downwards touches the deep perineal fascia anteriorly and surrounds the prostatic part and the membranous part of the prostatic urethra. The base is at the neck of the urinary bladder and is directed downwards.

The anterior surface lying 2cms behind the pubic symphysis is narrow and convex. It is made up of fibrous tissue and the urethra pierces its lower end. Superiorly it is connected by the puboprostatic ligaments to the pubic bones.

The posterior surface is flat sideways and is triangular in shape. The fascia of Denonvilliers separates the gland from the rectum.<sup>9</sup> It is pierced on either side at its upper border by the ejaculatory ducts. These ejaculatory ducts trace downwards and forwards, opening into the prostatic urethra.

The inferolateral surfaces are related to the walls of the pelvis and enclosed by the anterior fibers of the levator ani muscles.

There are two capsules of the prostate. The peripheral part of the gland condenses and forms the true capsule. It is fibromuscular in structure and is in continuity with the stroma of the gland. The false capsule formed by the endopelvic fascia lies outside the true capsule. It is in continuity anteriorly with the puboprostatic ligaments and posteriorly in continuity with the fascia of Denonvilliers. A plexus of veins lies between the two capsules.

The four ligaments supporting the prostatic gland are a pair of medial and a pair of lateral puboprostatic ligaments. These ligaments are seen extending to the pubis from the false capsule.

### **LOBES OF PROSTATE**

Initially the prostate was described to have five lobes which were anterior, posterior, median and two lateral lobes. Presently the glandular part of the prostate consists of three lobes which are median and two lateral lobes.<sup>6</sup>

The middle lobe is a transverse band lying in between the two lateral lobes. In early stages of life, it is small, lying below the neck of bladder behind the origin of the urethra. With advancing age, it enlarges projecting into the bladder and impeding urine passage.

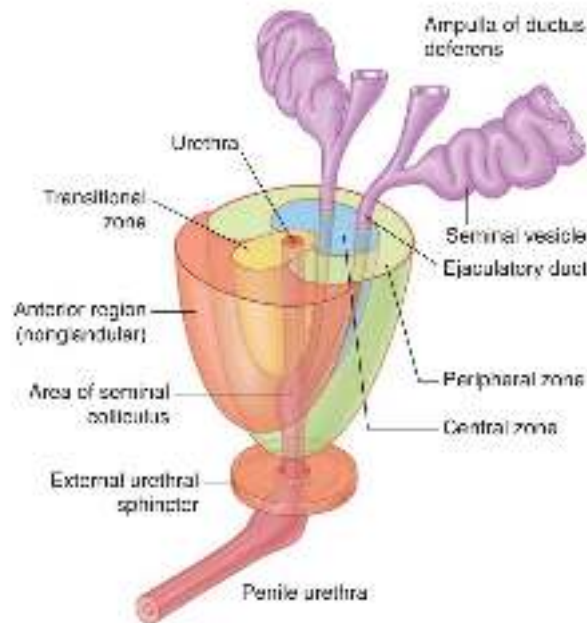
The two lateral lobes are separated posteriorly by a deep notch and their upper and under surfaces are marked by a furrow.

### **ZONES OF THE PROSTATE**

According to McNeal prostate is divided into four zones.<sup>10</sup>

1. Central zone: It forms 25% of the glandular part of the gland. It originates from the Wolffian duct and is situated behind the urethral lumen and above the ejaculatory ducts.
2. Peripheral zone: It forms 70% of the prostatic glandular part and is the site of carcinoma prostate.
3. Transition zone: It is periurethral and forms 5% of the glandular portion of prostate
4. Anterior fibromuscular stroma: One -third of prostate is occupied by it.

The central zone and transition zone together form the central gland.



**Figure 2- Zones of Prostate<sup>6</sup>**

### **BLOOD SUPPLY**

The arterial supply of prostate is derived from the internal pudendal, middle rectal and inferior vesicle arteries. These major arteries further form the subcapsular plexus which supplies majority of the gland and a small periurethral plexus.

The veins supplying the prostate form a plexus at the base and side of the gland which communicates with the vesicle plexus then draining into internal iliac veins. The prostatic plexus and vertebral plexus have a valveless communication through which carcinoma prostate can spread to the skull and the vertebral column.

### **LYMPHATIC DRAINAGE**

Intraprostatic trunks pierce the prostatic capsule forming periprostatic lymphatic plexus. The obturator and external iliac nodes are primary sites of lymphatic drainage of prostate.

### **NERVE SUPPLY**

Prostate is supplied by both the parasympathetic and sympathetic nerves which on stimulation lead to discharge of prostatic secretions.<sup>6</sup>

## **PHYSIOLOGY OF PROSTATE**

The prostate produces a thin alkaline fluid necessary for fertilization. It is rich in citric acid and fibrinolysins which liquefies the coagulated semen post ejaculation. This fluid neutralizes the acidity of the vagina and ductus deferens and thus increases the sperm motility and fertility.<sup>11</sup>

## **HISTOLOGY OF PROSTATE**

On histological sectioning the prostate shows two concentric zones which are separated by an irregular capsule instead of a lobular pattern.

The outer zone which is large is made up of tubulo-acinar glands which are branched and are embedded into a fibromuscular stroma.<sup>12</sup> A part of prostate which is made up of only collagen and muscle fibers lies anterior to the gland and also at the apex which is known as the anterior fibromuscular stroma. The ducts of these glands drain into prostatic sinuses and this zone is the site of carcinoma.

The inner smaller zone consists of mucosal glands surrounding the urethra at its upper portion and also consists of submucosal gland whose ducts open into prostatic sinuses.

The prostatic gland consists of epithelial cells and stromal cells. The epithelium of the glands is thrown into folds and the glands are arranged either in a papillary or a convoluted pattern. The glands consist of ducts which terminate as acini. These ducts are tubular in structure whereas acini are grouped in a lobular arrangement.

The glandular epithelium is bi-layered. The luminal cells are tall columnar with a pale cytoplasm and prominent round basally located nucleus. The cells are secretory. These cells show positive staining with prostate-specific antigen and also

with prostate specific acid phosphatase but they show negative immunoreactivity with high molecular weight cytokeratin.

The peripheral cells are small and flat with ovoid nucleus and are located at the basement membrane. These basal cells are stem cells and are seen to be the prominent cells in prostatic hyperplasia. They are absent in cancer prostate. Basal cells show immunoreactivity with high molecular weight cytokeratin.

The fibromuscular stroma also known as the supporting stroma is formed by collagen and muscle fibers.

Sometimes inspissated secretions can accumulate in the glandular lumen known as corpora amylacea. They form spherical concretions which as the age advances are seen in increased amount and have a tendency to get calcified.

### **PROSTATIC CARCINOMA**

Prostatic carcinoma is one of the most common malignancies and it is the second common cause of cancer mortality in males.<sup>1</sup> The incidence of prostate cancer worldwide is 25.3 per 100,000.<sup>13</sup>

The susceptibility of cancer prostate is highest in black population followed by whites with Asians having the lowest susceptibility.<sup>14</sup> Most of the men diagnosed with this cancer are older men above the age of 65 years and older.

### **INCIDENCE OF CANCER PROSTATE**

The incidence of cancer prostate increases with advancing age. Pudasaini S et al found prostatic adenocarcinoma in 17.6% of the patients out the 51 cases that were studied.<sup>15</sup> Mohammed G et al gave an incidence of 43% of cancer prostate out of the 348 patients studied with mean age of the patient being 68.4 years.<sup>16</sup> Jayapradeep D et al studied 170 prostatic biopsies and observed an incidence of 18.2% of carcinoma prostate.<sup>17</sup>

## **DEFINITIONS**

As mentioned by Franks<sup>18</sup> cancer prostate is classified into three types

1. Latent carcinoma: It is found incidentally as a small focus of cancer during autopsy studies.
2. Occult carcinoma: In this type the patient depicts no symptoms of cancer prostate but on investigations and clinically it is detected by evidence of metastasis.
3. Clinical carcinoma: Carcinoma which is confirmed by biopsy examination under microscope comes under this category.

## **ETIOLOGY**

1. Hereditary factors: Prostatic cancer susceptibility gene increases risk of cancer 20 times.
2. Environmental factors: Increased fat intake in diet and polycyclic aromatic hydrocarbon exposure increase the risk. On the other hand, antioxidants, lycopene and soy products reduce the risk.
3. Racial factor: African Americans have high risk followed by Americans and Asians.
4. Endocrine factors:
  - a. Orchiectomy stops the progression of metastatic cancer.
  - b. Regression of carcinoma occurs on administration of oestrogen.
  - c. In Klinefelter's syndrome it is rare to develop carcinoma.

## **PATHOGENESIS**

Androgens influence the growth and survival of cancer cells in prostatic carcinoma. They bind to androgen receptors and lead to expression of pro-growth and survival signals.

Increased expression of PI3K/AKT signalling leads to progression of carcinoma.<sup>4</sup> It is also seen in patients who have become resistant to antiandrogen therapy.

Patients with germline mutations of the tumour suppressor gene BRCA-2 and HOXB-13 which encodes transcription factors regulating cancer development have increased susceptibility to cancer prostate.<sup>19</sup> When the ETS transcription factors get overexpressed, they make the normal epithelial cells of prostatic glands to be more invasive.

Amplification and deletions of oncogenes have been implicated commonly in cancer prostate. These include amplification involving MYC oncogene, deletion involving PTEN tumour suppressor gene and in later stages of cancer, loss of TP53 and RB gene are commonly seen.<sup>19</sup> Epigenetic alterations most commonly leading to hypermethylation of GSTP1 gene is also one of the causes in pathogenesis of carcinoma prostate.

## **SPREAD OF CANCER PROSTATE**

1. Direct spread: This occurs within the gland into the capsule of prostate and in later stages extends to the neck of bladder, trigone and ureteral openings.

2. Metastatic spread:

- a. Hematogenous spread: It occurs by retrograde spread by the venous plexus of prostate and spreads to lumbar spine and pelvic bones leading to osseous

osteoblastic lesions. Metastasis to lung, kidney and brain also occurs through this route.

- b. Lymphatic spread: By this route metastasis occurs first to the obturator lymph nodes. Sacral, iliac and para-aortic lymph nodes can be involved.

## **CLINICAL FEATURES**

### **SYMPTOMS**

Majority of the cases are asymptomatic if diagnosed in the early stages. Later stages of carcinoma which are advanced and metastatic show presence of symptoms.

If the symptoms are present, they are as follows:

- a. Weak flow of urine
- b. Difficulty in initiating urination
- c. A feeling of presence of residual urine
- d. Dribbling of urine post urination
- e. Increased frequency of urination

These are collectively called lower urinary tract obstruction symptoms. If metastasis occurs then the symptoms are that of back pain or hip pain and unexplained weight loss.

### **SIGNS**

On digital rectal examination induration and enlargement of the gland can be detected. With locally advanced disease regional lymphadenopathy can be seen and in metastatic lesions signs of cord compression are evident.

**WHO CLASSIFICATION OF PROSTATIC TUMOURS 2016<sup>14</sup>**

**1. EPITHELIAL TUMOURS**

- Glandular neoplasms
  
- Acinar adenocarcinoma
  - ✓ Atrophic
  - ✓ Pseudohyperplastic
  - ✓ Microcystic
  - ✓ Foamy gland
  - ✓ Mucinous (colloid)
  - ✓ Signet ring like cell
  - ✓ Pleomorphic giant cell
  - ✓ Sarcomatoid
  
- Prostatic intraepithelial neoplasm
  - ✓ High grade
  
- Intraductal carcinoma
  
- Ductal adenocarcinoma
  - ✓ Cribriform
  - ✓ Papillary
  - ✓ Solid
  
- Urothelial carcinoma

- Squamous neoplasm
  - ✓ Adenosquamous carcinoma
  - ✓ Squamous cell carcinoma
- Basal cell carcinoma

## **2. NEUROENDOCRINE TUMOURS**

- Adenocarcinoma with neuroendocrine differentiation
- Well, differentiated neuroendocrine tumour
- Small cell and large cell neuroendocrine neoplasm

## **3. MESENCHYMAL TUMOURS**

- Stromal tumour of uncertain malignant potential
- Stromal sarcoma
- Leiomyosarcoma
- Rhabdomyosarcoma
- Leiomyoma
- Angiosarcoma
- Synovial sarcoma
- Inflammatory myofibroblastic tumour
- Osteosarcoma
- Undifferentiated pleomorphic sarcoma

- Solitary fibrous tumour
- Solitary fibrous tumour, malignant
- Haemangioma
- Granular cell tumour

#### **4. HEMATOLYMPHOID TUMOURS**

#### **5. MISCELLANEOUS TUMOURS**

#### **6. METASTATIC TUMOURS**

### **MORPHOLOGY OF PROSTATIC ADENOCARCINOMA**

Prostate cancer histologically is of two types acinar and ductal.

#### **GROSS**

Majority of carcinomas are seen to occur in the peripheral zone in the posterior lobe of the gland. The tumour is seen as firm, fibrous and ill defined. On cut section it appears homogeneous with irregular yellow areas. Usually, residual cancer is not detectable on gross and even on microscopy when a positive biopsy is done and it is called a vanishing cancer phenomenon and can be avoided by methodically limited targeted approach.<sup>20</sup>

## **MICROSCOPIC FEATURES**

- **ARCHITECTURAL**

Foci of crowded glands can be seen with luminal borders which are sharp. These glands are arranged in a back-to-back manner or are in seen placed haphazardly with absence of intervening stroma.

There can be small glands lying next to larger glands which are benign. Benign glands can be identified by them having a larger size, branching and papillary infolding. Infiltrative pattern if seen will show atypical glands arranged perpendicular to each other in the biopsy cores.

Well-formed glands will be seen in well differentiated tumours. Moderately differentiated tumours show a fenestrated glandular pattern. Poorly differentiated tumours can have solid sheets of tumour cells or the tumour cells can be single scattered or can be arranged in a cribriform pattern. Malignant glands have almost little to absent intervening fibromuscular stroma.

## **ACINAR ADENOCARCINOMA**

Well-defined glandular patterns are seen in majority prostate cancers. A variety of cytoarchitectural patterns have been observed with the major ones being:

- Medium sized glands:** The glands are arranged in a closely spaced arrangement having a luminal surface that is smooth and the luminal cells have prominent cytoplasm with the nuclei oriented against the basement membrane. The intervening stroma is scanty.
- Small glands:** The glands are round, regular and small in size. They are present as expansive nodules or as infiltrative glands present in between benign glands.

Both the above patterns show nuclear abnormalities which are seen in the form of nuclear hyperchromasia, nuclear enlargement and macronucleoli. Macronucleoli measure more than one micron in diameter. Mitoses is rarely observed with above patterns.

c. **Diffuse individual cell infiltration**

d. **Cribriform:** Small to medium sized glands with intraluminal proliferations which are complex are seen.

e. **Poorly formed glands**

f. **Glomeruloid:** Intraluminal clusters of malignant cells are seen in this pattern.<sup>21</sup>

Squamous metaplasia can be seen in high grade prostatic carcinoma and is associated with poor prognosis. The above-mentioned patterns can be seen in combination.

- **LUMINAL**

There is lack of corpora amylacea in the lumen. Comedonecrosis is usually seen in high grade tumours. Wispy mucin can be present intraluminally which is blue tinged. Crystalloids which are rhomboid in shape may be detected in foci of adenosis. Their presence is inversely related to Gleason grade. Granular eosinophilic secretions may also be seen.

- **NUCLEAR FEATURES**

Nuclear enlargement along with hyperchromasia is seen. There is chromatin clearing seen often. Prominent nucleoli is an important diagnostic nuclear feature when there are multiple eccentrically located nucleoli. Mitosis may be seen.

It was observed by Aihara et al that Gleason grade correlated with mitotic figures.<sup>22</sup> Montironi et al found that apoptotic bodies increased from small acinar carcinoma to cribriform carcinoma.<sup>23</sup>

- **CYTOPLASMIC**

Compared to the pale cytoplasm of the surrounding benign glands, the cytoplasm of the malignant glands is more amphophilic. There is lack of lipofuscin.

Three types of cells may be seen which are clear cells, dark cells or eosinophilic cells. The clear cells are mostly cuboidal to columnar and have a foamy cytoplasm. The dark cells show a homogeneous basophilic cytoplasm and granular cytoplasm is observed in the eosinophilic cells. Outer basal cell layer is lost in carcinoma.

## **PATHOGNOMONIC FEATURES**<sup>18</sup>

### **1. Mucinous fibroplasia (MF)**

The wispy blue mucin is seen intermingled with hyalinized eosinophilic material. Also called as collagenous micronodules which show an ingrowth of fibroblasts in a delicate network of fibrous tissue. These secretions sometimes displace the epithelium leading to atrophy of the cytoplasm and a pyknotic nucleus which is small in size.

### **2. Glomerulations**

Cribriform luminal proliferations are seen in the glands with a crescentic space surrounding them which resemble the renal glomeruli. They are attached to only one edge of the malignant glands.

### **3. Perineural invasion (PNI)**

Malignant glands are seen surrounding the nerve. It is seen in 20% of prostatic adenocarcinomas obtained on needle biopsy.<sup>24</sup> It needs to be differentiated from the perineural indentation caused by hyperplastic benign glands.

## **VARIANTS OF ACINAR ADENOCARCINOMA**

### **1. Foamy gland cancer**

The tumour cells are cuboidal to columnar and they have cytoplasm which is finely granular. Sometimes the cytoplasm is clear or foamy or xanthogranulomatous due to accumulation of lipids.<sup>25</sup> The nucleus is small and pyknotic with an inconspicuous nucleolus. Most of these cancers have a low Gleason score.<sup>26</sup>

### **2. Adenocarcinoma with atrophy**

The tumour cells are arranged in an infiltrative pattern with atrophic cytoplasm and the nucleus occupying almost entire cell. Nuclear enlargement with macronucleoli is also seen.<sup>27</sup>

### **3. Pseudohyperplastic type**

Hyperplastic glands are seen arranged in a microcystic pattern with papillary infoldings. There is presence of nuclear enlargement, macronucleoli, mitosis and crystalloids in the lumen.<sup>28</sup>

### **4. PIN like adenocarcinoma**

This is a rare variant with medium to large sized glands with a stratified epithelium arranged in a crowded manner. The epithelium can also be pseudostratified columnar.<sup>29</sup>

### **5. Aberrant p63 expressing cancer**

The neoplastic cells have a scant cytoplasm, eosinophilic secretions in the lumen and slit like luminal space. The nucleus of the neoplastic secretory cells expresses p63.<sup>30</sup>

### **6. Mucinous (mucin secreting)**

On gross examination these tumours have a gelatinous surface. 25% of the tumour is composed of intracellular and extracellular mucin.<sup>31</sup> Different patterns are seen which include cribriform, microglandular, comedo, solid and hypernephroid. Occasionally these tumours can have Penneith like neuroendocrine cells. Compared to the common acinar type they lack bony metastasis and hormone dependency. These have a poor prognosis.

### **7. Signet ring carcinoma**

The tumour cells are arranged in a single file, solid or acinar form. On ultrastructure examination the intracytoplasmic lumen is lined by micro villi. Due to accumulation of intracytoplasmic mucin the nucleus is pushed to one side giving the cells a signet ring appearance. Remmele et al mentioned that the presence of >50% of signet ring cells is a diagnostic criterion.<sup>32</sup>

### **8. Sarcomatoid carcinoma**

A sarcomatoid component is seen in addition to the malignant glands. Spindle cell or giant cell features differentiating in cartilage, bone and muscle are seen. Pleomorphic giant cell adenocarcinoma is its subtype.

## **DUCTAL ADENOCARCINOMA**

It occurs often in the periurethral zone. It occurs in <1% of total prostate cancers.<sup>33</sup> On gross it is seen in the form of a polypoidal, villous or infiltrative component. Some present as polyps.

## **MICROSCOPY**

It is often seen involving the central ducts of prostate. It is arranged in a papillary or cribriform pattern lined by columnar pseudostratified epithelium. Basal cells are retained.

## **OTHER MICROSCOPIC TYPES**

### **1. Intraductal carcinoma**

It is associated with a high grade and stage and a poor survival rate. It occurs as a result of intraductal spread of an aggressive cancer. They are seen in two patterns:

- a loose cribriform or micropapillary pattern
- solid cribriform pattern

The malignant cells fill acini and ducts along with nuclear enlargement and comedonecrosis. They are cuboidal cells with a round nucleus.

### **2. Intraepithelial proliferative neoplasm**

Most diagnosed cases are high grade prostatic intraepithelial neoplasia (PIN). The cells have an increased nuclear-cytoplasmic ratio, crowding of nuclei, an amphophilic cytoplasm and prominent nucleoli and are arranged in either tufting, micropapillary, cribriform or atrophic pattern.

Netto et al mentioned that there is a 39% risk of detecting cancer prostate in a repeat biopsy if there was a high grade in the patient on the original biopsy.<sup>34</sup>

### **3. Squamous cell cancer**

It is a rare type seen after hormonal treatment which is seen as a well circumscribed nodule in the transition zone of prostate.

### **4. Adenosquamous carcinoma**

It occurs post hormonal or radiational therapy. It can occur due to three reasons

- Deviation from pluripotent cells
- Adenocarcinoma cells undergoing metaplastic transformation
- It being a collision type of tumour

### **5. Basal cell carcinoma**

It occurs as a result of MYB rearrangement which is highly aggressive with features of basaloid carcinoma.

### **6. Adenoid basal cell carcinoma**

It is seen in various patterns like cribriform or multinodular with a fibromyxoid stroma.

### **7. Prostatic adenocarcinoma with a neuroendocrine differentiation**

The Paneth cell variant has a diffuse pattern of arrangement of cells which have a eosinophilic granular cytoplasm. The arrangement can also be in the form of nests or can be present in the form of single cells.

The small cell neuroendocrine carcinoma has a large number of apoptotic cells and has an aggressive behaviour. The large cell neuroendocrine cancer occurs as a result of long-term hormonal treatment for cancer prostate.

### **STAGING AND GRADING**

Out of the many grading systems like the WHO system, M.D Anderson system and the Gleason system, the Gleason grading is used along with the International Society of Urological Pathology (ISUP) 2019 for the microscopic grading of prostatic adenocarcinoma.<sup>35</sup>

The Gleason histological grading system works on two features<sup>35</sup>

- The degree of glandular architectural distribution and differentiation
- The pattern of growth of the tumour in relation to the intervening stroma.

Grade and stage are the best prognostic predictors in carcinoma prostate and hence grading is staging is of utmost importance.<sup>36</sup>

On low power examination the of the above two features, primary and secondary patterns are observed. The primary pattern is the most predominant tumour pattern and is graded from 1 to 5 and the secondary pattern is the other pattern if seen. Both the primary and secondary patterns are added with the sum indicating the Gleason score.

Sometimes a third pattern can be seen which is the minor pattern. If in tumors where there is only one pattern, the observed value is doubled to get the score.

The scoring is different for biopsy and prostatectomy specimens.

1. **In biopsy specimens**-The primary pattern is the most predominant pattern and the secondary pattern is one with the worst prognosis.

2. **In radical prostatectomy specimens**- Primary pattern is the most predominant pattern. If the other pattern involves less than 5% of tumour then it should be considered minor pattern (tertiary) but if it constitutes more than or equal to 5% of tumour then it becomes the secondary pattern and is included in the Gleason scoring. A high-grade minor pattern more than or equal to 5% of tumour is taken as the secondary pattern.

Well differentiated tumours have a Gleason grade of 1 while the most poorly differentiated tumours have a Gleason grade of 5. The score ranges as:

- Well differentiated tumours- Gleason score 2 to 4
- Moderately differentiated tumors-Gleason score 5 to 7
- Poorly differentiated tumors - Gleason score 8 to 10

WHO has developed a Grade group system for better categorisation with the Grade group ranging from 1 to 5.

Gleason's grading in case of 12 cores or multiple cores biopsy: On a given core, the highest Gleason score correlates better with stage than the most frequently seen or average grade among the cores.<sup>37</sup> It is preferred to grade each core separately and a core which has the highest tumour grade is selected to plan treatment. Other than giving separate individual scores for each core, giving a final overall score is optional.

**Original Gleason System: 1966 and 1967<sup>38-41</sup>**

**Pattern 1:** Very well differentiated, small, closely packed, uniform glands. Essentially circumscribed masses.

**Pattern 2:** Similar (to pattern 1) but with moderate variation in size and shape of glands. Cribriform pattern may be present, still essentially circumscribed, but more loosely arranged.

**Pattern 3:** Similar to pattern 2 but marked irregularity in size and shape of glands. Tiny glands or individual cells invading stroma away from circumscribed mass. Solid cords and masses with easily identifiable glandular differentiation. Includes poorly formed individual glands.

**Pattern 4:** Large, clear cells growing in a diffuse pattern resembling hypernephroma. May show gland formation.

**Pattern 5:** Very poorly differentiated tumors. Usually, solid masses or diffuse growth with little or no differentiation into glands.

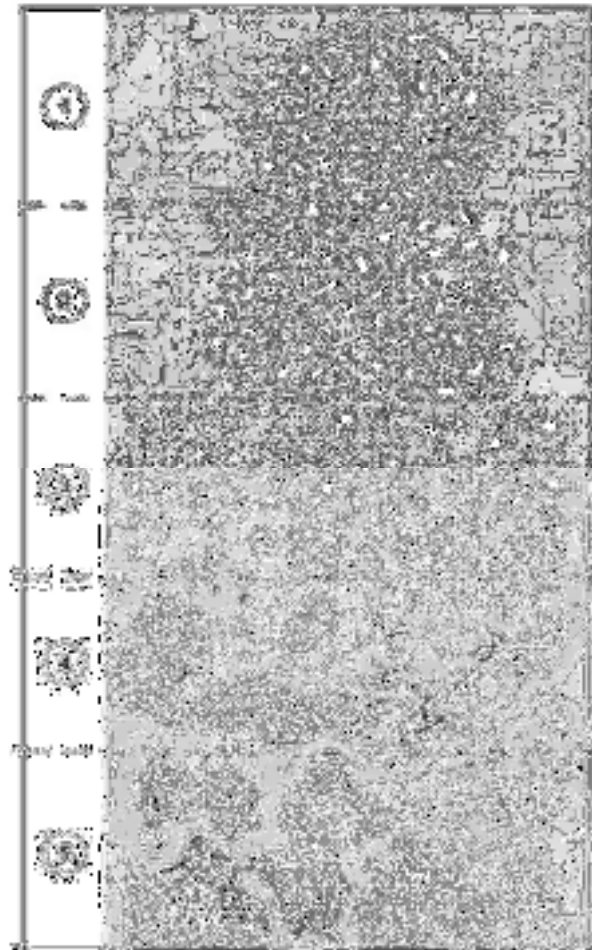
**Gleason's Modifications: 1974 and 1977<sup>38-41</sup>**

**Patterns 1 and 2:** Unchanged

**Pattern 3:** (additions) may be papillary or cribriform (1974), which vary in size and may be quite large, but the essential feature is the smooth and usually rounded edge around all the circumscribed masses of tumor (1977).

**Pattern 4:** (additions) raggedly infiltrating, fused-glandular tumor (1974). Glands are not single and separate but coalesce and branch (1977).

**Pattern 5:** (additions) can resemble comedocarcinoma of the breast (1977). Almost absent gland pattern with few tiny glands or signet cells (1977)



**Figure 3. Gleason grading system-schematic diagram ISUP 2005<sup>42</sup>**

**Pattern description of adenocarcinoma prostate ISUP 2005<sup>43</sup> for figure 3.**

**Pattern 1:** Circumscribed nodule of closely packed but separate, uniform, rounded to oval, medium-sized acini (larger glands than pattern 3).

**Pattern 2:**

- Like pattern 1, fairly circumscribed, yet at the edge of the tumour nodule, there may be minimal infiltration.
- Glands are more loosely arranged and not quite as uniform as Gleason pattern 1.

**Pattern 3:** Discrete glandular units. Typically, smaller glands than seen in Gleason pattern 1 or 2. Infiltrates in and among nonneoplastic prostate acini. Marked variation in size and shape

**Pattern 4:**

- Fused microacinar glands.
- Ill-defined glands with poorly formed glandular lumina.
- Cribriform glands
- Hypernephromatoid

**Pattern 5:**

- Essentially no glandular differentiation, composed of solid sheets, cords, or single cells.
- Comedocarcinoma with central necrosis surrounded by papillary, cribriform, or solid masses.

**2014 ISUP consensus<sup>44</sup>**

**New Modified Gleason Grading System**

- Gleason patterns 1–3 - distinct, discrete, and individual glands.
- Gleason pattern 4 - fused, cribriform, or poorly-formed glands, or glomerulation.
- Gleason pattern 5 - comedonecrosis, cords, sheets, solid nests, and single cells.

**New Prognostic grade group**

- Gleason score  $\leq 6$ : Prognostic Grade group I
- Gleason score  $3+4=7$ : Prognostic Grade group II

- Gleason score 4+3=7: Prognostic Grade group III
- Gleason score 4+4=8, 3+5=8, 5+3=8: Prognostic Grade group IV
- Gleason score 4+5=9, 5+4=9, 5+5=10: Prognostic Grade group V

**Table 1. 2014 Modified Gleason grading along with grade group<sup>18</sup>**

<b>GRADE GROUP</b>	<b>GLEASON SCORE</b>	<b>HISTOLOGICAL FEATURES</b>
1	$\leq 3 + 3 = 6$	Only individual well-formed and discrete glands
2	$3 + 4 = 7$	Predominantly well-formed glands with lesser component of poorly formed glands, fused glands, glomerulations or cribriform glands
3	$4 + 3 = 7$	Predominantly poorly formed glands, fused glands, glomerulations or cribriform glands with lesser component of well-formed glands (>5%)
4	$4 + 4 = 8$ $3 + 5 = 8$ $5 + 3 = 8$	-Only poorly formed glands, fused glands, glomerulations or cribriform glands. -Predominantly well-formed glands with lesser component of sheets, cribriform glands with comedonecrosis or single cells. -Predominantly sheets, cribriform glands with comedonecrosis or Single cells with lesser component of well-formed glands (>5%)
5	$\geq 4 + 5 = 9$	Only sheets, single cells or cribriform glands with comedonecrosis

In the present scenario the Gleason grade pattern 1 and 2 are not used as their outcome is similar to pattern 3.<sup>45</sup>

**Current International Society of Urological Pathology Modified Gleason System**

**2019 updates<sup>46,47</sup>**

- To report in biopsies the percentage of Gleason's pattern 4 for all GS 7 (ISUP Grade group-GG 2 and 3) in every core with > 5% being significant for prognosis.
- For radical prostatectomies, to include the presence of tertiary or minor Gleason's patterns 4 and 5 in the score if it is constituting >5% of tumour volume.
- To report in radical prostatectomies presence of tertiary/minor Gleason patterns 4 and 5.
- To not grade invasive ductal carcinoma (IDC) without invasive cancer.
- To include the grade of IDC into the score when invasive cancer is present.
- To comment on the presence and significance of IDC in biopsy and radical prostatectomy specimens.
- To comment on the presence of invasive cribriform cancer in biopsy and radical prostatectomy specimens.
- To report in biopsies a separate Gleason's score (ISUP GG) for each biopsy site.

**Table 2. TNM staging of prostatic adenocarcinoma 2010<sup>48</sup>**

<b>TNM DESIGNATION</b>	<b>ANATOMIC FINDINGS</b>
<b>EXTENT OF PRIMARY TUMOR (T)</b>	
T1	Clinically inapparent lesion on palpation/imaging
T1a	Involvement of $\leq 5\%$ of resected tissue
T1b	Involvement of $> 5\%$ of resected tissue
T1c	Carcinoma present on needle biopsy with prior levels of elevated PSA
T2	Palpable or visible cancer confined to prostate
T2a	Involvement of $\leq 5\%$ of one lobe
T2b	Involvement of $> 5\%$ of one lobe but unilateral
T2c	Involvement of both the lobes
T3	Local extraprostatic extension
T3a	Extracapsular extension
T3b	Seminal vesical invasion
T4	Invasion of contiguous organs or supporting structures like the bladder neck, rectum, external sphincter, levator muscle and pelvic floor
<b>STATUS OF REGIONAL LYMPH NODES (N)</b>	
N0	No regional nodal metastasis
N1	Metastases in regional lymph nodes
<b>DISTANT METASTASES (M)</b>	
M0	No distant metastases
M1	Distant metastases present
M1a	Metastases to distant lymph nodes
M1b	Bony metastases
M1c	Other distant metastases

## **DIAGNOSIS**

Triple approach is utilized for the diagnosis of carcinoma prostate with:

1. Digital rectal exam or a transrectal ultrasound
2. Levels of serum Prostate Specific Antigen
3. Transrectal ultrasound guided core needle biopsy

## **DIGITAL RECTAL EXAMINATION (DRE)**

In a case of carcinoma findings such as asymmetry, induration and hard nodules which are discrete are obtained. Majority of the cancers are usually non palpable. An abnormal DRE has a positive predictive value of 22 to 36%.<sup>49</sup>

## **TRANSRECTAL ULTRASOUND (TRUS)**

It is the most commonly used imaging modality in diagnosis of carcinoma prostate. The cancers on TRUS appear as hypoechoic when compared to the normal peripheral zone but it can also appear hyperechoic or isoechoic in some instances. Due to its poor sensitivity and specificity TRUS has now been used for two instances<sup>50,51</sup>:

- ✓ Major role is in assisting needle core biopsies of prostate
- ✓ In estimating prostatic volume such that PSA density can be calculated

Two more techniques have recently come up for clinical staging of carcinoma prostate which are endorectal Magnetic Resonance Imaging and multiparametric MRI (mpMRI). mpMRI is use in conjunction with modalities like diffusion weighted

images, dynamic contrast enhanced images (DCE0 and spectroscopy. It has the best sensitivity and specificity compared to other imaging modalities.

Another important modality is positron emission tomography (PET) tracers. The tracers using choline are useful in early-stage detection of cancer prostate. The tracers using FDG and sodium F18-fluoride are used in detection of advanced cases and also for assessing bony metastasis.

### **NEEDLE BIOPSY TECHNIQUE:**

Ultrasound guided systematic sextant biopsies are done in which sampling of prostate was done from the parasagittal midlobe region. Now the biopsies from the following have been added:

1. The transition zone: This is done if the first biopsy was benign even when there was a suspicion of cancer
2. Enlarged prostate: Studies shows that there is a decreased detection of cancer in larger prostates<sup>52-54</sup>
3. Non transition zone

The use of the routine sextant biopsies along with posterolateral needle biopsies now a days has increased the cancer detection rates and an accurate pathological stage and Gleason score.<sup>55-57</sup> 14 core biopsies are done with 12 cores from the sextant and posterolateral biopsies and additional 2 cores from the transition zone.

### **PROSTATE SPECIFIC ANTIGEN (PSA)**

It is also called gamma-seminoprotein or kallikrein-3 and belongs to the kallikrein related peptidases. It is produced by the epithelium of prostatic ducts and acini and is found in normal, hyperplastic and malignant conditions of the prostatic gland. The entry into the sera is by its diffusion from the luminal cells of prostatic glands to the capillary basement membranes in the stroma. It acts on the proteins semenogelin and fibronectin in the semen post ejaculation and liquefies the coagulum releasing the spermatozoa.<sup>58</sup>

PSA is present in the serum in very low concentration in bound and unbound forms with the normal value ranging from 1.0 to 4.0 ng/ml.<sup>59</sup> Small concentration of PSA is also produced by breast, renal and adrenal malignancies.

### **FACTORS INFLUENCING PSA LEVELS**

It is under the influence of androgens and varies with age, race and the prostatic volume. It correlates directly with testosterone levels and is detected at puberty.

PSA levels are found to be elevated in serum when there is loss of cellular architecture of prostate caused by disruption of the barrier formed by the basal cells and basement membranes. This can occur in conditions like BPH, cancer prostate or prostatitis where there is disease of the prostate or when there is prostatic manipulation like in case of prostate massage or prostate biopsy.

5alpha reductase inhibitors cause artificial drop in PSA levels by 50%. Due to haemodilution the patients of high BMI have lower values of PSA. The most important factor affecting PSA levels of serum is prostatic disease.

## **PSA SCREENING**

It is a good measure in terms of diagnosis of disease recurrence and to monitor response after treatment. Aim of screening is to decrease deaths due to cancer.

4ng/ml is the most commonly used cut off for PSA levels. The cancer detection rate on a prostate biopsy when the DRE is normal and the PSA levels are between 4-10 ng/ml is 25% but with levels more than 10ng/ml in a similar setting increases the rate of 67%.<sup>46</sup> Yearly screening is recommended in men with age group of 40-50 years.

## **MOLECULAR FORMS OF PSA**

Majority of PSA is bound to alpha 1 antichymotrypsin (ACT) and the rest is bound to alpha 1 antitrypsin and alpha 2 macroglobin also called complexed PSA. A smaller portion of PSA is not bound and is free. The PSA bound to ACT is immunoreactive and hence is important in diagnosis of cancer. Total serum PSA is thus combination of the complexed form and the free form.

PSA is released in the form of a precursor which is pro PSA which is inactive. In prostate cancer this inactive form is the major part of the free PSA. A ratio of free and total PSA levels was found to be a good indicator of predicting the Gleason's scores.<sup>60</sup>

## **AGE ADJUSTED REFERENCE RANGE OF PSA**

Such ranges are being used as it increases the sensitivity of detection in young men and in older men it increases the specificity of cancer prostate.

In older men prostate is found to be large due to hyperplasia, therefore higher PSA values are permissible but this has a disadvantage that with higher cut off large number of clinically important cancer can be missed.

The advantage of these ranges is that the number of biopsies performed on older men will be less with slightly higher PSA level than cut off and the number of biopsies in young will be more with slightly lower PSA levels.

**Table 3. Age specific PSA reference ranges<sup>46</sup>**

<b>UPPER REFERENCE RANGE (ng/ml)</b>	<b>AGE IN YEARS</b>
2.5	40 – 49
3.5	50 – 59
4.5	60 – 69
6.5	70 – 79

### **PSA DERIVATIVES**

These have been developed and used to decrease the number of false positive cases and to increase the specificity of the test. It includes PSA velocity and PSA density.

### **PSA VELOCITY**

It is the rate of change of serum PSA value. Patients with prostate cancer have an increase rate of change of PSA as compared to those without cancer with the PSA velocity being 0.75 ng/ml per year. For this derivative to be valid at least three PSA values must be available in a period of two years.

Another derivative used is PSA doubling time which is the amount of time required for PSA to double. It is used along with PSA velocity and the combined term is PSA dynamics.

### **PSA DENSITY**

This derivative gives the amount of PSA produced per gram of prostatic tissue.

PSA density = Total serum PSA/ Estimated gland volume

The gland volume is measured by transrectal ultrasound. A higher value of PSA density suggests an increased risk of carcinoma prostate. The cut off value to perform biopsy is 0.15 ng/ml/mm<sup>3</sup>.

PSA density has been found to be increased in patients with aggressive cancer prostate.<sup>61,62</sup> It has also been shown that PSAD is an independent predictor of an upgraded Gleason score in repeat biopsies and radical prostatectomies.<sup>63,64</sup>

John Hopkins criteria and the PRIAS protocol use PSAD in the inclusion criteria for active surveillance in selecting prostate cancer patients due to the role of PSAD in identifying low risk disease. A study done recommends use of the PRIAS protocol with PSAD cut off being 0.2 ng/ml/cumm for active surveillance of cancer prostate.<sup>65</sup>

A study done in Taiwan stated that PSAD when compared to free PSA was a better predictor of a positive result in a repeat biopsy of prostate.<sup>66</sup>

Feedland et al found that in comparison to PSA, PSAD is a better predictor of extracapsular disease, positive surgical margin, seminal vesical invasion and biochemical recurrence.<sup>67</sup>

### **POST TREATMENT PSA MONITORING**

It done to decide if post treatment biopsy is needed. After the therapy of a localised cancer PSA starts decreasing to upto  $<0.2$  ng/ml. It takes 6 weeks for the decline in radical prostatectomies and takes 27 weeks post radiation therapy.<sup>68</sup> If the PSA value increases after treatment, then it is called as biochemical recurrence.

### **CORRELATION OF PSA WITH HISTOPATHOLOGICAL FINDINGS**

Many studies have been done which have shown that serum PSA levels correlate positively with the Gleason score that is as the Gleason score increases PSA values also are found to increase.<sup>2</sup> A significant positive correlation is present between primary Gleason grade and PSA concentration too.<sup>1,69</sup>

A study done by Ferro et al showed that histological grade shows a statistically significant correlation with pre-treatment PSA levels.<sup>70</sup>

Compared to the well differentiated cancer prostate the poorly differentiated ones secrete PSA in large quantities and higher the Gleason group more significantly high amounts of PSA is produced in comparison to low Gleason groups.<sup>71</sup>

In prostate cancer patients, a high Gleason score correlates with increased amounts of total PSA and correlates with decreased amounts of free PSA and hence free to total PSA ratio is useful for predicting the Gleason score.<sup>60,72-73</sup>

As there is an increase in Gleason grade and score along with increasing anaplasia, the PSA levels are seen to increase.<sup>74</sup>

## **IMMUNOHISTOCHEMISTRY**

It is a valuable adjunct to routine microscopy in case of prostate biopsy. PSA and prostate specific acid phosphatase are older markers which can be used to confirm the prostatic origin of an acinar lesion and also can be used to differentiate from mimickers.

Two important markers used are:

- Basal cell specific markers
- Racemase (AMACR).

### **Basal cell markers-**

The basal cell specific markers include high molecular weight cytokeratin, cytokeratin 5/6 and p63. These markers can be used in combination as a cocktail of markers with a double or triple markers. Loss of basal cells is considered as the hallmark of malignancy.

High molecular weight cytokeratin is also called 34betaE12 and is a widely used basal cell marker and it helps in detecting presence or absence of basal cells in presence of atypical prostatic glands. p63 is a nuclear staining marker and is expressed by the basal or the myoepithelial cells of prostate.

### **Racemase-**

It is also called as P504S or AMACR (alphanmethyl-acyl-CoA). It is a cytoplasmic stain which stains the luminal cells of prostatic glands and its involvement is observed in beta-oxidation of branched chain fatty acids and its derivatives.<sup>75</sup>

Overexpression of this marker is seen in tumours of the colon, mammary gland, malignant melanoma and renal carcinoma (papillary type). It shows a high positivity rate in prostate carcinomas and high-grade PIN.<sup>76</sup>

During the procedure of immunohistochemistry there should be internal non - neoplastic glands so that a comparison can be made. The benign glands show strong basal cell staining which will not be seen in malignant glands as they lack basal cells. A comparison is always done with haematoxylin and eosin-stained slides.

**PHOSPHORYLATED Akt (p-Akt)**

Akt is also called as protein kinase B. It is a central signalling serine or threonine kinase regulating important effector molecules in apoptosis, cell cycle progression and anoikis.

The interaction of tyrosine kinase receptors along with binding of RAS to the catalytic subunit activates the Akt pathway. This activity can be negated by PTEN.<sup>77</sup>

Once Akt pathway is activated there is phosphorylation and inactivation of the BAD protein which is a pro-apoptotic factor ultimately causing a change in the transcriptional activity of androgen receptors.<sup>78</sup>

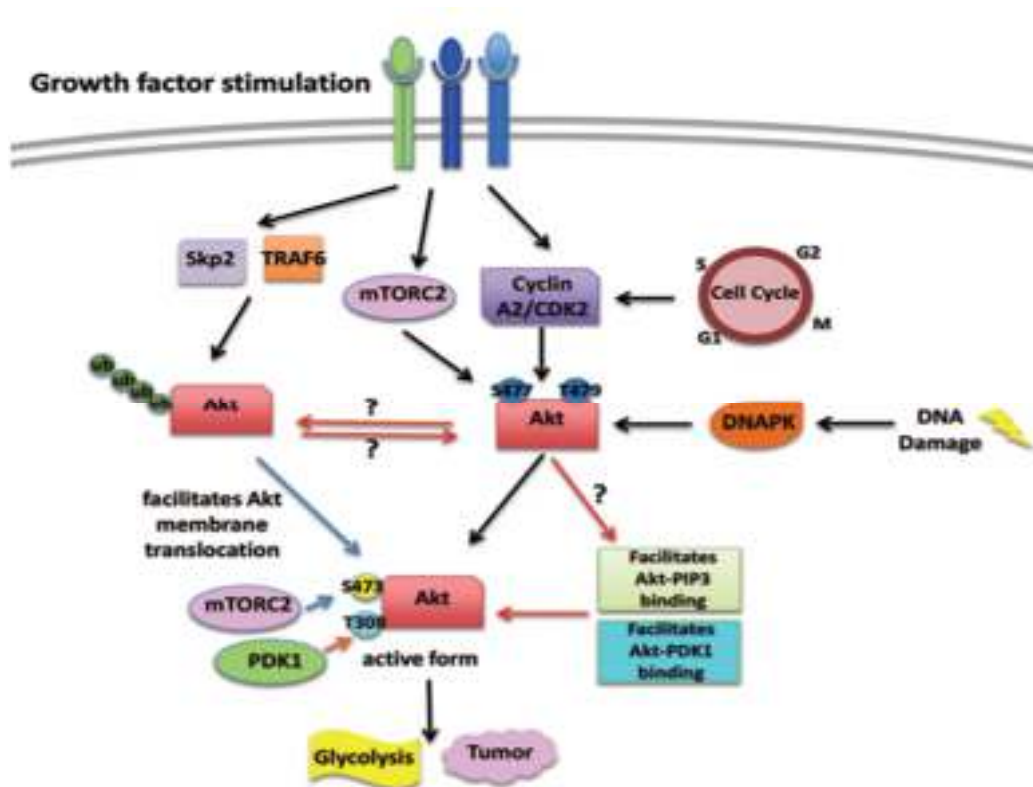


Figure 4 – Akt activation by phosphorylation<sup>79</sup>

It is implicated in genesis and also the progression of many tumours.<sup>80</sup> Once the Akt pathway is activated it suppresses the apoptotic response and enhances production of key growth factors and survival factors and such alterations lead to progression of prostatic cancer.<sup>81-84</sup> Overexpression of Akt triggers release from cell cycle block and a shift to autonomous production of proteins from usual dependence on paracrine growth factors.<sup>85</sup>

There are 3 isoforms of Akt which are Akt1, Akt2, Akt3. On performing RT-PCR on the isoforms, Akt 1 and Akt2 mRNA expression was seen unequivocally in normal as well as carcinoma prostate.<sup>86</sup> Akt 3 expression is more in advanced prostate cancers which are hormone independent.

It was observed that intensity of expression of Akt was stronger in malignant prostatic tissue compared to benign tissue with the amount of Akt expression being of greater importance in tumour progression when compared to number of Akt positive cells.<sup>4</sup>

Higher levels of Akt are observed in prostate cancers having a higher Gleason grade and score with it being more increased in higher Gleason scores than lower Gleason scores. When Gleason scores 6 and 7 were studied, the prognostic value of Akt was retained.<sup>87</sup>

Also, there is a significant correlation between pre-operative PSA levels and Akt immunoreactivity. Akt expression is stronger by the basal cells with the luminal cells showing weaker expression.<sup>4</sup> It is localised mainly to the cytoplasm but in tumours it is localised close to the plasma membrane and in rare case to the nucleus.

In cancers with high Gleason grade there is loss of the tumour suppressor gene PTEN which implies that increases expression of Akt seen in poorly differentiated cancer occurs as a result of loss of PTEN.<sup>88</sup>

A study done in Houston observed that Phospho- Akt 1 expression was stronger in Gleason grades of 8 to 10 believing that it can be a therapeutic target. It also found that higher levels of Akt1 were associated with earlier recurrence with it being an independent prognostic indicator of the biochemical recurrence free survival.<sup>3</sup>

### **INTEPRETATION OF PHOSPHO-Akt STAINING**

Positive tissue reactions based on staining intensity are scored into<sup>4</sup>:

1. Negative
2. Weak
3. Moderate
4. Strong

Positive tissue reaction based on percentage of positive cells is classified into<sup>89</sup>:

1. 0% cells take stain
2. 1-30 %
3. 31-60%
4. 61-100%

Immunoreactivity score =staining intensity x percentage of positive cells<sup>4</sup>

## **TREATMENT OF CARCINOMA PROSTATE**

For early disease curative treatment is given. Active surveillance is done with 3-6 monthly DRE and PSA levels.

Radical prostatectomy is done in cases of localised disease with a life expectancy of more than 10 years with exclusion of metastasis with a bone scan and chest xray.

External beam radiotherapy is done for locally advanced cancer prostate. Erectile dysfunction is a common complication. Brachytherapy is another option for low volume low grade disease.

Androgen ablation therapy is given for patients locally advanced or metastatic cancer without symptoms. Bilateral orchidectomy is performed. Luteinizing hormone releasing hormone (LHRH) agonist have been approved for prostate cancer treatment. A complete androgen blockade with a combination of LHRH agonist or orchidectomy with an antiandrogen can be administered.

Castration gives better survival in patients with metastatic disease but high dose anti androgen monotherapy is used as an alternative to castration in metastatic and locally advanced cases where erectile function and libido is to be maintained.

## **METHODOLOGY**

This study was done at the Department of Pathology, KAHER'S Jawaharlal Nehru Medical College and Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. Institutional ethical clearance was obtained from the ethics committee.

**STUDY DESIGN:** Hospital based descriptive study

**STUDY PERIOD:** January 2019 - December 2020

- One year prospective (1<sup>st</sup> January 2020 to 31<sup>st</sup> December 2020).
- One year retrospective (1<sup>st</sup> January 2019 to 31<sup>st</sup> December 2019).

**STUDY POPULATION:** All the specimens of prostatic adenocarcinoma from patients admitted to KAHER'S JNMC and DR. PRABHAKAR KORE HOSPITAL from 1<sup>st</sup> January 2019 to 31<sup>st</sup> December 2020.

### **INCLUSION CRITERIA**

1. TRUS (transrectal ultrasound) guided biopsy of prostate including needle biopsy specimens and Tru-Cut biopsy specimens.
2. TURP (transurethral resection of prostate) specimens including prostatic chips.

**EXCLUSION CRITERIA:** Malignancies other than prostatic adenocarcinoma in prostate.

**SAMPLE SIZE:** 50 was taken based on universal sampling. Average number of specimens of the included lesions obtained at Histopathology laboratory at JN Medical College and Dr. Prabhakar Kore Hospital, Belagavi in two years was around 50.

**DATA COLLECTION:** After obtaining permission from the hospital authorities, the participants were briefed about the study and informed consent was taken from the prospective cases. Information regarding the retrospective cases was obtained from the medical records department (MRD).

The data was collected using a predesigned proforma. The information about the clinical parameters were obtained from the patient's records from MRD, Department of Pathology, Dr. Prabhakar Kore Hospital and Medical Research Centre.

### **FIXATION OF SPECIMENS**

The specimens obtained were fixed in neutral buffered formalin for a duration of 12-24 hours. The specimens were weighed and the gross findings were recorded as per the department of pathology format. Entire specimen was processed for tru cut and core biopsies.

### **STEPS OF PROCESSING**<sup>90</sup> (Procedure-Annexure IV)

The specimens were processed in an automated processor by Leica TP 1020. Sections were made with an automated rotary microtome set at 3-5 microns and were stained by hematoxylin and eosin<sup>90</sup> (Procedure-Annexure V).

### **TISSUE CHARACTERISTICS**

The carcinoma was identified and subjected to Gleason's scoring and Grade grouping based on the Current International Society of Urological Pathology Modified Gleason System 2019.<sup>46,47</sup>

Presence of perineural invasion, eosinophilic and basophilic luminal secretions, glomeruloid bodies and mucinous fibroplasia was noted.

**IMMUNOHISTOCHEMICAL (IHC) STAINING** (Procedure-Annexure VI)

For IHC staining the mounting of sections were done on poly-L-lysine coated slides. **Primary Antibody**-Rabbit monoclonal [EPR18405] to AKT1 + AKT2.

Kit used- DAKO Polymer Kit. Indirect or sandwich method was used for IHC staining. The chromogen was 3,3 diaminobenzidine.

Positive control- Human gastric adenocarcinoma.

All the slides were observed under penta-head compound microscope (Labkron).

Scoring system included

- Intensity of staining
- Percentage or fraction of cells taking up the stain

Each of the above were graded and the product of the grades gave the immunoreactivity score

**INTEPRETATION OF PHOSPHO-Akt STAINING**

Positive tissue reactions based on staining intensity are scored into<sup>4</sup>:

1. Negative
2. Weak
3. Moderate
4. Strong

Positive tissue reaction based on percentage of positive cells is classified into<sup>89</sup>:

1. 0% cells take stain
2. 1-30 %
3. 31-60%
4. 61-100%

Immunoreactivity score =staining intensity x percentage of positive cells<sup>4</sup>

## **DATA ANALYSIS**

Data was analyzed using SPSS 16 software. Mean, median, percentages and standard deviation are used to describe the patients according to age, type of specimens, Gleason's grade and score.

Prostate specific antigen levels done before the biopsy were obtained from patient records.

Regression and Correlation analysis was used to find:

- Correlation of PSA levels with Gleason's score, Grade group, other histopathological characteristics and p-Akt immunoeexpression.
- Correlation of IHC expression of p-Akt with Gleason's score, Grade group and other histopathological characteristics.

P value of less than 0.05 was taken as significant.

## **RESULTS**

In a span of two years, 50 cases of adenocarcinoma of prostate were analysed. In these 50 cases, majority cases were 12 cores biopsy specimens (34), 11 cases of TURP specimens, 04 cases of TRUS guided biopsy specimens and 01 case of 18 cores biopsy. The pre-biopsy total serum PSA levels were obtained.

**Table 4. Age distribution**

<b>Age(years)</b>	<b>No. of Patients</b>	<b>Percentage</b>
50-59	03	06 %
60-69	11	22 %
70-79	28	56 %
80-89	07	14 %
90-99	01	02 %
Total	50	100

The age distribution the patients ranged from 50-99 years with majority of the cases in the age group of 70-79 years (28 cases,56%) followed by 60-69 years. (11 cases,22%).

**Table 5. Distribution of Gleason's Score**

Gleason's Score	No. of Patients	Percentage (%)
Score 2-5	00	00
Score 6	02	04
Score 7	20	40
Score 8	14	28
Score 9	12	24
Score 10	02	04
Total	50	100

On histopathological analysis of the cases, more than one Gleason's pattern was observed in all the cases. The commonest Gleason's pattern was pattern 4 followed by pattern 3.

Gleason's score was obtained by addition of the two Gleason patterns. The commonest Gleason's score was 7 (20 cases,40%) followed by 8(14 cases,28%). Combination pattern of 3+4 and 4+3 was seen in majority (11 cases,22%) each.

**Table 6. Distribution of Grade Group**

Grade group	No. of Patients	Percentage(%)
1	02	04
2	11	22
3	09	18
4	14	28
5	14	28
Total	50	100

The new Grade group system was used with the commonest Grade group being 4 (14 cases, 28%) and 5 (14 cases, 28%).

**Table 7. Distribution based on age and average total serum PSA levels.**

Age (years)	Average total serum PSA (ng/ml) [range]
50-59	121.33 [60-204]
60-69	157.18 [11-117]
70-79	330.85 [11-3049]
80-89	33.37 [7-69]
90-99	1000 [1000]

The PSA values ranged from 7 to 3049 ng/ml. Average total PSA levels was highest in the age group of 90-99 years (1000 ng/ml). None of the cases had PSA levels in the normal range.

**Table 8. Distribution of average total serum PSA levels**

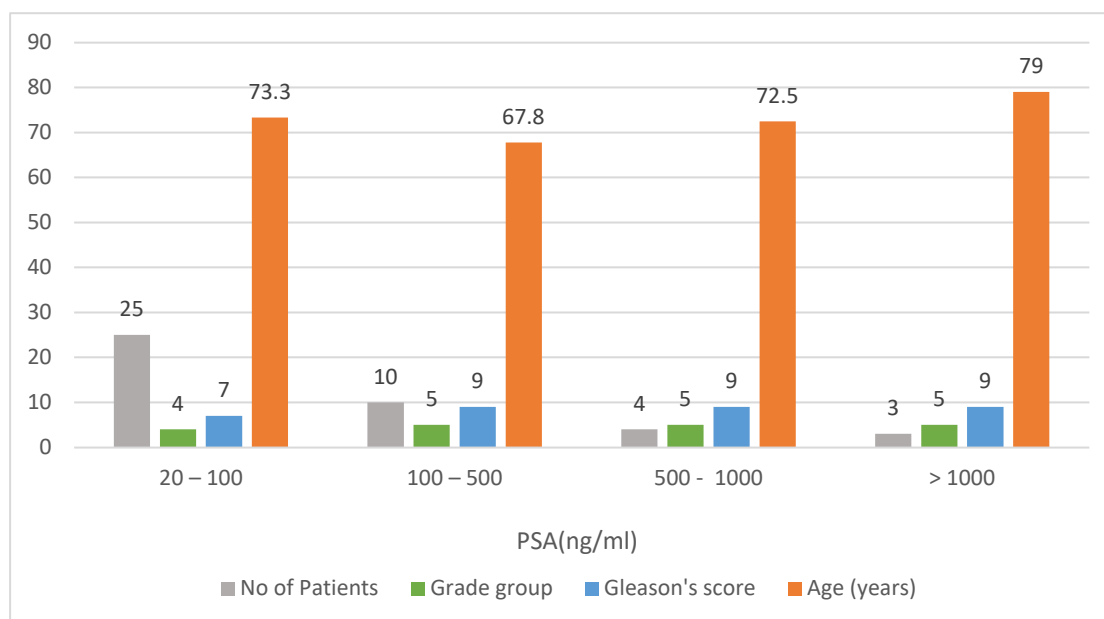
PSA ( ng/ml)	No. of Patients with cancer	Percentages
0.01 – 4	00	00 %
4 – 10	02	04 %
10 – 20	06	12 %
> 20	42	84 %
Total	50	100

Majority of the cases (42,84%) had PSA levels more than 20ng/ml, followed by 06 cases (12%) with 10-20 ng/ml PSA and just 02 cases (4%) in the range of 4-10 ng/ml.

**Table 9. Distribution of 42 cases with PSA levels more than 20 ng/ml.**

PSA (ng/ml)	No of Patients	Percentage(%)	Grade group	Gleason's score	Age (years)
20 – 100	25	59.5	4	7,8	73.3
100 – 500	10	23.8	5,4	9	67.8
500 - 1000	04	9.5	5,4	9	72.5
> 1000	03	7.1	5	9	79
Total	42	100			

**Graph 1. Distribution of 42 cases with PSA levels more than 20 ng/ml**



Out of the 42 cases(84%) with PSA levels more than 20 ng/ml, higher levels of PSA were observed in higher Gleason's scores and Grade groups.

Cases of lower Grade group (4) and lower score (7,8) showed a comparatively lower PSA levels whereas cases with higher Grade group (5) and higher score (9) had

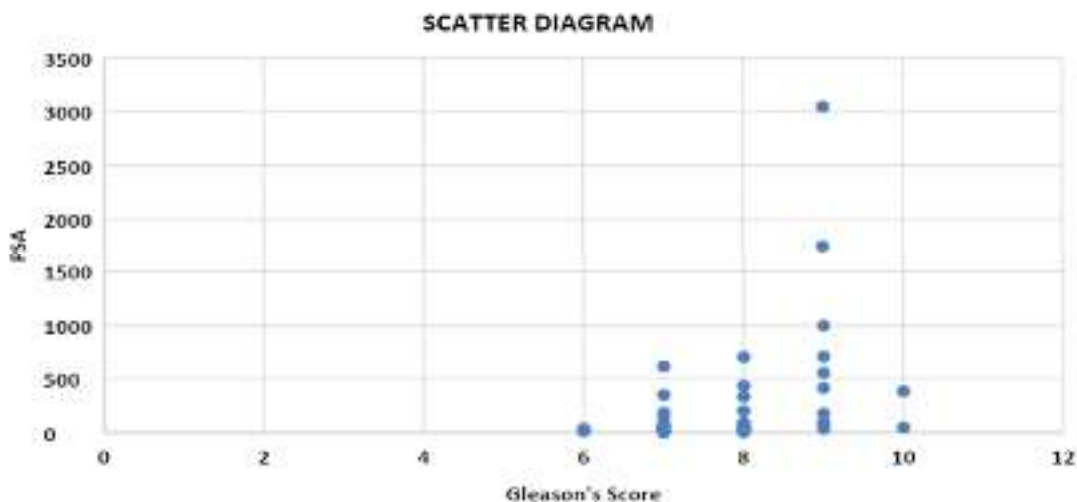
higher levels of PSA upto 1000 ng/ml. This shows a linear correlation between PSA levels and Gleason's score and Grade group.

**Table 10. Distribution of PSA levels and Gleason's score**

Average total serum PSA(ng/ml)	Gleason's Score
00	2 – 5
47	6
96.05	7
153	8
670.25	9
219.5	10

A linear increase in PSA levels is seen with increase in Gleason's score but a dip in PSA levels was noted beyond Gleason's score 9.

**Graph 2. Scatter diagram for correlation of PSA and Gleason’s score**



A scatter diagram was made to find the correlation between PSA levels and Gleason’s score. The Gleason’s scores of 6-8 were seen in majority and a rise in PSA levels is noted with increased score.

**Table 11. Correlation between Gleason’s score and PSA levels**

Correlation coefficient between	R	R <sup>2</sup>	P value	Inference
Gleason’s score and PSA	0.3811	0.1452	0.0068	Statistically Significant

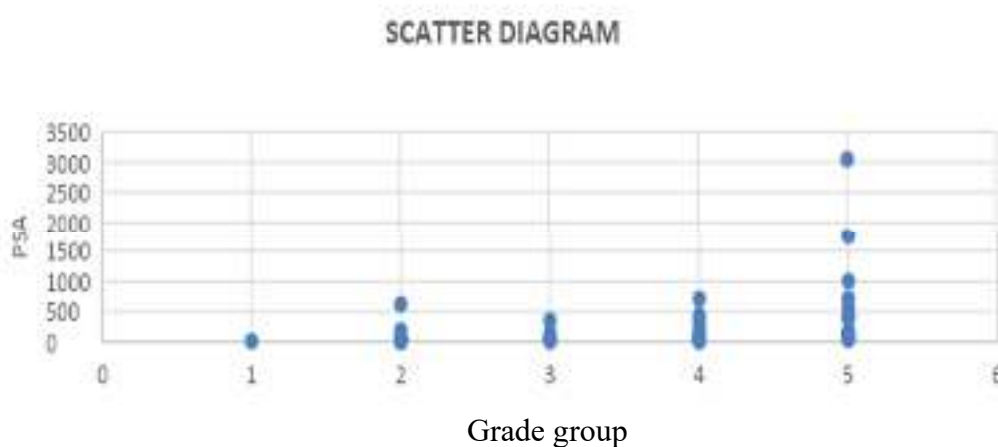
With the P value being less than 0.05 there is a statistically significant correlation between serum PSA levels and Gleason’s score. Higher PSA levels are seen with increasing scores.

Table 12. Distribution of PSA levels and Grade group

Average total serum PSA (ng/ml)	Grade group
26.57	1
102.09	2
88.33	3
163.23	4
638.43	5

A linear increase in PSA levels is seen with higher Grade groups.

Graph 3. Scatter diagram for correlation of PSA and Grade groups



Scatter diagram was made to find correlation between the serum PSA levels and Grade group. Higher PSA levels were observed in higher Grade group.

**Table 13. Correlation between Grade group and PSA levels**

Correlation coefficient between	R	R <sup>2</sup>	P value	Inference
Grade group and PSA levels	0.3893	0.1516	0.0056	Statistically Significant

With the P value being less than 0.05, a statistically significant correlation is seen between serum PSA levels and Grade group with higher PSA levels seen in patients with higher Grade groups.

**Table 14. Pattern of immunohistochemical staining with p-Akt**

	CYTOPLASMIC	MEMBRANOUS	NUCLEAR
No. of cases	50	23	01
Percentage(%)	100	46	02
Total	50	50	50

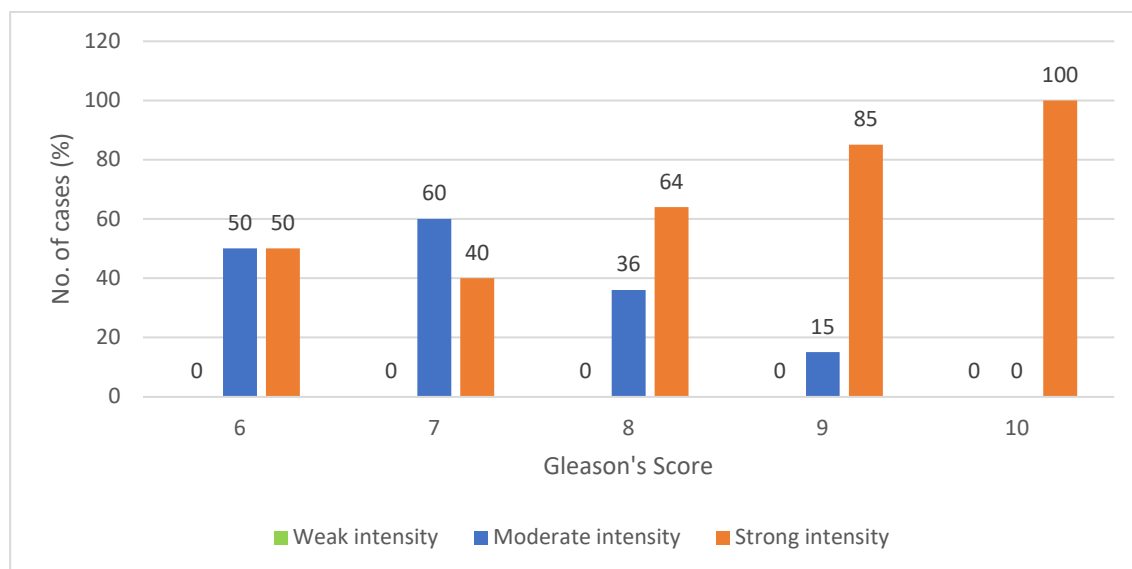
All the cases showed cytoplasmic staining with p-Akt with 23 cases (46%) showing membranous staining and 01 case (02%) showing nuclear staining.

**Table 15. Distribution of Intensity of Immunohistochemical staining with Gleason’s score**

Gleason’s score	No. of cases	No staining Score 0 Cases (%)	Weak intensity Score 1 Cases (%)	Moderate intensity Score 2 Cases (%)	Strong intensity Score 3 Cases (%)
6	02	0 (0)	0 (0)	01 (50)	01 (50)
7	22	0 (0)	0 (0)	13 (60)	09 (40)
8	11	0 (0)	0 (0)	04 (36)	07 (63)
9	14	0 (0)	0 (0)	02 (14)	12 (85)
10	01	0 (0)	0 (0)	00 (0)	01 (100)
Total	50	0	0	20	30

It was observed that all the cases showed moderate to strong intensity of immunireaction with p-Akt. With increasing Gleason’s score the intensity of immunoreaction progressed from moderate to strong.

**Graph 4. Bar diagram showing various IHC intensities in Gleason’s score**



The bar diagram shows the findings of the above table 15.

**Table 16. Distribution of IHC staining intensity with carcinoma grades**

Gleason's score-Grading	No. of cases	No staining Score 0 Cases (%)	Weak intensity Score 1 Cases (%)	Moderate intensity Score 2 Cases (%)	Strong intensity Score 3 Cases (%)
Low grade (6)	02	0 (0)	0 (0)	01 (50)	01 (50)
Medium grade (7)	22	0 (0)	0 (0)	13 (59)	09 (41)
High grade (8-10)	26	0 (0)	0 (0)	06 (23)	20 (77)
Total	50	0	0	20	30

High grade prostate adenocarcinoma shows increased intensity of immunoreaction.

**Table 17. P value for intensity of p-Akt IHC and Gleason's score**

Chi square statistic	P value
6.52	<b>0.038</b>

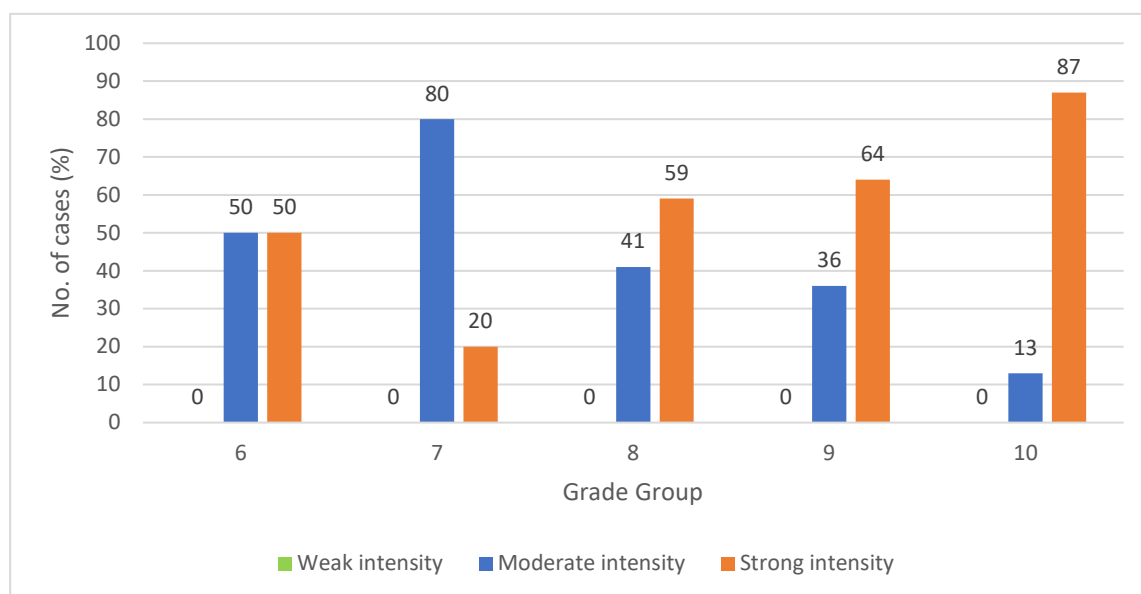
The P value is < 0.05 and the correlation between intensity of immunoreaction and Gleason's score is statistically significant. As the scores increased intensity progressed from moderate to strong with strongest intensity being seen in high grade cancers of score 8-10.

**Table 18. Distribution of Intensity of Immunohistochemical staining with Grade Group**

Grade Group	No. of cases	No staining Score 0 Cases (%)	Weak intensity Score 1 Cases (%)	Moderate Intensity Score 2 Cases (%)	Strong intensity Score 3 Cases (%)
1	02	0 (0)	0 (0)	01 (50)	01 (50)
2	10	0 (0)	0 (0)	08 (80)	02 (20)
3	12	0 (0)	0 (0)	05 (41)	07 (59)
4	11	0 (0)	0 (0)	04 (36)	07 (64)
5	15	0 (0)	0 (0)	02 (13)	13 (87)
Total	50	0	0	20	30

All the cases showed moderate to strong intensity of immunireaction with p-Akt. With increasing Grade group the intensity of immunoreaction progressed from moderate to strong.

**Graph 5. Bar diagram showing various IHC intensities in Grade group**



The bar diagram shows findings of table 18.

**Table 19. P value for intensity of p-Akt IHC and Grade group**

Chi square statistic	P value
11.26	<b>0.023</b>

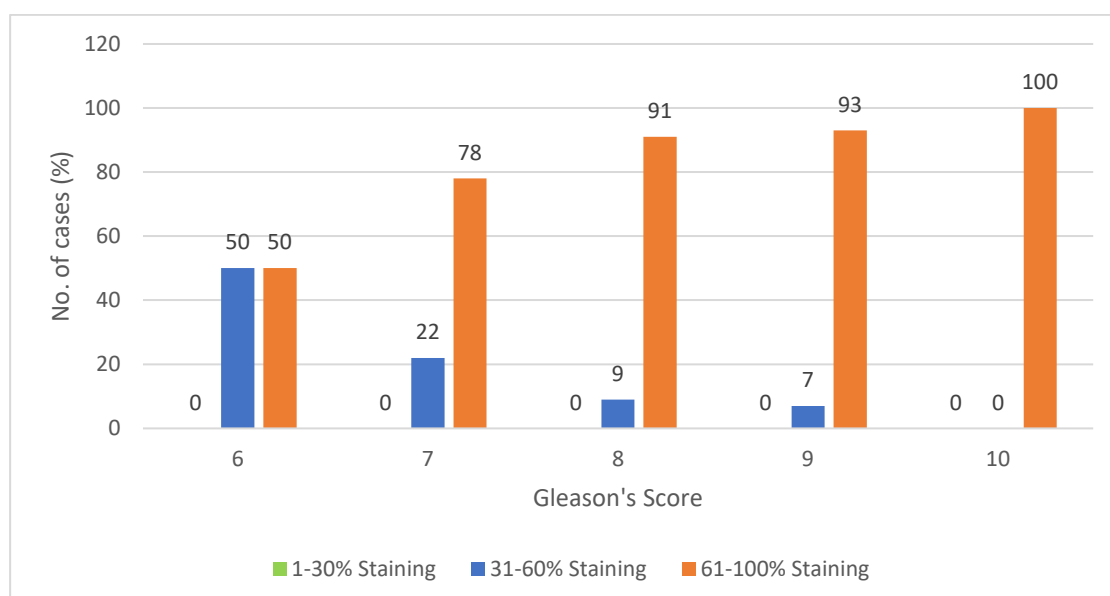
The P value is  $< 0.05$  and the correlation between intensity of immunoreaction and Grade group is statistically significant. As the Grade group increased the intensity was found to progress to stronger intensity.

**Table 20. Distribution of percentage of positive cells with Gleason’s score**

Gleason’s score	No of cases	No cell take stain Score 0 Cases (%)	1-30% staining Score 1 Cases (%)	31-60% staining Score 2 Cases (%)	61-100% staining Score 3 Cases (%)
6	02	0 (0)	0 (0)	01 (50)	01 (50)
7	22	0 (0)	0 (0)	05 (22)	17 (78)
8	11	0 (0)	0 (0)	01 (09)	10 (91)
9	14	0 (0)	0 (0)	01 (07)	13 (93)
10	01	0 (0)	0 (0)	00 (00)	01 (100)
Total	50	0	0	8	42

With increasing Gleason’s score, the percentage of cells showing immunoreaction with p-Akt showed an increase.

**Graph 6. Bar diagram showing % of positive cells in various Gleason’s scores**



The above bar diagram describes findings of table 20.

**Table 21. Distribution of % of positive cells for p-Akt with carcinoma grades**

Grade (Gleason's score)	No of cases	No cells take stain Score 0 Cases (%)	1-30% staining Score 1 Cases (%)	31-60% staining Score 2 Cases (%)	61-100% staining Score 3 Cases (%)
Low grade (6)	02	0 (0)	0 (0)	01 (50)	01 (50)
Medium grade (7)	22	0 (0)	0 (0)	05 (22)	17 (78)
High grade (8-10)	26	0 (0)	0 (0)	02 (07)	24 (93)
Total	50	0	0	8	42

Higher grades of prostate adenocarcinoma show increased percentage of positive cells.

**Table 22. P value for % of positive cells for p-Akt IHC and Gleason's score**

Chi square statistic	P value
3.79	0.14

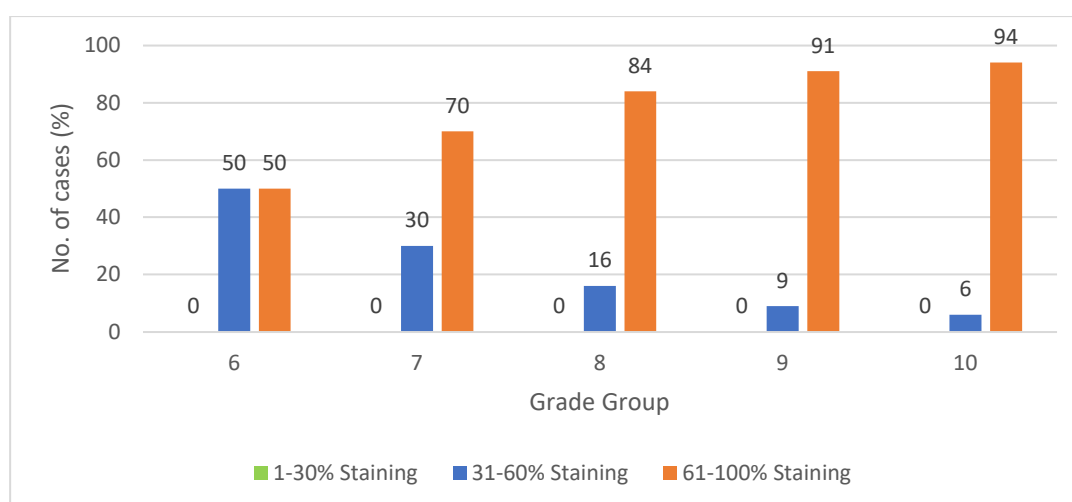
The P value is not < 0.05 and the correlation between Gleason's score and percentage of positive cells is not statistically significant.

**Table 23. Distribution of percentage of positive cells with Grade Group**

Grade Group	No of cases	No cells take stain Score 0	1-30% staining Score 1	31-60% staining Score 2	61-100% staining Score 3
		Cases (%)	Cases (%)	Cases (%)	Cases (%)
1	02	0 (0)	0 (0)	01 (50)	01 (50)
2	10	0 (0)	0 (0)	03 (30)	07 (70)
3	12	0 (0)	0 (0)	02 (16)	10 (84)
4	11	0 (0)	0 (0)	01 (09)	10 (91)
5	15	0 (0)	0 (0)	01 (06)	14 (94)
Total	50	0	0	8	42

As the Grade group increased there was an increase in the percentage of positive cells.

**Graph 7. Bar diagram showing % of positive cells in various Grade groups**



Above bar diagram shows findings of table 23.

**Table 24. P value for % of positive cells for p-Akt IHC and Grade group**

Chi square statistic	P value
4.55	0.33

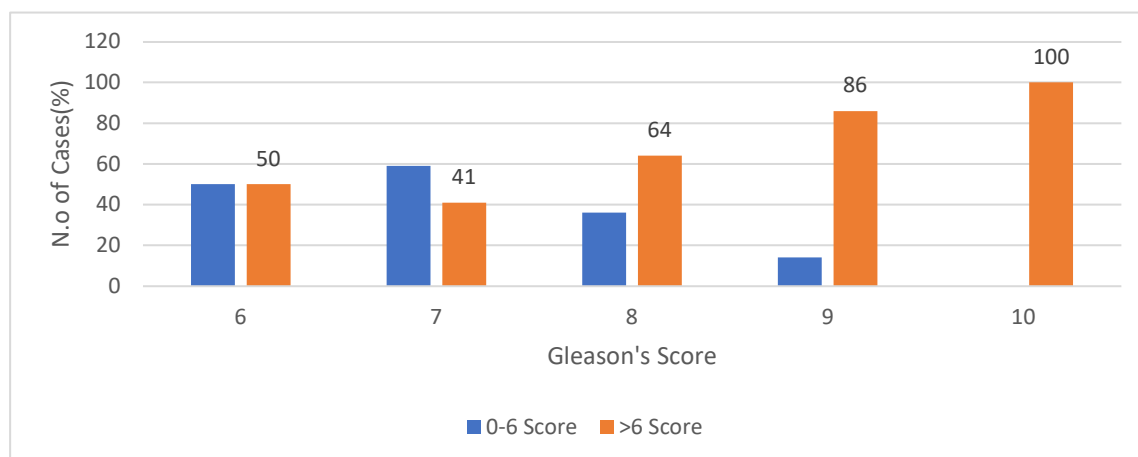
The P value is not  $< 0.05$  and the correlation between Grade group and percentage of positive cells is not statistically significant.

**Table 25. Distribution of Immunoreactivity score with Gleason’s score**

Gleason’s score	No of cases	0-6 score Cases (%)	>6 score Cases (%)
6	02	01 (50)	01 (50)
7	22	13 (59)	09 (41)
8	11	04 (36)	07 (64)
9	14	02 (14)	12 (86)
10	01	00 (00)	01 (100)
Total	50	20	30

With increase in Gleason’s score there was an increase in the immunoreactivity score.

**Graph 8. Bar diagram showing distribution of Immunoreactivity score with Gleason’s score**



Findings of table 25 are shown in this bar diagram.

**Table 26. Distribution of immunoreactivity score and carcinoma grades**

<b>Grade (Gleason's score)</b>	<b>No of cases</b>	<b>0-6 score Cases (%)</b>	<b>&gt;6 score Cases (%)</b>
Low grade (6)	02	01 (50)	01 (50)
Medium grade (7)	22	13 (59)	09 (41)
High grade (8-10)	26	06 (23)	20 (77)
Total	50	20	30

Higher grades of prostate adenocarcinoma are associated with higher immunoreactivity score of p-Akt.

**Table 27. P value for immunoreactivity score for p-Akt IHC and Gleason's score**

<b>Chi square statistic</b>	<b>P value</b>
6.03	<b>0.048</b>

The P value is < 0.05 and the correlation between Gleason's score and immunoreactivity score is statistically significant. Immunoreactivity score was found to be higher in higher grade or higher Gleason's scores with highest value in score 8-10.

**Table 28. Distribution of Immunoreactivity score with Grade Group**

Grade Group	No of cases	0-6 score Cases (%)	>6 score Cases (%)
1	02	01 (50)	01 (50)
2	10	08 (80)	02 (20)
3	12	05 (41)	07 (59)
4	11	04 (36)	07 (64)
5	15	02 (13)	13 (87)
Total	50	20	30

Immunoreactivity was found to be higher with increasing Grade groups.

**Graph 9. Bar diagram showing distribution of Immunoreactivity score with Grade group**

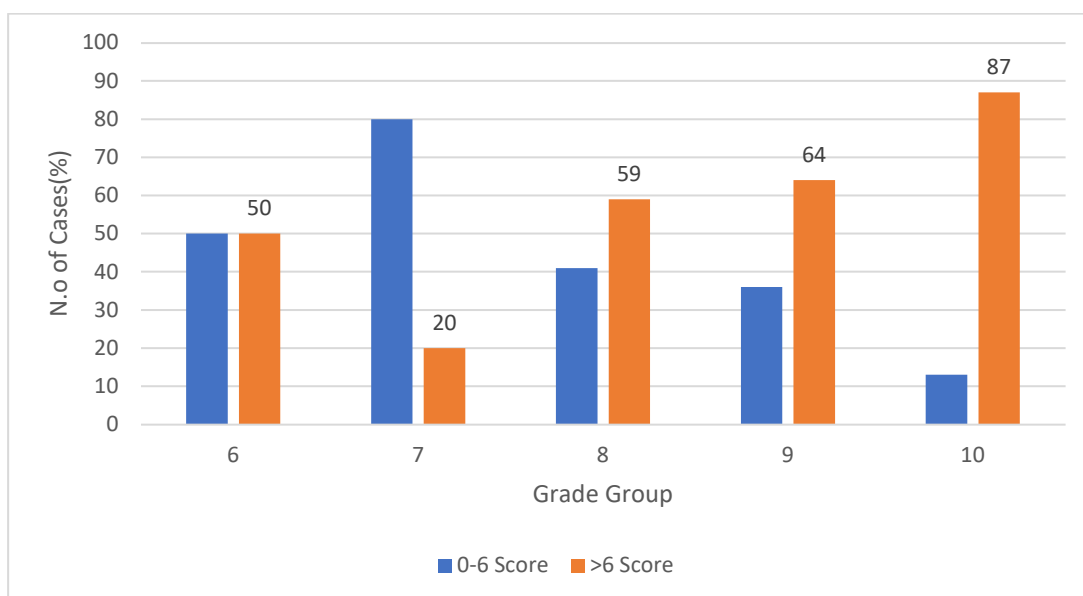


Table 28 findings are depicted by the above bar diagram.

**Table 29. P value for immunoreactivity score for p-Akt IHC and Grade group**

Chi square statistic	P value
11.26	<b>0.023</b>

The P value is < 0.05 and the correlation between Grade group and immunoreactivity score is statistically significant. A high value of immunoreactivity score was seen in cases with higher Grade groups.

**Table 30. Correlation of Histopathological features with PSA, Gleason's score and p-Akt IHC**

	Perineural invasion (cases)	Mucinous fibroplasia (cases)	Eosinophilic secretions (cases)	Basophilic secretions (cases)
<b>GLEASON'S SCORE</b>				
6	1	0	0	1
7	9	8	4	4
8	10	1	2	0
9	10	3	2	2
10	1	0	0	0
<b>P value</b>	0.960	0.703	0.760	0.530
<b>PSA (ng/ml)</b>				
4-10	1	0	1	0
10-20	2	1	0	1
20-100	17	7	5	4
100-500	5	3	2	2
500-1000	4	1	0	0
>1000	1	0	0	0
<b>P value</b>	0.242	0.262	0.263	0.266

<b>p-Akt IHC intensity</b>				
1	1	0	0	0
2	15	6	4	3
3	5	6	4	4
<b>P value</b>	0.330	0.333	0.331	0.179
<b>p-Akt IHC % of staining</b>				
1	0	0	0	0
2	5	2	1	0
3	26	10	7	7
<b>P value</b>	0.217	0.212	0.249	0.333
<b>p-Akt IHC immunoreactivity score</b>				
≤ 6	16	7	5	3
>6	5	5	3	4
<b>P value</b>	0.150	0.200	0.250	0.210

Histopathological features showed no statistically significant correlation with Gleason's score, PSA and p-Akt immunohistochemical expression. But perineural invasion was found in cases showing higher Gleason's scores, higher PSA above 20ng/ml, in stronger intensity and with increased % of staining with p-Akt. PNI was seen in majority in the age group of 70-79 years.

Table 31. Correlation of PSA and p-Akt IHC

PSA (ng/ml)	4-10	10-20	20-100	100-500	500-1000	> 1000
<b>p-Akt IHC intensity</b>						
Score 1 (cases)	1	0	2	0	0	0
Score 2 (cases)	1	1	11	8	2	1
Score 3 (cases)	0	4	14	3	1	1
<i>P value</i>	0.640					
<b>p-Akt IHC % of staining</b>						
Score 1 (cases)	0	0	0	0	0	0
Score 2 (cases)	1	1	6	0	1	0
Score 3 (cases)	1	3	23	10	2	2
<i>P value</i>	0.710					
<b>p-Akt IHC immunoreactivity score</b>						
Score $\leq$ 6 (cases)	2	1	14	8	1	2
Score > 6 (cases)	0	3	15	2	1	1
<i>P value</i>	0.050					

PSA showed no statistically significant correlation with p-Akt IHC but majority cases with strong intensity, increased % of staining and high immunoreactivity score had PSA in the range of 20-100 ng/ml.

## DISCUSSION

Carcinoma prostate is one of the most common malignancies and its biological activity is related to the histological appearance.

The histological appearance has been graded as well differentiated, moderately differentiated and poorly differentiated. Gleason's scoring and Grade grouping is done on hematoxylin and eosin-stained sections of prostate biopsies.

Gleason's scoring helps in predicting the metastasis and is used to evaluate the prognosis. Grade group is a new contemporary prostate cancer grading system used along with Gleason's scoring.

Pre biopsy PSA levels have been used as a biochemical tumour marker for prostate cancer. The levels of PSA have been correlated with Gleason's score and Grade group which in turn helps in diagnosing cancer and the cancer recurrence.

A newer immunohistochemical marker for prostate cancer has been under study lately called p-Akt. It is a protein kinase- B and very limited studies have been done on this marker.

A correlation has been studied between the immunoreaction of P-Akt and the Gleason's score and it has been found that cancers with higher grades show a strong immunoreaction with p-Akt.

A correlation between P-Akt and Grade group has not been done previously which was done in this study.

### Age Incidence in Carcinoma Prostate

The age group in this study ranged from 50-99 years with peak incidence of adenocarcinoma prostate in the 8<sup>th</sup> decade of life. Similar finding was seen in a study done by Pudasaini et al.<sup>15</sup>

**Table 32. Studies showing age distribution in prostate cancer**

STUDY	MEAN AGE (yrs)
Mwakyoma HA <sup>91</sup>	75.6
Di Silviero F <sup>92</sup>	68.9
Pinto PA <sup>93</sup>	68.5
Jayapradeep DP et al <sup>17</sup>	68.8
Present study	74.3

The mean age in this study was 74.3 years. The mean age of this study was similar to that obtained by Mwakyoma HA<sup>91</sup> but slightly different than that obtained by three other studies.

**Table 33. Studies showing age distribution > 65 years**

STUDY	AGE > 65 yrs CASES (%)
Xie et al <sup>94</sup>	84.2
Shimada et al <sup>95</sup>	75
Present study	92

It was observed that majority cases in this study had an age of > 65 years and similar findings were seen in study done by Xie et al<sup>95</sup> and Shimada et al.<sup>95</sup>

The increased incidence of carcinoma with age could be as a result directly or indirectly due to the process of aging.<sup>96</sup>

**Distribution of Gleason’s score and Grade group in Cancer Prostate**

The least common pattern was pattern 1 which correlated with study done by Jose L et al.<sup>1</sup>

**Table 34. Studies showing common Gleason’s patterns**

STUDY	COMMON GLEASON’S PATTERN
Jose L et al <sup>1</sup>	3
Pudasaini S et al <sup>15</sup>	3
Present study	4

The most common Gleason’s pattern was 4 followed by 3. Other studies showed pattern 3 being more common as seen in the table 29.

The most common pattern combination was 3+4 and 4+3.

**Table 35. Studies showing common Gleason’s score**

STUDY	COMMON GLEASON’S SCORE
Jose L et al <sup>1</sup>	7
Jayapradeep DP et al <sup>17</sup>	7
Present study	7

Gleason’s score 7 was found to be the commonest and similar findings were seen in many other studies as seen in table 35.

**Table 36. Studies showing Gleason's score distribution**

STUDY	GLEASON'S SCORE (CASES, %)		
	2-4	5-7	8-10
Divrik et al <sup>98</sup>	9.7	76.7	13.6
Mwakyom & Mabandi <sup>99</sup>	5.3	61	33.6
Present study	0	44	52

When the distribution of Gleason's score was studied it was found that no cases were in the score range of 2-4, few cases were in the score of 5-7 and majority were in range of 8-10. This was different when compared to two other studies.

**Table 37. Studies showing commonest Grade groups**

STUDY	COMMON GRADE GROUP
Yelda D et al <sup>99</sup>	2
Pudasaini S et al <sup>15</sup>	4
Present study	4

Grade group 4 was the commonest also seen in study done by Pudasaini et al.<sup>15</sup>

**Correlation of PSA levels in Cancer Prostate**

None of the cases had PSA levels in the normal range of 0-4 ng/ml.

**Table 38. Studies showing PSA distribution in prostate cancer**

STUDY	CASES WITH PSA> 20 ng/ml (%)
Pudasaini S et al <sup>15</sup>	88.9
Hirachand S et al <sup>100</sup>	99
Present study	84

4% patients of prostate adenocarcinoma had PSA levels in the range of 4-10 ng/ml, few had PSA levels between 10-20 ng/ml.

84% cases had PSA more than 20 ng/ml which on further study showed majority cases in 20-100 ng/ml range.

Many other studies found a similar result with their PSA levels more 20 ng/ml. PSA values are found on a higher level as the age advances.<sup>101</sup>

**Table 39. Studies showing PSA correlation with Gleason’s score and Grade group**

STUDY	PSA correlation with GS and GG
Jose L et al <sup>1</sup>	Statistically significant correlation (P value= 0.031)
Blackwell KL et al <sup>69</sup>	Statistically significant correlation (P value< 0.001)
Ferro et al <sup>70</sup>	Statistically significant correlation (P value<0.001)
Present study	Statistically significant correlation (P value=0.006)

In this study when a correlation was done between PSA and Gleason's score it was found that with increasing score the PSA value was found to be of a higher range and this correlation was statistically significant.

Similar observation was made in the correlation between PSA and Grade group, higher Grade groups had higher PSA levels. This correlation was statistically significant.

This correlation of PSA is attributed to cancer cells of prostate cancer producing more amounts of PSA as they progress from lower to higher grades.<sup>70</sup>

A slight dip in PSA was seen in score 10 in the present study. Similar decrease in PSA in score 10 tumours was seen by a study done by Partin et al<sup>102</sup> and their explanation for this dip was that in higher grades (score 10) of cancer less PSA is produced by cancer cells.

In the present study both Gleason's score and Grade group showed a statistically significant correlation with PSA levels which is in agreement with a study done by Ferro et al.<sup>70</sup> Their study found a significant positive correlation between histological grade and preoperative serum PSA levels.

Similar results were also obtained in a study done by Blackwell et al<sup>69</sup> where they found a significant positive correlation between serum PSA levels and Gleason score.

**Immunohistochemical expression of p-Akt in Prostate Cancer**

All the cases showed cytoplasmic localisation of p-Akt with 46% cases showing membranous and 02% cases showing nuclear localisation.

Similar result was seen in a study done by Liao Y et al<sup>4</sup> whereas study by Malik S et al<sup>103</sup> shows majority cases showing membranous staining.

Akt is present in the cytoplasm normally and on phosphorylation gets recruited to the plasma membrane and on complete phosphorylation dissociates from the membrane and can move to nucleus or any other cell component.<sup>104</sup>

**Table 40. Studies showing localization of p-Akt stain in cells**

STUDY	P-Akt LOCALISATION
Liao Y et al <sup>4</sup>	Cytoplasmic
Malik S et al <sup>103</sup>	Membranous
Present study	Cytoplasmic

The immunohistochemical expression was studied as intensity of immunoreaction, percentage of positive cells and the immunoreactivity score.

**Table 41. Studies showing correlation of IHC of p-Akt with Gleason’s score**

STUDY	CORRELATION OF INTENSITY OF IMMUNOREACTION AND GLEASON’S SCORE
Ayala G et al <sup>87</sup>	Statistically significant correlation (P value=0.035)
Malik S et al <sup>103</sup>	Statistically significant correlation (P value≤ 0.001)
Paweletz et al <sup>105</sup>	Statistically significant correlation (P value< 0.03)
Present study	Statistically significant correlation (P value= 0.038)

The intensity of immunoreaction in this study was found to be moderate in intensity in low grades of prostatic adenocarcinoma and the intensity progressed from moderate to strong as the score increased with strongest intensity in higher grades of cancer (8-10) and the correlation was statistically significant in this study.

This was in concordance by a study done by Malik S et al<sup>103</sup> and many other studies.

Intensity of immunoreaction was also seen to progress from moderate to strong with higher Grade groups and this correlation was statistically significant.

Literature search did not find any study comparing Grade group with IHC of p-Akt. In this study a correlation was done between Grade groups and p-Akt immunoreaction and the correlation between intensity of immunoreaction and Grade groups was statistically significant.

Percentage of positive cells for p-Akt showed a positive correlation with Gleason's score and Grade group but it was not statistically significant.

Immunoreactivity score showed a statistically significant correlation with both Gleason's score and Grade group.

The correlation of p-Akt with Gleason's score and Grade group may be seen as a result of the activation of the p-Akt pathway.<sup>88</sup> Higher grades of prostate adenocarcinoma show loss of tumour suppressor gene which further activates the Akt pathway leading to disease progression.<sup>88</sup>

A correlation was done in our study between histopathological features like PNI, MF and eosinophilic and basophilic luminal secretions with Gleason's score, PSA and p-Akt IHC and it was not statistically significant but PNI was seen in higher scores and was associated with high values of PSA. This association of PNI with

higher scores can be because the perineural space can provide as microenvironment promoting growth and spread of carcinoma.<sup>106</sup>

All the above histopathological features were seen in majority in the age group of 70-79 years, in Gleason's score 7 and in PSA levels in range of 20-100 ng/ml.

Correlation of PSA with p-Akt IHC was statistically not significant in the current study although a significant correlation was found in study done by Liao Y et al.<sup>4</sup>

In this study majority cases having a strong intensity, increased % of staining and a high immunoreactivity score had PSA in the range of 20-100 ng/ml.

## **CONCLUSION**

This study found a statistically significant correlation of IHC expression of p-Akt with Gleason's score and Grade group.

The intensity of immunoreaction of p-Akt in prostate adenocarcinoma was found to increase with progression to higher grades and poorly differentiated carcinoma i.e Gleason's scores 8-10 and the correlation was statistically significant.

A statistically significant correlation was also found between the new Grade groups and the intensity of p-Akt immunoreaction.

Percentage of cells showing immunoreaction with p-Akt was also found to increase with increased Gleason's scores and Grade groups but the correlation was not statistically significant.

The final immunoreactivity score also showed an increase with higher Gleason's score and Grade groups and the correlation was statistically significant. 30% of cases had immunoreactivity score of  $> 6$  and 20% cases had immunoreactivity score of  $\leq 6$ .

This study also suggests that in patients of Prostatic adenocarcinoma there is a positive correlation between Gleason's score and grade group with PSA levels and this correlation is statistically significant.

## **SUMMARY**

The present study was done over a span of two years from January 2019 to December 2020 in the Department of Pathology, KAHER'S Jawaharlal Nehru Medical College and Dr Prabhakar Kore Hospital, Belagavi.

50 cases of prostatic adenocarcinoma were studied and their Gleason's score and Grade group was noted. PSA correlation was done with both, the score and Grade group.

Immunohistochemical expression of p-Akt was studied and correlation was done with Gleason's score, Grade group and PSA using SPSS 16 software with P value of < 0.05 being statistically significant.

The commonest age group in the study belonged to the 8<sup>th</sup> decade with the mean age being 74.3 years. The commonest Gleason's pattern was 4 with score 7 being the commonest score. Grade group 4 and 5 were found in majority.

The average PSA levels ranged from 121 to 1000 ng/ml with 84% cases showing PSA levels > 20 ng/ml. There was a statistically significant positive correlation of PSA with both Gleason's score (**P value= 0.006**) and Grade group (**P value=0.005**).

On studying the immunohistochemical expression of p-Akt it was found that all the cases showed cytoplasmic localization of p-Akt.

A statistically significant positive correlation was found between the intensity of immunoreaction of p-Akt with both Gleason's score (**P value = 0.038**) and Grade group (**P value= 0.023**).

Percentage of positive cells for p-Akt also showed a positive correlation with both Gleason's score (P value=0.140) and Grade group (P value= 0.330) but it was not statistically significant.

Immunoreactivity score showed a statistically significant positive correlation with Gleason's score (**P value= 0.048**) and Grade group (**P value= 0.023**).

No statistically significant correlation was seen between:

- PSA and p-Akt IHC (P value was > 0.05)
- Histopathological features with Gleason's score, PSA and p-Akt IHC. (P value was > 0.05)

## **LIMITATIONS**

- As the sample size was low the correlation of other histopathological features like PNI, Mucinous fibroplasia, Eosinophilic and basophilic secretions with Gleason's score, PSA and p-AKT IHC could not be studied adequately.
- Role of p-Akt as an indicator of cancer recurrence could not be evaluated due to inability to follow-up patients.

## **SCOPE**

- More research can be done to see significance of p-Akt as a therapeutic agent and anti p-Akt therapies can be brought into use which can target cancer.
- More studies testing p-Akt as a prognostic indicator for prostate carcinoma recurrence need to be done.

**BIBLIOGRAPHY**

- 1) Jose L, Suma MT, Sadasivan S, Souda VM. Gleason's Grading of Carcinoma Prostate with PSA Correlation. *Journal of Medical Science and Clinical Research*.2017;5(9):27634-9.
- 2) Shih WJ, Gross K, Mitchell B, Collins J, Wierzbinski B, Magoun S, Ryo UY. Prostate adenocarcinoma using Gleason scores correlates with prostate-specific antigen and prostate acid phosphatase measurements. *J Natl Med Assoc*. 1992;84(12):1049-50.
- 3) Hara M, Kimura H. Two prostate-specific antigens, gamma-seminoprotein and beta-microseminoprotein. *J Lab Clin Med*. 1989;113(5):541-8.
- 4) Liao Y, Grobholz R, Abel U, Trojan L, Michel MS, Angel P et al. Increase of AKT/PKB expression correlates with gleason pattern in human prostate cancer. *Int J Cancer*. 2003 N;107(4):676-80.
- 5) Singh I. *Human Embryology*. 11<sup>th</sup> ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2018.
- 6) Standring S, Gray H. *Gray's anatomy*. Edinburgh: Churchill Livingstone/Elsevier; 2008.
- 7) Shah R, Zhou M. *Prostate biopsy interpretation*. 2<sup>nd</sup> ed. Switzerland: Springer Nature; 2019. Chapter 1, Anatomy and normal histology of the prostate pertinent to biopsy interpretation; p. 1-10.
- 8) Chaurasia B, Garg K. B. D. *Chaurasia's human anatomy*. New Delhi: CBS Publishers & Distributors; 2004.
- 9) Snell RS. *Clinical anatomy by regions*. Philadelphia: Lippincott Williams & Wilkins;2008.

- 10) McNeal JE, Bostwick DG. Intraductal dysplasia: a premalignant lesion of the prostate. *Hum Pathol.* 1986; 17(1):64-71.
- 11) Hall JE. *Guyton and hall textbook of medical physiology.* 13<sup>th</sup> ed. Philadelphia: Elsevier inc; 2016. Chapter 81, Reproductive and Hormonal functions of the male; p. 1020-35.
- 12) Young B, O'Dowd G, Woodford P. *Wheater's functional histology.* 6<sup>th</sup> ed. Philadelphia: Churchill Livingstone; 2015. Chapter 18, Male reproductive system; p. 337-50.
- 13) Ramon J, Denis LJ. *Prostate cancer.* Springer; 2007.
- 14) Moch H, Humphrey PA, Ulbright TM, Reuter VE. *WHO Classification of Tumours of the Urinary System and Male Genital Organs.* Switzerland: IARC; 2016.
- 15) Pudasaini S, Subedi N, Shrestha N. Evaluation of Prostate specific antigen levels and its correlation with histopathological findings. *Journal of Pathology of Nepal.* 2019; 9(1):1485-89.
- 16) Abu Farsakh MG, Abu Farsakh HA. Serum prostate-specific antigen, radiologic findings and Gleason score in prostate biopsies in Jordan. *Hematol Oncol Stem Cell Ther.* 2008;1(3):171-4.
- 17) Jayapradeep DP, Prakash VB, Philipose TR, Pai MR. Histomorphological Correlation of PSA Levels in Prostatic Pathology. *National Journal of Laboratory Medicine.* 2017;6(4):28-32.
- 18) Franks LM. Etiology, epidemiology and pathology of prostate cancer. *Cancer.* 1973; 5: 1092-95.
- 19) Epstein JI, Lotan TL. The lower urinary tract and male genital system. In: Kumar V, Abbas Ak, Fausto N, editors. *Robbins and Cotran pathological basis of disease,*

- South Asia edition, Haryana: Reed Elsevier India Private Limited; 2014. p. 859-990.
- 20) Goldstein NS, Bégin LR, Grody WW, Novak JM, Qian J, Bostwick DG. Minimal or no cancer in radical prostatectomy specimens. Report of 13 cases of the "vanishing cancer phenomenon". *Am J Surg Pathol.* 1995;19(9):1002-9.
- 21) Pacelli A, Lopez-Beltran A, Egan AJ, Bostwick DG. Prostatic adenocarcinoma with glomeruloid features. *Hum Pathol.* 1998;29(5):543-46.
- 22) Aihara M, Truong LD, Dunn JK, Wheeler TM, Scardino PT, Thompson TC. Frequency of apoptotic bodies positively correlates with Gleason grade in prostate cancer. *Hum Pathol.* 1994;25(8):797-801.
- 23) Montironi R, Magi Galluzzi C, Scarpelli M, Giannulis I, Diamanti L. Occurrence of cell death (apoptosis) in prostatic intra-epithelial neoplasia. *Virchows Arch A Pathol Anat Histopathol.* 1993;423(5):351-7.
- 24) Bastacky SI, Walsh PC, Epstein JI. Relationship between perineural tumor invasion on needle biopsy and radical prostatectomy capsular penetration in clinical stage B adenocarcinoma of the prostate. *Am J Surg Pathol.* 1993; 17:336–41.
- 25) Nelson RS, Epstein JI. Prostatic carcinoma with abundant xanthomatous cytoplasm. Foamy gland carcinoma. *Am J Surg Pathol.* 1996;20(4):419-26.
- 26) Zhao J, Epstein JI. High-grade foamy gland prostatic adenocarcinoma on biopsy or transurethral resection: a morphologic study of 55 cases. *Am J Surg Pathol.* 2009;33(4):583-90.
- 27) Cina SJ, Epstein JI. Adenocarcinoma of the prostate with atrophic features. *Am J Surg Pathol.* 1997;21(3):289-95.

- 28) Humphrey PA, Kaleem Z, Swanson PE, Vollmer RT. Pseudohyperplastic prostatic adenocarcinoma. *Am J Surg Pathol.* 1998;22(10):1239-46.
- 29) Epstein JI. Diagnostic criteria of limited adenocarcinoma of the prostate on needle biopsy. *Hum Pathol.* 1995;26(2):223-29.
- 30) Giannico GA, Ross HM, Lotan T, Epstein JI. Aberrant expression of p63 in adenocarcinoma of the prostate: a radical prostatectomy study. *Am J Surg Pathol.* 2013;37(9):1401-6.
- 31) McNeal JE, Alroy J, Villers A, Redwine EA, Freiha FS, Stamey TA. Mucinous differentiation in prostatic adenocarcinoma. *Hum Pathol.* 1991;22(10):979-88.
- 32) Remmele W, Weber A, Harding P. Primary signet-ring cell carcinoma of the prostate. *Hum Pathol.* 1988; 19(4):478-80.
- 33) Netto GJ, Epstein JI. Widespread high-grade prostatic intraepithelial neoplasia on prostatic needle biopsy: a significant likelihood of subsequently diagnosed adenocarcinoma. *Am J Surg Pathol.* 2006;30(9):1184-8.
- 34) Melicow MM, Pachter MR. Endometrial carcinoma of the prostatic utricle (uterus masculinus). *Cancer.* 1976; 20(10):1715–21.
- 35) Gleason DF. Histologic grading of prostate cancer: a perspective. *Hum Pathol.* 1992;23(3):273-9.
- 36) Rosai J, Ackerman L. Rosai and Ackerman's surgical pathology. Edinburgh: Mosby/ Elsevier; 2011.
- 37) Gettman MT, Pacelli A, Slezak J, Bergstralh EJ, Blute M, Zincke H et al. Role of microvessel density in predicting recurrence in pathologic Stage T3 prostatic adenocarcinoma. *Urology.* 1999;54(3):479-85.
- 38) Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol.* 1974 ;111(1):58-64.

- 39) Amin A, Partin A, Epstein JI. Gleason score 7 prostate cancer on needle biopsy: relation of primary pattern 3 or 4 to pathological stage and progression after radical prostatectomy. *J Urol.* 2011; 186:1286–90.
- 40) Mellinger GT, Gleason D, Bailar J. The histology and prognosis of prostatic cancer. *J Urol.* 1967 ;97(2):331-7.
- 41) Bailar JC, Mellinger GT, Gleason DF. Survival rates of patients with prostatic cancer, tumor stage, and differentiation--preliminary report. *Cancer Chemother Rep.* 1966;50(3):129-36.
- 42) Freedland SJ, Wieder JA, Jack GS, Dorey F, deKernion JB, Aronson WJ. Improved risk stratification for biochemical recurrence after radical prostatectomy using a novel risk group system based on prostate specific antigen density and biopsy Gleason score. *J Urol.* 2002;168(1):110-5.
- 43) Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL. ISUP Grading Committee. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. *Am J Surg Pathol.* 2005 ;29(9):1228-42.
- 44) Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA; Grading Committee. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. *Am J Surg Pathol.* 2016;40(2):244-52.
- 45) Pessoa R, Werahera PN, Fernando JK. Prostate Cancer. In: Harken AH, Moore EE, editors. *Abernathy's Surgical Secrets.* California: Elsevier;2018. p. 450-51.
- 46) Epstein J, Netto G. Biopsy interpretation of the Prostate. 5<sup>th</sup> ed. Philadelphia: Wolters Kluwer Health;2015. Chapter 1, Clinical correlates with biopsy: serum

- prostate specific antigen, digital rectal examination and imaging techniques; p. 1-7.
- 47) van Leenders GJ, van der Kwast TH, Grignon DJ, Evans AJ, Kristiansen G, Kweldam CF et al. ISUP Grading Workshop Panel Members. The 2019 International Society of Urological Pathology (ISUP) Consensus Conference on Grading of Prostatic Carcinoma. *Am J Surg Pathol.* 2020;44(8):87-99.
- 48) Eble JN, Sauter G, Epstein JI, Sesterhenn IA. World Health Organization. Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press;2004.
- 49) Scardino PT, Weaver R, Hudson MA. Early detection of prostate cancer. *Hum Pathol.* 1992;23(3):211–22.
- 50) Carter HB, Hamper UM, Sheth S, Sanders RC, Epstein JI, Walsh PC. Evaluation of transrectal ultrasound in the early detection of prostate cancer. *J Urol.* 1989;142(4):1008-10.
- 51) Coffield KS, Speights VO, Brawn PN, Riggs MW. Ultrasound detection of prostate cancer in postmortem specimens with histological correlation. *J Urol.* 1992;147(3):822-6.
- 52) Trabulsi EJ, Merriam WG, Gomella LG. New imaging techniques in prostate cancer. *Curr Urol Rep.* 2006; 7:175–80.
- 53) Wu LM, Xu JR, Ye YQ, Lu Q, Hu JN. The clinical value of diffusion-weighted imaging in combination with T2-weighted imaging in diagnosing prostate carcinoma: a systematic review and meta-analysis. *AJR Am J Roentgenol.* 2012; 199:103–10.
- 54) Delongchamps NB, Rouanne M, Flam T, Beuvon F, Liberatore M, Zerbib M et al. Multiparametric magnetic resonance imaging for the detection and localization of

- prostate cancer: combination of T2-weighted, dynamic contrast-enhanced and diffusion-weighted imaging. *BJU Int.* 2011;107(9):1411-8.
- 55) Epstein JI, Walsh PC, Carter HB. Importance of posterolateral needle biopsies in the detection of prostate cancer. *Urology.* 2001;57(6):1112–6.
- 56) Terris MK, Wallen EM, Stamey TA. Comparison of mid-lobe versus lateral systematic sextant biopsies in the detection of prostate cancer. *Urol Int.* 1997;59(4):239-42.
- 57) Eskew LA, Woodruff RD, Bare RL, McCullough DL. Prostate cancer diagnosed by the 5-region biopsy method is significant disease. *J Urol.* 1998 Sep;160(3):794-6.
- 58) Gretzer MB, Partin AW. PSA markers in prostate cancer detection. *Urol Clin North Am.* 2003;30(3):677–86.
- 59) Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med.* 1991;324(17):1156-61.
- 60) Sakai I, Harada K, Hara I, Eto H, Miyake H. Limited usefulness of the free-to-total prostate-specific antigen ratio for the diagnosis and staging of prostate cancer in Japanese men. *Int J Clin Oncol.* 2004;9(1):64-7.
- 61) Kundu SD, Roehl KA, Yu X, Antenor JA, Suarez BK, Catalona WJ. Prostate specific antigen density correlates with features of prostate cancer aggressiveness. *J Urol.* 2007;177(2):505–9.
- 62) Min SH, Park YH, Lee SB, Ku JH, Kwak C, Kim HH. Impact of prostate size on pathologic outcomes and prognosis after radical prostatectomy. *Korean J Urol.* 2012;53(7):463-6.

- 63) Magheli A, Hinz S, Hege C, Stephan C, Jung K, Miller K et al. Prostate specific antigen density to predict prostate cancer upgrading in a contemporary radical prostatectomy series: a single center experience. *J Urol.* 2010;183(1):126-31.
- 64) Oh JJ, Hong SK, Lee JK, Lee BK, Lee S, Kwon OS et al. Prostate-specific antigen vs prostate-specific antigen density as a predictor of upgrading in men diagnosed with Gleason 6 prostate cancer by contemporary multicore prostate biopsy. *BJU Int.* 2012;110(11):494-9.
- 65) Tsang CF, Lai TC, Lam W, Ho BS, Ng AT, Ma WK et al. Is prostate specific antigen (PSA) density necessary in selecting prostate cancer patients for active surveillance and what should be the cutoff in the Asian population? *Prostate Int.* 2019;7(2):73-7.
- 66) Chen CS, Wang SS, Li JR, Cheng CL, Yang CR, Chen WM et al. PSA density as a better predictor of prostate cancer than percent-free PSA in a repeat biopsy. *J Chin Med Assoc.* 2011;74(12):552-5.
- 67) Freedland SJ, Wieder JA, Jack GS, Dorey F, deKernion JB, Aronson WJ. Improved risk stratification for biochemical recurrence after radical prostatectomy using a novel risk group system based on prostate specific antigen density and biopsy Gleason score. *J Urol.* 2002;168(1):110-5.
- 68) Shen S, Lepor H, Yaffee R, Taneja SS. Ultrasensitive serum prostate specific antigen nadir accurately predicts the risk of early relapse after radical prostatectomy. *J Urol.* 2005;173(3):777-80.
- 69) Blackwell KL, Bostwick DG, Myers RP, Zincke H, Oesterling JE. Combining prostate specific antigen with cancer and gland volume to predict more reliably pathological stage: the influence of prostate specific antigen cancer density. *J Urol.* 1994;151(6):1565-70.

- 70) Ferro MA, Barnes I, Roberts JB, Smith PJ. Tumour markers in prostatic carcinoma. A comparison of prostate-specific antigen with acid phosphatase. *Br J Urol.* 1987;60(1):69-73.
- 71) Zivkovic S. Correlation between prostate specific Antigen and histopathological difference of CA prostate. *Arch Oncol.* 2004; 12 (3): 148-151.
- 72) Ceylan C, Gazel E, Keleş İ, Doluoğlu Ö, Yiğman M. Can the Free/Total PSA Ratio Predict the Gleason Score Before Prostate Biopsy? *Curr Urol.* 2016;9(1):24-7.
- 73) Aus G, Becker C, Lilja H, Khatami A, Pihl CG, Hugosson J. Free-to-total prostate-specific antigen ratio as a predictor of non-organ-confined prostate cancer (stage pT3). *Scand J Urol Nephrol.* 2003;37(6):466-70.
- 74) Issac AS, Mah EB, Pai KP. A study of biological determinants of serum prostate specific antigen level in prostatic adenocarcinoma with normal, borderline, and high serum PSA levels. *Arch Med Health Sci.* 2014;2(1):8-11.
- 75) Amery L, Fransen M, De Nys K, Mannaerts GP, Van Veldhoven PP. Mitochondrial and peroxisomal targeting of 2-methylacyl-CoA racemase in humans. *J Lipid Res.* 2000 ;41(11):1752-9.
- 76) Molinié V, Fromont G, Sibony M, Vieillefond A, Vassiliu V, Cochand-Priollet B et al. Diagnostic utility of a p63/alpha-methyl-CoA-racemase (p504s) cocktail in atypical foci in the prostate. *Mod Pathol.* 2004;17(10):1180-90.
- 77) Makarov DV, Trock BJ, Humphreys EB, Mangold LA, Walsh PC, Epstein JI et al. Updated nomogram to predict pathologic stage of prostate cancer given prostate-specific antigen level, clinical stage, and biopsy Gleason score (Partin tables) based on cases from 2000 to 2005. *Urology.* 2007;69(6):1095-101.

- 78) Lin HK, Yeh S, Kang HY, Chang C. Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor. *Proc Natl Acad Sci U S A.* 2001;98(13):7200-5.
- 79) Liu P, Begley M, Michowski W, Inuzuka H, Ginzberg M, Gao D et al. Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. *Nature.* 2014;508(7497):541-5.
- 80) Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci USA* 1999;96(8):4240–5.
- 81) Coffey PJ, Jin J, Woodgett JR. Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem J.* 1998;335(1):1–13.
- 82) Medema RH, Kopd GJ, Bos JL, Burgering BM. AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. *Nature.* 2000;404(6779):782–7.
- 83) Gringras AC, Raught B, Sonenberg N. Regulation of translation initiation by FRAP/mTOR. *Genes Dev.* 2001;15(7):807–26.
- 84) Isaacs JT. The biology of hormone refractory prostate cancer. Why does it develop? *Urol Clin North Am.* 1999;26(2):263–73.
- 85) Agus DB, Cordon-Cardo C, Fox W, Drobnyak M, Koff A, Golde DW et al. Prostate cancer cell cycle regulators: response to androgen withdrawal and development of androgen independence. *J Natl Cancer Inst* 1999;91(21):1869 – 76.

- 86) Zinda MJ, Johnson MA, Paul JD, Horn C, Konicek BW, Lu ZH et al. AKT-1, -2, and -3 are expressed in both normal and tumor tissues of the lung, breast, prostate, and colon. *Clin Cancer Res.*2001; 7:2475–9.
- 87) Ayala G, Thompson T, Yang G, Frolov A, Li R, Scardino P et al. High levels of phosphorylated form of Akt-1 in prostate cancer and non-neoplastic prostate tissues are strong predictors of biochemical recurrence. *Clin Cancer Res.* 2004;10(19):6572-8.
- 88) McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res.* 1999;59(17):4291-6.
- 89) Molinie V, Fromont G, Sibony M, Vieillefond A, Vassiliu V, Priollet BC, et al. Diagnostic utility of a p63/-methyl-CoA racemase(p504S) cocktail in atypical foci in the prostate. *Mod Pathol.*2004;17(10):1180–90.
- 90) Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques.* Philadelphia: Churchill Livingstone;2008.
- 91) Mwakyoma HA. The Prevalence of High Grade Prostatic Intraepithelial Neoplasia in Prostatic Biopsies Diagnosed as Benign Prostatic Hyperplasia at Muhimbili National Hospital, Dar es Salaam. *Tanzania Medical Journal.* 2008;23(1):1-4.
- 92) Di Silverio F, Gentile V, Matteis DA, Mariotti G, Giuseppe V, Luigi PA et al. Distribution of inflammation, pre-malignant lesions, incidental carcinoma in histologically confirmed benign prostatic hyperplasia: a retrospective analysis. *Eur Urol.* 2003;43(2):164- 75.
- 93) Pinto PA, Chung PH, Rastinehad AR, Baccala AA, Kruecker J, Benjamin CJ et al. Magnetic resonance imaging/ultrasound fusion guided prostate biopsy improves

- cancer detection following transrectal ultrasound biopsy and correlates with multiparametric magnetic resonance imaging. *J Urol.* 2011;186(4):1281-85.
- 94) Xie LP, Qin J, Zheng XY, Shen HF, Chen ZD, Cai SL et al. Age and pathological features of 481 prostate cancer patients. *Zhonghua Nan Ke Xue.*2005;11(6):428-30.
- 95) Shimada H, Misugi K, Sasaki Y, Iizuka A, Nishihira H. Carcinoma of the prostate in childhood and adolescence. Report of a case and review of the literature. *Cancer.* 1980; 46:2534-42.
- 96) Ghosh PM, Malik S, Bedolla R, Kreisberg JI. Akt in prostate cancer: possible role in androgen-independence. *Curr Drug Metab.* 2003 ;4(6):487-96.
- 97) Divrik RT, Eroglu A, Sahin A, Zorlu F, Ozen H. Increasing the number of biopsies increases the concordance of Gleason's scores of needle biopsies and prostatectomy specimens. *Urol Oncol Semin.* 2007; 25:376-82.
- 98) Mwakyoma HA, Mabandi JL. Correlation of Gleason's score and pretreatment prostate specific antigen in patients. *Professional Med J.*2010;17(2):235-40.
- 99) Dere Y, Akarken I, Sahin H. The role of preoperative PSA level in prostate cancer. *Int J Adv Med.* 2018 ;5(4):780-3.
- 100) Hirachand S, Dangol U, Pradhanang S, Acharya S. Study of prostatic pathology and its correlation with prostate specific antigen level. *Journal of Pathology of Nepal.*2017; 7(1):1074–77.
- 101) Constantinou J, Feneley MR. PSA testing: an evolving relationship with prostate cancer screening. *Prostate Cancer Prostatic Dis.* 2006;9(1):6-13.
- 102) Partin AW, Carter HB, Chan DW, Epstein JI, Oesterling JE, Rock RC et al. Prostate specific antigen in the staging of localized prostate cancer: influence of

- tumor differentiation, tumor volume and benign hyperplasia. *J Urol.* 1990;143(4):747-52.
- 103) Malik SN, Brattain M, Ghosh PM, Troyer DA, Prihoda T, Bedolla R et al. Immunohistochemical Demonstration of Phospho-Akt in High Gleason Grade Prostate Cancer. *Clinical Cancer Research.* 2002; 8:1168-71.
- 104) Li L, Ittmann MM, Ayala G, Tsai MJ, Amato RJ, Wheeler TM et al. The emerging role of the PI3-K-Akt pathway in prostate cancer progression. *Prostate Cancer Prostatic Dis.* 2005;8(2):108-18.
- 105) Paweletz CP, Charboneau L, Bichsel VE, Simone NL, Chen T, Gillespie JW et al. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene.* 2001;20(16):1981-9.
- 106) Zareba P, Flavin R, Isikbay M, Rider JR, Gerke TA, Finn S et al. Perineural Invasion and Risk of Lethal Prostate Cancer. *Cancer Epidemiol Biomarkers Prev.* 2017 ;26(5):719-26.

**ANNEXURE I**  
**INFORMED CONSENT**

**Purpose of the study:** You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo TRUS guided biopsy/TURP for prostatic malignancy you will be included in this study.

The purpose of this study is to study the expression of the IHC marker, Akt in prostatic adenocarcinoma and to determine the correlation of histological grading of prostatic adenocarcinoma with PSA levels.

**Procedure:** During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. The principal investigator of the study is Dr. Savoikar Shalaka Narayan under the guidance of Dr. Vijayalaxmi Dhorigol<sup>MD</sup> (guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

**Risks and benefits:** There are no risks involved in taking part in this study and benefit is we will be able to know a better way to diagnose prostatic adenocarcinoma which is essential for providing appropriate treatment.

**Alternatives:** Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

**Privacy and confidentiality:** All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be

published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

**Financial incentives for participation:** You will not be paid / offered any gift /incentives for participating in this study.

**Authorization to publish results:** The results of this study would be forwarded to the KAHER, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

**Questions:** In case you have any questions related to the study in future you can contact:

1. **REG. NO.: BN0119010**, Department of Pathology, J.N. Medical College.
2. \_\_\_\_\_, Department of Pathology, J.N. Medical College.
3. If you have any queries about your rights as a study subject, you may call Dr. Roopa Bellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi

**CONSENT STATEMENT**

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

**Principal Investigator: REG. NO.: BN0119010**

**Guide** : Dr. \_\_\_\_\_

Name of the participant:

(signature/thumbprint)

Name of the witness: (signature)

Name of the investigator: (signature)

Date:

Address:

Phone no:

**ANNEXURE II. ETHICAL CLEARANCE.**



K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH  
(Deemed to be University)

Accredited 'A' Grade by NAAC (2<sup>nd</sup> Cycle) Placed in Category 'A' by MHRD (Govt)

**JAWAHARLAL NEHRU MEDICAL COLLEGE,**  
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: <http://www.jnmc.edu>  
E-Mail : [dnmc@jnmc.edu](mailto:dnmc@jnmc.edu)

Phone: (+91-0831) Office : 2472550  
Principal: 2471701  
Fax No. :+91 (0)831 - 2470739

Ref: MDC/DOME/ 269.

Date: 24/12/2019

To,

**REG. NO.: BN0119010**

PG student in Pathology,  
J.N.Medical College,  
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled "STUDY OF IMMUNOHISTOCHEMICAL EXPRESSION OF AKT (PROTEIN KINASE B) IN PROSTATIC ADENOCARCINOMA – DESCRIPTIVE STUDY AT TERTIARY CARE HOSPITAL, BELAGAVI", is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dahal)  
Member Secretary  
JNMC Institutional Ethics Committee  
on Human Subjects Research,  
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)  
Chairman,  
JNMC Institutional Ethics Committee  
on Human Subjects Research,  
J.N.Medical College, Belagavi.

**ANNEXURE III**

**PROFORMA**

**Patient History**

Name:

Age:

I.P number:

Total Serum PSA (Prostate specific antigen) Levels:

Surgery done:

Nature of the specimen:

**Brief Clinical History**

**Clinical Diagnosis**

**Histopathological Diagnosis**

**Hematoxylin and eosin staining**

- Gleason's score:
- Grade grouping:

**Histopathological features**

- PNI
- Mucinous fibroplasia
- Eosinophilic and basophilic secretions

**Immunohistochemical Staining**

- Intensity of immunoreaction
- Percentage of cells with positive immunoreaction
- Immunoreactivity score

**ANNEXURE IV**

**STEPS OF TISSUE PROCESSING**

Automated tissue processor Leica TP 1020 was used for processing the specimens and includes following steps:

- Two changes of 10% formalin for 3 hours in one followed by 1 hour in the second container.
- Dehydration with graded concentrations of alcohol (50%, 70%, 80%, 95%) for one hour each followed by two changes of absolute alcohol for 30 minutes each.
- Clearing by xylene- two changes for 1 hour each.
- Paraffin wax impregnation – two changes for 2 hours each.
- Embedding in paraffin wax followed by making block.
- Sections were cut using a semi-automated microtome at a setting of 3-5 microns.
- Sections were floated in a water bath at 60<sup>0</sup> C.
- Mounting of sections done on egg albumin coated slides for hematoxylin and eosin staining and mounted on poly-l-lysine coated slides for IHC.

**ANNEXURE V**

**HAEMATOXYLIN & EOSIN STAINING PROTOCOL**

1. Deparaffinize slides in 3 changes of xylene for 5 minutes each.
2. Rehydrate using 2 changes of Absolute Ethanol 100% for 5 minutes each.
3. Rinse in distilled water for 5 minutes.
4. Rinse in running tap water for 5 minutes.
5. Staining is done in Harris's haematoxylin by progressive method for 2 minutes.  
The stain should be freshly prepared and filtered.
6. Rinse in running tap water for 20 minutes.
7. Decolorize in 1% acid alcohol for 1 second or one dip.
8. Rinse well in tap water for 5 minutes.
9. Immerse in hot water bath at 55<sup>0</sup> C for bluing for 3 Seconds.
10. Rinse in tap water for 5 minutes.
11. Counterstaining is done in Eosin for 15 seconds.
12. Dehydrate in absolute alcohol 100 % with 2-4 dips.
13. Clear in two changes of xylene for 5 minutes each.
14. Mounting is done with DPX.

**Stock Solutions – EOSIN:**

Stock – 1% aqueous Eosin-Y

Stock – 1% aqueous Phloxin B

**Working Solutions – Eosin:**

Stock Eosin	100 ml
Stock Phloxin B	10 ml
95% Ethanol	780 ml
Glacial Acetic Acid	4ml

**Working Solution: - Haematoxylin**

Harris Haematoxylin	1 Litre
---------------------	---------

**Working Solution: - 0.25% Acid Alcohol**

95% Ethanol	2578 ml
dH <sub>2</sub> O	950ml
HCL	9ml

**Result:**

Nuclei – Blue,  
Cytoplasm – Pink,  
RBCs – Red.

**Reference:** Bancroft D, Layton C. The haematoxylin and eosin, In: Kim SS Ed, Bancroft's Theory and practice of histopathological techniques. 7<sup>th</sup> Ed., China, Churchill Livingstone; 2013: p173-87.

**ANNEXURE VI**

**IMMUNOHISTOCHEMICAL STAINING FOR P-Akt**

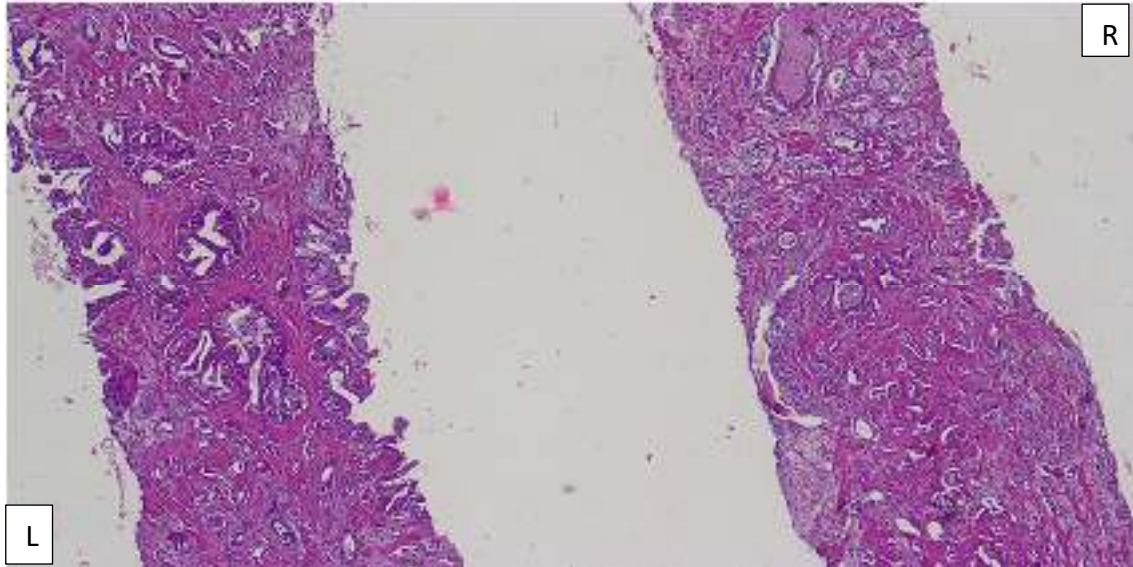
- 2-4 microns thick sections were taken on poly-L-lysine coated slides.
- Staining was done on a section obtained from one block per patient.
- Slides were baked at 80 degrees Celsius for 30 minutes.
- Sections were deparaffinized in 3 changes of xylene for 5 minutes each and hydrated with graded concentration of alcohol (100%, 90%, 70% ethyl alcohol) for 5 minutes each.
- Slides were washed in running tap water for 5 minutes followed by soaking in PBS buffer for 2 minutes.
- Antigen retrieval was done using a pressure cooker method. Sections were retrieved under pressure using Biocare's Decloaking Chamber by placing slides in antigen retrieval buffer solution.
- It was done with a citrate buffer at a pH of 6 for a duration of 20 minutes.
- After cooling for about 20mins, the slides were rinsed in PBS 3 times.
- The endogenous peroxidase activity was blocked by incubation with 3% peroxide for 10 minutes.
- Rinsing was done in PBS, 3 times.
- The sections were then incubated in the primary antibody (**Primary Antibody-** Rabbit monoclonal [EPR18405] to Akt1 + Akt2) for 60 minutes.
- This was followed by rinse in PBS.
- The sections were then incubated with linker for 20 minutes followed by rinsing with PBS.
- Incubation with polymer (Envision) was done for 30 minutes followed by rinse with PBS.

- Incubation was done with chromogen substrate and 3,3' diaminobenzidine enhancer for 10 minutes followed by rinsing in PBS.
- Counterstaining was done with haematoxylin for 3 minutes.
- Slides were then dehydrated, cleared and mounted with DPX.
- Incubation in nonimmune rabbit IgG was done in place of primary antibody for negative controls.
- Human Gastric adenocarcinoma was used as positive control.

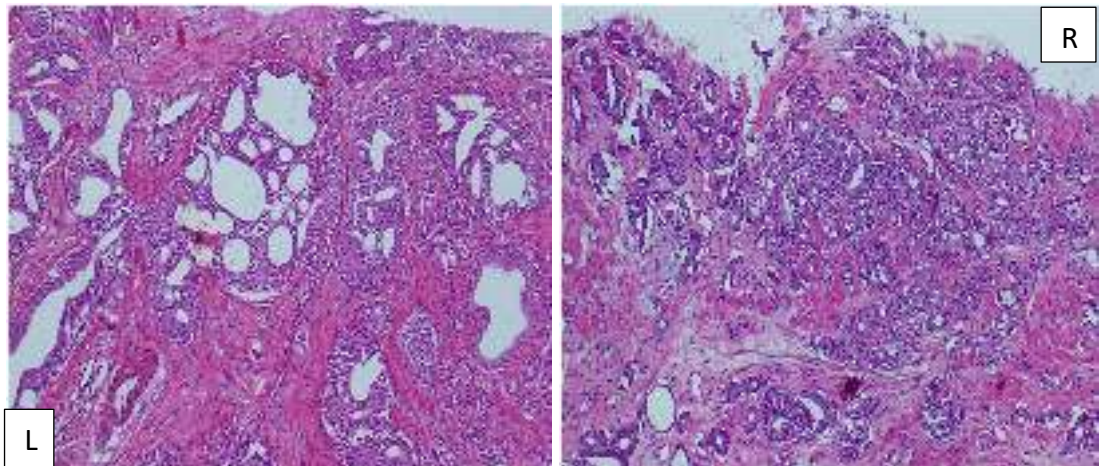
## **RESULT**

Stain is localized in the cytoplasm and/or membrane of neoplastic cells rarely nucleus.

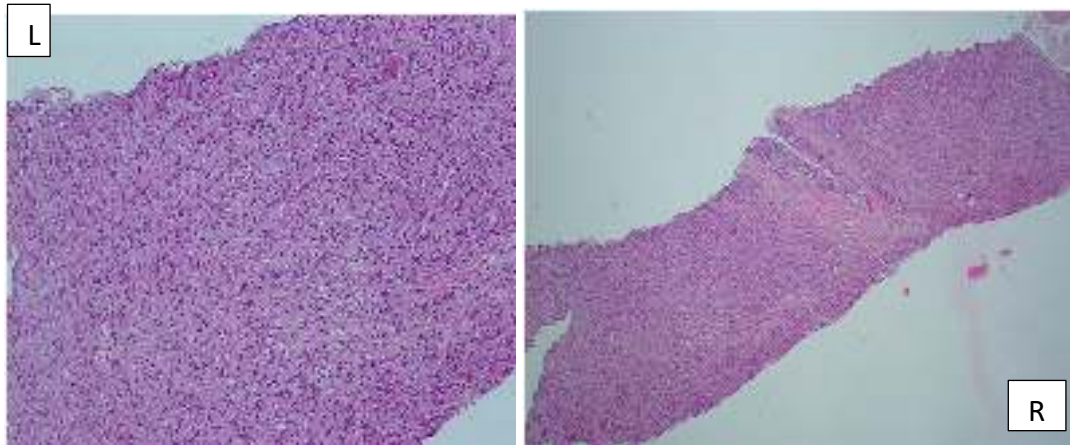
**ANNEXURE VII**  
**PHOTOMICROGRAPHS**



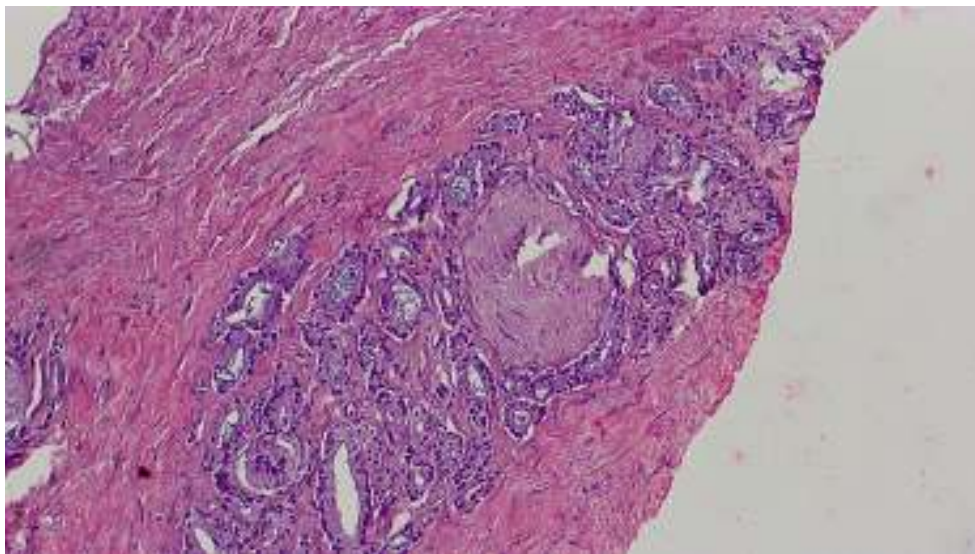
**Photomicrograph 1. Gleason's score  $4(L)+3(R)=7$ , Grade Group 3 (H&E, X40)**



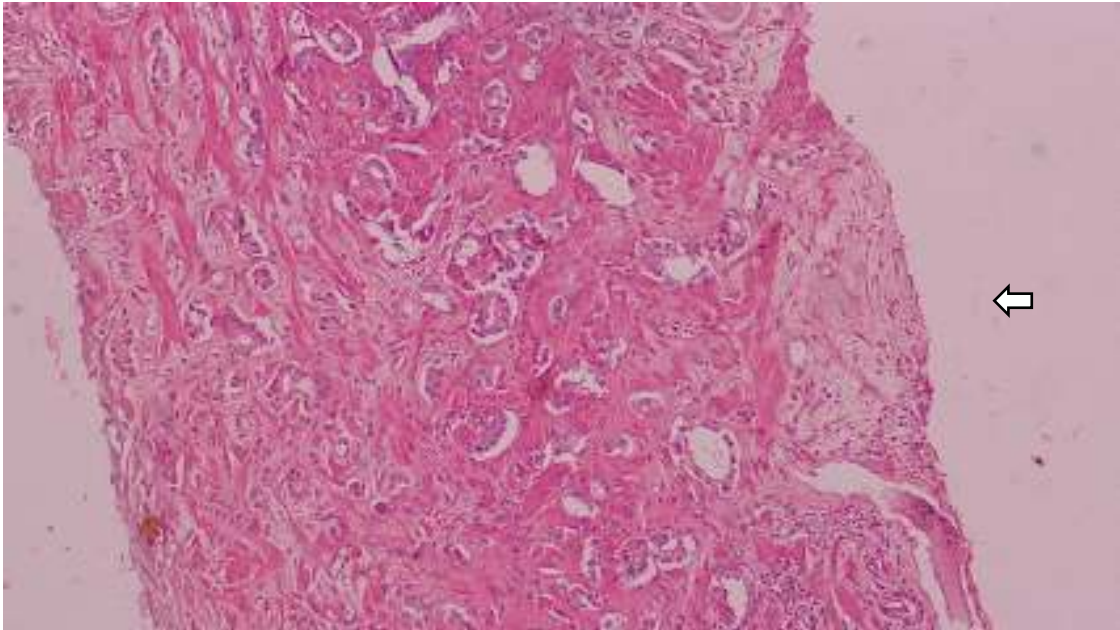
**Photomicrograph 2. Gleason's score  $4(L)+4(R)=8$ , Grade Group 4 (H&E, X100)**



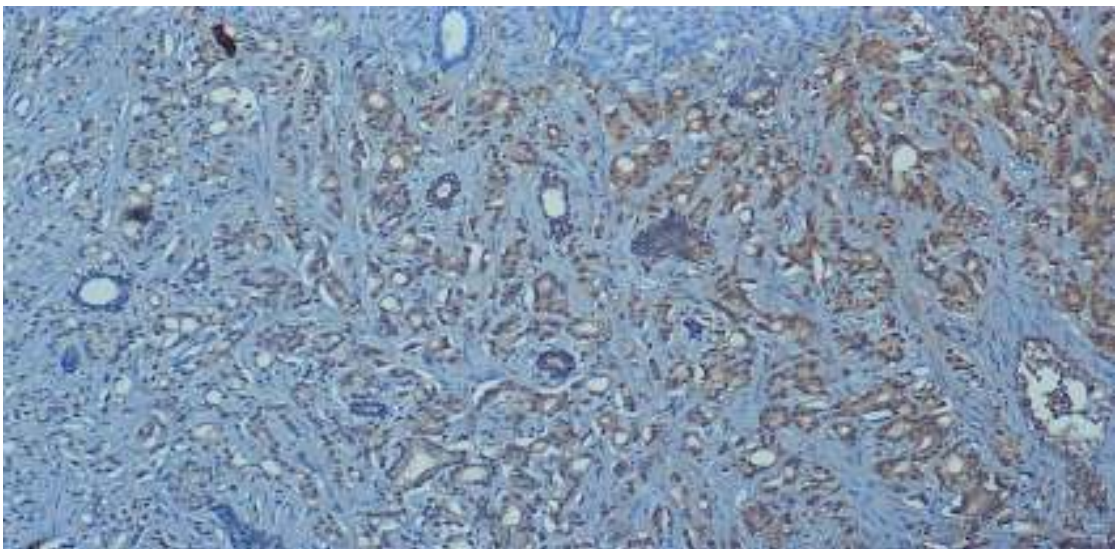
**Photomicrograph 3. Gleason's score 5(L)+5(R)=10, Grade group 5 (H&E, L-X100, R-X40)**



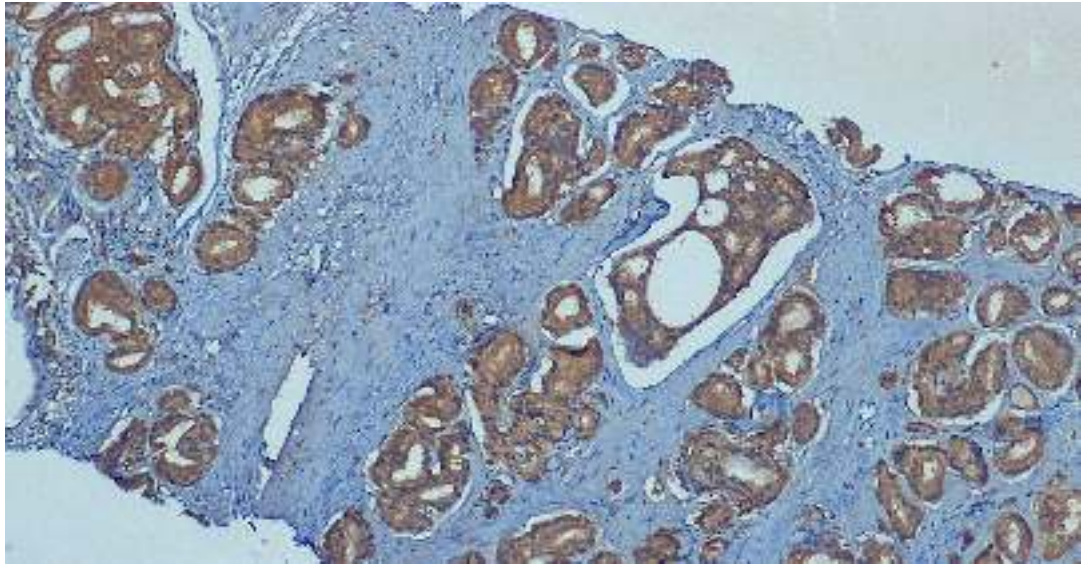
**Photomicrograph 4. Perineural invasion with basophilic luminal secretions (H&E, X100)**



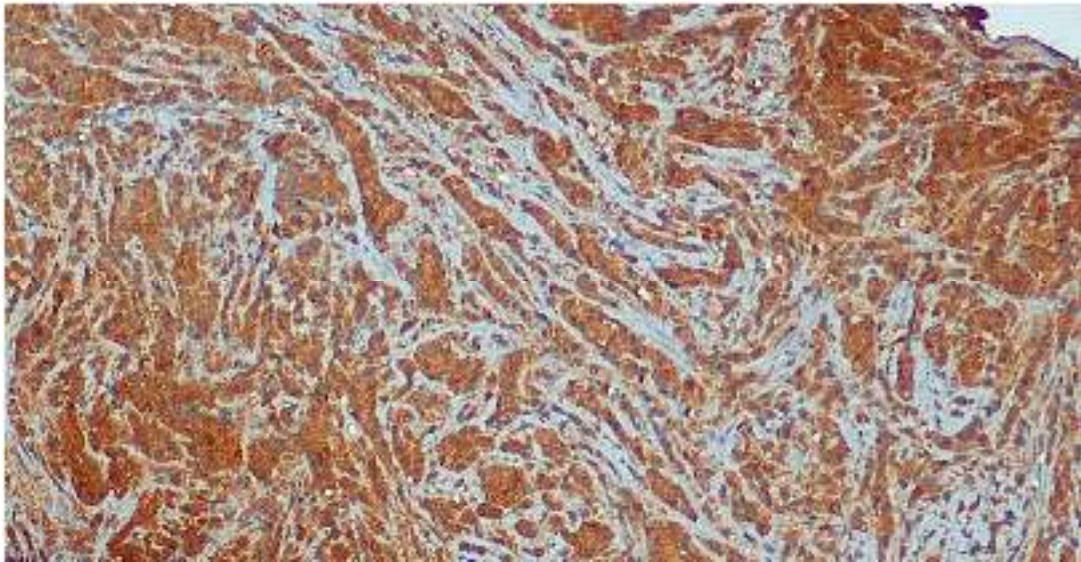
**Photomicrograph 5. Mucinous fibroplasia shown with arrow (H&E, X100)**



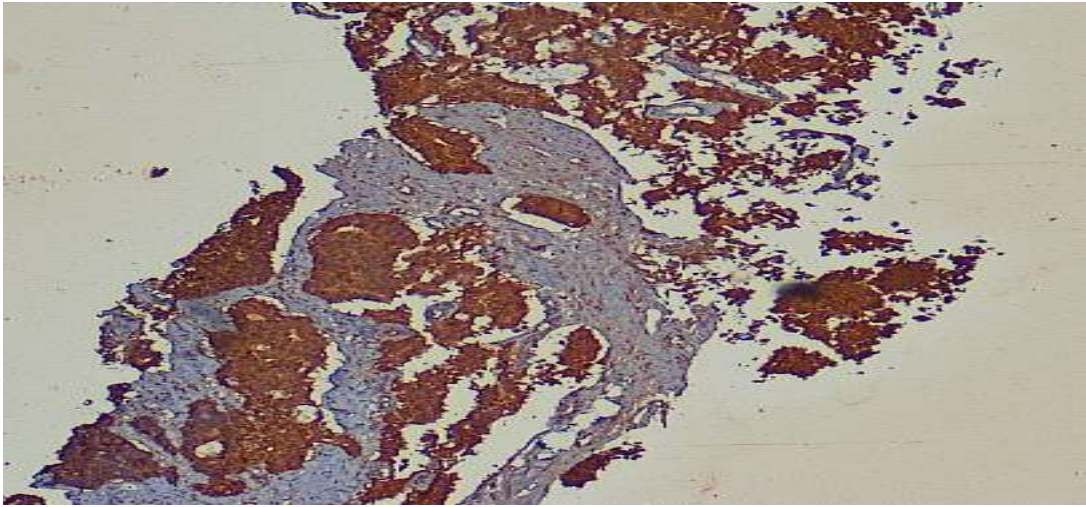
**Photomicrograph 6. IHC expression of p-Akt in Gleason's score 4+3=7, Grade group 3 with intensity- score 1, 31-60% positive cells-score 2 (IHC, X100)**



**Photomicrograph 7. IHC expression of p-Akt in Gleason's score 4+4 =8, Grade group 4 with intensity- score 2, 31-60% positive cells-score 2 (IHC, X40)**



**Photomicrograph 8. IHC expression of p-Akt in Gleason's score 4+5=9, Grade group 5 with intensity- score 2, 61-100% positive cells- score 3 (IHC, X100)**



**Photomicrograph 9. IHC expression of p-Akt in Gleason's score 5+5=10, Grade group 5 with intensity -score 3, 61-100% positive cells- score 3 (IHC, X40)**

**ANNEXURE VIII**

**KEY TO MASTER CHART**

Bx	Biopsy
IS	Intraluminal Secretions
No	Number
%	Percentage
PNI	Perineural invasion
PSA	Prostate specific antigen
TRUS	Transrectal ultrasound
TURP	Transurethral resection of prostate

**ANNEXURE IX – MASTERCHART**

Serial no.	Sample No	Age (years)	Biopsy	Gleason's score	Grade group	PSA(ng/ml)	IHC Expression of p-Akt						Pathognomonic Features				
							% of cells	Intensity	Immunoreactivity score	Cytoplasmic staining	Nuclear staining	Membranous staining	PNI	Mucinous fibroplasia	Basophilic intraluminal secretions	Eosinophilic IS	Glomeruloid bodies
1	77/19	85	12 cores	3+4=7	2	69	3	3	9	present	absent	present	absent	absent	absent	absent	absent
2	121/19	77	12 cores	4+3=7	3	13	3	3	9	present	absent	absent	present	absent	absent	absent	absent
3	127/19	75	12 cores	4+5=9	5	1750	3	3	9	present	absent	present	present	absent	absent	absent	absent
4	158/19	79	12 cores	4+4=8	4	707	2	2	4	present	absent	absent	present	absent	absent	absent	absent
5	518/19	77	12 cores	4+3=7	3	353	3	2	6	present	absent	absent	absent	absent	absent	absent	absent
6	1242/19	75	TRUS Bx	5+4=9	5	89	3	2	6	present	present	present	present	absent	absent	present	absent
7	1296/19	66	18 cores	5+4=9	5	88	2	2	4	present	absent	absent	present	absent	absent	absent	absent
8	1293/19	63	12 cores	3+4=7	2	11	3	3	9	present	absent	present	absent	present	absent	absent	absent
9	1317/19	68	12 cores	5+4=9	5	60	3	3	9	present	absent	present	present	absent	absent	absent	absent
10	1574/19	72	12 cores	4+5=9	5	3049	3	2	6	present	absent	absent	absent	absent	absent	absent	absent
11	1729/19	68	TURP	4+3=7	3	60	3	2	6	present	absent	present	absent	present	present	absent	absent
12	2379/19	75	12 cores	4+3=7	3	65	3	3	9	present	absent	present	present	absent	absent	absent	absent
13	2464/19	73	12 cores	5+4=9	5	70	3	3	9	present	absent	present	present	absent	absent	absent	absent
14	2509/19	80	Trucut	4+4=8	4	7	3	2	6	present	absent	absent	present	absent	absent	present	absent
15	2553/19	64	12 cores	4+3=7	3	23	3	3	9	present	absent	present	present	absent	absent	present	absent
16	2570/19	74	12 cores	4+4=8	4	42	3	3	9	present	absent	present	present	absent	absent	present	absent
17	2596/19	58	TURP	3+5=8	4	60	2	2	4	present	absent	absent	present	present	absent	absent	absent
18	2612/19	67	12 cores	4+5=9	5	180	3	3	9	present	absent	absent	present	present	absent	absent	absent
19	2630/19	70	12 cores	3+5=8	4	35	3	2	6	present	absent	absent	present	absent	absent	absent	absent
20	2869/19	75	12 cores	5+4=9	5	39	2	3	6	present	absent	present	absent	present	absent	present	absent
21	2984/19	71	12 cores	4+3=7	3	150	3	2	6	present	absent	absent	absent	present	absent	present	absent
22	3148/19	72	12 cores	3+4=7	2	11	2	2	4	present	absent	absent	absent	absent	absent	absent	absent
23	3198/19	69	12 cores	3+4=7	2	61	3	2	6	present	absent	absent	absent	present	present	absent	absent
24	3391/19	90	12 cores	5+4=9	5	1000	3	2	6	present	absent	absent	present	present	absent	absent	absent
25	3701/19	69	TURP	4+4=8	4	94	3	2	6	present	absent	present	absent	absent	absent	absent	absent

Serial no.	Sample No	Age (years)	Biopsy	Gleason's score	Grade group	PSA (ng/ml)	IHC Expression of p-Akt						Pathognomonic Features				
							% of cells	Intensity	Immunoreactivity score	Cytoplasmic staining	Nuclear staining	Membranous staining	PNI	Mucinous fibroplasia	Basophilic intraluminal secretions	Eosinophilic IS	Glomeruloid bodies
26	3748/19	74	12 cores	5+4=9	5	400	% of cells	Intensity	Immunoreactivity score	Cytoplasmic staining	Nuclear staining	Membranous staining	PNI	Mucinous fibroplasia	Basophilic intraluminal secretions	Eosinophilic IS	Glomeruloid bodies
27	3749/19	65	12 cores	4+4=8	4	438	3	2	6	present	absent	absent	present	absent	absent	absent	absent
28	3815/19	75	13 cores	3+4=7	2	189	3	2	6	present	absent	present	absent	present	absent	present	absent
29	4128/19	74	12 cores	4+3=7	3	80	3	1	3	present	absent	absent	present	absent	absent	absent	absent
30	4543/19	76	Trucut	5+4=9	5	419	3	2	6	present	absent	present	present	absent	present	absent	absent
31	4665/19	85	12 cores	4+4=8	4	36	3	3	9	present	absent	present	present	absent	absent	absent	absent
32	4669/19	50	12 cores	4+4=8	4	204	3	2	6	present	absent	present	present	absent	absent	absent	absent
33	4728/19	77	13 cores	4+3=7	3	34	2	2	4	present	absent	present	present	absent	absent	absent	absent
34	4711/19	73	12 cores	3+4=7	2	70	3	3	9	present	absent	absent	absent	absent	absent	absent	absent
35	18/20	70	12 cores	4+3=7	3	52	3	3	9	present	absent	present	absent	absent	absent	absent	absent
36	324/20	77	12 cores	3+3=6	1	14	3	3	9	present	absent	present	present	absent	present	absent	absent
37	570/20	77	13 cores	4+3=7	3	353	3	3	9	present	absent	present	present	absent	absent	absent	absent
38	708/20	70	12 cores	3+4=7	2	23	3	3	9	present	absent	absent	absent	absent	present	absent	absent
39	827/20	77	12 cores	3+4=7	2	48	3	3	9	present	absent	absent	present	present	absent	absent	absent
40	983/20	60	12 cores	3+4=7	2	14	3	3	9	present	absent	absent	present	absent	absent	present	absent
41	1086/20	70	Trucut	3+4=7	3	47	3	3	9	present	absent	present	present	present	present	absent	absent
42	1301/20	84	Trans rectal Bx	4+3=7	3	37	3	3	9	present	absent	absent	absent	present	absent	absent	absent
43	1308/20	65	TURP	5+5=10	5	51	3	2	6	present	absent	absent	absent	absent	absent	absent	absent
44	1462/20	54	12 cores	4+5=9	5	100	3	3	9	present	absent	absent	absent	absent	present	absent	absent
45	1514/20	82	12 cores	3+3=6	1	39	2	1	2	present	absent	absent	absent	absent	absent	absent	absent
46	1516/20	79	12 cores	4+5=9	5	57	3	2	6	present	absent	absent	present	absent	absent	absent	absent
47	1607/20	86	12 cores	4+4=8	4	38	2	2	4	present	absent	present	present	absent	absent	absent	absent
48	1804/20	82	12 cores	3+4=7	2	8	2	1	2	present	absent	present	absent	absent	absent	absent	absent
49	1805/20	73	12 cores	4+4=8	4	337	3	2	6	present	absent	absent	present	absent	absent	absent	absent
50	2111/20	65	12 cores	4+5=9	5	711	3	3	9	present	absent	absent	present	absent	absent	absent	absent