"STUDY OF IMMUNOHISTOCHEMICAL EXPRESSION OF KI67 IN PROSTATE ADENOCARCINOMA- A CROSS SECTIONAL HOSPITAL BASED STUDY, BELAGAVI"

By

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LIST OF ABBREVIATIONS USED

Sr. No.	Abbreviation	Expansion
1.	H&E	Hematoxylin and Eosin
2.	IHC	Immunohistochemistry
3.	PZ	Peripheral Zone
4.	CZ	Central Zone
5.	TZ	Transitional Zone
6.	PSA	Prostate-Specific Antigen
7.	LUTS	Lower Urinary Tract Symptoms
8.	TURP	Transurethral Resection of the Prostate
9.	TRUS	Transrectal Ultrasound
10.	AR	Androgen Receptor
11.	CK5/6	Cytokeratin 5/6
12.	AMACR	Alpha-Methylacyl-CoA Racemase
13.	HMWCK	High Molecular Weight Cytokeratin
14.	PSAP	Prostatic-Specific Acid Phosphatase
15.	WHO	World Health Organization
16.	AJCC	American Joint Committee on Cancer
17.	NCCN	National Comprehensive Cancer Network
18.	CAP	College of American Pathologists

ABSTRACT

TITLE: Study Of Immunohistochemical Expression Of Ki67 In Prostate Adenocarcinoma- A Cross Sectional Hospital Based Study, Belagavi.

INTRODUCTION: Prostatic adenocarcinoma is one of the most common cancers worldwide and the cause of death of thousands of men every year. Gleason's scoring system is used to grade prostate cancer based on degree of glandular architecture and growth pattern of tumor. Ki67 is a classic cell proliferation marker that can be used to predict the aggressiveness of this cancer. Extent of mortality can be reduced by PSA screening in the elderly.

OBJECTIVE: To study the correlation of Ki67 expression with PSA and histological grading of adenocarcinoma of prostate using Gleason's score and PSA levels.

METHODOLOGY: Specimens received of adenocarcinoma prostate in Department of Pathology at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi over 2 years. 35 cases of prostatic adenocarcinoma were studied and correlated. Ki67 and H and E stained slides were used to detect its expression in tumor area.

RESULTS: This study identifies, 71-80 age group as the most prevalent. PSA levels vary widely, from 0.79 to 5000 ng/ml, with 87.1% showing elevated levels. High Ki-67 index was observed in majority of prostate adenocarcinoma cases and was associated with high Gleason's score and elevated PSA levels.

CONCLUSION: High Ki67 expression is significantly associated with higher Gleason scores and elevated PSA levels, reflecting its potential as a prognostic marker in prostatic adenocarcinoma. Ki67 expression is high with increased Gleason's score reflecting its crucial relationship.

KEY WORDS: Adenocarcinoma Prostate, Ki-67, Gleason's score.

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INTRODUCTION

The prostate was initially described by Venetian anatomist Niccolò Massa in his 1536 work Anatomiae libri introductorius (Introduction to Anatomy), and it was illustrated by Flemish anatomist Andreas Vesalius in his 1538 publication Tabulae anatomicae sex (Six Anatomical Tables).[1] During that period, Du Laurens described what was believed to be a pair of organs rather than a single two-lobed structure.[2] The Ancient Greek word parastatai, which referred to the seminal vesicles, was actually mistranslated as prostatae in Latin.[2] The figurative term prostatae was first used in 1600 by the French physician Du Laurens Prostate cancer was first identified in 1853 by surgeon John Adams in a speech to the Medical and Chirurgical Society of London.[3] Hugh H. Young carried out the first radical perineal prostatectomy, or surgical removal of the prostate for cancer, at Johns Hopkins Hospital in 1904.[4]

Prostate adenocarcinoma is the most common cancer in men in the United States; 174,650 new cases were recorded in 2019, accounting for 20% of all male cancers. .[5]It is the second leading cause of cancer-related deaths in men and accounts for 95% of prostate malignancies.[5] As Asian diets become more influenced by Western eating habits, the incidence of clinical prostate cancer in the region seems to be on the rise.[6] Epidemiological data suggests that adenocarcinoma of the prostate is most commonly observed in Black individuals, followed by White individuals, while Asians have a comparatively lower risk.[6]

Age- Age raises the risk of prostate cancer, with men 65 and older accounting for around three-quarters of cases globally. [7]

The disease develops as a result of both environmental and hereditary factors. The development of prostate cancer is significantly influenced by oestrogen receptors and androgen exposure.[8] Other risk factors include smoking, a high-fat diet, alcohol consumption, obesity, and prostatic intraepithelial neoplasia (PIN). Prostate cancer risk is, however, known to be decreased by green tea, lycopene, soy, and vitamin D.[9] Measuring PSA levels is important but not always specific to prostate cancer. PSA screening can help reduce prostate cancer mortality by enabling early detection.[10] Histopathological grading and diagnosis are crucial in the management of prostate cancer.[10]

Immunohistochemical (IHC) markers like Ki-67 are useful in determining tumor grade. A strong correlation has been observed between a high Gleason score and increased Ki-67 expression. [11]

Based on the tumour's growth pattern in respect to the stroma and the degree of glandular differentiation, prostate cancer is graded using the Gleason method. Tumours graded between 8 and 10 are categorized as poorly differentiated malignancies, and the scale goes from 2 to 10.[12]

AIMS AND OBJECTIVES

Primary

1. To study the expression of Ki67 in Adenocarcinoma prostate.

Secondary

1. To study the correlation of Ki67 expression with histological grading of adenocarcinoma prostate and prostate-specific antigen (PSA) levels where ever available.

REVIEW OF LITERATURE

EMBRYOLOGY

Comprising both glandular and fibromuscular components, the prostate is a hard, pear-shaped retroperitoneal exocrine gland. It weighs about 20 grams and has dimensions of about 4 by 3 by 2 cm in an adult male.[13]

The endodermal urethral epithelium produces a number of tubular outgrowths that eventually become the prostate gland during the twelfth week of embryonic development. The fetal testes production of androgenic hormones influences this process, which takes place at the level of the pelvic part of the urogenital sinus as well as the primitive urethra.[14]

The fibromuscular stroma of the prostate is the result of these outgrowths developing into the surrounding mesenchymal tissue. As the prostatic utricle develops, the remaining fused Müllerian ducts are likewise enclosed by the prostate gland.[13]

Until the twenty-fourth week of pregnancy, when the initially sparse glandular structures become firm cords, this process continues. There are more glands as the pregnancy goes on, and they start to produce lumina.[13]

Rather than the epithelium, there is compelling evidence that the mesenchyme is the main focus of androgenic impact.[14]

In order to guide the development, branching, and anatomical arrangement of the entire ductal system, the mesenchyme plays a crucial inductive role. The degree of

mesenchymal cell proliferation in response to androgenic stimulation thus determines the eventual volume of the prostate gland.[15]

The creation of distinct glands with 30–50 tubules and 15–30 secretory ducts is the outcome of these developmental alterations. These components make up the tubulo-acinar units, which comprise the majority of the prostate gland and are embedded in the fibromuscular stroma.[16] The stromal cells' inductive and proliferative abilities are permanently silenced after birth. The adult epithelium can still react to the right signals, though.[17] Prostate growth is mostly regulated by the androgen secreted by Leydig cells during development.[17]

ANATOMY

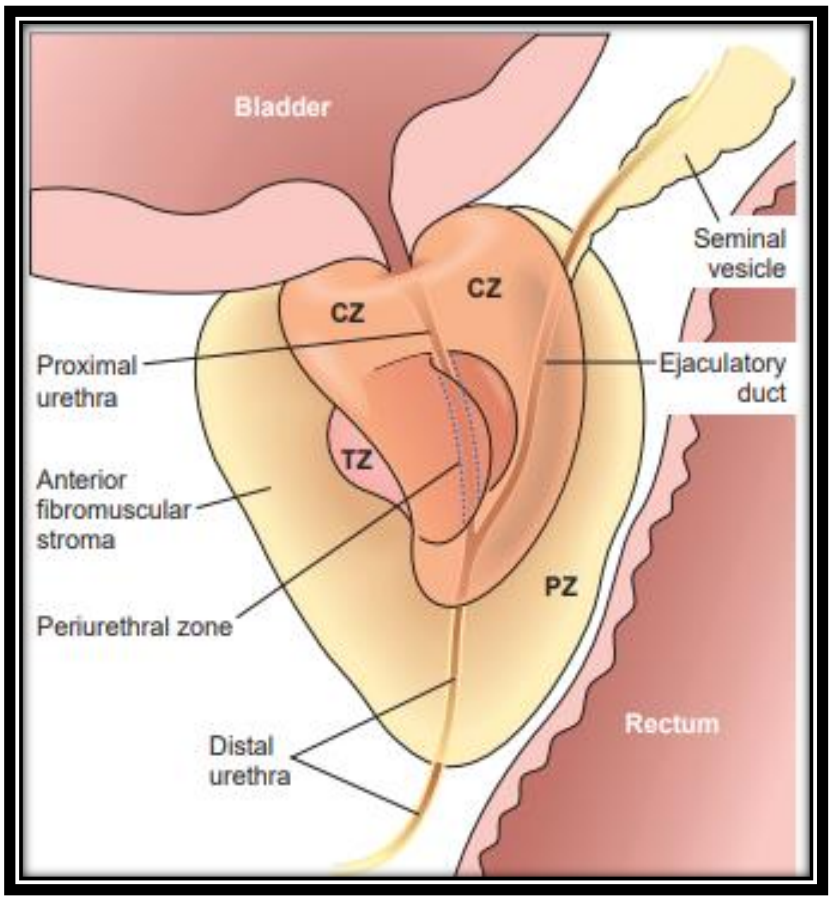


Figure 1: Diagrammatic representation of Anatomy of Normal Prostate Gland [22]

The human prostate gland is one of the male accessory sex organs, along with the bulbo-urethral glands and seminal vesicles.[18] The prostate is a pyramidal fibromuscular gland that encircles the prostatic urethra from the base of the bladder to the membranous urethra.

A robust, slender capsule of connective tissue envelops it.[19] It is palpable behind the neck of the bladder, in front of the rectal ampulla, behind the inferior border of the pubic area and symphysis pubis, and deep within the lesser pelvis. On average, a healthy adult prostate weighs around 20 grams.[19]

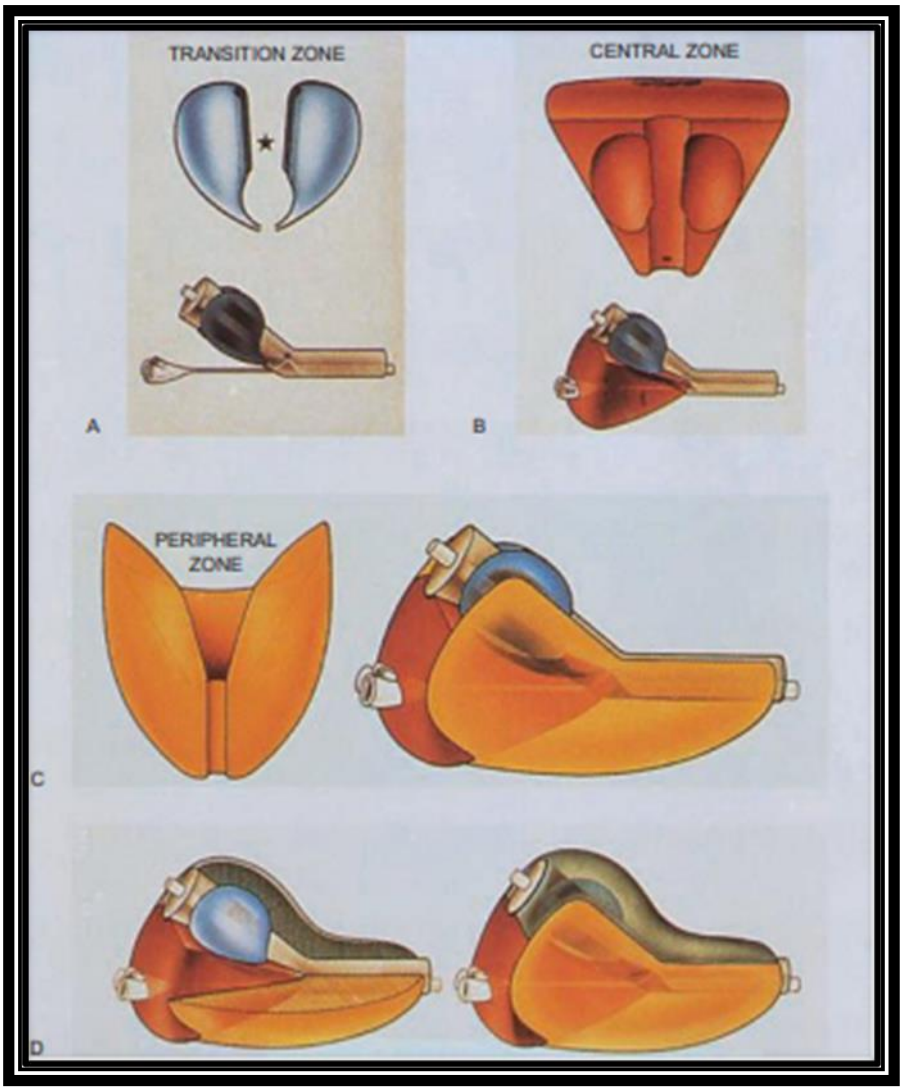
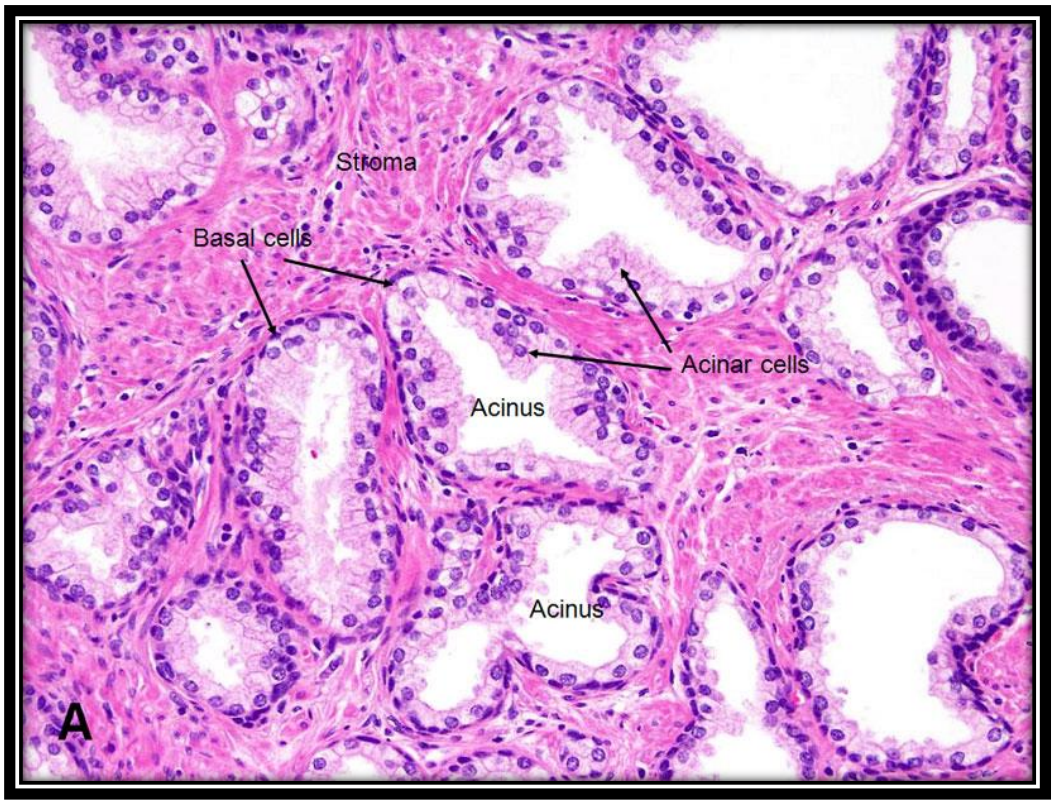


Figure 2: Zonal anatomy of the prostate [23]

The anterior, posterior, median, and two lateral lobes are the five lobes that make up the prostate anatomically. McNeal's findings served as the foundation for the current division of the prostate into various zones.[20] The peripheral zone (PZ), which makes up over 70% of the prostate and is mainly located posteriorly, is the most prevalent location for the development of cancer. McNeal divided the prostate into various zones. Additionally, he recognized the central zone (CZ), which makes up about 25% of the prostate and is situated above the ejaculatory ducts and posterior to the urethra.[20]Furthermore, the most frequent location for benign prostatic hyperplasia (BPH) is the periurethral and transitional zone (TZ), which makes up around 5% of the prostate's volume. An exterior layer of collagen and an interior layer of smooth muscle make up the prostate capsule. The glandular components are few near the apex, and the capsule, which is made up of a mixture of smooth muscle, striated muscle, and fibrous connective tissue, is less distinct.[20] Consequently, rather than being a real capsule, the prostatic capsule is regarded as a pseudocapsule. Accurately evaluating prostatic biopsy specimens from transurethral resection of the prostate (TURP) and Trucut needle biopsy requires knowledge of the anatomical lobes and surgical zones. [20] Branches of the internal pudendal, middle rectal, and inferior vesical arteries give blood to the prostate. The vesical and internal iliac veins are the routes via which venous drainage takes place.[21]The prostatic plexus is made up of the veins that round the prostate and empties into the internal iliac veins. The internal iliac, sacral, and obturator lymph nodes are responsible for the lymphatic drainage of the prostate. [21] Autonomic fibers from the inferior hypogastric plexus innervate the prostate gland, whereas the hypogastric nerve provides sympathetic and the pelvic nerve provides parasympathetic input.[21]

HISTOLOGY [24]

The prostate is composed of both glandular tissue and stroma, which includes fibroblasts and smooth muscle cells. It consists of approximately 70% glandular tissue and 30% fibromuscular stroma. The prostate contains multiple individual glands, organized into 30–50 lobules, which connect to 15–30 secretory ducts that open into the urethra. These ducts form elongated, branching tubular structures that end in rounded acini. The glandular lining consists of three distinct epithelial cell types: secretory cells, basal cells, and neuroendocrine cells.



Photomicrograph 1: Normal Prostate Histology

The cytoplasm of luminal secretory cells is clear to pale, and their shapes vary from cuboidal to columnar. They had positive results for a number of enzymes, including prostate-specific antigen and prostatic acid phosphatase. Furthermore,

melanin, lipofuscin, and acid and neutral mucins may be present in non-neoplastic secretory cells.

Peripherally, between the foundation membrane and the secretory cells, are the glands' basal cells. Their long axis lies parallel to the foundation membrane, giving them a cigar-like shape. These cells have a uniformly dispersed, coarsely granular chromatin pattern. They may also demonstrate nuclear reactivity for the IHC marker p63 or cytoplasmic staining with high molecular weight keratin. They are thought to symbolize the prostate's stem cell compartment.

It is difficult to identify neuroendocrine cells without the use of specific staining techniques since they are unevenly distributed throughout the ducts and acini.

Secretory cells are located along the glandular lumen and typically have a columnar shape, except when they are atrophic. Their nucleoplasm appears dark purple under a microscope. [25]

Basal cells function as a barrier between the secretory cells and the basement membrane. They are composed of low cuboidal epithelial cells and columnar mucus-secreting cells. Their nuclei have a lighter blue appearance and may feature distinct nuclear grooves and vacuoles.[25] They act as reserve (stem) cells, capable of undergoing myoepithelial metaplasia, though they are not classified as myoepithelial cells. When basal cells undergo hyperplasia, they can form multiple layers and exhibit prominent nucleoli, especially in reactive or inflamed prostates. The presence of basal cells helps differentiate benign conditions from well-differentiated adenocarcinomas, which lack these cells.[25] However, conditions such as prostatic intraepithelial neoplasia and intraductal carcinoma may retain focal basal cells, and in some cases, even invasive prostate cancer can exhibit rare basal cells.[25]

PHYSIOLOGY

Numerous hormones, such as androgens, estrogen, progesterone, and prolactin, operate through their distinct receptors to affect the physiological activity, development, and differentiation of prostatic tissue. Within the prostatic nerve plexus, hormones and the dual autonomic sympathetic and parasympathetic nerves directly affect secretions.[26] Both the sympathetic and parasympathetic nervous systems are in charge of ejaculating the seminal fluid into the urethra and the secretory functions of the epithelial cells, respectively. The endocrine hypothalamic-pituitary-testicular-prostatic axis releases hormones that affect the physiologic homeostatic balance of the prostate glands.[26] Progesterone, estrogen, and androgen are among the hormones. The hypothalamus releases luteinizing hormone-releasing hormone, which causes the anterior pituitary to release luteinizing hormone. This then triggers the production of testosterone by the testes' Leydig cells, which is then converted to dihydrotestosterone by the prostatic epithelial cells' 5-alpha reductase enzyme. After entering the cell, dihydrotestosterone attaches itself to the nuclear receptor to promote protein synthesis and cell division. [27]According to research by Jon Kindblom, the non-androgenic hormone prolactin is linked to the etiology of nodular hyperplasia and prostatic cancer as well as the control of prostatic growth and development. The transformation of normal prostatic cells into hyperplastic, pre-malignant, and ultimately malignant cells is currently linked to a pathophysiologically complex multi-factorial process that involves interactions between environmental factors, androgenic receptor modifications, and sequential genetic alteration.[27]

PSA - Prostate Specific Antigen (PSA)- Discovery -1979

The seminal vesicle and prostatic secretory epithelium both secrete PSA, a glycoprotein enzyme.[28]

PSA works by chemically shortening and dissolving the big proteins in semen into smaller molecules over time, which lowers the viscosity of the seminal fluid.[28]

PSA often diffuses into the bloodstream in small amounts. PSA diffuses into extracellular space and causes higher levels in conditions like trauma and prostatic illnesses like cancer. Although it is not specific to prostate cancer, a raised PSA is sensitive to it.[28]

Malignant cells produce less PSA than healthy cells do, yet when PSA leaks from cancer cells, plasma levels remain elevated.[28]

Less than or equal to 4ng/ml is typically regarded as the normal PSA value.[30]

PSA is useful for diagnosing metastatic carcinomas of unknown origin and differentiating prostatic adenocarcinomas from other neoplasms that affect the prostate secondary to the original tumour.[29]Additionally, PSA is useful in ruling out benign prostatic carcinoma mimics including granulomatous prostatitis, Cowper's glands, and nephrogenic adenoma. A member of the human glandular kallikrein family, PSA is a serine protease.[29] Gel-forming proteins found in the seminal fluid serve to capture spermatozoa during ejaculation. PSA enters the seminal plasma via being secreted into the lumina of the prostatic ducts.[29] It diffuses from the luminal cells through the stroma and epithelial basement membranes before entering the serum and passing through the capillary basement membranes. By proteolyzing the gel-forming proteins into smaller, more soluble fragments, PSA dissolves the coagulum and breaks down the seminal clot, releasing the spermatozoa.[29]

The age specific reference ranges [30]

Benign prostatic hyperplasia causes men's prostates to expand as they age. based on blood PSA level measurements in a sizable cohort of males without prostate cancer of various ages

Following are the age specific upper reference ranges for serum PSA are as follows:

2.5 ng/ml for men aged 40–49

3.5 ng/ml for males aged 50–59

For men aged 60-69, 4.5ng/ml

For men aged 70-79, 6.5ng/ml

Prostatic Specimens

Prostatic needle biopsies, Trans Urethral Resection of Prostate (TURP) chips, and occasionally radical and suprapubic prostatectomy are among the specimens obtained.

Prostatic needle biopsy [31]

Typically, prostate needle biopsies are performed as outpatient procedures. Urologists perform biopsies on patients who may have prostatic disease. It is generally accepted that men who have an elevated PSA (>4.0 ng/ml), an abnormal Digital Rectal Examination (DRE), or a PSA velocity (rate of PSA change) >0.4 to 0.75 ng/ml/year should undergo a TRUS guided prostate needle biopsy. [31]Additionally, it is advised that men who had a prior prostate needle biopsy and were found to have high-grade prostatic intraepithelial neoplasia (PIN) or atypia undergo a follow-up biopsy three to twelve months later.[31]

Usually, a spring-action automated biopsy device outfitted with an 18-gauge biopsy needle is used to acquire multiple 1.5 cm prostate biopsy specimens...[31]

Prostate biopsies guided by TRUS have been carried out in a systematic manner in the past.

Six distinct sites' sextant biopsies are sampled and labelled independently. In 1989, Hodge et al. instructed the biopsies to be performed in both hypoechogenic areas (Bx6C) and six conventional quadrants.[32] Out of 136 patients, 62% had prostate cancer, according to this conventional method. Typically thin, biopsies taken with a "biopsy gun" are processed as single cores in separate cassettes.[32] To detect severe lesions, three stages are necessary. Additional slides may show prostate cancer if focal glandular atypia is found in the first three.[32]

Trans Urethral Resection of Prostate (TURP)

TURP is typically performed with a resectoscope. It is a thin metal tube with a wire loop, light, and camera inside. The surgeon inserts the resectoscope into the urethra and uses a light and camera to guide it to the prostate location.33 Following the treatment, a catheter is used to pump liquids into the bladder, which flushes away the excised prostate tissue. To remove obstruction, several pieces are removed from the prostate's core and transitional zones. If the complete specimen is analyzed, carcinoma may be detected in 14% to 19% of TURP, compared to 7% to 8% with limited sample. [33]

Suprapubic Prostatectomy [34]

Rarely, when the prostate is greatly enlarged or when transurethral surgery is not suitable because of other diseases (such urethral illness or bladder diverticula), enucleation procedures are performed. The specimen may be in two or more pieces, but it typically resembles a large apple with a wedge cut off one side. Typically, there are no orienting components. Since the entire prostate is not removed, margin is pointless. Serial slices should be 3 to 4 mm thick, show areas of necrosis or bleeding, and show the parenchyma's color (white/tan, yellow, gray), as well as its consistency

(stiff, hard, soft, indurated). Compared to hyperplastic nodules, cancers may be more hard and yellow.[34]

Send in a minimum of eight cassettes from various locations, such as the left and right lobes, the capsule, the urethra (if identifiable), and any other regions that seem worrisome for tumors. [34]

Radical Prostatectomy

In the modern period, radical prostatectomies are not done. There have been several suggested procedures for presenting tissue, ranging from limited sampling using standard slides to submitting the full specimen in whole mount specimens. The extent, grade, stage, and margin status of the carcinoma should all be assessed using any technique. [34]

To evaluate malignancy or other pathology, prostate samples are commonly paraffin embedded, preserved in 10% formalin, and stained with hematoxylin and eosin (H&E).[34]

COMPLICATIONS OF BIOPSY

The most frequent side effect is persistent hematuria. Additionally, vasovagal episodes and other intraoperative problems may arise. Other concerning symptoms include hematospermia (blood in the semen) and hematochezia (rectal bleeding), albeit the majority go away on their own. [35]

WHO CLASSIFICATION OF PROSTATIC TUMOURS 2016 [36]

1. EPITHELIAL TUMOURS

- Glandular neoplasms
- Acinar adenocarcinoma
- Atrophic

- Psedohyperplastic
- 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

CARCINOMA OF THE PROSTATE

1) Epidemiology

The most prevalent disease in males is prostate cancer, which accounts for 33% of all malignant tumors in men and accounts for 9% of cancer-related deaths, the third-highest rate among men after colorectal and lung cancers. Death and incidence rates have gone up globally, including in India, in recent decades. Older males over 50 are the disease's primary victims.[37]

2) Etiology

The five main factors implicated as aetiologic agents are:

Age :

As one ages, the risk of prostate cancer increases significantly. Clinical illness incidence is modest until age 50, after which it rises quickly. Compared to those aged 50–60, the age group of 70–79 years has the highest incidence of various epithelial malignancies. [38]

Race:

Black Americans are more likely than white Americans to be affected. In contrast to the 50–60% prevalence rate among white people in the US, the prevalence rate among Japanese is between 3 and 4%, and among Chinese in Hong Kong, it is only 1%. [38]

Endocrine factors:

When testosterone enters the gland, it is converted into the more metabolically active form, dihydrotestosterone (DHT), by the enzyme steroid 5alpha reductase type II. The androgen receptor is where DHT and testosterone bind to DNA and activate genes that include androgen-responsive elements, like those that control cell division. Next, the receptor-ligand combination enters the nucleus. [38]

Environmental influences:

Environmental factors (exposure at work) and behavioral factors (sexual life) don't seem to have a big influence. There is no proof that blood type, height, weight, cigarette smoking, alcohol use, or hair distribution are associated with prostatic cancer. [39]

Dietary and Hereditary factors:

Prostate cancer has been strongly linked in studies to consumption of animal products, particularly red meat17, as well as exposure to cadmium and deficiencies in vitamins A and D. Men who have two or more afflicted first-degree relatives are five to eleven times more likely to be at risk.. [39]

Location:

The periurethral region is spared, with the exception of the late stages of the disease, and the majority of cancers found on TURP are in the peripheral zone, whether posteriorly, laterally, or anteriorly. Modern TURP specimens reveal cancer in 8% of cases. [39]

Clinical features [40]:

Prostate cancer is typically clinically silent and does not show any particular symptoms, despite the fact that it typically causes widespread lower urinary tract discomfort.

For example,

1) Lower urinary tract symptoms (LUTS) such as difficulty in voiding, urinary retention, dysuria
2) Poor stream of urine, hesitancy, intermittency.
3) Urinary incontinence, dribbling, etc.

When tumours expand locally, they can cause bleeding, rectal blockage, or pubic pain. Compression of the cord, stiffness, and bone pain are signs of metastatic illness. On occasion, prostate cancer may present as a paraneoplastic condition.[40]

Methods for screening :[41]

1. Rectal examination using digital technology
2. PSA level measurement in the serum
3. Ultrasonic transrectal (TRUS)

DRE detection rates range from 0.8% to 2.7%. Transurethral resection is used to find the majority of carcinomas (93,97). Abnormal PSA increase may be the initial finding in many cases. A PSA value more than 4ng/ml is considered abnormal for a male over 50. There may be hypoechogenicity areas on TRUS. However, in certain instances, no anomalies are observed. It is possible to acquire directed biopsies of aberrant locations. If no anomalies are discovered, a number of sites are routinely biopsied. Both sides, with or without the transition zone, have biopsies taken at the gland's apical and basal midsections.

Histologically, prostate adenocarcinoma is identified by the presence of tiny, irregularly shaped, infiltrative glands that do not have a basal cell layer. This can be verified by immunohistochemical markers such high-molecular-weight cytokeratin (HMWCK) and p63.[42] Usually packed, these cancerous glands lack branching or papillary features and frequently have a fused or angulated appearance. The tumor cells have nuclear atypia, which includes hyperchromasia, larger nuclei, and conspicuous nucleoli, while mitotic figures are typically scarce. Perineural invasion, a crucial diagnostic hint, is one of the defining characteristics, where tumor cells encircle or infiltrate nerve fibers.[43] Depending on the tumour grade, prostate cancer exhibits different architectural patterns. Small, distinct glands that are still comparatively well-formed but devoid of basal cells are a feature of well-differentiated tumours (Gleason pattern 3). Tumours classified as intermediate to poorly differentiated (Gleason pattern 4) exhibit cribriform features, in which malignant cells form vast, irregular glandular gaps with perforations, glandular fusion, or poorly formed glands. An aggressive phenotype is indicated by solid tumour cell sheets, single-cell invasion, or comedonecrosis in high-grade tumours (Gleason pattern 5). [43]

GLEASONS MICROSCOPIC GRADING SYSTEM OF PROSTATIC CARCINOMA

Overview of History:

Figure 3: Donald F. Gleason (1920-2008) [44]

Over 2,900 patients participated in a groundbreaking randomized, carefully monitored, prospective trial conducted by the US Veterans Administration in the 1960s and 1970s that gave rise to the Gleason grading system for prostate cancer. [42]The histological growth patterns (grades) of prostate cancer were described in detail by Dr. Donald Gleason, who also examined the association between these findings and clinical information including staging and prognosis. [44]. The Gleason grading system was approved by the WHO in 2004 for the classification of prostate cancer, and it is now incorporated into the AJCC/UICC staging system and the NCCN guidelines as one of the crucial factors [along with staging and serum prostate specific antigen, or PSA] in treatment decisions.[44]

The 2014 consensus states that the modified Gleason system, which was based on the 2005 consensus and subsequent developments, essentially eliminated Gleason grade 1 and severely limited Gleason pattern 2. In the event that no higher-grade patterns are found, Gleason 3 would be the lowest grade given. Numerous modifications were made to Gleason pattern 3, most notably the relocation of clusters of poorly formed glands and the majority of the original cribriform structures into Gleason 4.[44]

Current International Society of Urological Pathology Modified Gleason System 2005 [47]

Pattern description of adenocarcinoma prostate ISUP 2005[47] for

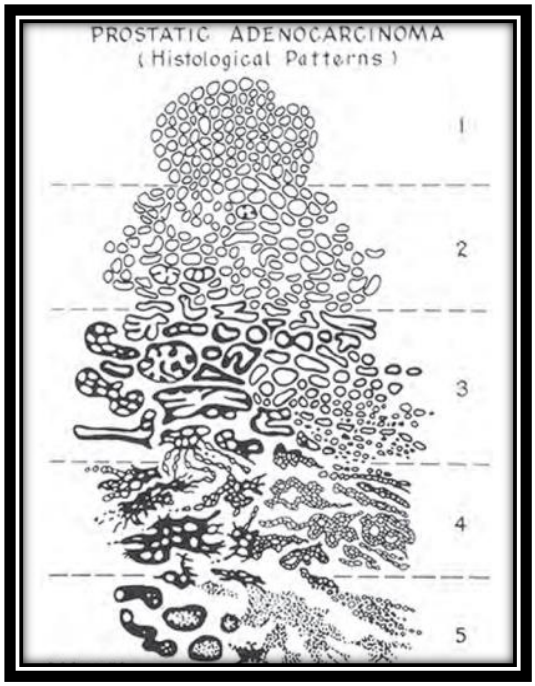
Pattern 1: A circumscribed nodule of homogeneous, rounded to oval, medium-sized, closely packed yet distinct acini (bigger glands than pattern 3).

Pattern 2: Compared to Gleason pattern 1, glands are less uniform and more loosely structured. It is fairly limited, just like pattern 1, but there might not be much infiltration near the edge of the tumor nodule.Various glandular components comprise

Pattern 3. glands that are usually smaller than those found in Gleason patterns 1 or 2. invades and spreads throughout nonneoplastic prostate acini. noticeable differences in shape and size.

Pattern 4: Microacinar glands fused together. Poorly developed glandular lumina and ill-defined glands. Cribriform glands- hypernephromatoid

Pattern 5: Made up of solid sheets, cords, or single cells, there is essentially no glandular differentiation. Comedocarcinoma with solid, cribriform, or papillary masses encircling the central necrosis



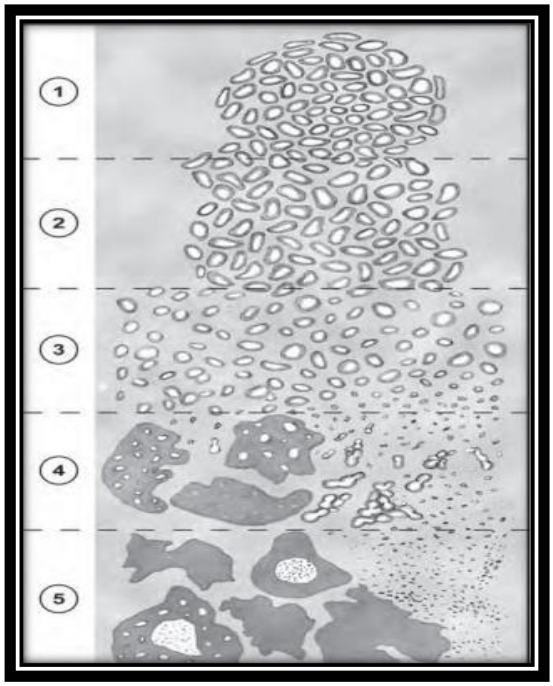


Figure 4: Original grading diagram [46] Figure 5: Modified Gleason Pattern diagram [46]

Gleason Pattern 1 and 2: [47]

Previously, a score of 1+1=2 was used to identify single, separate, closely packed, homogenous spherical glands gathered in a circumscribed nodule with pushing borders.

Gleason Pattern 3:

Single, separate glands

Either minute or large cyst like.

Although they seem badly formed, tangentially cut glands are not scored as 4 unless they are fused and poorly formed on multiple layers.

Patterns –

The most typical pattern is the infiltration of well-formed, somewhat homogenous glands between benign glands.

Small glands with pinpoint lumina

Medium sized glands with undulating luminal contours

Large sized glands with pseudo-atrophic appearance

Gleason Pattern 4:

Coalescent or fused glands with more than one lumen and absence of intervening stroma is key finding.

Patterns –

According to the 2014 consensus, all cribriform carcinoma should be graded Gleason grade 4 because its existence and amount carry a significantly unfavorable prognosis for recurrence and cancer-related death. This contains the Cribriform pattern and tiny acinar structures that fuse into cords or chains, some of which have well-formed lumina. pattern of hypernephroid, with clear cell nests that resemble renal cell carcinoma When combined with invasive cancer, intraductal carcinoma should be classified as Gleason grade 4. With the exception of a single site of attachment, the tuft of cells in the glomeruruloid pattern is isolated from the surrounding duct area.

Gleason Pattern 5:

It is mostly made up of single cells, solid sheets, or cords and does not have glandular differentiation.

Comedonecrosis with solid, cribriform, or papillary masses encircling the central necrosis.

Single cells without a glandular lumen that may form cords (signet ring cells).

To get the Gleason score or sum, the predominant tumor pattern, often known as the "primary," is scored from 3 to 5, and the "secondary" pattern, if present, is graded similarly. The final score is calculated by multiplying the number by two if the tumor exhibits consistent patterns throughout, meaning it only has a "primary" pattern. [47]

Table 1: 2014 Modified Gleason grading and Grade group (CAP Version 4.2.0 November 2021) [48]		
Grade group	Gleason Score	Definition
1	Less than or equal to 6	Only distinct, well-formed glands that are individual.
2	3+4=7	A smaller percentage of poorly formed, fused, or cribriform glands than primarily well-formed ones.
3	4+3=7	Mostly cribriform, fused, or poorly formed glands with a smaller percentage of well-formed glands.
4	4+4=8 3+5=8	Only glands that are cribriform, fused, or poorly developed.
		Mostly well-formed glands with a smaller percentage of necrotic or poorly formed, fused, or cribriform glands.
	5+3=8	A higher proportion of missing or necrotic glands and a lower proportion of well-formed glands.
5	>/=4+5=9 5+4=9 5+5=10	Necrosis or lack of gland formation, with or without poorly developed, fused, or cribriform glands.

IHC Markers for prostatic adenocarcinoma [49] –

Immunohistochemistry (IHC) is essential for diagnosing prostate adenocarcinoma. Common markers include **PSA** and **PSAP**, which are prostate-specific, while **NKX3.1** and **protein (P501S)** help confirm prostate origin. **AMACR (P504S)** is often positive in cancer, while **basal cell markers** like **p63, HMWCK, and CK5/6** are absent in adenocarcinoma but present in benign glands. **ERG** is expressed in some cancers with genetic fusion, and **AR (Androgen Receptor)** is used in metastatic cases

Ki67 [50] –

Ki-67 is a well-known proliferation marker initially identified as an antigen in proliferating cell nuclei. It is not present in resting (G0) cells, but it is expressed in the late G1, S, G2, and M phases. Ki-67 staining typically appears nucleolar or perinuclear and provides a higher labeling index compared to other antibodies, with strong inter-reader reproducibility. While Ki-67 is a recognized prognostic marker, its application in prostate cancer remains limited. It is increasingly used in clinical decision-making to differentiate between indolent and aggressive prostate cancer. However, variations in cutoff values, scoring methods, and interobserver variability present challenges to its routine use. Standardization of Ki-67 assessment is needed to improve its integration into clinical practice for better patient management.[50]

Ki-67 is a key proliferation marker used to assess tumour aggressiveness in prostate adenocarcinoma. It is expressed in actively dividing cells but absent in resting (G0) cells, making it a useful indicator of tumour growth. Higher Ki-67 expression is associated with poor prognosis, including higher tumour grade, increased risk of recurrence, and reduced survival rates. Despite its prognostic significance, variations in scoring methods and cutoff values limit its widespread clinical application. Standardization of Ki-67 assessment could enhance its role in risk stratification and treatment planning for prostate cancer patients. [51]

MATERIALS AND METHODS

This is a hospital based study in which 35 cases of histopathologically diagnosed adenocarcinoma of prostate were studied. This study was done for period of 2 years from January 2023 to December 2024.

Study design: Cross sectional study.

Study population and data collection: All the specimens of adenocarcinoma prostate received at the Department of Pathology, KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, a teaching hospital attached to Jawaharlal Nehru Medical College, Belagavi.

Study Period: 2 years from January 2023 to December 2024 (2 year Prospective study)

Sample Size: 35 cases were taken on the basis of universal sampling technique. Average number of specimens of the included lesions obtained at Histopathology laboratory at JN Medical College and KLE's Dr Prabhakar Kore Hospital, Belagavi was around 35.

Sampling technique: Universal sampling technique.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human Subjects Research

Inclusion Criteria:

All the histologically diagnosed cases of Adenocarcinoma of prostate that include:

1. Needle Biopsies
2. Prostatic chips (TURP)
3. Resected specimens (Prostatectomy).

Exclusion Criteria:

1. Benign lesions of prostate.
2. Metastatic lesions.
3. Improperly preserved samples.
4. Inadequate specimens.

Method of data collection:

Informed consent was obtained from all respective cases and data was collected from Department of Urology. The gross examination of specimen was performed.

Histopathological evaluation:

Tissue specimens of adenocarcinoma prostate obtained were collected and fixed in 10% neutral buffered formalin and processed.

Paraffin embedded blocks were prepared.

Processing of specimen was done in automated tissue processor by Leica TP 1020. Sections were cut using automated rotary microtome set at 3-5 microns and were stained by haematoxylin and eosin. The slides were evaluated and Gleasons

scoring and pattern based on Current International Society of Urology Pathology Modified Gleason System 2019 was done. Immunohistochemistry (IHC) was performed using on formalin-fixed, paraffin-embedded tissue sections (3-5 µm). The primary antibody used in this study was SP6, a rabbit monoclonal antibody known for its specificity in detecting ki67 proliferation marker. The Ki-67 SP6 kit from Vitro Diagnostics was used for detection. The sections were baked, deparaffinized with xylene and alcohol, and antigen retrieval was carried out using Tris-EDTA buffer in a pressure cooker. After blocking endogenous peroxidase activity with 3% hydrogen peroxide, the primary antibody was incubated for 45–60 minutes. After washing, polymer HRP was applied for 25-30 minutes, followed by DAB chromogen for 10 minutes to visualize the antigen. The slides were then counterstained with haematoxylin, blued in warm water, cleared in xylene, and mounted with DPX for microscopic examination.

Immunohistochemical analysis:

Immunohistochemical staining with Ki67 was performed on slides by the standard method described above and slides were evaluated for its expression. All slides were observed on penta-head compound microscope (Labkron).

Immunohistochemical staining evaluation:

The slides were analysed by counting the number of cells in the tumour hot spot area, which is the field of maximum staining. Brown nuclear stain was regarded as positive. Any nuclear staining, regardless of its intensity, was considered as positive.

Ki-67 activity was assessed using the labelling index, which is the proportion of cells that tested positive for Ki-67 out of all the cells that were counted.

The formula for the labeling index is= (Number of Ki-67stained cells / Total number of cells counted) × 100. [52]

Cell counting is performed under high-power magnification (40X) to ensure accuracy in assessing proliferative activity. [52]

Ki-67 expression was categorized into three groups based on established grading criteria: [51]

Low expression was defined as ≤5%, indicating minimal proliferative activity.

Intermediate expression ranged from >5% to ≤10%, representing a moderate level of cellular proliferation.

High expression was classified as >10%, signifying increased tumour aggressiveness. This classification aids in assessing tumour behaviour and potential prognosis.

Data processing and analysis/ statistical analysis:

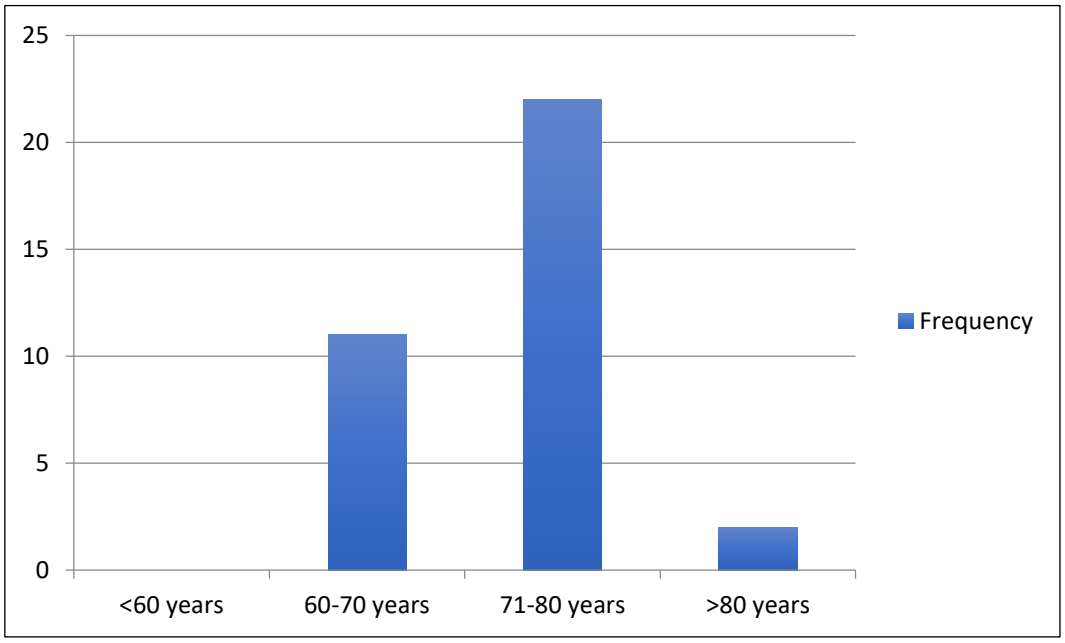
After entering the data into Microsoft Excel, SPSS software (version 27) and the proper statistical procedure were used to analyse it. Averages like mean / median were calculated for quantitative estimates of ki67 expression and clinicopathological parameters. PSA levels were done before the samples was obtained from patient records. Comparative analysis was done using descriptive statistics and chi-square test and p-value was calculated. A p-value of less than 0.05 was regarded as statistically significant.

RESULTS

Table 2: Age distribution of the cases studied

Age group	Frequency (n=35)	Percentage
<60 years	0	0
60-70 years	11	31.43
71-80 years	22	62.86
>80 years	2	5.71
Total	35	100.00
Mean Age	72.74±5.64	

Graph 1: Age group Wise distribution of cases studied.



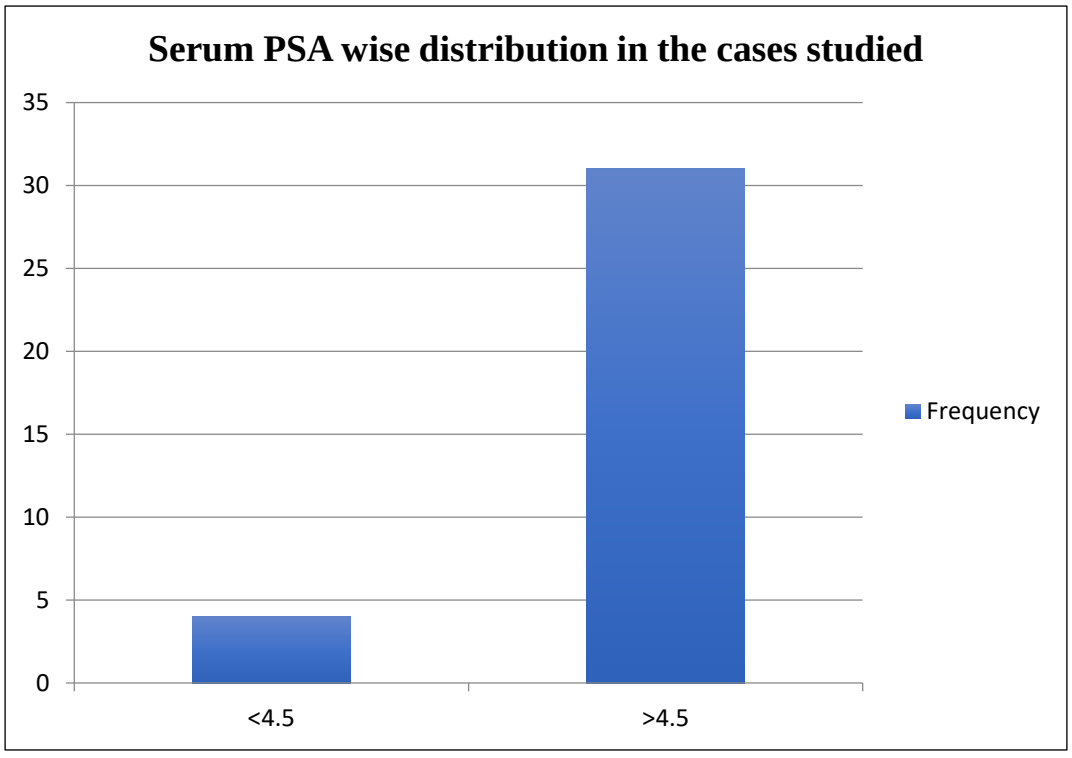
The study included 35 participants, with an average age of 72.74 ± 5.64 years. All subjects were above 60 years old. Most participants (62.86%) were between 71 and 80 years old, while 31.43% were in the 60–70 age group. A small percentage (5.71%) were over 80 years old.

Table 3: Distribution of Serum PSA levels in the cases studied

PSA level ng/ml	Frequency (n=35)	Percentage
<4.5	4	11.43
>4.5	31	88.57
Chi square value	20.82	

*if p<0.05 find significant

Graph 2: Serum PSA wise distribution in the cases studied

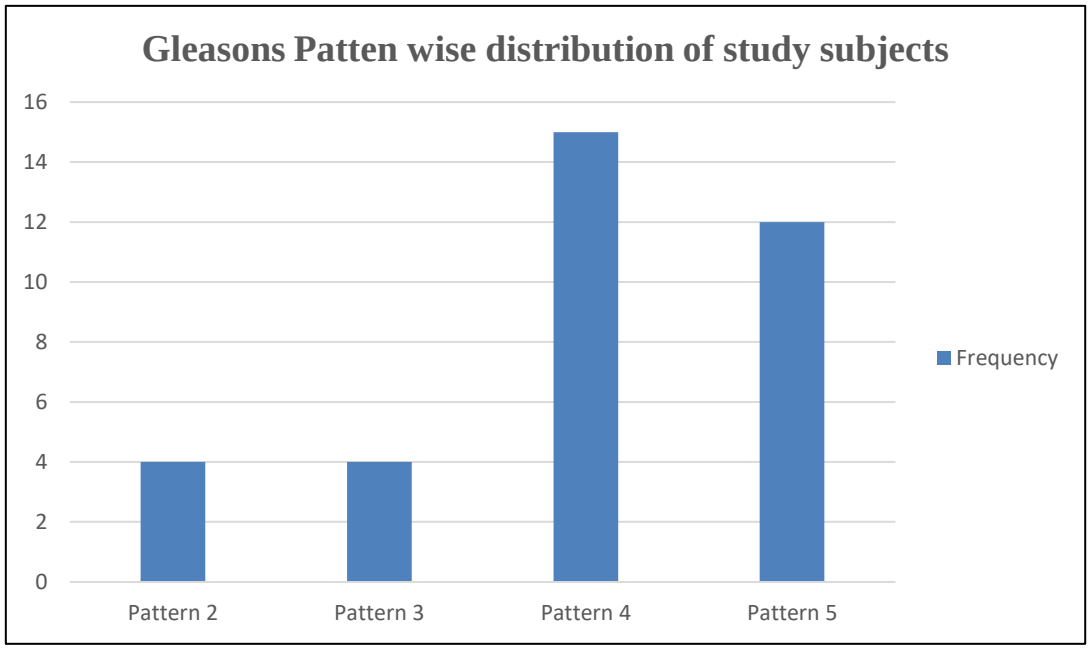


In this study, 88.57% of participants had PSA levels above 4.5 ng/ml, while only 11.43% had levels below this threshold. 31 cases showed elevated serum PSA levels, suggesting that elevated PSA levels are associated with adenocarcinoma of prostate.

Table 4: Gleason Pattern Distribution Among Study Subjects

Gleasons Pattern	Frequency (n=35)	Percentage	Chi square
Pattern 2	4	11.43	10.82
Pattern 3	4	11.43	
Pattern 4	15	42.86	
Pattern 5	12	34.29	

Graph 3: Gleasons Patten wise distribution of study subjects

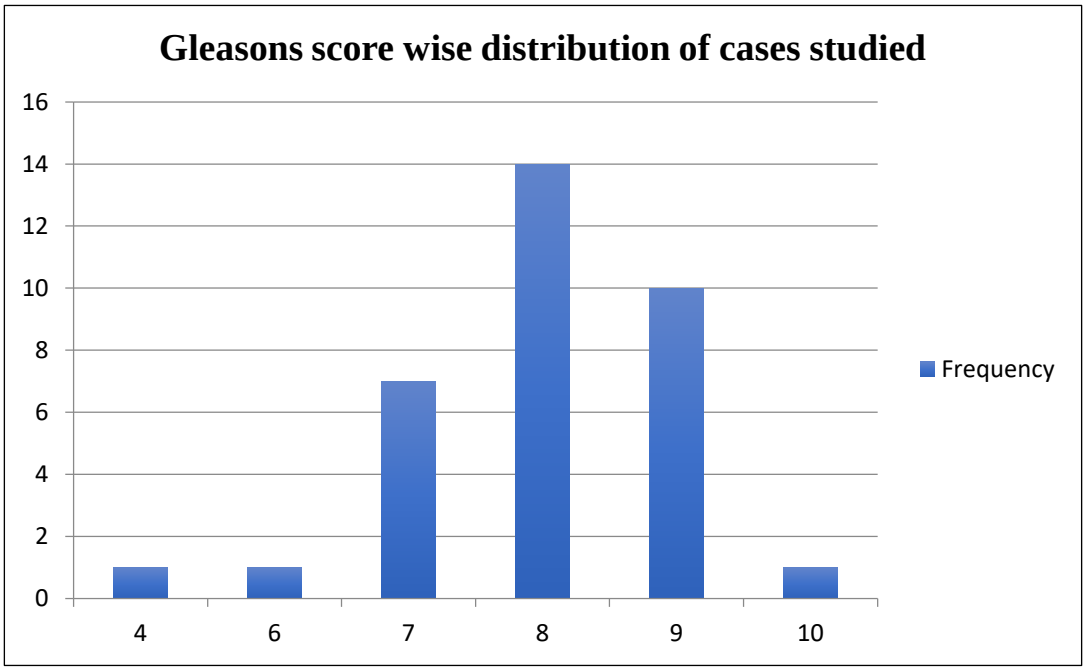


Gleason Pattern helps classify prostate adenocarcinoma based on tumour differentiation. In this study, Pattern 4 was the most common pattern, observed in 42.86% of cases, followed by Pattern 5 in 34.29%. Pattern 2 and 3 were less frequent, each accounting for 11.43% of cases. The distribution of Gleason Patterns in this study highlights the importance of early detection, as higher patterns are linked to more aggressive disease progression.

Table 5: Distribution of Gleason Scores Among Cases Studied

Gleasons score	Frequency (n=35)	Percentage	Chi square
4	1	2.86	29.62
6	1	2.86	
7	7	20.00	
8	15	42.86	
9	10	28.57	
10	1	2.86	

Graph 4: Gleasons score wise distribution of cases studied

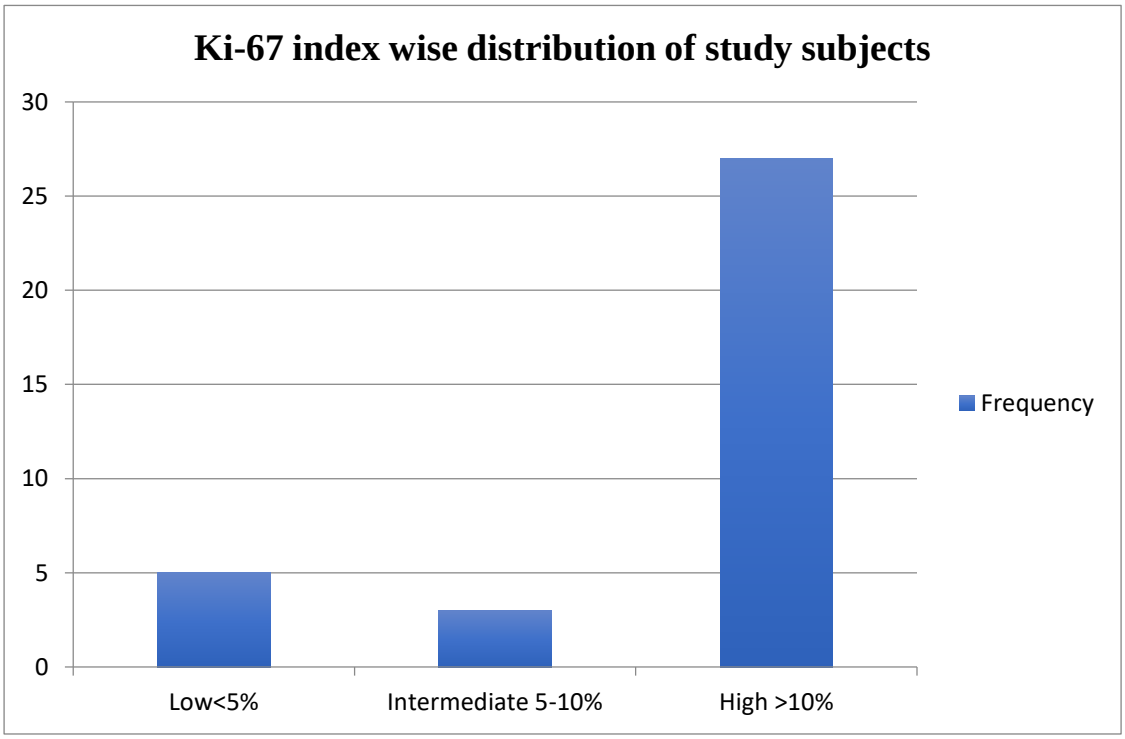


The Gleason score is a crucial grading system used to assess the aggressiveness of adenocarcinoma of the prostate. In this study, the most common score was 8 (42.86%), followed by 9 (28.57%) and 7 (20.00%) showing that most patients had high-risk form of the disease. Lower scores of 4 and 6 were less frequent, each observed in 2.86% of participants. A lower score suggests a less aggressive cancer, while a higher score suggests a more advanced and fast-growing tumour.

Table 6: Distribution of Ki-67 Index Among Cases Studied

Ki-67 index	Frequency(n=35)	Percentage	Chi square
Low<5%	5	14.29	30.4
Intermediate 5-10%	3	8.57	
High >10%	27	77.14	

Graph 5: Ki-67 index wise distribution of study subjects

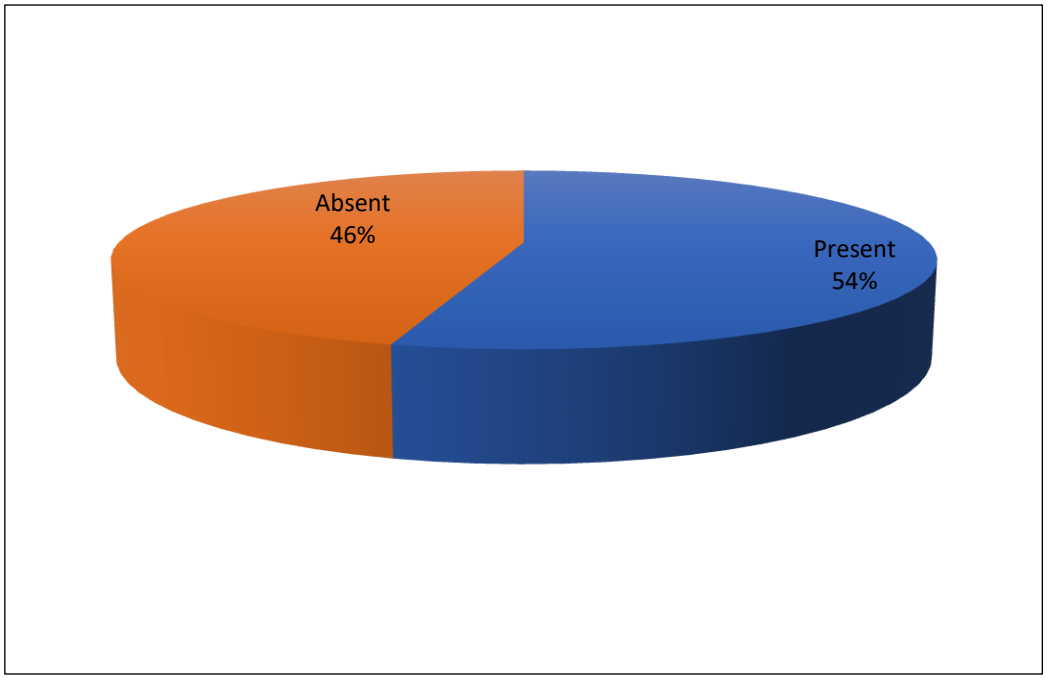


The Ki-67 index, a marker of tumour proliferation, is categorized into three groups. Most subjects (77.14%) have a high Ki-67 index (>10%), while 14.29% fall in the low (<5%) category, and 8.57% in the intermediate (5–10%) range. The findings in present suggest that the majority of subjects exhibit high tumour proliferation activity, indicating aggressive disease.

Table 7: Distribution of Perineural Invasion Among the Cases Studied

PNI (Perineural invasion	Frequency(n=35)	Percentage
Present	19	54.29
Absent	16	45.71

Graph 6: PNI (Perineural invasion) wise distribution of the cases studied



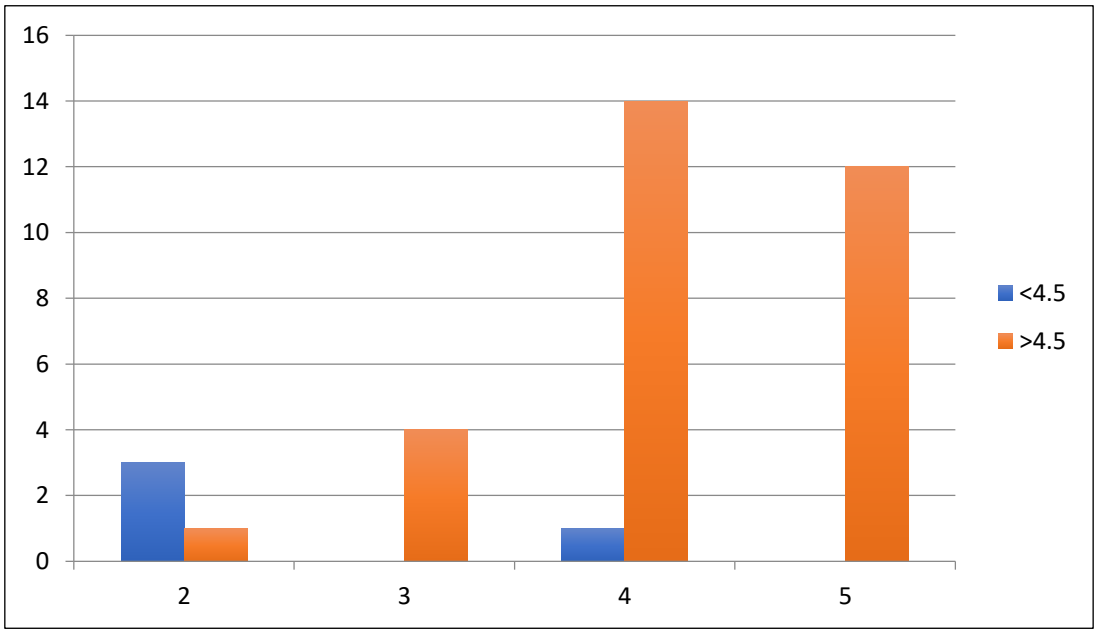
Among the study subjects, 54.29% exhibit perineural invasion, whereas 45.71% do not. This suggests that a significant proportion of subjects have PNI, which is associated with increased cancer progression and poorer prognosis.

Table 8: PSA and Gleasons Pattern wise distribution of cases studied

PSA level ng/ml	Gleasons Pattern	Frequency (n=35)	Percentage	Chi square	P value
<4.5	2	3	75.00	30.4	<0.001*
	4	1	25.00		
>4.5	2	1	3.23		
	3	4	12.90		
	4	14	45.16		
	5	12	38.71		

*if p<0.05 find significant

Graph 7: PSA and Gleasons Pattern wise distribution of cases studied



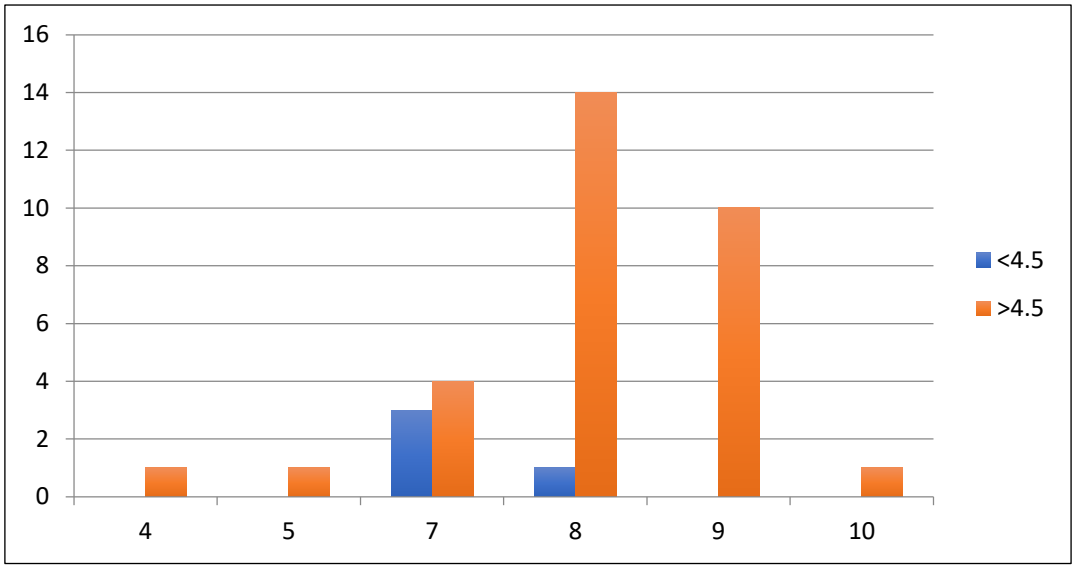
The Gleason pattern distribution changes according to PSA levels. With PSA levels < 4.5 ng/ml, 75% of subjects have Gleason pattern 2, and 25% have Pattern 4. However, the distribution is more varied among patients with PSA levels above 4.5 ng/ml, with Pattern 4 accounting for 45.16% and Pattern 5 for 38.71%. Gleason's pattern and PSA levels are strongly correlated, as seen by the chi-square test result of 30.4 and a very significant p-value of <0.001 (p<0.05).

Table 9: Comparison of Gleason Scores and PSA Levels

Gleasons score	PSA<4.5	>4.5	Chi Square	P value
4	0	1	8.84	0.115
5	0	1		
7	3	4		
8	1	14		
9	0	10		
10	0	1		

*if p<0.05 find significant

Graph 8: Gleasons score and PSA wise distribution of cases studied



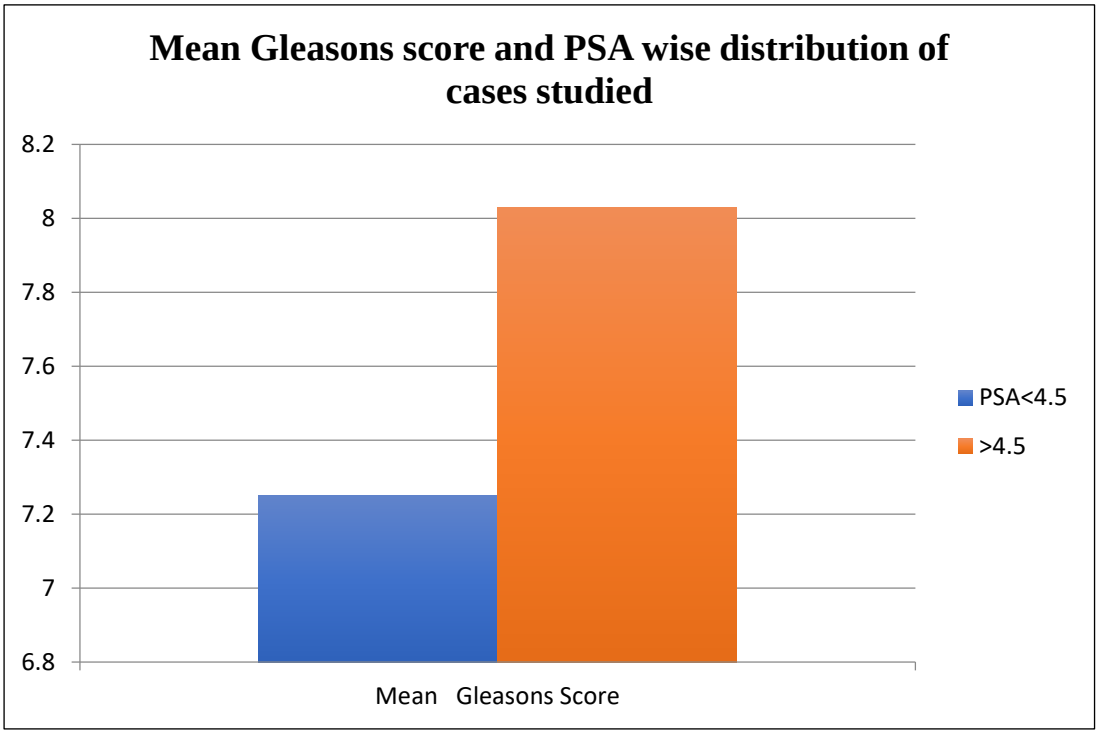
The Gleason score distribution differs for patients with PSA values above and below 4.5 ng/ml. In individuals with PSA values above 4.5 ng/ml, higher Gleason scores (8 and 9) are more frequently seen, but lower scores (4 and 5) are less common in this population. There is no statistically significant correlation between PSA levels and Gleason scores, despite this pattern, according to the chi-square value of 8.84 and the p-value of 0.115.

Table 10: Mean Gleason Score Distribution in Relation to PSA Levels

Gleasons Score	PSA<4.5 (n=4)	PSA>4.5 (n=31)	t value	P value
Mean	7.25	8.03	1.28	0.208
SD	0.5	1.19		

*if p<0.05 find significant

Graph 9: Mean Gleasons score and PSA wise distribution of cases studied



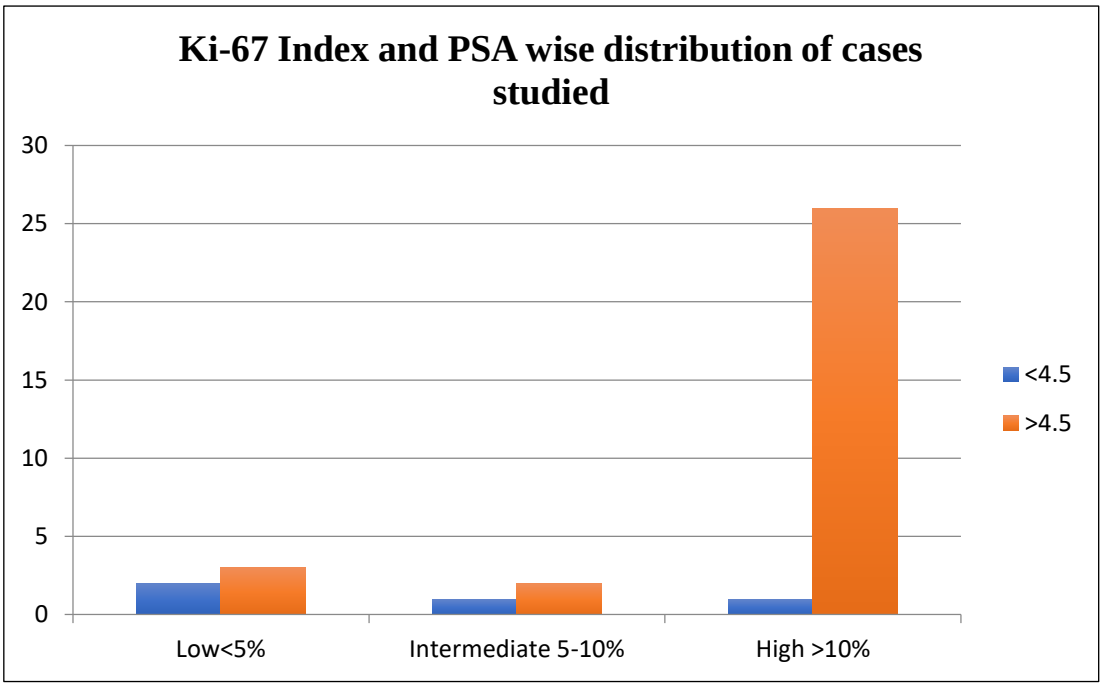
The mean Gleason score is slightly higher in patients with PSA levels >4.5 ng/ml (8.03 ± 1.19) compared to those with PSA <4.5 ng/ml (7.25 ± 0.5). However, t-value of 1.28 with p-value of 0.208 indicate no significant difference between the two groups.

Table 11: Correlation Between Ki-67 Index and PSA Levels

Ki-67 Index	PSA<4.5	PSA>4.5	Chi Square	P value
Low<5%	2	3	7.04	0.02*
Intermediate 5-10%	1	2		
High >10%	1	26		

*if p<0.05 find significant

Graph 10: Ki-67 Index and PSA wise distribution of cases studied



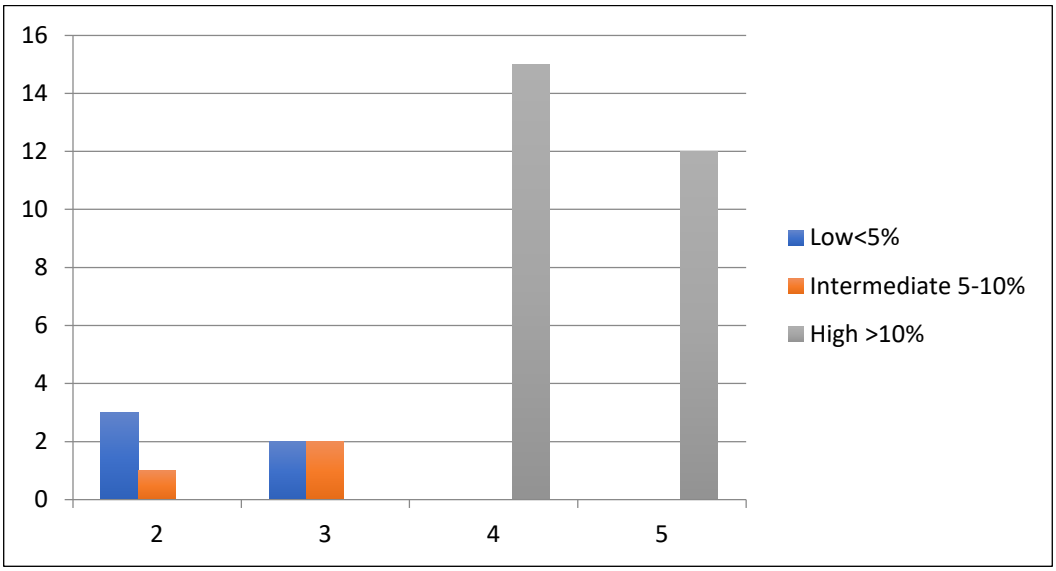
The distribution of Ki-67 index across PSA levels shows that patients with a low Ki-67 index are more likely to have PSA levels below 4.5 ng/ml. In contrast, those with intermediate or high Ki-67 indices predominantly have PSA levels above 4.5 ng/ml. The chi-square test result of 7.04 and a significant p-value of 0.02 suggest a meaningful correlation, indicating that higher PSA levels are associated with increased tumour proliferation.

Table 12: Analysis of Ki-67 Index in Relation to Gleason Patterns

Ki-67 Index	Gleasons Pattern	Frequency	Percentage	Chi square	P value
Low<5%	2	3	60.00	0.61	0.735
	3	2	40.00		
Intermediate 5-10%	2	1	3.33		
	3	2	6.67		
High >10%	4	15	50.00		
	5	12	40.00		

*if p<0.05 find significant

Graph 11: Ki-67 Index and Gleasons Pattern wise distribution of cases studied



The analysis of Ki-67 index and Gleason pattern shows that among patients with a low Ki-67 index, 60% have Pattern 2, while 40% have Pattern 3. In the intermediate Ki-67 group, 3.33% have Pattern 2, and 6.67% have Pattern 3. Among those with a high Ki-67 index, the majority (50%) have Pattern 4, and 40% have Pattern 5. The p-value (0.735) and chi-square value (0.61), however, show no discernible correlation between the Gleason pattern and the Ki-67 index.

Table 13: Correlation between Ki-67 and Gleasons score

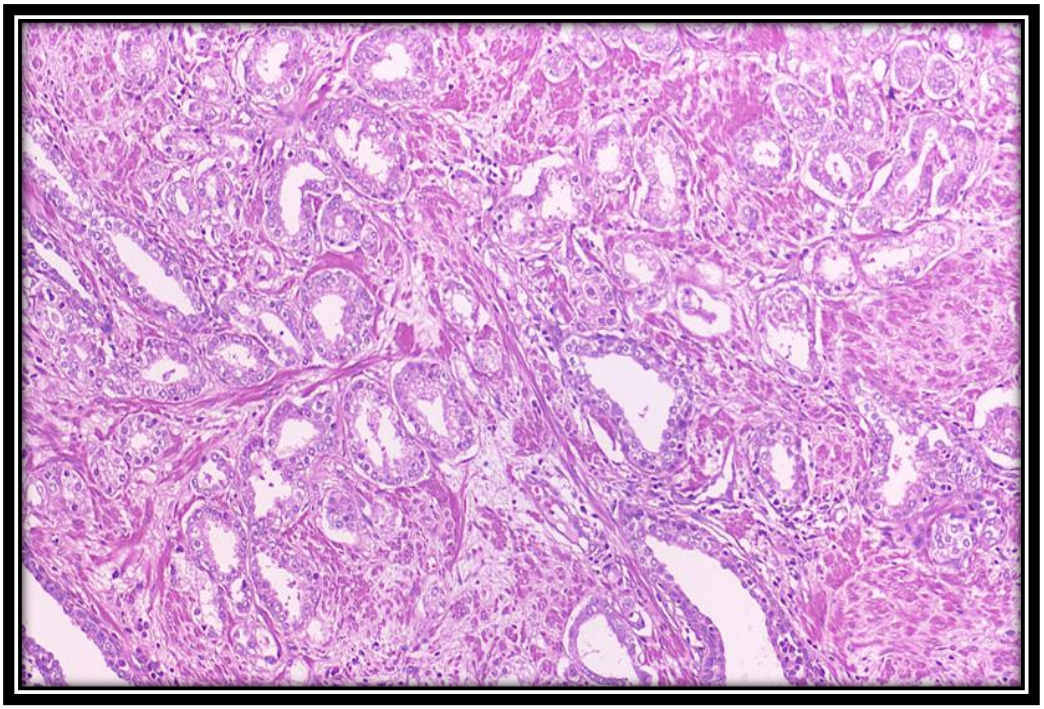
Ki-67	Gleasons score	Frequency	Percentage
Low (>5%)	7	5	14.28
Intermediate (>5% and <10%)	7	2	5.71
	8	1	2.85
High (>10%)	4	1	2.85
	5	1	2.85
	8	12	22.85
	9	9	25.71
	10	1	2.85

The data shows that higher Ki-67 levels are associated with higher Gleason scores, indicating a potential correlation between increased tumour proliferation and more aggressive prostate cancer.

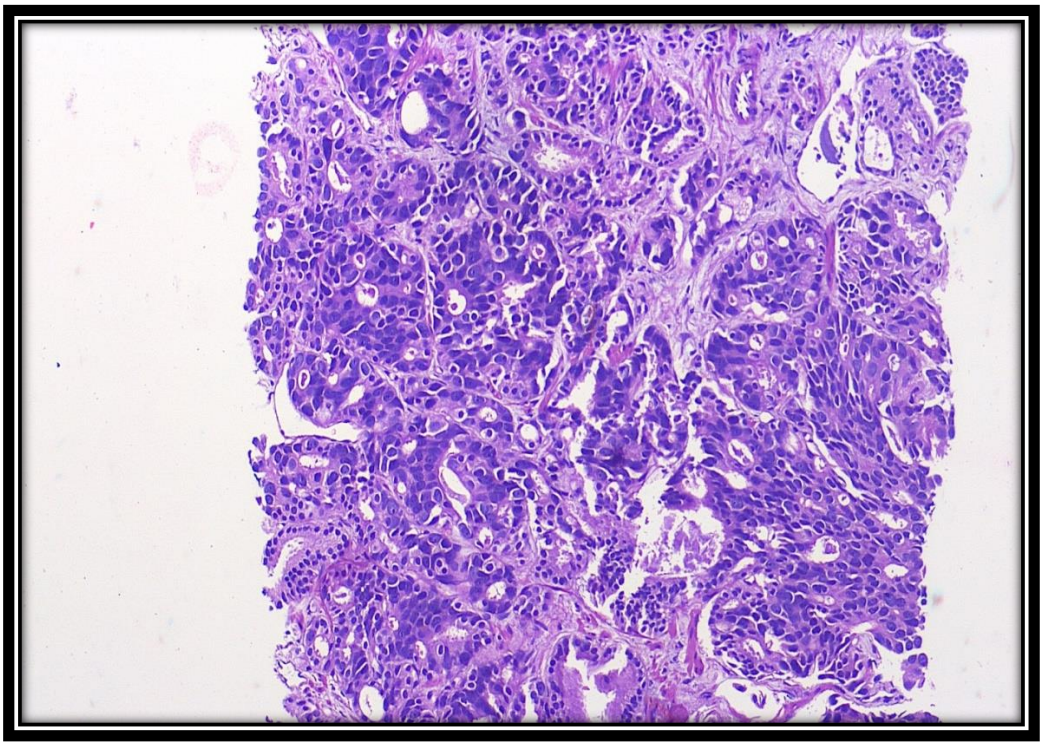
The "Low" Ki-67 category (≤5%) is primarily observed with Gleason scores of 7, suggesting that lower proliferation rates are more common in moderately aggressive tumours.

The "Intermediate" Ki-67 category (>5% and <10%) is rare, appearing in only a few cases with Gleason scores of 7 and 8, implying a transitional phase in tumour aggressiveness.

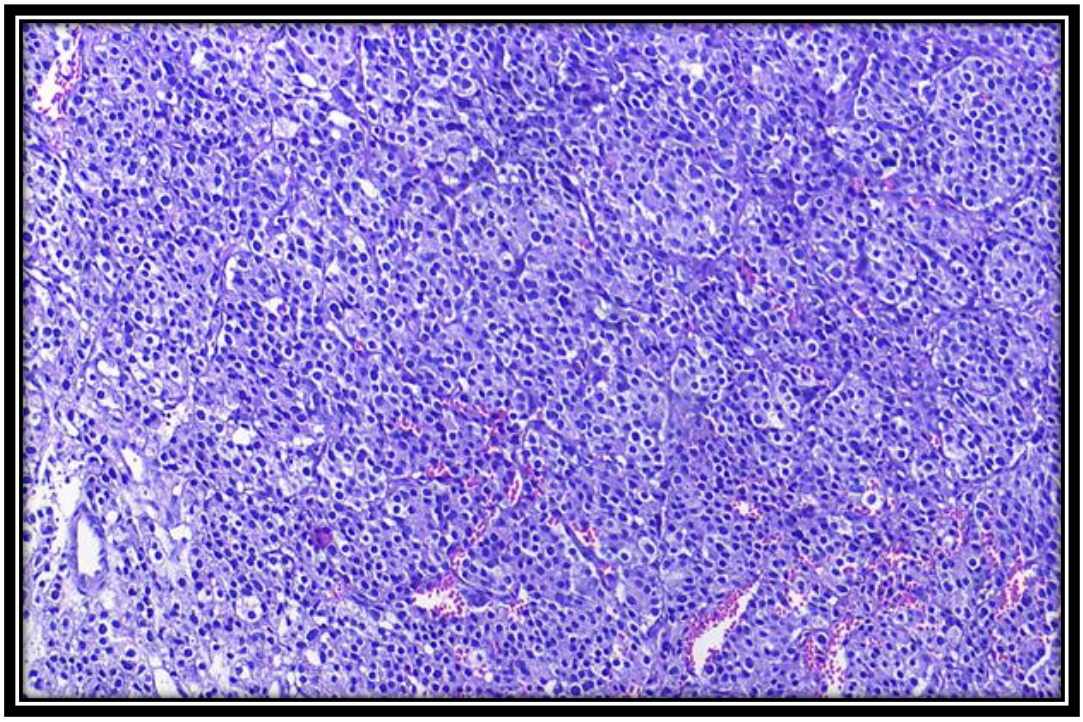
The "High" Ki-67 category (>10%) is predominantly linked to higher Gleason scores (8–10), particularly with Gleason 8 and 9, which together account for nearly 50% of cases, reinforcing the association between high proliferation and aggressive disease. According to the findings of the Chi-Square test, the p-value is 0.00013 and the chi-square statistic is 22.91. This suggests that there is a substantial correlation between more aggressive prostate cancer (higher Gleason scores) and higher Ki-67 expression, as there is a statistically significant correlation between the two ($p < 0.05$).



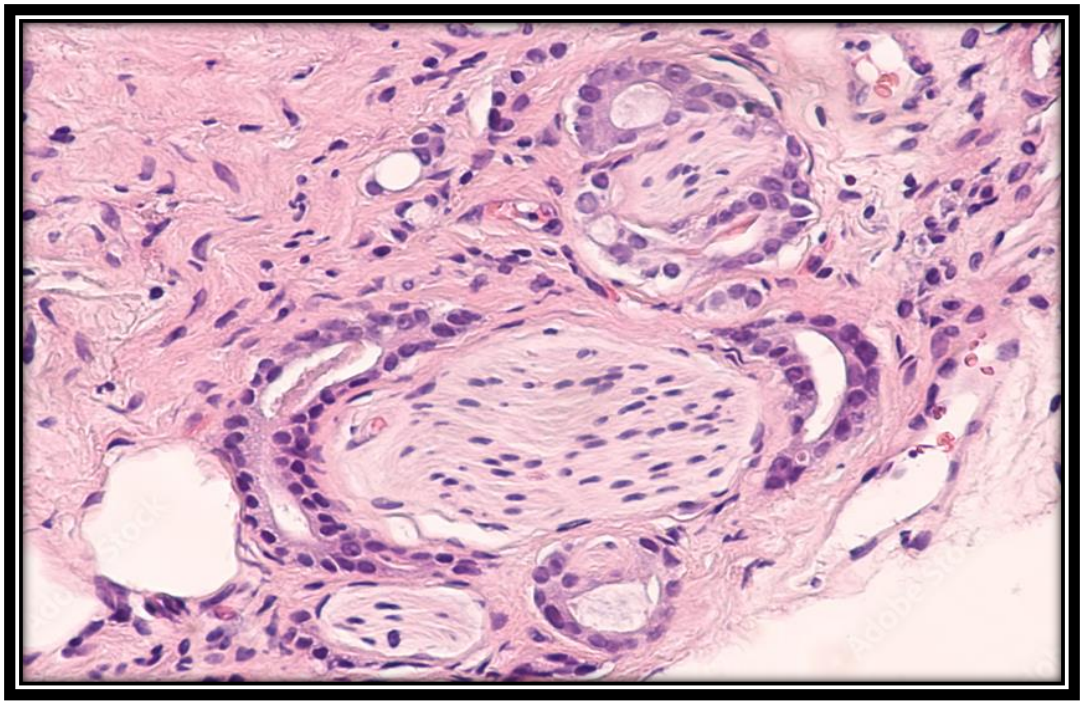
Photomicrograph 2: Gleason's Pattern 3 of prostatic biopsy specimen in 20X



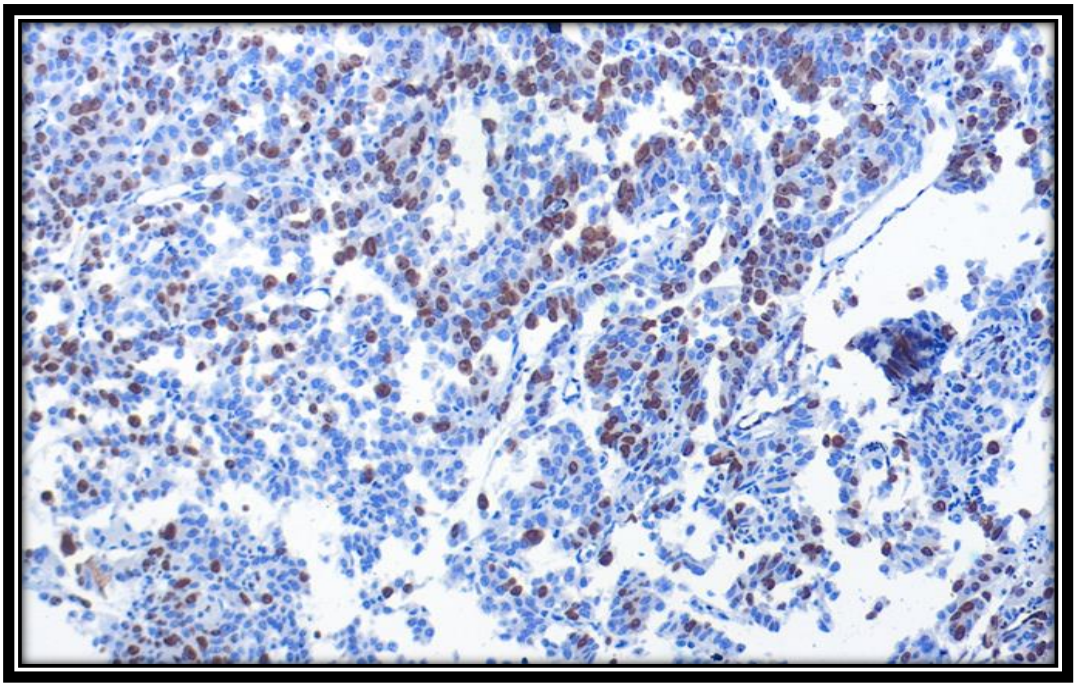
Photomicrograph 3: Gleason's Pattern 4 in 40X in TURP chips. The most common in present study



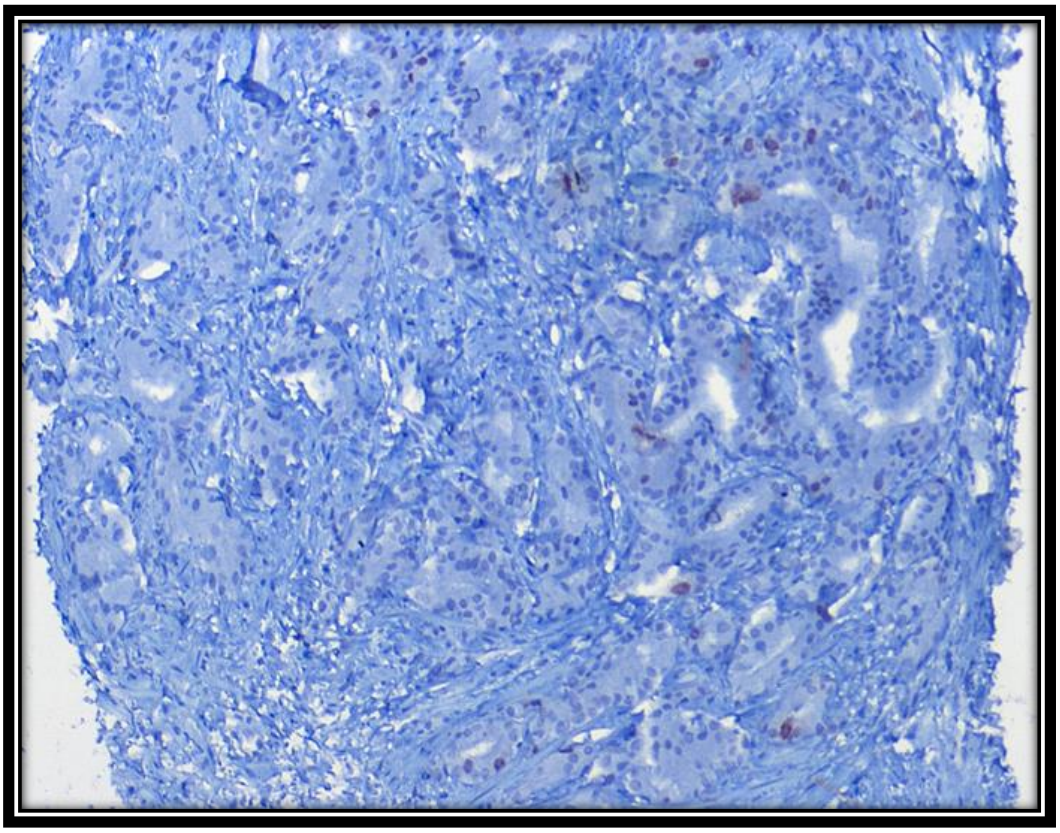
Photomicrograph 4: Gleason's Pattern 5 in proctectomy specimen in 40X



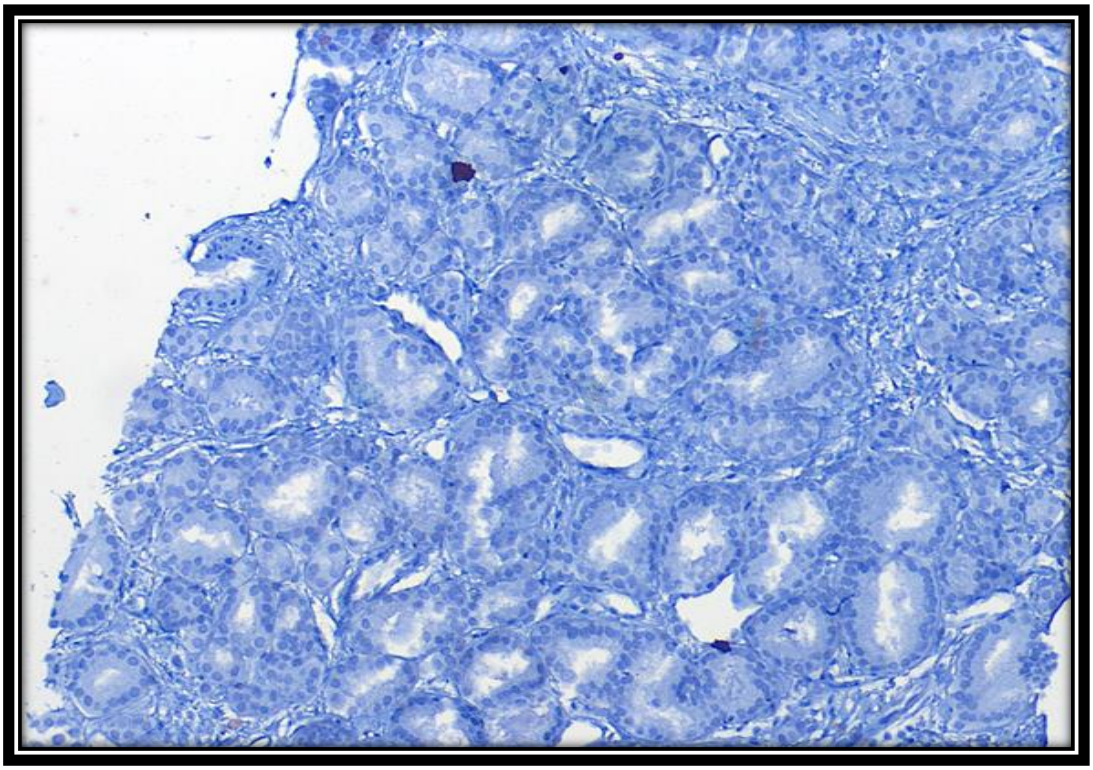
Photomicrograph 5: Perineural Invasion seen under 40X magnification



Photomicrograph 6: High Ki67 Index (>10 %)



Photomicrograph 7: Intermediate Ki-67 Index (5-10% Positivity)



Photomicrograph 8: Low Ki67 Index <5% Positivity

DISCUSSION

One of the most prevalent cancers in men identified globally, prostate carcinoma contributes significantly to cancer-related death.[53] The disease primarily affects older males, with risk factors including age, genetic predisposition, and environmental influences.

Prostate-specific antigen (PSA) levels, histopathological grading using the Gleason score, and tumour proliferation markers such as Ki-67 are crucial in assessing disease severity and guiding treatment decisions. Understanding the correlations between these factors can aid in predicting prognosis and optimizing patient management.[54]

Adenocarcinoma of the prostate is a significant concern in elderly populations (71-80 years in present study), often diagnosed through elevated PSA levels and histopathological grading.[55] This study examines the clinical and pathological characteristics of prostate adenocarcinoma, emphasizing PSA levels, Gleason scores, Ki-67 indices, and perineural invasion (PNI).

The majority of the study population (88.57%) exhibited PSA levels above 4.5 ng/ml, reinforcing its association between elevated PSA and prostate malignancy. Given PSA's role in screening, these findings align with existing literature that associates higher PSA levels with increased risk of prostate cancer detection and progression.

PNI was observed in 54.29% of subjects, indicating a substantial proportion of cases with potential for aggressive disease and perineural spread. PNI is well-

documented in literature as a key factor in prostate cancer progression and recurrence, further validating its prognostic importance in this study.

Gleason scores provide crucial prognostic insights. In this study, the predominant Gleason score was 8 (42.86%), followed by 9 (28.57%) and 7 (20.00%). These findings align with known trends in advanced prostate cancer, where higher Gleason scores correlate with worse prognoses. Despite the observed trends, no statistically significant association was found between PSA levels and Gleason scores (chi-square 8.84, p=0.115). Similarly, the mean Gleason scores for PSA <4.5 ng/ml (7.25) and PSA >4.5 ng/ml (8.03) were not significantly different (p=0.208). This suggests that while PSA is a useful screening tool, it may not reliably predict histological aggressiveness, necessitating complementary markers for prognosis.

A significant relationship was found between PSA levels and Gleason patterns (chi-square 30.4, p<0.001), with higher PSA levels corresponding to higher tumour pattern (Gleason Pattern 4 and 5). Additionally, PSA levels were significantly associated with Ki-67 proliferation index (chi-square 7.04, p=0.02), suggesting that elevated PSA correlates with increased tumour proliferation, reinforcing its role as a marker of disease severity. However, Ki-67 did not show any statistical association with Gleasons pattern. Although, pattern 4 and 5 were seen in majority of the cases and showed High ki67 index.

Histological grading revealed that Pattern 4 (42.86%) and Pattern 5 (34.29%) were most common. The presence of higher Gleason patterns (4 and 5) correlates with increased tumour aggressiveness and poorer clinical outcomes.

Ki-67, a key marker of tumour proliferation, was significantly elevated in 77.14% of subjects (>10%). High Ki-67 expression is widely recognized as an indicator of rapid tumour growth and poorer prognosis, further emphasizing the aggressive nature and poor prognosis of prostate adenocarcinoma in this study.

Smith et al (2021) – Studied 50 prostate carcinoma patients and found that 85% had PSA levels above 4.0 ng/ml. While they reported a weak but significant correlation between PSA and Gleason score (p=0.048), our study found no significant correlation (p=0.115), suggesting PSA's prognostic value may vary among cohorts.[57]

Lee et al (2020) – Conducted a meta-analysis of 15 studies on PSA and prostate cancer detection. They concluded that while PSA >4.0 ng/ml is a sensitive marker for detecting prostate carcinoma, its specificity for predicting Gleason score remains inconsistent, aligning with our findings.[58]

Williams et al (2021) – Investigated PSA levels and tumour stage in 80 prostate cancer patients. They found that PSA >10 ng/ml was significantly associated with advanced tumour stages (T3 and T4), reinforcing PSA’s predictive value.[59]

Sharma et al (2023) – Found no statistically significant association between PSA levels and Gleason patterns (p=0.234), similar to our findings (p=0.115).[60]

Patel et al (2022) – Examined 100 prostate cancer cases and found that Gleason Grade 4 (45%) and Grade 5 (32%) were most common, closely matching our findings (42.86% and 34.29%). They also noted that higher Gleason patterns correlated with advanced disease stages.[61]

Zhang et al. (2021) – Reported perineural invasion (PNI) in 58% of cases, comparable to our study's 54.29%. They also emphasized that PNI presence was linked to increased biochemical recurrence, highlighting its prognostic value.[62]

Johnson et al (2022) – Analysed 60 prostate cancer cases and found that 70% of those with Gleason score ≥8 had a high Ki-67 index (>10%), aligning with our study (77.14%) and reinforcing Ki-67's role in tumour aggressiveness.[63]

Thomas et al (2023) – Reported that high Ki-67 expression (>10%) was associated with increased metastasis risk. Although our study did not assess metastasis, the high Ki-67 prevalence in our cohort supports its role as an aggressiveness indicator.[64]

Singh et al (2002) A Study in Eastern Uttar Pradesh – Found a significant association between Ki-67 and Gleason grade but no correlation between Ki-67 and PSA levels.[65]

Jha et al. (2024) A study in the Himalayan Foothills – Analysed 102 biopsy specimens and found significant links between Ki-67 expression and clinicopathological factors, further validating its role in risk stratification and treatment decisions.[66]

Table 13: Comparison of the below mentioned studies with the present study

Study	Age (years)	PSA levels	Gleasons Pattern	Gleasons Score	Ki-67 levels	Significant Correlation
Smith et al.[57] (n=50)	65.4 ± 6.2	85% > 4.0 ng/ml	-	Significant correlation (p=0.048)	-	Present (PSA & Gleason Score)
Lee et al.[58] (n=15)	-	PSA >4.0 ng/ml common	Not consistent	Not consistent	-	Not present (PSA & Gleason Score)
Williams et al.[59] (n=80)	68.0 ± 6.8	PSA >10 ng/ml = Advanced Tumour Stages	-	-	-	Yes (PSA & Tumour Stage)
Sharma et al[60] (n=100)	69.8± 5.7	-	-	-	Significant correlation with PNI	Yes (Ki67 and PNI)
Patel et al.[61] (n=100)	66.3 ± 5.9	-	Pattern 4 (45%), Pattern 5 (32%)	High correlation with advanced disease	-	Yes (Gleason Grade & Disease Stage)
Zhang et al.[62] (n=171)	68.5 ± 6.4	-	-	-	-	Yes (PNI & Recurrence)
Johnson et al.[63] (n=60)	67.8 ± 5.7	-	-	70% ≥ 8	70% >10%	Yes (Gleason Score & Ki-67)
Thomas et al.[64] (n=199)	70.2 ± 5.5	-	-	-	High Ki-67 linked to metastasis	Yes (Ki-67 & Metastasis)
Singh et al[65] (n=50)	68.2 ± 6.5	-	-	Significant association	No correlation with PSA	Yes (Ki-67 & Gleason Pattern)
Jha et al[66] (n=102)	69.5 ± 5.8	-	-	Significant correlation	Ki-67 significant for prognosis	Yes (Ki-67 & Prognosis)
Present study	72.74 ± 5.64	88.57% >4.5 ng/ml (p=0.000)	Pattern 4 (42.86%), Grade 5 (34.29%)	Gleason Score 8 (42.86%), 9 (28.57%)	High Ki-67 (>10%) in 77.14% (p<0.001)	Yes (Ki67 correlated with PSA level, Gleasons score)

SUMMARY

The The present study was done over a span of two years from January 2023 to December 2024 in the Department of Pathology, KLE's Jawaharlal Nehru Medical College and Dr Prabhakar Kore Hospital, Belagavi.

- A total of 35 cases of prostatic adenocarcinoma were studied, with the highest prevalence observed in the 71–80year age group.
- Majority (88.57%) of patients had PSA levels above 4.5 ng/ml, indicating a higher risk of prostate adenocarcinoma.
- Gleason's Pattern 4 (42.86%) and Pattern 5 (34.29%) dominated the study, indicating poor prognosis.
- Gleason scores of 8 (42.86%) was the most common followed by Gleasons score 9 (28.57%), and 7 (20.00%) indicating a high prevalence of aggressive tumours.
- PNI was observed in 54.29% of cases, indicating a higher risk of disease progression.
- Ki-67 index > 10 was observed in 77.14% cases, demonstrating high tumour proliferation and aggressive nature of the tumour.
- PSA levels correlated with Gleason pattern ($p<0.001$) and Ki-67 index ($p=0.02$), reinforcing its role as a marker of disease severity. No significant association was found between PSA levels and overall Gleason scores ($p=0.115$), suggesting PSA alone may not reliably predict tumour aggressiveness. These findings highlight the importance of using multiple biomarkers for prognosis rather than relying solely on PSA.

- This study showed correlation of the Ki-67 index with PSA levels and Gleason's score, but not with Gleason's pattern, indicating that Ki-67 is more closely associated with overall tumour aggressiveness and prognosis rather than specific architectural patterns.

- This study contributes to understanding prostate adenocarcinoma's biological behaviour, aiding in improved diagnostics and treatment strategies.

CONCLUSION

From our study results, we can conclude that,

- A Our study found that a higher Ki-67 index was associated with elevated PSA levels and a higher Gleason score. Elevated serum PSA levels was associated with higher Gleason patterns and an increased Ki-67 index. This association was found to be statistically significant.
- These findings suggest that Ki-67, in conjunction with Gleason Pattern and PSA levels, could serve as valuable predictive prognostic markers for aggressive nature of prostate cancer.
- The high prevalence of PNI further highlights the potential for advanced disease progression.
- These insights contribute to a better understanding of prostate adenocarcinoma's biological behaviour, aiding in improved diagnostic and therapeutic strategies.

LIMITATIONS

- Because of the limited sample size, the results may not be as applicable to larger populations.
- This study was conducted in one geographic region, potentially leading to selection bias and limiting applicability to diverse populations.
- Molecular and genetic markers were not analysed, which could have provided additional insights into tumour behaviour. Being a single-institution study, the results may not reflect broader population trends.

FURTHER SCOPE OF THE STUDY

- Expanding the study with a larger sample size across multiple institutions to enhance generalizability.
- Incorporating molecular and genetic markers to better understand tumour biology and aggressiveness.
- Studying the impact of personalized medicine and targeted therapies in high-risk prostate cancer patients.

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ANNEXURES

ANNEXURE –I PROFORMA

Patient History

Name:

Age:

I.P number:

Total Serum PSA (Prostate specific antigen) Levels:

Clinical symptoms-

Medical history-(diabetes, hypertension, prior malignancies, family history)

Surgical & specimen details:

- Type of surgery (TURP, radical prostatectomy, biopsy):
- Nature of specimen (TURP chips, needle biopsy, prostatectomy):
- Number of specimens received:

Brief Clinical History

- Chief complaints & duration:
- History of present illness:
- Personal history (smoking, alcohol, lifestyle, occupation):
- Family history of malignancies:
- Previous biopsy results:

Clinical Diagnosis

- Preliminary clinical impression:
- DRE findings (nodularity, induration, asymmetry):
- Imaging findings (MRI, TRUS, bone scan):

Histopathological Diagnosis

Hematoxylin and eosin staining –

- Gleason's pattern:
- Gleason's score:

Histopathological features –

- Glandular arrangement (well-formed, cribriform, fused, single cells):
- Perineural invasion (PNI): present/absent
- Lymphovascular invasion (LVI): present/absent
- Necrosis: present/absent, type

Immunohistochemical Staining –

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

ANNEXURE - II

INFORMED CONSENT FORM

"Study Of Immunohistochemical Expression Of Ki67 in Prostate Adenocarcinoma- A Cross Sectional Hospital Based Study, Belagavi "

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Introduction: Prostate cancer is 5th leading cause of cancer related death in men worldwide. The purpose of this study is to evaluate the expression of Ki67 in prostate carcinoma.

Explanation of procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer the best of your knowledge. If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations. The blocks will be taken and studied for the expression of Ki67 marker.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will not get any benefits by participating in this study. The data gathered will help population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data

collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the **principal investigator.**

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"Study Of Immunohistochemical Expression Of Ki67 in Prostate Adenocarcinoma- A Cross Sectional Hospital Based Study, Belagavi ".** My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE – III

HEMATOXYLIN AND EOSIN STAIN REAGENTS

1. Erhlich's Haematoxylin solution
2. Eosin Y solution 1%
3. 1% acid alcohol solution

HEMATOXYLIN AND EOSIN STAIN – PROCEDURE

1. Deparaffinise the tissue sections in xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
2. Subject the tissue section to water through reducing grades of alcohol (90% alcohol for 5 mins + 70% alcohol for 5 mins)
3. Keep it in hematoxylin for 8 to 10 minutes
4. Rinse it in tap water for 2 mins
5. Differentiate with 1% acid alcohol for 10 sec
6. For bluing - place in tap water for about 10 minutes
7. Counter stain by eosin 1-2 minutes
8. Rinse in water
9. Dehydration increasing grades of alcohol (70% alcohol for 30 sec + 90% alcohol for 30 sec)
10. Clearing is done by Xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
11. Mount it with Dibutylphalate Polystyrene Xylene (DPX).

ANNEXURE IV

IHC MANUAL STAINING PROTOCOL –

1. About 3-5 micrometre thick sections were taken from the formalin fixed paraffin embedded block from each case.
2. Bake the section overnight at 37 degrees Celsius.
3. Before test bake it at 60 degree Celsius.
4. Deparaffinise steps- Xylene I for 10mins, Xylene II for 10mins, Absolute alcohol I for 10 mins, Absolute alcohol II for 10mins, Rinse in water for 5mins, Rinse in distilled water for 1 min.
5. Antigen Retrieval – (Tris Buffer + EDTA)- Prepare the required amount of buffer and cook the slides in pressure cooker for three whistles. Then allow it to cool to room temperature for 1 hour. Wash with buffer 3 times with a gap of 2mins each. Apply 3% Hydrogen peroxide -8-10mins. Wash with wash buffer 3 times with gap of 2minutes each.
6. Primary Antibody to be incubated for 45 to 60 mins in closed chamber at room temperature.
7. Wash with wash buffer 3 times with gap o f 2 mins.
8. Apply polymer HRP for 25-30mins (Pink colour reagent in closed chamber)
9. Wash with wash buffer 3 times with gap o f 2 mins.
10. Apply DAB chromogen for 10mins.
11. Wash slowly under running tap water from back side.
12. Counter stain with Haematoxylin – 2mins.
13. Blueing in warm water- 1min.
14. Clear in Xylene and mount with DPX.

ANNEXURE V

KEY TO MASTER CHART-

Bx- Biopsy

No- Number

PSA- Prostate specific antigen

TURP- Transurethral resection of prostate

RP- Radical Prostatectomy

C/f- Clinical Features

GP- Gleason's Pattern

GS-Gleasons Score

PNI- Perineural Invasion

LVI- Lymphovascular Invasion

ANNEXURE VI MASTER CHART-

Sr no	Bx no.	Age(Male)	12 Core Bx	TURP	RP	C/f-oligouria	Hesitancy	Urgency	Hematruria	Noturia	PSA (ng/dl)	GP- 2	GP-3	GP-4	GP-5	GS	Ki-67-Low	Intermediate	High	PNI	LVI	Metastasis
1	567/24	72	YES	NO	NO	YES	YES	YES	NO	YES	5.1	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
2	698/24	75	YES	NO	NO	YES	YES	YES	NO	NO	173	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
3	1103/24	72	YES	NO	NO	YES	YES	NO	NO	NO	20.6	NO	NO	YES	NO	9(4+5)	NO	NO	YES	NO	NO	NO
4	1154/24	68	NO	YES	NO	YES	YES	YES	NO	YES	40	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	YES	NO
5	1640/24	61	NO	YES	NO	YES	YES	YES	YES	YES	36.7	NO	NO	NO	YES	9(4+5)	NO	NO	YES	NO	NO	YES
6	1779/24	72	YES	NO	NO	YES	YES	YES	NO	YES	380	NO	YES	NO	NO	7(4+3)	NO	YES	NO	YES	NO	NO
7	1867/24	85	NO	YES	NO	YES	NO	NO	NO	YES	65	NO	YES	NO	NO	7(4+3)	YES	NO	NO	NO	NO	NO
8	4079/24	71	YES	NO	NO	YES	YES	YES	YES	YES	144	NO	NO	NO	YES	10(5+5)	NO	NO	YES	YES	YES	NO
9	2457/24	73	YES	NO	NO	NO	YES	YES	NO	NO	0.79	YES	NO	NO	NO	7(3+4)	YES	NO	NO	NO	NO	NO
10	2527/24	72	NO	YES	NO	YES	YES	YES	YES	YES	38	NO	NO	NO	YES	10(5+5)	NO	NO	YES	NO	NO	NO
11	2592/24	74	YES	NO	NO	YES	YES	YES	NO	YES	8.38	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
12	2723/24	78	YES	NO	NO	YES	YES	YES	NO	YES	66.5	NO	YES	NO	NO	7(3+5)	YES	NO	NO	NO	NO	NO
13	3452/24	75	YES	NO	NO	YES	YES	YES	YES	YES	73.4	NO	NO	NO	YES	9(4+5)	NO	NO	YES	YES	NO	NO
14	3584/24	73	NO	NO	YES	YES	YES	YES	YES	YES	78	YES	NO	NO	NO	7(3+4)	YES	NO	NO	NO	YES	YES
15	3121/24	70	NO	YES	NO	NO	NO	YES	NO	YES	3.3	YES	NO	NO	NO	7(3+4)	YES	NO	NO	NO	NO	NO
16	4585/23	63	YES	NO	NO	YES	YES	YES	NO	YES	159	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
17	4607/23	70	YES	NO	NO	YES	YES	NO	NO	YES	1.32	NO	NO	YES	NO	8(3+5)	NO	NO	YES	NO	NO	NO
18	4640/23	70	YES	NO	NO	YES	YES	YES	NO	YES	29	NO	YES	NO	NO	8(3+5)	NO	YES	NO	NO	NO	NO
19	4724/23	63	NO	YES	NO	YES	YES	YES	YES	YES	550	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	YES	NO
20	4787/23	66	YES	NO	NO	YES	NO	YES	NO	YES	70.7	NO	NO	NO	YES	9(4+5)	NO	NO	YES	YES	YES	NO
21	5434/23	75	YES	NO	NO	YES	YES	YES	YES	YES	47	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
22	3884/23	62	NO	YES	NO	YES	YES	YES	NO	YES	19.2	NO	NO	YES	NO	8(4+4)	NO	NO	YES	NO	NO	NO
23	4809/23	86	YES	NO	NO	YES	YES	YES	YES	YES	30.5	NO	NO	YES	NO	8(4+4)	NO	NO	YES	NO	NO	NO
24	5000/23	77	YES	NO	NO	YES	YES	YES	YES	YES	100.2	NO	NO	NO	YES	9(4+5)	NO	NO	YES	YES	NO	NO
25	5002/23	76	YES	NO	NO	YES	YES	YES	YES	YES	100	NO	NO	NO	YES	9(4+5)	NO	NO	YES	YES	NO	NO
26	5007/23	63	YES	NO	NO	YES	YES	YES	YES	YES	5000	NO	NO	NO	YES	9(4+5)	NO	NO	YES	NO	NO	NO
27	4584/23	75	YES	NO	NO	YES	YES	YES	NO	YES	100	NO	NO	NO	YES	8(4+5)	NO	NO	YES	NO	NO	NO
28	5364/23	75	YES	NO	NO	YES	YES	YES	NO	YES	70	NO	NO	NO	YES	9(4+5)	NO	NO	YES	YES	NO	NO
29	3864/24	75	YES	NO	NO	YES	YES	NO	NO	YES	0.143	YES	NO	NO	NO	7(3+4)	NO	YES	NO	NO	NO	NO
30	370/23	80	NO	YES	NO	YES	YES	YES	YES	YES	25	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
31	2564/23	76	YES	NO	NO	YES	YES	YES	YES	YES	58.3	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
32	2662/23	78	YES	NO	NO	YES	YES	YES	NO	YES	11.2	NO	NO	NO	YES	9(4+5)	NO	NO	YES	NO	YES	NO
33	5465/24	74	YES	NO	NO	YES	YES	YES	NO	YES	12.6	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO
34	5287/24	69	YES	NO	NO	YES	YES	YES	NO	NO	10	NO	NO	NO	YES	5(4+5)	NO	NO	YES	YES	NO	NO
35	6075/24	76	YES	NO	NO	YES	YES	YES	YES	YES	605	NO	NO	YES	NO	8(4+4)	NO	NO	YES	YES	NO	NO