"ANDROGEN RECEPTOR AND P63 EXPRESSION IN PROSTATIC ADENOCARCINOMA – A HOSPITAL-BASED CROSS-SECTIONAL STUDY"

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LIST OF ABBREVIATIONS

AR	Androgen receptor
ARS	Antigen Retrieval Solution
BPH	Benign Prostatic Hyperplasia
DAB	3,3'- diaminobenzidine
DHT	5-dihydrotestosterone
DPX	Dibutylphthalate Polystyrene Xylene
DRE	Digital rectal examination
EPCA	Early prostate cancer antigen
GG	Grade group
GS	Gleason score
H&E	Haematoxylin and Eosin
HIER	Heat Induced Epitope Retrieval
hK2	Human kallikrein 2
HRP	Horse Radish Peroxidase
IGF-1	Insulin-like growth factor-1
IGFPBs	Insulin-like growth factor binding proteins
IHC	Immunohistochemistry
IL-6	Interleukin-6
PAP	Prostatic acid phosphatase

PCa	Prostate cancer
PSA	Prostate-specific antigen
RT-PCR	Reverse transcriptase-polymerase chain reaction
T	Testosterone
TGF-β1	Transforming growth factor-β1
TNM	Tumor, node and metastases
TRUS	Transrectal ultrasound
TSG	Tumor suppressor gene
TURP	Transurethral Resection of the Prostate
TZ	Transition Zone
UGS	Urogenital sinus
WHO	World health organization

ABSTRACT

"ANDROGEN RECEPTOR AND P63 EXPRESSION IN PROSTATIC ADENOCARCINOMA – A HOSPITAL-BASED CROSS-SECTIONAL STUDY"

Prostate cancer (PCa) ranks among the most prevalent malignancies affecting men around the world. In 2020, prostate cancer was the second most commonly diagnosed cancer in men worldwide, with approximately 1.4 million new cases and 375,000 deaths recorded globally. Androgens are primary sex steroids present in men and have a crucial part in the evolution of the prostate. They are also essential for sexual maturity during puberty. The tumor suppressor gene p63 is a homolog of p53 and serves as a basal cell marker. The study assesses the correlation between tumor cell and stromal androgen receptor (AR) expression with clinicopathological factors and Gleason grade groups. The expression of p63 in PCa, the absence of which is used as an indicator of malignancy, is also evaluated. The AR's quantitative immunohistochemistry (IHC) expression in combination with p63 has not been studied as a predictive indicator for PCa. Hence, we have undertaken the present study.

OBJECTIVES

• Primary

- To evaluate the expression of P63 in prostatic adenocarcinoma.
- To evaluate the expression of the Androgen Receptor in tumor cells and stromal cells of prostatic adenocarcinoma.

• Secondary

- To correlate Tumor cell AR positivity with Gleason grade group & clinical parameters.
- To correlate Stromal AR positivity with Gleason grade group & clinical parameters.

MATERIALS AND METHOD

Spanning two years (January 2023 – December 2024), this hospital-based cross-sectional study included 30 core biopsy samples. Hematoxylin and eosin (H&E) slides were studied for Gleason score (GS). Myoepithelial cell absence using p63 immunohistochemistry was done. For the immunohistochemical analysis of AR in tumor cells, intensity of staining and percentage positivity were noted. The histological score (H Score) for AR was evaluated by multiplying the intensity of staining with the percentage positivity of cells. The immunohistochemical analysis of AR in stromal cells was semi-quantitatively assessed using the Allred Score, which is given by adding the intensity of staining and the proportion of positive cells. A P-value of less than 0.05 was considered statistically significant**.**

RESULTS

A total of 30 cases were studied, where PCa was more common in individuals aged 66 to 75 years, with a mean age of 70.87 ± 6.31 years. The most common grade group observed was Grade Group 4 (GG4), followed by GG5. Perineural invasion (PNI) was positive in 26 cases, mainly in GG4 and GG5. However, it was not statistically significant. P63 expression (basal cell marker) was absent in all cases. AR expression was studied in all cases and was positive but differed in intensity and percentage

positivity. GG4 and GG5 had strong AR intensity of staining in tumor cells and showed a significant P value (0.0227). The H-score was employed to assess AR expression in tumor cells across all cases and showed that 63.33% exhibited low expression, and the remaining (36.67%) showed high expression. The higher AR expression was predominantly observed in higher-grade groups, and a significant correlation was identified between them (p-value = 0.019). There was no significant correlation between tumor cell AR expression and factors such as age, Digital Rectal Examination (DRE) findings, approximate tumor volume, or the presence of PNI. A higher Allred score was observed in GG 2, while lower scores were seen in GG 4 and 5, showing a statistically significant correlation (P value:0.022). There was no significant correlation between stromal AR expression and factors such as age, approximate tumor volume, or the presence of PNI.

CONCLUSION

P63 expression was negative in all cases suggesting absence of basal layer. Strong AR expression in tumor cells was more common in higher-grade tumors, showing a significant correlation (P=0.0227). Stromal AR expression was higher in Grade Group 2 and lower in Grade Groups 4 and 5, also showing a meaningful association (P=0.022). This study highlights the potential role of combined tumor cell and stromal cells androgen receptor's quantitative immunohistochemistry (IHC) expression with p63 as a predictive marker for Pca.

KEYWORDS: Prostate cancer, Androgen Receptor, P63, Adenocarcinoma, Gleason Score, Gleason Grade Group.

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INTRODUCTION

Prostate cancer (PCa) ranks among the most prevalent malignancies affecting men around the world.[1,2]. In 2020, prostate cancer was the second most commonly diagnosed cancer in men worldwide and the fifth leading cause of cancer-related mortality, with approximately 1.4 million new cases and 375,000 deaths recorded globally.[3] By 2030, the frequency of prostate cancer worldwide could reach approximately 1.7 million, with nearly 500,000 deaths expected as a result.[4] Fortunately, PCa is better treated and has a reduced fatality rate when it is detected early. PCa is categorized based on its response to androgens. It can be either androgen-sensitive or androgen-insensitive, which determines whether testosterone stimulates its growth and influences potential treatment options. [5]

Androgens are primary sex steroids present in men and have a crucial part, in the evolution of the prostate. They are also essential for sexual maturity during puberty. In addition to their impact on reproduction, androgens influence other tissues, such as mesenchymal tissues and brain.[6] The effects of androgens, particularly testosterone (T) and 5-dihydrotestosterone (DHT), are largely mediated via androgen receptor (AR). [7,8]

The tumor suppressor gene p63 is a homologue of p53 and serves as a basal cell marker. Unlike the p53 gene, p63 produces at least six major isoforms. Different epithelial tissues express p63 only in the basal cells.[9] On prostatic core biopsies, it is frequently utilized to immunostain for p63 to separate benign from malignant tumours.[9,10]

The p63 gene is from the p53 family of transcription factors. It is situated at chromosome 3q27-28.[11] Diagnosing prostatic carcinoma through routine biopsies, such as Trucut and Transurethral Resection of the Prostate (TURP), can be challenging. Difficulties arise when tissue samples are insufficient or when small carcinoma foci are present. Additionally, benign conditions that resemble prostate cancer, such as atrophy, atypical adenomatous hyperplasia, and basal cell hyperplasia, can complicate the diagnosis. Immunohistochemistry (IHC) is a valuable tool for confirming prostate cancer and differentiating it from its benign counterparts. Strong nuclear p63 immunostaining is observed in the basal cells of normal and benign prostate lesions, whereas it is absent in malignant ones. [11,12,13]

The study is to assess the correlation of both tumor cell and stromal AR expression with clinicopathological factors and Gleason grade groups (GG). Additionally, the expression of p63 in PCa, the absence of which is used as an indicator of malignancy is also evaluated. The androgen receptor's quantitative IHC expression in combination with p63 has not been studied as a predictive indicator for PCa. Hence, we have undertaken this study.

OBJECTIVES

- **Primary**
  - To evaluate the expression of P63 in prostatic adenocarcinoma.
  - To evaluate the expression of the Androgen Receptor in tumor cells and stromal cells of prostatic adenocarcinoma.
- **Secondary**
  - To correlate Tumor cell AR positivity with Gleason grade group & clinical parameters.
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REVIEW OF LITERATURE

The prostate gland is the male reproductive organ located in the true pelvis, anterior to the rectum between the bladder and penis.[14] It is pivotal in supporting the reproductive system in men; the primary functions are to secrete an alkaline fluid that helps neutralize vaginal Ph. This alkalinity extends the lifespan of sperm, providing them with optimal conditions for fertilization. The fluid also contains essential enzymes and proteins that promote sperm health. Prostatic fluid, along with sperm and seminal fluid, also aids in the smooth propulsion of semen along the urethra.[14,15]

Embryology of the prostate

Epithelial buds from the wall of the urogenital sinus initiate lateral growth of prostate gland. These buds split off to form solid cords, which canalize to create the ducts and acini. The interfascicular fibroblasts and the smooth muscle of the prostate are formed in the urogenital sinus mesenchyme surrounding it.[15] The prostatic Mullerian mesenchyma expresses androgen receptors, which are stimulated by androgens to produce the prostate. The primary regulator of prostate growth during development is androgen production by Leydig cells.[16]

Anatomy of the prostate

Prostate gland is located anterior to the rectum, between the perineal membrane and the bladder neck. A pathologic zonal architecture is generally used to describe the gland. The anterior fibromuscular stroma, free of glandular elements, is further divided into the transition, central, periurethral, and peripheral zones. The apex and base, both of which are directed upward to the inferior border of the bladder, are further divisions of the prostate. [17,18,19]

The average prostate gland measures 3 cm by 3 cm by 5 cm, with a volume of 25 ml. The peripheral zone is where 70% of all PCa are found, 20% are found in transition zone (TZ) and 10% in the central zone. A preferred route for cancer dissemination is the neurovascular bundle, which, bilaterally, traverses parallel to the posterolateral portion of the prostate. (figure 1). [20]

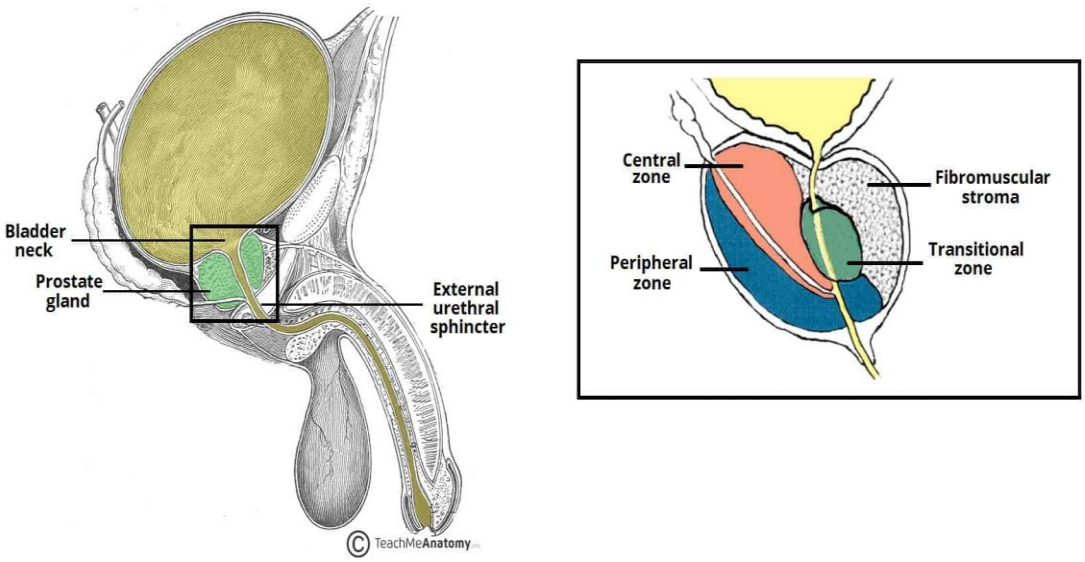


Figure 1. Anatomy of the prostate.[20]

Blood supply

The prostate is supplied by the inferior vesical artery, which shares the bladder's blood supply due to its proximity. Although the internal pudendal and middle haemorrhoidal arteries deliver to the inferior gland region, the inferior vesical artery provides the most arterial blood flow. The end arteries join together in a succession of arteries distributed uniformly throughout the prostate. One set of these arteries is internal or urethral; another group is exterior or capsular. The former mostly supplies the prostate's inner region, particularly the middle lobe when it is

present, and the bladder neck and lateral lobes of prostate gland are supplied by vessels, which run along the postero-lateral surface of the gland. [21,22]

Prostate venous drainage drains into an internal iliac vein, and a dorsal vein of the penis is received at the apex of the prostate gland. Veins draining the bladder wall and seminal vesicles converge here to form the plexus of veins (plexus of Santorini). Hence, the plexus becomes Y-shaped.[21,22]

Lymphatic drainage

Lymphatic drainage from the lower part of the prostate travels from the base of the gland along the posterior surface. They subsequently travel to the hypogastric nodes through the main hemorrhoidal artery's prostatic branches. Two or three draining lymphatics for the posterior prostate go down the rectovesical fascia before ending in para-sacral nodes. Notably, the lymphatic veins between the rectum and the prostate are lined with numerous lymph nodes. The lymphatics of the rectum and the prostate are connected, and the pararectal lymph nodes are also involved. Unquestionably, there are little lymphatic branches in every area where lymphatic vessels pass. [23,24]

Histology of prostate

The luminal cells and basal cells line the acini and ducts that make up the prostate glandular epithelium (Fig 2). The central zone has a considerably greater degree of this papillary shape. Columnar in shape, the luminal cells have spherical nuclei close to the base and pale eosinophilic cytoplasm. The luminal cells secrete seminal fluid. Prostate-specific antigen (PSA), which is present in seminal fluid, is immunohistochemically detected in luminal cells. With the ovoid nuclei and discrete

cytoplasm, myoepithelial cells are found abreast of the basement membrane. The number of myoepithelial cells in a single prostate can differ among glands. They can typically be found by carefully examining standard H&E sections, but IHC for p63, high-molecular cytokeratin makes it simpler to find them. Within the lumens of the acini, rounded eosinophilic corpora amylacea are often seen within the acini.

Stroma of prostate is fibromuscular and contains fibroblasts, blood vessels, nerves, and a large number of smooth muscle cells. The prostate doesn't have any adipose tissue. Compared to the mouse prostate's comparatively thin fibromuscular stroma, this stroma is much more noticeable. [25,26]

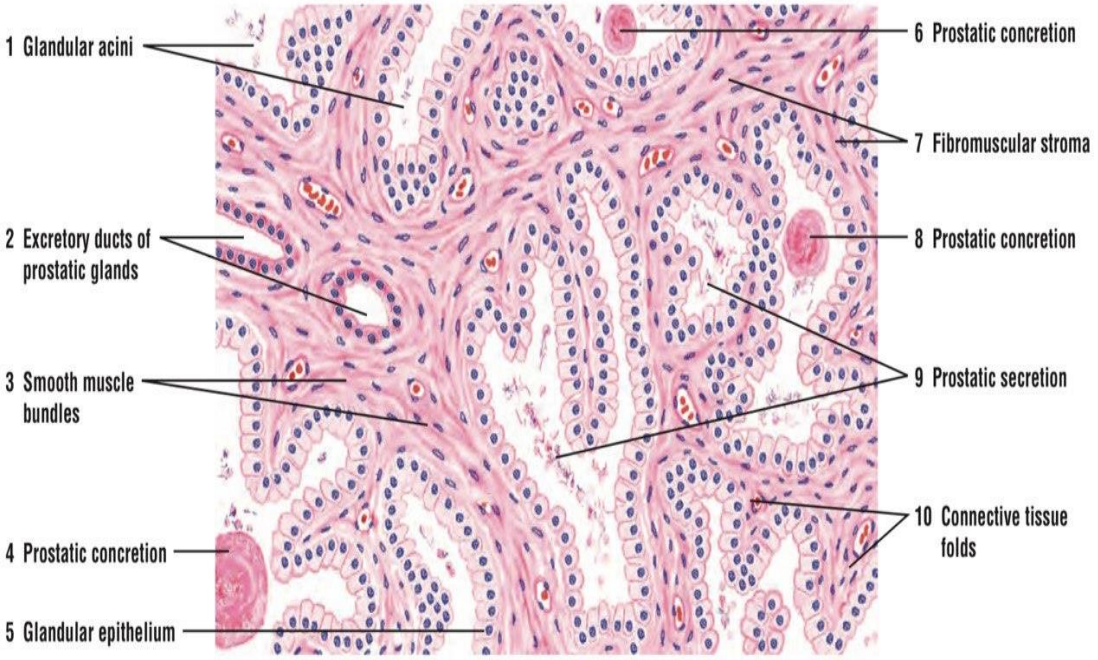


Figure 2: Histology of Prostate Gland: H&E, Medium magnification [25]

<u>Various lesions affecting the prostate</u>

Prostate disorders account for a large percentage of male cases handled by urologists and primarily result in morbidity and death in adult males globally. Prostatitis, BPH, tumors (premalignant and malignant lesions) are the pathologies that

primarily affect the prostate gland. BPH is the most prevalent of these three, and it happens so frequently in later life that it is practically considered to be a "normal" aspect of aging. By the age of 40, 20% of men have histologic evidence of BPH; by the ages of 60 to 80, this percentage rises to 70% to 90%, respectively. The pathogenesis of BPH remains obscure. The availability of active testosterone and DHT in the body is necessary to develop histologic characteristics of BPH. Several modifiable risk factors, such as obesity, diabetes, heavy alcohol use, and inactivity, are additional risk factors. Additionally, Black versus white racial differences are thought to be significant but are not yet fully understood.[27]

Prostatitis

Prostatitis involves inflammation in the prostate and its surrounding tissues.

Types of Prostatitis [28]-in Annexure

Benign Prostatic Hyperplasia (BPH)

The hyperplasia of prostatic tissue is a primary reason for urinary symptoms in males. The advancement of benign prostatic hyperplasia results from the excessive growth of stromal and epithelial cells within the prostate's transition zone, encircling the urethra.

As the condition advances, it leads to urethral compression, resulting in obstruction to the outflow from the bladder. [29]

Prostate cancer

Patients of PCa can have a localized or advanced form of the illness. Prostate tumors are often low-grade, slow-growing, generally low-risk, and not particularly

aggressive.[14] In early stages of PCa, it often remains asymptomatic and progresses at a slow rate. As a result, active surveillance is typically required rather than immediate treatment. Although some patients may not have any symptoms, the most frequent complaints are nocturia, frequency, and difficulties in urination. Currently, most PCa's are identified when the serum PSA level is elevated and are connected to palpably normal prostates.[30]

Epidemiology

PCa incidence and fatality rates vary greatly between countries. As per GLOBOCAN 2020, 3,75,304 (3.8%) global deaths and 14,14259 (7.3%) new cases of PCa in men.[2] More than 60%of PCa cases of south cental Asia are from India, where these have been 34,540 incidences and 16,783 deaths.[31] Cancer is a global health issue due to its rising prevalence, financial cost, and mortality. Out of 170 cases of genitourinary tract cancers, 31 (18.23%) persons had PCa; according to Belbase et al.[32] PCa occurs at a rate of 9.6 per 100,000 people in Iran.[33]

Upon 50 years, benign prostatic hyperplasia remains a widespread condition that affects 210 million men worldwide. According to the national cancer registry, PCa is the 2nd to 10th most prevalent cancer in males across different metropolises in India.[34, 35]

WHO histologic classification [36,37] in Annexure

Pathophysiology

The prostatic tissue secretes a fluid that accounts for 25% to 30% of semen, which helps sustain an elevated pH. Testosterone ensures optimal functioning of prostate. This explains the effectiveness of hormonal therapy. It is suggested that castrate-resistant tumors generate androgens within their cells.[38,39,40]

Healthy prostate glandular cells undergo initial mutations, typically beginning in the basal cells of the peripheral region.[40,41] Prostate cancer (PCa) is usually detected in the region of the prostate that can be examined through a digital rectal examination (DRE). Since it primarily originates in the glandular section of the prostate and exhibits distinct glandular architecture under a microscope, PCa is classified as an adenocarcinoma. Cancer cells grow and multiply, initially invading the surrounding prostate tissue and forms a tumor nodule. This tumor may remain confined for years or extend beyond the prostate capsule, spreading to nearby areas. PCa metastases usually to the bone and lymph nodes. It is believed that the prostatic venous plexus, which drains into the vertebral veins, contributes to the spread of cancer to the bones.[41,42]

Histopathology

Criteria for Diagnosis of Prostatic Adenocarcinoma [37] in Annexure

Gleason scoring system for prostate cancer

In the 1960s, Dr. Donald Gleason developed the scoring system of PCa.[43] Unlike cellular features used in other malignancies, the histological pattern forms the basis of the Gleason score (GS).

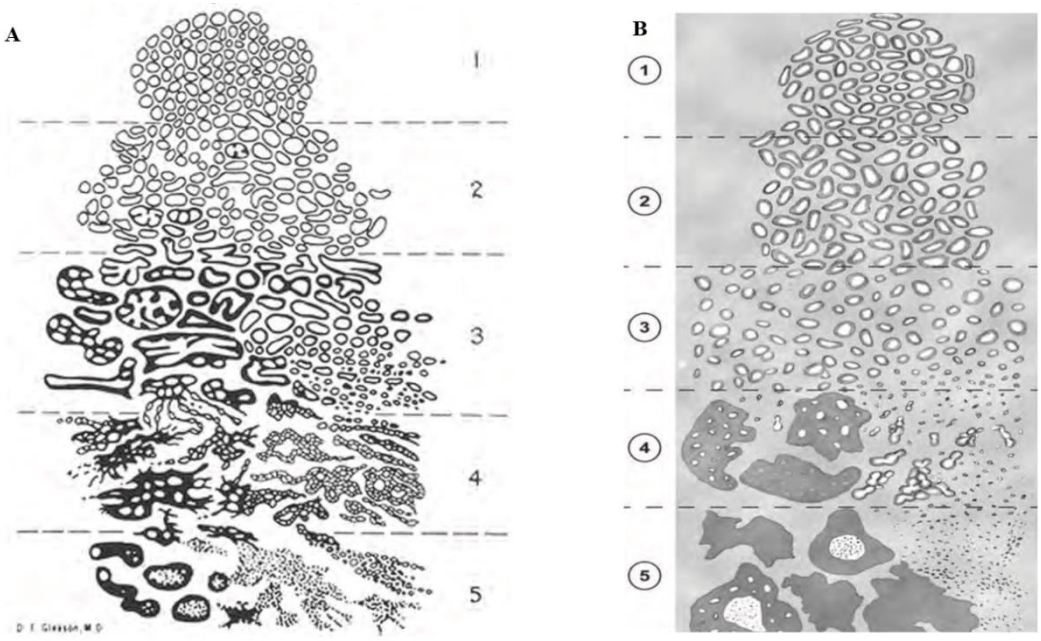


Figure 3: A. Conventional gleason pattern diagram, B. Modified Gleason pattern diagram. [43]

The grading scale ranges from 1 to 5, where a score of 1 indicates a microscopic glandular structure that closely resembles normal tissue. In contrast, a score of 5 signifies a complete loss of glandular architecture, with only clusters of abnormal cancer cells present.[44,45] 2 patterns are added to get a final score in GS. The first number, graded from 1 to 5, is the predominant pattern, followed by 2nd, which is a minor pattern.[46] If only a single Gleason pattern is present, the score consists of that grade repeated, such as Gleason 3+3=6.[46]

High-grade cancer is defined as 4+3=7 or higher on the Gleason scale.[46]

Tumors with a GS of 3+3=6 or lower are categorized as low-grade. For intermediate-grade tumors, the GS is typically 3+4=7, signifying that the majority of the tumor consists of Gleason grade 3 cells, with a smaller portion exhibiting the more advanced grade 4 characteristics.[47,48]

According to the 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma, the main conclusions were noted:[49]

- Cribriform and Glomeruloid glands should be graded as pattern 4.
- Mucinous PCa must be graded according to its fundamental growth pattern rather than uniformly classified as pattern 4.
- Intraductal PCa without invasive features must not be classified as pattern 4 or assigned a Gleason grade.[49]

Proposal for new grading system

Epstein JI first suggested a novel grading system. The updated (2005 and 2014) GS groups are used as the grading system's basis, producing 5 prognostically different grade groups. Grade group 1 differs from Gleason patterns 1 to 3 in that each separate gland is present independent of the well-defined/ invasive pattern, the regularity of the size and shape of the gland, or the volume of the stroma. To validate the new grade method, a multi-organization study was corporated [50]

The new grade grouping system[49,50] in Annexure

Histologic grading system[49,50]

The patient's prognosis depends largely on the Gleason grade. It is frequently used clinically in nomograms to predict pathologic stage, responsiveness to therapy, and outcome, together with blood PSA level, clinical, or pathologic stage. Additionally, the Gleason score is utilized to create risk groups that direct patient therapy. In addition to Gleason score, so-called "grade group," which is according to Gleason score, should be reported. Except for those that are post-hormonal therapy or

post-radiotherapy (when the radiation effect is obvious), all prostate adenocarcinomas should be graded. Cytologic atypia and mitotic activity are not taken into account in the grading process; only the architecture is considered. The two frequent patterns are totalled to produce the score (on a scale of 2 to 10) at low magnification (x100).

Recent recommendations in the application of the Gleason system include the following

- Do not (or rarely) assign a well-differentiated Gleason score of 2 to 4 for carcinoma in a core biopsy; this almost always represents under-grading.
- When three grades are present in core biopsies, give frequent grades with worst grades. In prostatectomy, give the most frequent and second most common grades, but if there is a minor (less than 5%) tertiary high-grade 4 or 5 patterns, this should be noted.
- If there is 95% of pattern 4 or 5 with 5% or less pattern 2 or 3, ignore the lower-grade component.
- Pattern (4 or 5) must be integrated in core biopsy. Thus, 98% of pattern 3 and 2% of pattern 4 on core biopsy is GG 3 + 4 = score of 7, with a comment indicating the percentage of pattern 4.
- If three Gleason patterns are present and the tumor consists mostly of low-grade adenocarcinoma, the minor high-grade component is included as a "minor pattern" (previously called "tertiary pattern");
- Variants of prostatic adenocarcinoma can be graded.
- Cribriform adenocarcinomas are high-grade pattern 4.
- For needle biopsies, provide grade by clinically submitted container, even if several cores are within the container. It may also be helpful to provide the

Gleason grade for cores having maximum GS that is different from the overall Gleason score for cores from that container.

- Percentage of the tumor composed of Gleason patterns 4 and 5 should be reported; this is mandatory only for grade groups 2 and 3 but may be helpful in any case where high-grade patterns are present.[49,50]

TNM Staging [51] in Annexure

Markers used for prostate cancer

Prostatic acid phosphatase (PAP)

PAP was the earliest biochemical marker to be utilized to diagnose and stage PCa. Esters are hydrolyzed by PAP into inorganic phosphates in an acidic environment. Although PAP has been found in many organs, including the liver, brain, and lungs, the prostate has the largest concentration. PAP is secreted by prostate epithelial cells into the glandular lumen. It can be detected using an enzymatic or immunoassay.[52]

PAP levels in the prostates of patients with metastatic PCa were found to be high in a 1938 investigation.[53] A later investigation, however, revealed that PAP in PCa tissue was significantly lower than that found in BPH patients.[54] In addition, 84% cases with raised PAP later revealed metastasis / extracapsular extension in research that measured PAP levels in 102 individuals.[55] According to one study, only 5% of patients with increased PAP have diseases that can be surgically treated.[56] The value of evaluating PAP levels has decreased following the implementation of serum PSA assessment, in research included 460 patients who were sent to Johns Hopkins (Baltimore, Maryland, USA) regularly.[57]

Prostate Specific Antigen (PSA)

During the 1970s, researchers identified PSA, also called human kallikrein 3 (hK3), from prostatic extracts. This 33-kDa serine protease is a member of tissue kallikrein family. [58] Wang et al.[58] first identified PSA in human serum, and Papsidero et al.[59] then isolated PSA from prostate tissue). Male periurethral glands and the prostatic epithelium are the main sources of PSA, This is then released into seminal fluid and has the effect of liquifying the coagulum in the ejaculate.[60,61] All normal, BPH, and neoplastic conditions produce PSA, but BPH patients' prostatic transition zones have been found to have the highest PSA concentrations.[62] The majority of PSA exists unbound or free in serum. In prostate, conditions like inflammation, hyperplasia, and neoplasia cause physiological barriers to be breached, basement membrane permeability to increase, and PSA to be released into the bloodstream more often.[63]

Age-specific Values of PSA[64]- in Annexure

PCa screenings frequently employ PSA testing. DRE and transrectal ultrasound (TRUS) are more expensive than PSA screening, which can detect more prostate tumors and are specific than DRE alone.[64-66] According to estimates, 70–80% of PCa's now are organ-confined, due to screening of PSA levels.[67,68] However, using PSA alone to screen for PCa is not advised because it misses 18–28% of tumors that would have been found when PSA was used in concurrence with DRE at a PSA value of 4 ng/ml.[68]

Serum PSA remained inversely correlated with PCa volume, Gleason score, and stage, despite some studies showing that levels of PSA studied in PCa reduced as GS increases.[69, 70] This rise in serum PSA could be attributed to higher-grade tumours

having more prostatic epithelium that is disorganized, which results in greater PSA release.[71]

PSA is one of the most contentious PCa biomarkers, despite being the most widely used. The drawback of PSA in the early identification of the PCa was recently acknowledged by a study. It was discovered that to stop one death, numerous males must be checked, biopsied, and diagnosed.[72] The intention of study was to improve specificity of early PCa screening for deadly forms. The findings suggested that men who have PSA levels at the highest quartile should be the focus of screening for PCa in men between the ages of 50 and 60. It was discovered that the majority of the future cases of metastasis in this group involved men. To help with biopsy decision-making, it was also advised that four kallikrein markers in males with increased PSA be tested. This is just one of several research looking into how to make PSA screening more accurate. A more reliable marker is advised because of the numerous conflicting views on the proper application of PSA that have been produced by the extensive body of literature on this subject.[52]

Human kallikrein 2 (hK2)

The amino acid sequence of hK2 and PSA have 80% homology, making them members of the same family. Similar to PSA, prostatic tissue has the highest concentration of hK2.[73] Unlike PSA, hK2 is mostly present in the serum in a free, unbound condition.[74] According to Partin et al.[75], the likelihood of detecting PCa improved when greater hK2 levels were correlated with lower fPSA values.

According to a current study using a porous silicon antibody immunoassay technology to find total hK2 levels in serum. This more efficient approach requires 15 µl of serum, and the experiment takes around 3 hours to complete. According to

reports, the hK2 level, whether independently or alongside PSA, might help with PCa prophecy. The ability to diagnose PCa more precisely provided by this new approach may benefit clinical practice in the future.[76]

Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding proteins (IGFPBs)

While a previous study[77] demonstrated that IGF-1 could be detected in PCa and might be utilized in conjunction with PSA in early detection techniques, a subsequent study[78] was unable to identify any link between systemic IGF-1 levels and the existence of PCa.

IGFBP-had the lowest in bone metastasis patients, gradually increased in cases of localized illness, greatest in normal participants. On the other hand, levels of IGFBP-2 and IGFBP-3 in plasma were found to be linked with the disease process.[79] Lower concentration of IGFBP-2 were linked to disease progression while greater levels were linked to organ-confined disease.[79]

Transforming growth factor-β1 (TGF-β1)

TGF-1 family cytokines have been linked to multiple stages of tumor formation, and individuals with various malignancies have been reported to have higher levels of TGF-1. According to specific immunohistochemistry findings, malignant prostatic epithelium expresses TGF-1 more than normal prostate tissue.[52] PCa progression is positively correlated with raised serum levels of TGF-1 in several investigations. [80,81] Shariat et al. [82] studied 302 consecutive patients where radical prostatectomy for clinically confined illness had their preoperative serum TGF-1 assessed. The study found that in individuals with a tumor spread beyond the capsule

to the seminal vesicle and nodal metastases, TGF-1 plasma levels were considerably higher pre- and postoperatively.

Interleukin-6 (IL-6)

IL-6 is involved in several biological processes, which include the control of defense response and resorption of bone. Research utilizing IHC has shown that localized PCa tissue has an 18-fold increase in IL-6 protein concentrations when compared to normal prostate tissue. Also, IL-6 receptor concentrations were eight times higher in PCa tissue than in healthy tissue.[83] In another investigation, 120 cases of clinically confined PCa with prostatectomy had their serum IL-6 and IL-6 soluble receptors assessed.[84] Patients with metastatic cancer had the highest plasma IL-6 levels, while patients with bone metastases and involvement of local lymph nodes had the highest soluble IL-6 receptor concentrations. A recent study that measured TGF-1 levels, 302 patients with localized illness scheduled prostatectomy had their pre- and postoperative values of IL-6 and IL-6 receptors examined.[80] Whether or not a cure was achieved, levels of these two markers considerably dropped after prostatectomy.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

The heat-stable bacterial DNA polymerase and sequence-specific primers, the molecular technique known as PCR, may amplify trace amounts of DNA. Reverse transcriptase is used to convert the starting material, RNA, into DNA before the reaction starts. PSA, hK2, prostate-specific membrane antigen (PSMA) are tumor indicators with tissue-specific mRNA that can be detected by RT-PCR. A 100-kDa transmembrane glycoprotein known as PSMA has been found in all varieties of prostatic tissue. PSMA values were utilized in conjunction with RT-PCR with other techniques, using radionuclide tagging (ProstaScint) to find PCa cells in the blood.[52]

Early prostate cancer antigen (EPCA)

Using immunohistochemistry, it was discovered that people with prostate adenocarcinoma expressed EPCA, a nuclear structural protein linked to PCa, all over their prostates. The detection of PCa by EPCA was reported to have a sensitivity and specificity of 84% and 85%. According to the study, EPCA immunohistochemistry may be able to identify PCa five years sooner than current methods and reduce the burden of biopsies needed because of elevated PSA levels.[85]

Androgen receptor and prostate cancer

Androgen receptor (AR), a nuclear receptor; regulates differentiation, cell growth, metabolism, and programmed cell demise in both the prostate and PCa. [86,87]. PCa patients benefit greatly from inhibiting AR signalling. PCa invariably develops castration resistance through a variety of, still poorly understood, processes. Despite the resistance to castration, AR signalling continues to drive PCa.[88]

A cytoplasmic receptor called the AR is triggered when androgenic hormones like testosterone and DHT bind to it. Following androgen binding, the AR translocates into the nucleus. Following dimerization, AR binds to HREs, or hormone response elements, in DNA.[88,89] (Figure 4)

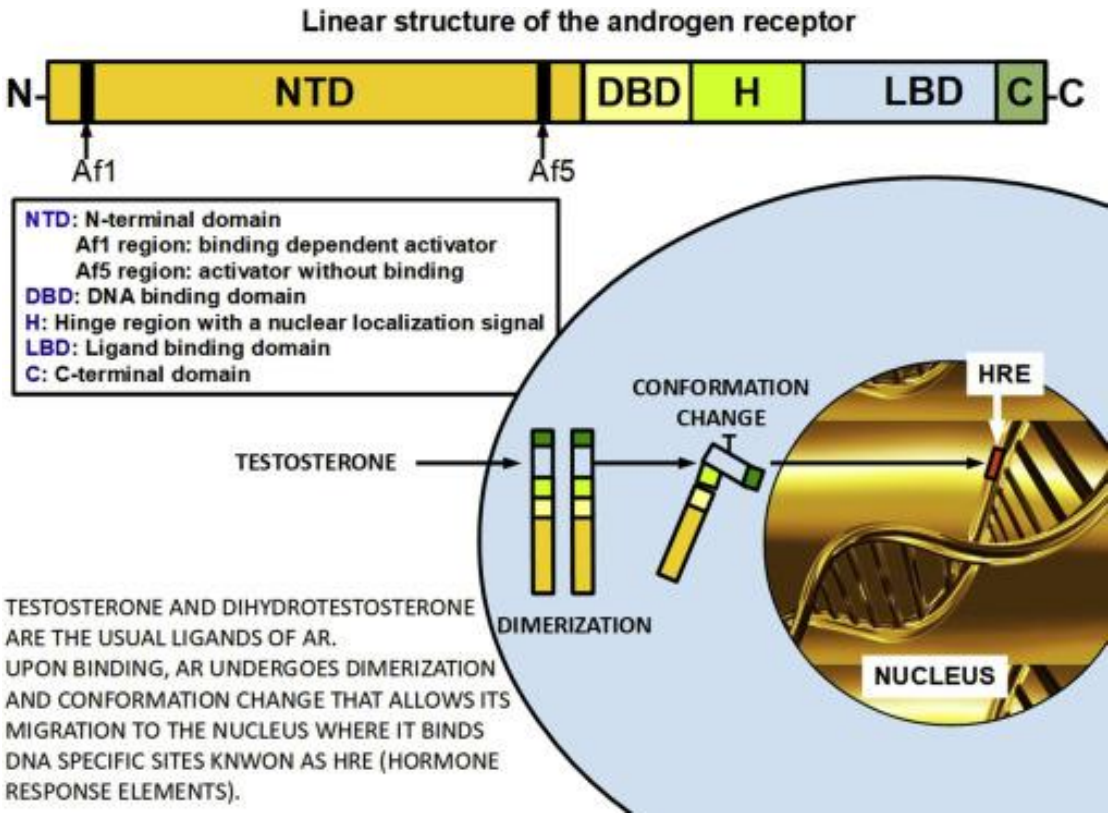


Figure 4. Structure of androgen receptor[88]

While few patients with metastases of PCa ultimately develop hormone resistance after androgen abstinence therapy, AR remains crucial. It plays a significant part in both early and advanced PCa stages. Hormonal therapy for prostate cancer aims to lower serum androgen levels, leading to a subsequent decrease in androgen receptor activity.[89,90] Nearly all primary prostate tumors express the AR to some extent. Research in both people and animals suggests a connection with AR at the cellular level in both localized and metastatic tumors and may influence disease advancement. [91,92]

The variability in PCa advocate that higher AR expression may not typically linked with the onset of disease. Additionally, hormone-resistant prostate cancers do not appear to be clonally chosen from AR-negative regions. However, animal models of prostate cancer indicate that an increase in AR expression may contribute to cancer

initiation or promote recurrent growth when androgen levels are low. It has been proposed that the AR gene amplification is a mechanism that makes PCa cells susceptible to lower concentrations of androgens following androgen deprivation therapy. Nevertheless, it is unknown whether hormone-resistant tumors with amplified AR genes also have higher quantities of AR protein.[93,94]

Co-regulators modify the AR by influencing its ligand selectivity. They also affect its ability to bind to DNA as well as other functional aspects of AR and also have an impact on the transcriptional activity of AR. These co-regulators have the power to either activate or repress AR function. Due to the widespread expression of AR in prostate tumors and their metastases,[95,96] Dysregulated AR activity, influenced by co-regulators may aid in the development of PCa or lead to anti-androgens acting as activators. PCa, for example, has variable expression of dual-specificity phosphatases from the Cdc25 family that promote progression of cell cycle.[95,96]

As prostate cancer progresses, stromal cells gradually shed androgen receptor (AR) expression, with this decline emerging during the stage of high-grade PIN. The reduction in stromal AR expression correlates with the level of differentiation, indicated by the Gleason grade, and the advancement of prostate cancer. The shift in stromal androgen receptor (AR) function, from promoting growth during fetal development to regulating specific tumor-suppressing mechanisms in cancer, plays a significant role. This transition helps explain why the loss of AR expression in stromal cells may be a key factor in the development of resistance to androgen ablation therapy for prostate cancer.[97]

The role of p63 in the prostate

Researchers recognised the p63 gene due to its similarity to the tumor suppressor gene (TSG) p53. Located on chromosome 3, p63 spans approximately 220 kb. The human p63 protein is encoded by the TP63 gene, which is located on chromosome 3q27–28.[98]

Prostate forms within the intermediate region of the primitive urogenital sinus (UGS). Histological analysis of tissues in the urethral region in newborn p63-deficient mice highlighted the pivotal function of the p63 gene in regulating prostate gland development during embryogenesis. [98,99,100]

The occurrence of prostate agenesis proposed, p63 is pivotal in the formation of stem cells of the prostate, which reside in the basal layer of the UGS epithelium. Progenitor/stem cells with the capacity to develop into basal and secretory cells can be found in the population of p63-expressing cells in the developing prostate. This finding suggests that adult stem cells, which maintain and repair the adult prostate epithelium, are present in p63-positive basal cells of the fully formed prostate.[98,99,100]

Similar studies

Din NU et al. (2011) study analyzed and compared p63 expression in both urothelial carcinomas and prostate adenocarcinomas. On 50 each of urothelial carcinoma and prostatic adenocarcinoma cases, IHC of p63 was used. Patients' names, ages, histological codes, tumor grade, and p63 expression levels were noted. P63 was negative in all prostatic adenocarcinomas. In combination with other indicators like PSA, p63 can be a good diagnostic to identify prostatic adenocarcinomas from urothelial carcinomas in challenging instances.[100]

Shokeir FA et al. (2019) have studied stromal AR expression in 65 cases of prostatic adenocarcinoma. They concluded that stromal AR expression related significantly in relation to the proportion of carcinoma in the specimen, tumor grading, presence of perineural invasion, and stage of cancer.[101]

Hashmi AA et al. (2019) conducted a study on androgen receptor expression in prostatic acinar adenocarcinoma, a strong correlation was observed between AR expression and the total Gleason score, WHO grade, and the percentage of tissue involvement. These factors are considered key indicators of tumor progression.[102]

Rathod SG et al. (2019) conducted a prospective study on 80 cases of prostate; the effectiveness of triple antibody staining (AMCAR, p63, and HMWCK) in diagnosing prostate neoplasms was evaluated. Findings suggested that utilizing AMCAR as a positive marker, along with HMWCK[103]

Hermien H et al. (2022) An observational analytical study was carried out to estimate the impact of AR expression in prostate adenocarcinoma. The results revealed a significant link between AR expression and WHO grade groups.[104]

Putriyuni A. et al. (2023) A study was performed to assess androgen receptor (AR) expression in 56 prostate cancer cases. The findings showed a significant statistical association between AR expression and the Gleason score; however, no association was observed with perineural invasion. [105]

MATERIALS AND METHODS

This hospital-based cross-sectional study was carried out on core biopsies of prostatic adenocarcinoma, in the Department of Pathology KAHER's JNMC and KLE's Dr Prabhakar Kore Hospital and Medical Research Center, Belagavi.

Study period: January 2023 to December 2024 (2 years duration).

Study Design: Hospital Based cross-sectional study.

Sample Size: On average, the pathology department receives 12 specimens of prostatic adenocarcinoma in a year. Therefore, the Sample size was 30.

Selection Criteria:

Inclusion Criteria:

Core biopsy samples from cases of prostatic adenocarcinoma.

Exclusion Criteria:

Improperly preserved or improperly fixed specimens

Ethical clearance:

The study commenced after receiving ethical clearance from the Institutional Ethics Committee of JNMC, Belagavi.

Methodology

A total of n= 30 subjects who met the inclusion criteria were enrolled to this study. This present study was conducted on all prostatic adenocarcinoma core biopsy specimens obtained in the Department of Pathology KAHER's JNMC &

Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. On acquiring permission from Hospital authorities, the information on the data for clinical parameters and relevant investigations was obtained from case records.

Histopathological evaluation:

Tissue specimens of prostate adenocarcinoma were collected and immediately preserved in 10% formalin solution and processed. Paraffin wax-embedded blocks were prepared. About 3-4-micron thin sections were taken, and a pathologist examined H & E-stained slides, and reporting was done along with Gleason Score, Grade grouping and approximate tumor volume.

Immunohistochemical analysis of p63:

About 3-to 4-micron sections were taken from the blocks of all 30 cases and stained with p63. Slides were evaluated for presence or absence of myoepithelial cells. Absence of p63 expression (myoepithelial marker) confirms the diagnosis of adenocarcinoma prostate.

Steps of P63 Immunohistochemistry in Annexure

Immunohistochemical staining for evaluation

The slides were examined under the Olympus BX41 microscope, and selected pictures were captured with the digital camera. The immunohistochemical staining for p63 was deciphered as positive or negative.

Steps of AR Immunohistochemistry in Annexure

Immunohistochemical analysis of AR in tumor cells:

The intensity of AR staining and the proportion of AR-positive tumor cells were recorded and graded from 0 to 3+ as described below,

0+: No staining

1+: Weak

2+: Intermediate

3+: Strong

The AR percentage positivity was evaluated as a continuous variable (0 to 100%). The histological score (H Score) for AR was evaluated by multiplying the intensity of AR staining with the percentage positivity in cells. The H Score ranged from 0-300. A cut-off point of 200 was used to group the AR expression into the low expression (<200) and high expression (>200).[103, 105]

Immunohistochemical analysis of AR in stromal cells:

Allred Score semi-quantitatively assessed the stromal AR expression. The Allred Score was given by summing the intensity of staining and the proportion of positively stained cells. The intensity of staining was scored as follows;

0: No staining

1: Mild

2: Moderate

3: Strong

The percentage of positive cells were scored as 0 to 5

0: 0%

1: <1%

2: 1 to 10%

3: 11 to 33%

4: 34 to 66%

5: 67 to 100%

The total score of is ranged in between 0 to 8 and 0 - 4 were considered low expressing and 5 - 8 were high expressing.[101]

AR IHC slides were examined using the Olympus BX41 microscope. Images were captured with the JENOPTIK SUBRA digital camera, utilizing the GRYPHAX software.

Statistical Analysis:

Data was entered using Microsoft Excel. The comprehensive analysis was conducted using SPSS Version 23 (Statistical Package for the Social Sciences). The study examined tumor cell and stromal cell AR expression and its relationship with clinicopathological parameters and histological grading. A p-value below 0.05 was regarded statistically meaningful.

RESULTS

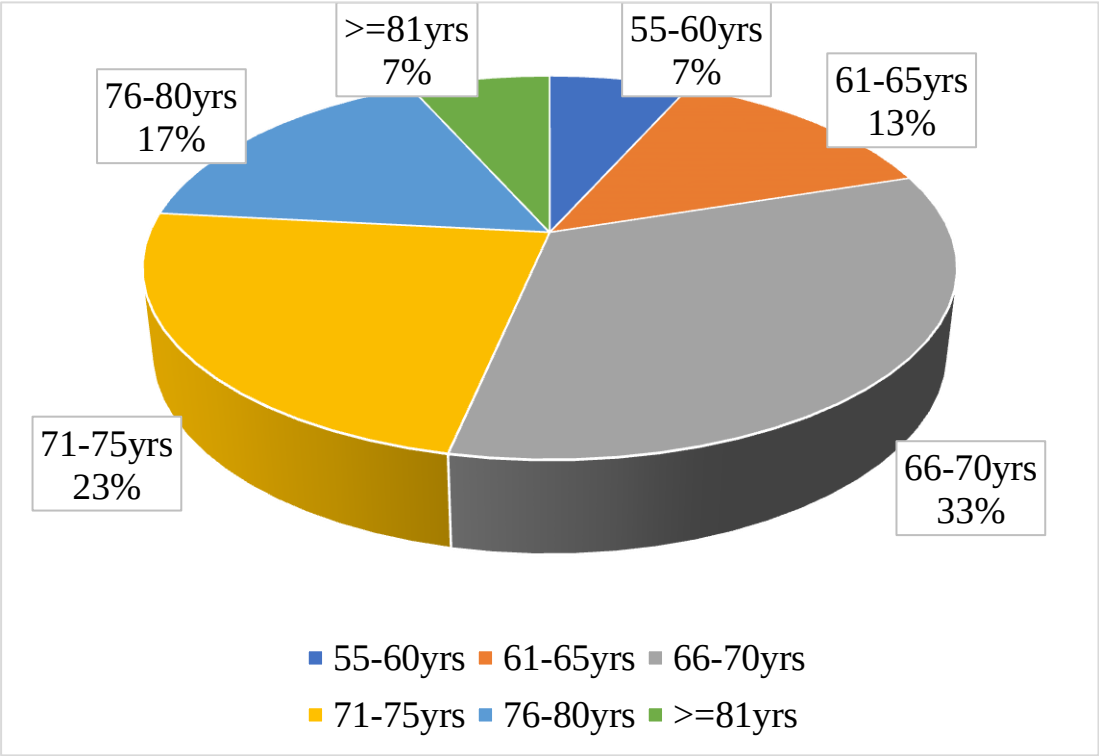
This present study was done at KAHER'S JNMC & KLE'S Dr Prabhakar Kore Hospital and Medical Research Center, Belagavi where 30 cases of prostatic adenocarcinoma were analyzed to evaluate P63 expression and tumor and stromal AR expression and their association with various clinicopathological factors.

In the study age of patients varied from 58 -86 years with a mean age of 70.87 ± 6.31. The youngest being 58 years and the oldest 86 years of age. Age group 66-70 years had the highest proportion (33%), followed by 71-75 years (23%) and 76-80 years (17%). Both 55-60 years and ≥81 years had the lowest percentage (7%). [Table 1, Graph 1]

Table 1: Age wise distribution

Age category	Number of cases	%
55-60yrs	2	6.67
61-65yrs	4	13.33
66-70yrs	10	33.33
71-75yrs	7	23.33
76-80yrs	5	16.67
≥ 81 yrs	2	6.67
Mean	70.87	
SD	6.31	

Graph 1: Age wise distribution



Prostate Specific Antigen (PSA) values were available in 24 of the 30 cases where mean PSA is 124.13 and median 86.2.

DRE revealed hard nodule in 18 cases (60%) out of 30 cases.

Table 2: Distribution of hard nodule on digital rectal examination (DRE)

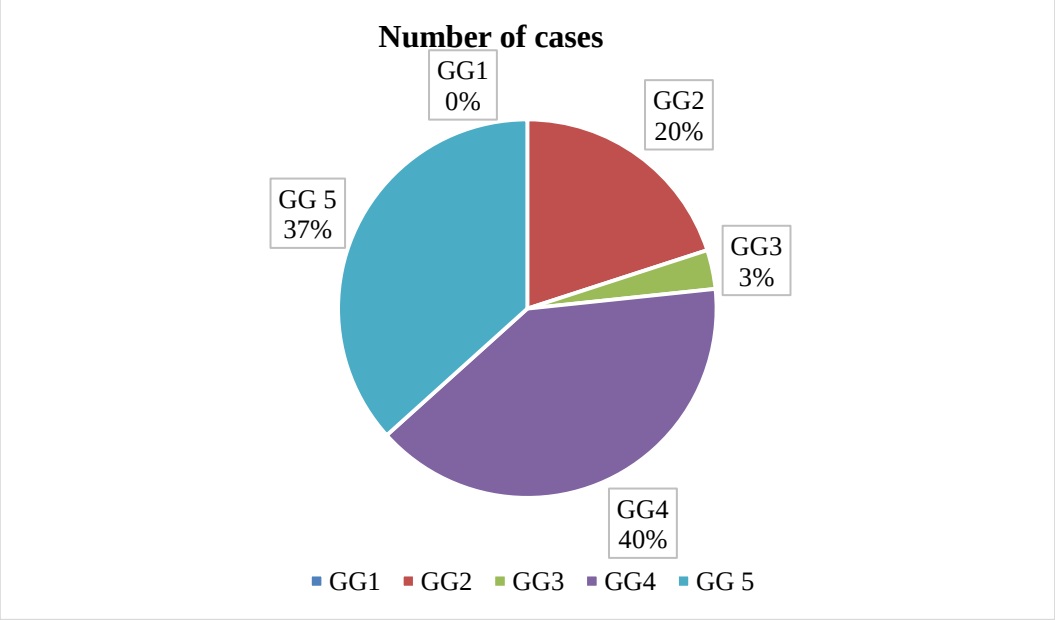
Hard nodule on DRE	Number of cases (%)
Present	18 (60%)
Absent	12 (40%)

In the study, the distribution of PCa across Gleason grade groups (GG) show maximum percentage in Grade Group 4 (40.00%), followed by Grade Group 5 (36.67%) and Grade Group 2 (20.00%). Grade Group 3 showed 3.33%, whereas no cases were found in Grade Group 1. [Table 3, Graph 2]

Table 3: Gleason grade group wise distribution

Gleason grade groups	Number of cases	%
GG1	0	0
GG2	6	20
GG3	1	3.33
GG4	12	40
GG 5	11	36.67
Total	30	100

Graph 2: Gleason grade group wise distribution

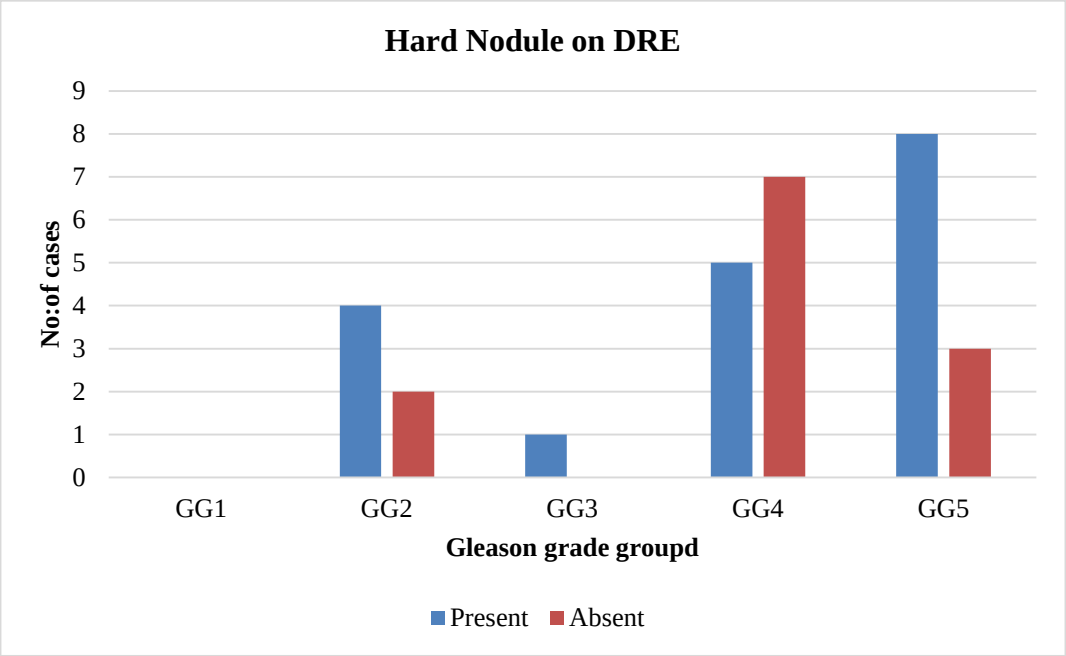


Association of presence or absence of hard nodule on digital rectal examination (DRE) with various Gleason grade groups was studies and no statistically significant correlation was found, p-value (0.524). [Table 4, Graph 3]

Table 4: Association of presence or absence of hard nodule on digital rectal examination (DRE) with various Gleason grade groups.

Hard nodule on DRE	GG1	GG2	GG3	GG4	GG5	Total	%
Present	0	4	1	5	8	18	60
Absent	0	2	0	7	3	12	40
Total	0	6	1	12	11	30	100
P value=0.52							

Graph 3: Association of presence or absence of hard nodule on DRE with various Gleason grade groups.

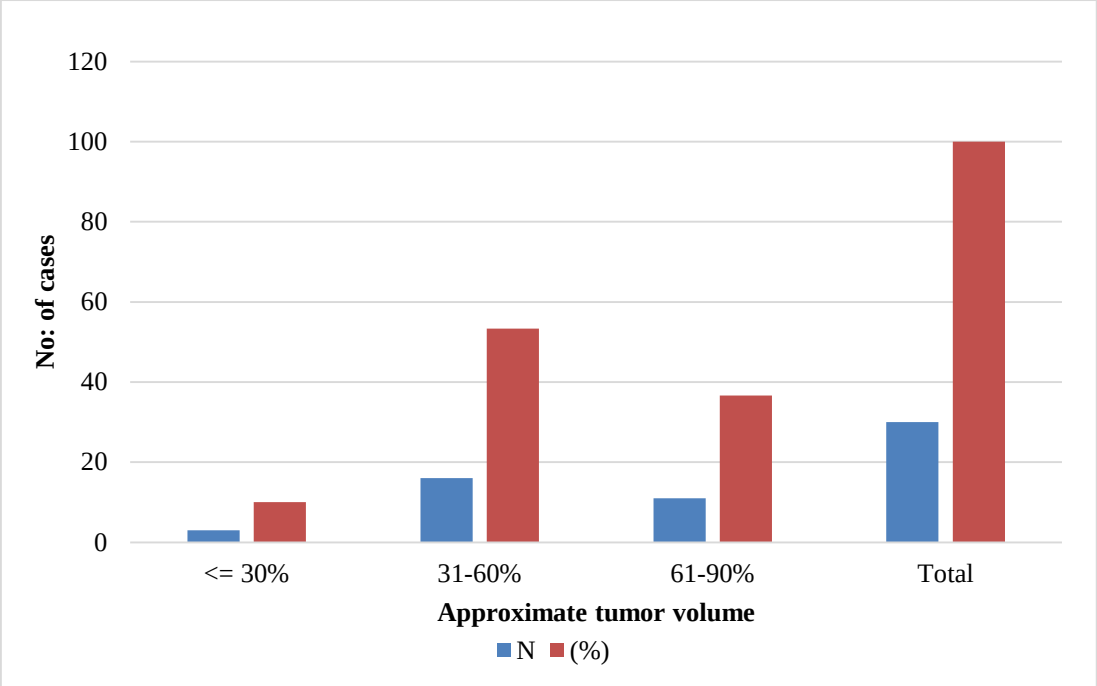


The distribution of the approximate tumor volume in each of the 30 cases showed that maximum number of cases (53.33%) had a volume of 31-60% followed by 61-90% and <30%. [Table 5, Graph 4]

Table 5: Distribution of approximate tumor volume

Approximate Tumor Volume	Number of cases	(%)
<= 30%	3	10
31-60%	16	53.33
61-90%	11	36.66
Total	30	100

Graph 4: Distribution of approximate tumor volume

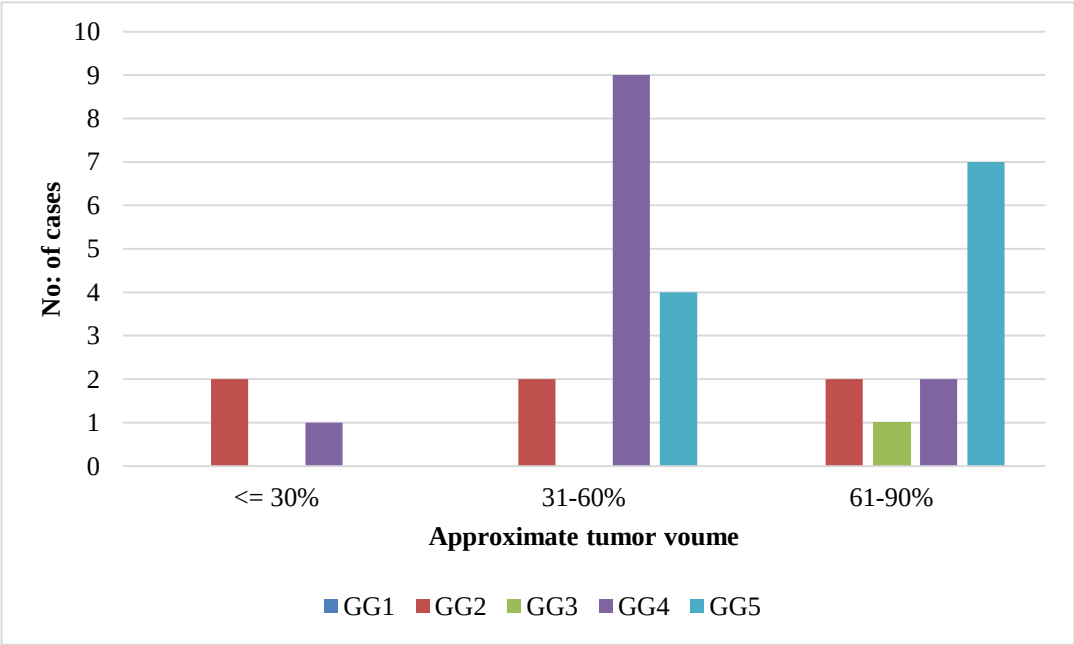


In the study the association of approximate tumor volume and Gleason grade grouping was evaluated. The highest number of cases was observed in GG4 and GG5, with the majority of cases having tumor volumes between 31-60% and 61-90%. The Chi-square test (4.40) and p-value (0.11) suggest that the association between tumor volume and Gleason grade groups is not statistically significant. [Table 6, Graph 5]

Table 6: Association of approximate tumor volume across various grade groups

Approximate tumor volume	GG1	GG2	GG3	GG4	GG5	Total
<= 30%	0	2	0	1	0	3
31-60%	0	2	0	9	4	16
61-90%	0	2	1	2	7	11
Total	0	6	1	12	11	30
Chi square =11.808 p valuep-value≈0.17						

Graph 5: Association of approximate tumor volume across various grade groups

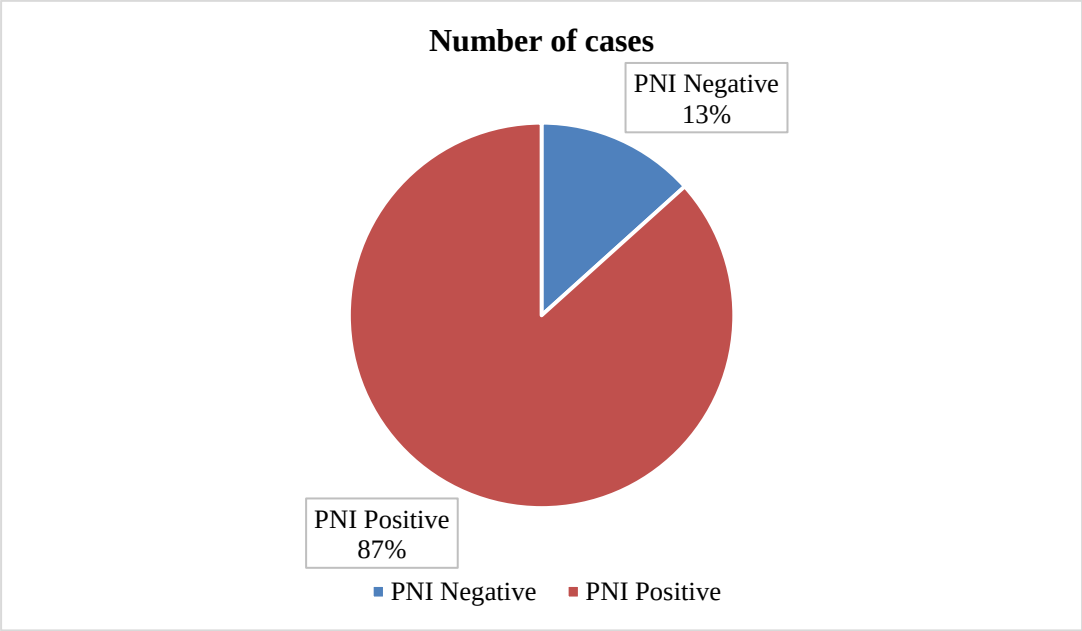


The Perineural Invasion (PNI) status among 30 cases were studied, with 86.67% (26 cases) being PNI-positive and the remaining were PNI-negative. Fisher's Exact Test yielded a p-value of 0.284 and was not statistically meaningful. [Table 7, Graph 6]

Table 7: Status of PNI-wise distribution

Status of PNI	Number of cases	%
PNI Negative	4	13.33
PNI Positive	26	86.67
Total cases	30	100.00

Graph 6: Distribution of Status of PNI in PCa patients.



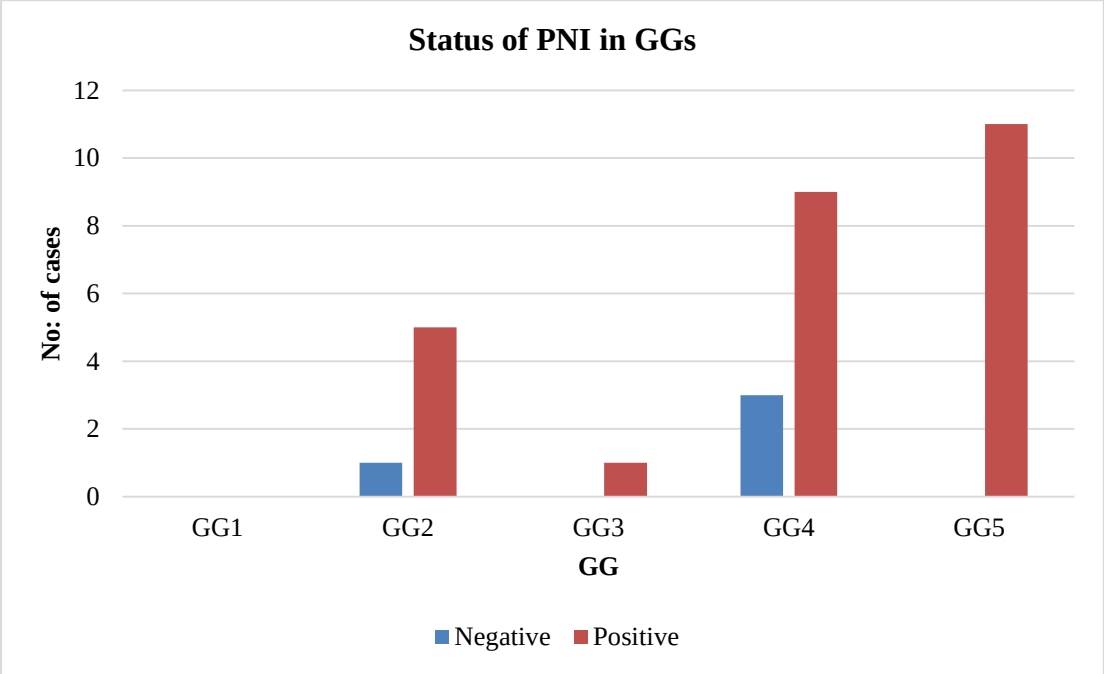
Perineural invasion (PNI) was seen more in higher Gleason grade groups (GG4 and GG5).

However, a p-value of 0.558 is not significant. [Table 8, Graph 7]

Table 8: Distribution of status of PNI in various grade groups

Status of PNI	GG1	GG2	GG3	GG4	GG5	Number	%
Negative	0	1	0	3	0	4	13.33
Positive	0	5	1	9	11	26	86.67
Total	0	6	1	12	11	30	100

Graph 7: Distribution of status of PNI in various grade groups

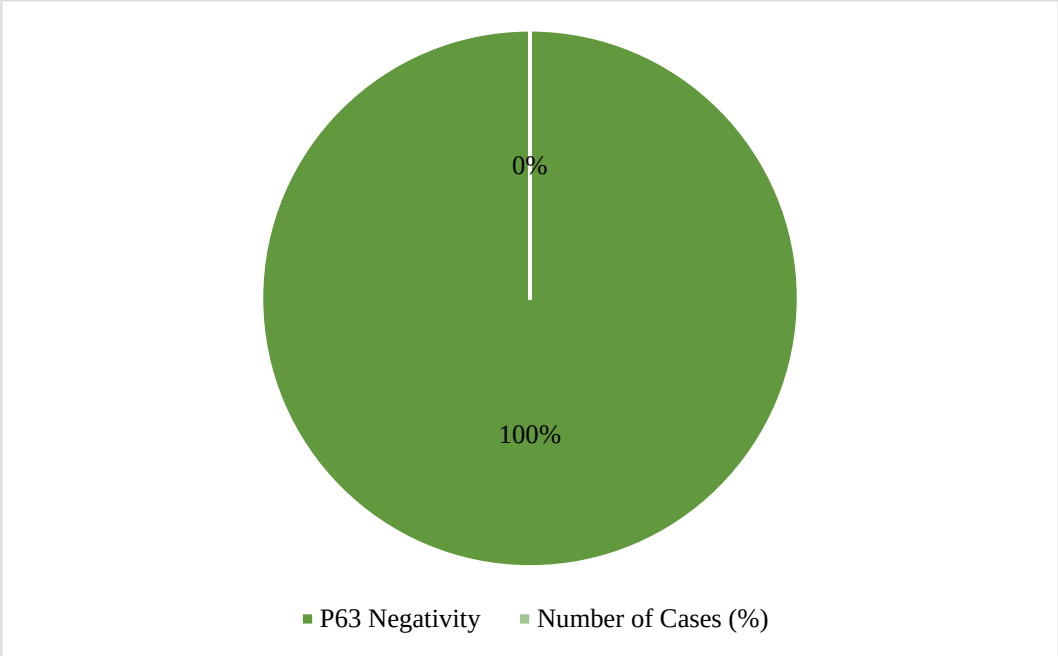


P63 expression was studied across all of the 30 cases and was found to be negative. [Table 9. Graph 8]

Table 9: Distribution status of P63 staining

P63 Negativity	Number of Cases (%)
30	30(100%)

Graph 8: Distribution status of P63 staining

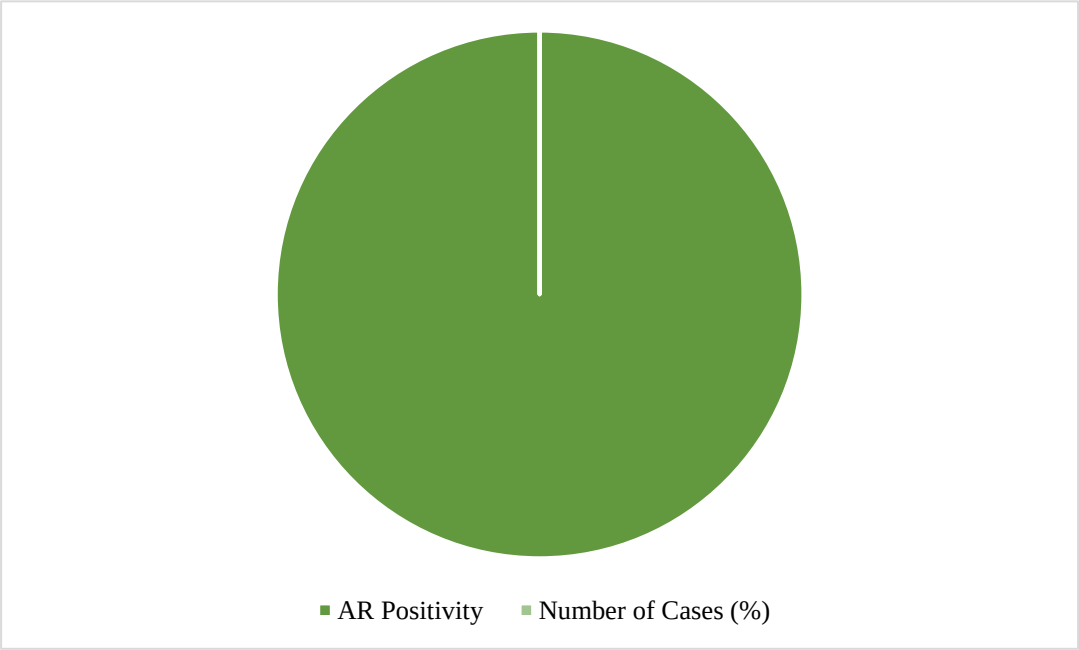


All 30 cases showed AR positivity in tumor cells.

Table 10: Distribution status of Tumor cell AR staining

AR Positivity	Number of Cases (%)
30	30(100%)

Graph 9: Distribution status of Tumor cell AR staining



The association between the Tumor cell Androgen receptor (AR) staining intensity and Gleason grade groups shows that, strong staining is mostly seen in higher-grade tumors, particularly in Grade Group 5 (30%). The p-value (0.0227) is significant, suggests a strong association between increasing AR staining intensity and higher Gleason grade. [Table 11]

Table11: Distribution of tumor cell AR intensity of staining across various Gleason grade groups

Gleason grade group	**AR-intensity of staining**				
	No staining(0)	**Weak staining (1+)**	**Moderate staining (2+)**	**Strong staining (3+)**	**Total**
GG 1	-	-	-	-	-
GG 2	-	1 (3.3%)	3 (10.0%)	2 (6.7%)	6 (20%)
GG 3	-	-	1 (3.3%)	-	1 (3.33%)
GG 4	-	4 (13.3%)	5 (16.7%)	3 (10.0%)	12 (40%)
GG 5	-	2 (6.7%)	-	9 (30.0%)	11 (36.66%)
Total		7 (23.33%)	9 (30%)	14 (46.66%)	30 (100%)
P value		**0.0227**			

In the present study, the histological score (H Score) for AR was evaluated by multiplying intensity of staining with the proportion positivity of cells. The Histologic Score varied from 0-300. A cut-off point of 200 was applied to group the AR expression as low expression (<200) and high expression (>200).[102,104] 63.33% cases showed low expression and 36.67% cases showed high expression. [Table 12]

Table 12: Distribution of H Score

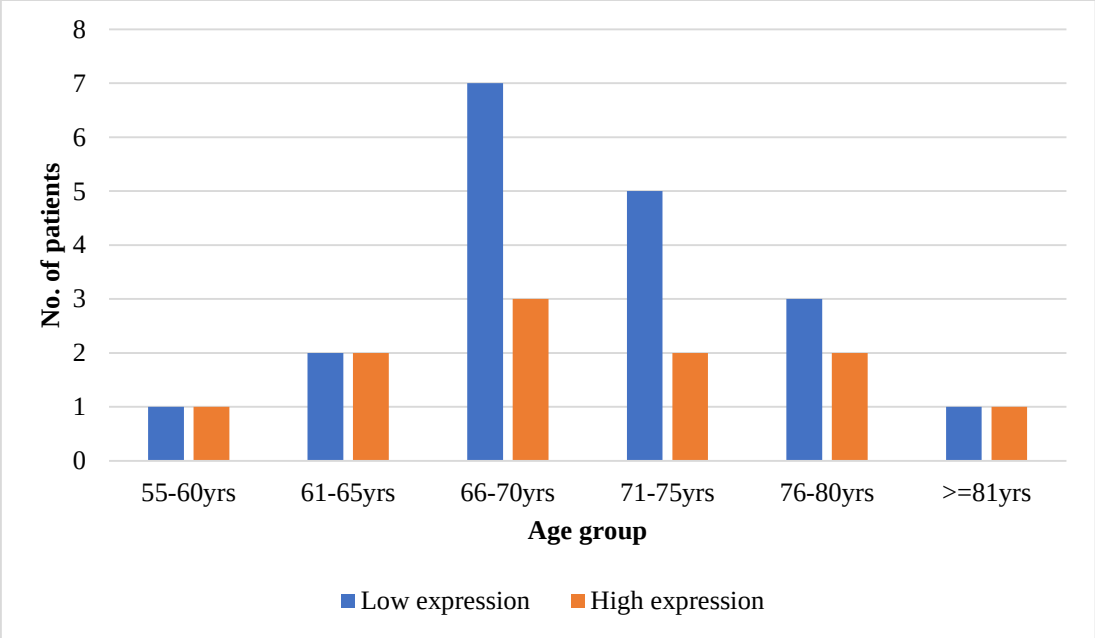
H Score	Number of cases (%)
Low (<200)	19 (63.33)
High (>200)	11 (36.67)

H Score was assessed in various age groups. Both low and high Tumor cell AR expression was observed in the age category of 66-70 years and not statistically significant. [Table 13, Graph 9]

Table 13: Association between Tumor cell AR Expression (H Score) with age groups

Age group	H Score		Total
	Low (H score< 200)	High (H score >200)	
55-60yrs	1 (3.33%)	1 (3.33%)	2 (6.67%)
61-65yrs	2 (6.67%)	2 (6.67%)	4 (13.33%)
66-70yrs	7 (23.33%)	3 (10.00%)	10 (33.33%)
71-75yrs	5 (16.67%)	2 (6.67%)	7 (23.33%)
76-80yrs	3 (10.00%)	2 (6.67%)	5 (16.67%)
≥81yrs	1 (3.33%)	1 (3.33%)	2 (6.67%)
Total	19 (63.33%)	11 (36.67%)	30 (100%)
P value	0.9748		

Graph 10: Association between Tumor cell AR Expression (H Score) with age groups



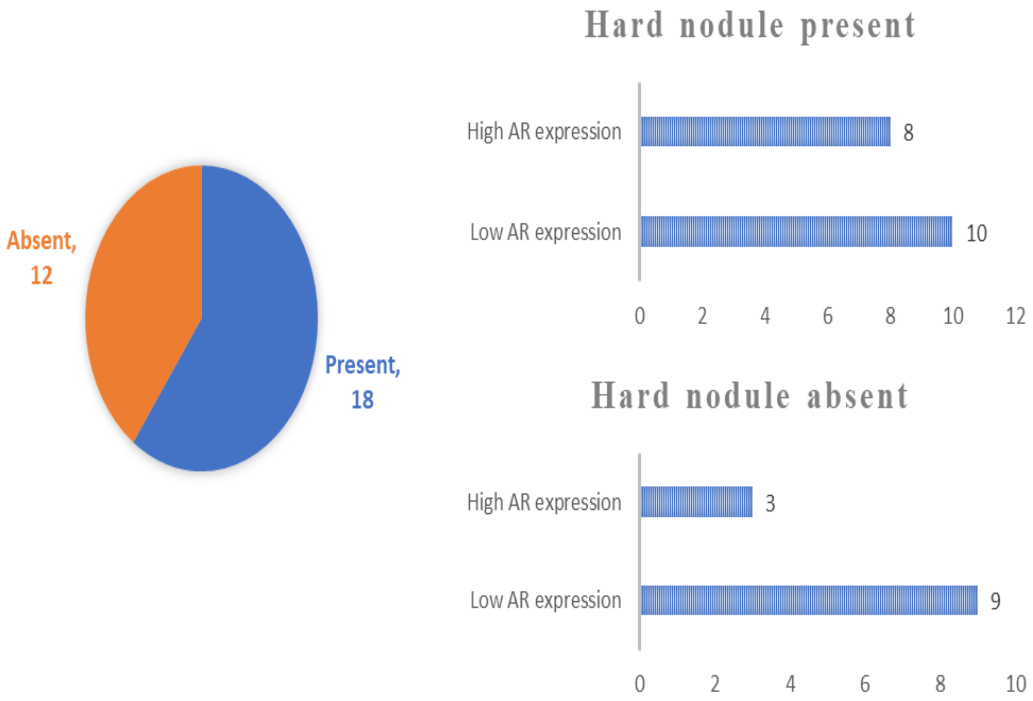
The association between a hard nodule on DRE and Tumor cell androgen receptor (AR) expression level was studied. Among the 18 cases with a hard nodule, 33.33% had low AR expression, and 26.66% showed high AR expression, while in the 12 cases without a hard nodule, 30% had low AR expression, and 10% had high AR expression, with a P-value =0.4425, indicating no statistical significance. [Table 14, Graph 11]

Table 14: Association of Hard nodule on DRE and Tumor Cell AR Expression

Hard nodule on DRE	**Number of cases (%)**	**H Score**	
		Low AR expression (h score< 200)	**High AR expression (h score >200)**
Present	18 (60%)	10 (33.33%)	8 (26.66%)
Absent	12 (40%)	9 (30%)	3 (10%)
P value	0.4425		

*Fishers G test done

Graph 11: Association of Hard nodule on DRE and Tumor cell AR expression

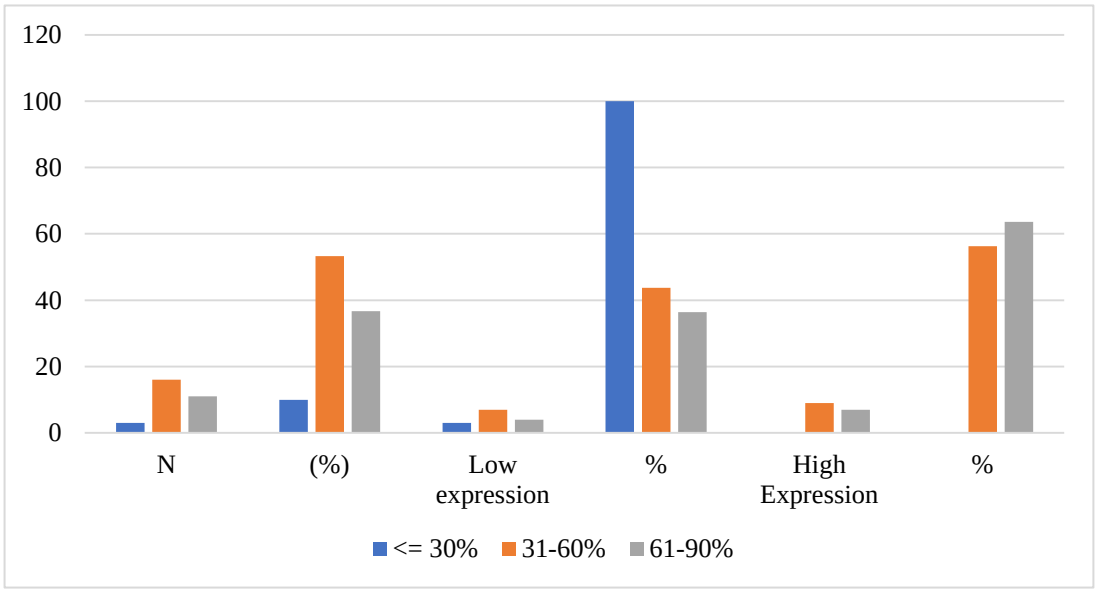


All cases (100%) with tumor volume ≤ 30% exhibited low androgen receptor (AR) expression. In contrast, as tumor volume increased, the proportion of higher AR expression cases also increased, with 56.25% in the 31-60% tumor volume group and 43.75% in the 61-90% tumor volume group. A p-value from Fisher's Exact Test is 0.090 was not statistically significant. [Table 15, Graph 12]

Table 15: Association of approximate tumor volume and Tumor cell AR expression

Approx. Tumor volume	Number of cases	%	H Score			
			Low expression (h score< 200)	%	High expression (h score >200)	%
≤30%	3	10	3	21.42	0	0
31-60%	16	53.33	7	50	9	56.25
61-90%	11	36.66	4	28.57	7	43.75
Total	30	100	14	100	16	100

Graph 12: Association of approximate tumor volume and Tumor cell AR expression



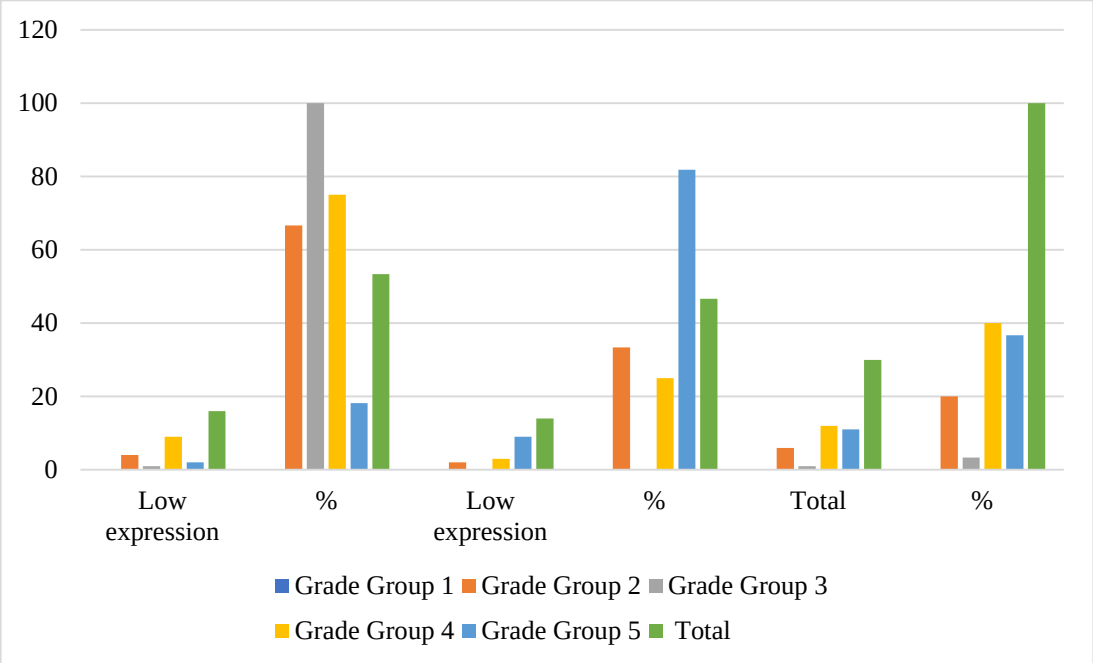
In the present study, the association of Tumor cell AR expression and Gleason grade groups was analysed. Higher expression was observed predominantly in Grade 5 (81.81%), while lower grade groups showed low AR expression. The chi-square value of 11.82 and p-value of 0.019 implies a statistically meaningful association between expression AR levels and Gleason grades. [Table 16, Graph 13]

Table 16: Association between levels of tumor cell AR expression with Gleason grade groups

Gleason grade group	H Score					
	LowExpression (h score< 200)	**%**	**HighExpression (h score >200)**	**%**	**Total**	**%**
GG 1	0	0.00	0	0.00	0	0.00
GG 2	4	66.67	2	33.33	6	20.00
GG 3	1	100	0	0	1	3.33
GG 4	9	75	3	25	12	40.00
GG 5	2	18.18	9	81.81	11	36.67
Total	16	53.33	14	46.67	30	100.00

Chi-square=11.82**., p=0.019**

Graph 13: Association between levels of tumor cell AR expression with Gleason grades



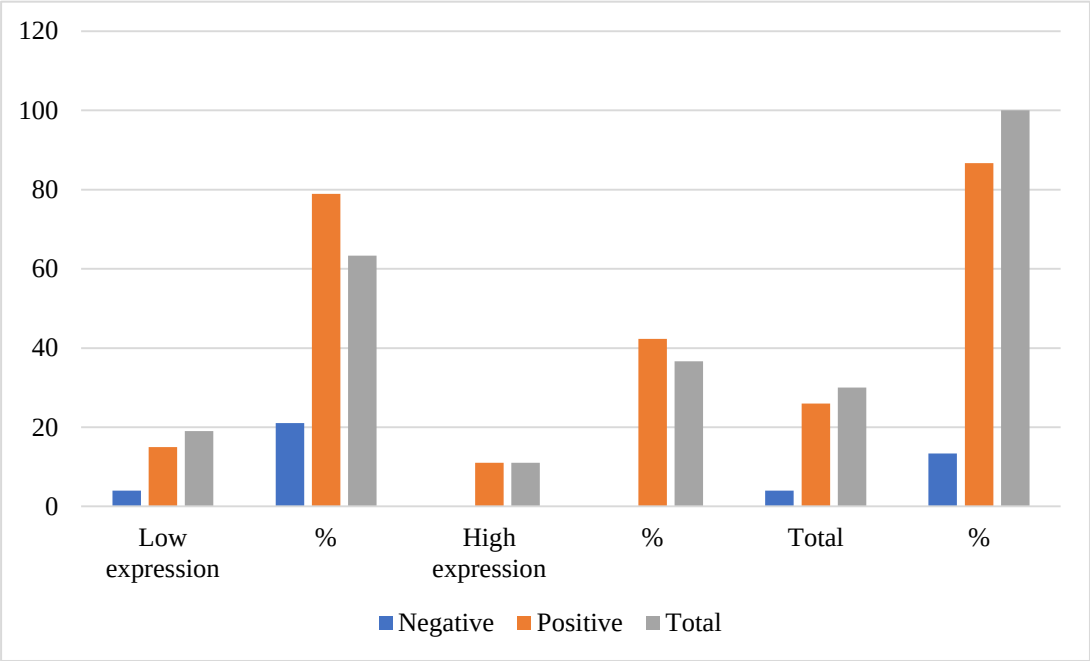
AR expression in the tumor cell were compared with the status of PNI in all the cases. Among them, 78.94 % had low androgen receptor (AR) expression and 42.30% having high AR expression. The chi-square value of 2.681 and p-value of 0.101 indicate no statistically significant association. [Table 17, Graph 14]

Table17: Association between tumor cell AR expression with Status of PNI

Status of PNI	H Score					
	Low Expression	%	High Expression	%	Total	%
Negative	4	21.05	0	0	4	13.33
Positive	15	78.94	11	42.30	26	86.67
Total	19	63.33	11	36.66	30	100.00

Chi-square=2.681, p≈ 0.101

Graph 14: Association between tumor cell AR expression with Status of PNI

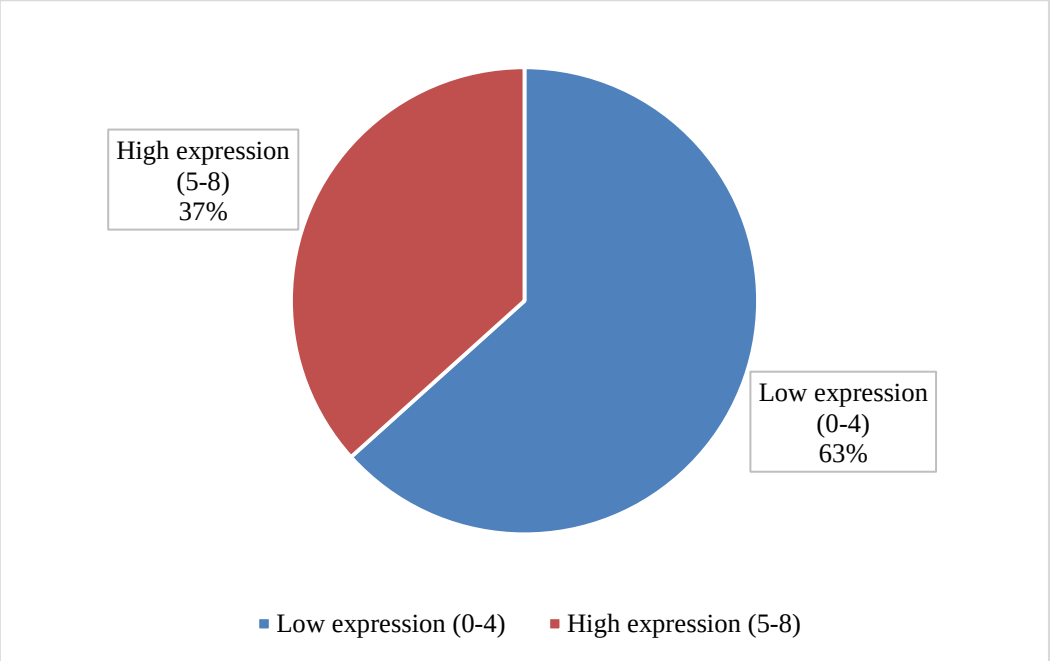


In this analysis, the Stromal AR expression was studied across all 30 cases. The stromal AR expression was semiquantitatively assessed. Allred score (Sum of intensity and proportion scores") was applied to interpret the AR staining. Staining intensity was score in a range of 0-3 and proportion of positive cell from 0-5. Final score ranged from 0-8 [9]. 0-4 is taken as Low expressing and 5-8 high expressing. [10]

Table18: Distribution of Allred Score (Stromal AR Expression).

Allred Score	Number of cases	%
Low expression (0-4)	19	63.33
High expression (5-8)	11	36.67

Graph 15: Distribution of Allred Score (Stromal AR Expression).

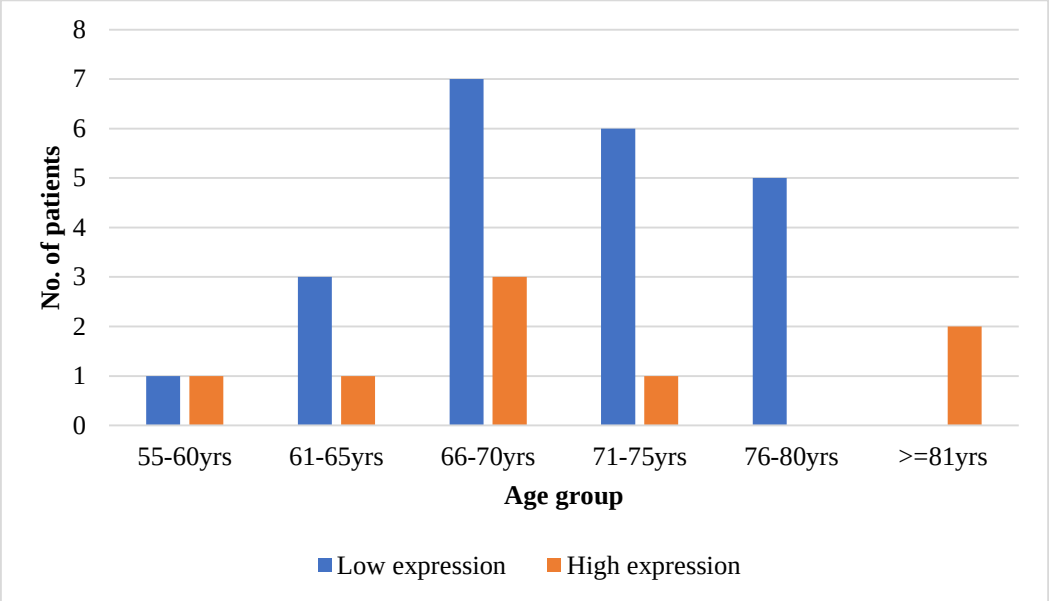


The association of stromal AR expression with different age groups studied shows that low expression is more common (63.33%) than high expression (36.67%) across all age groups. The p-value of 0.1365 suggests no significant relationship between stromal AR expression and age. [Table 19, Graph 16]

Table 19: Association between levels Stromal AR Expression with age groups

Age group	Stromal AR Expression		Total
	Low Expression (0-4)	High Expression (5-8)	
55-60yrs	1 (3.33%)	1 (3.33%)	2 (6.67%)
61-65yrs	3 (10.00%)	1 (3.33%)	4 (13.33%)
66-70yrs	7 (23.33%)	3 (10.00%)	10 (33.33%)
71-75yrs	6 (20.00%)	1 (3.33%)	7 (23.33%)
76-80yrs	5 (16.67%)	0 (0.00%)	5 (16.67%)
≥81yrs	0 (0.00%)	2 (6.67%)	2 (6.67%)
Total	19 (63.33%)	11 (36.67%)	30 (100%)
P value	0.1365		

Graph 16: Association between levels of Stromal AR Expression with age groups



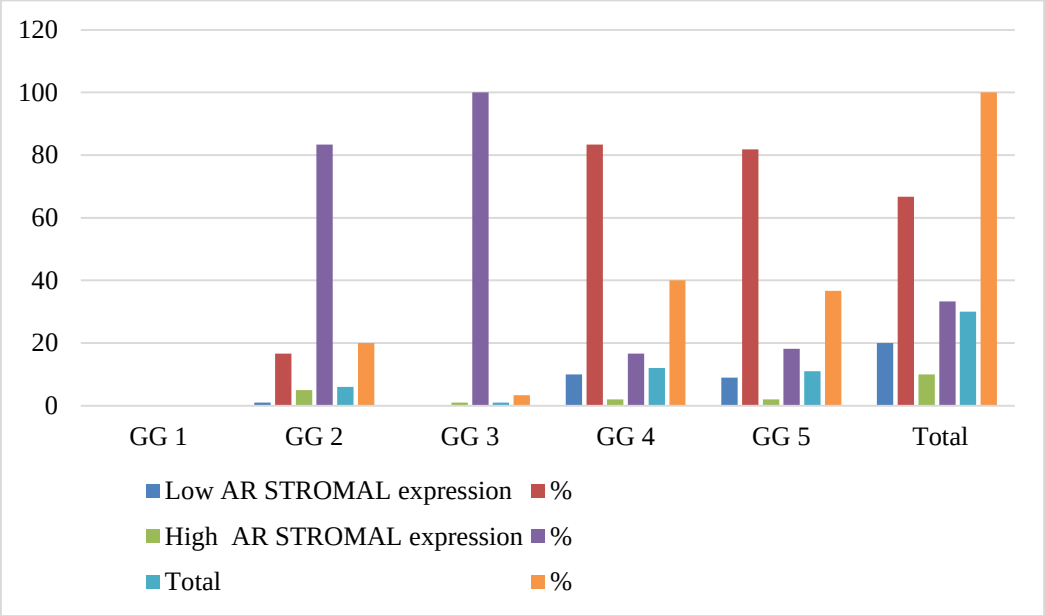
Stromal AR expression was studied in various grade groups. A higher Allred Score was found in GG2 and lower in GG4 and GG5. The chi-square value of 11.42 and a p-value of 0.022 suggest a statistically meaningful correlation. [Table 20, Graph 17]

Table 20: Association between levels of Stromal AR expression with Gleason grade group.

Gleason grade group	Stromal AR Expression					
	Low Expression (Allred score0-4)	%	High Expression (Allred score5-8)	%	Total	%
GG 1	0	0.00	0	0.00	0	0.00
GG 2	1	16.67	5	83.33	6	20.00
GG 3	0	0	1	100	1	3.33
GG 4	10	83.33	2	16.67	12	40.00
GG 5	9	81.81	2	18.18	11	36.67
Total	20	66.67	10	33.33	30	100.00

Chi-square=11.42**., p=0.022**

Graph 17: Association between levels of Stromal AR expression with Gleason grade group



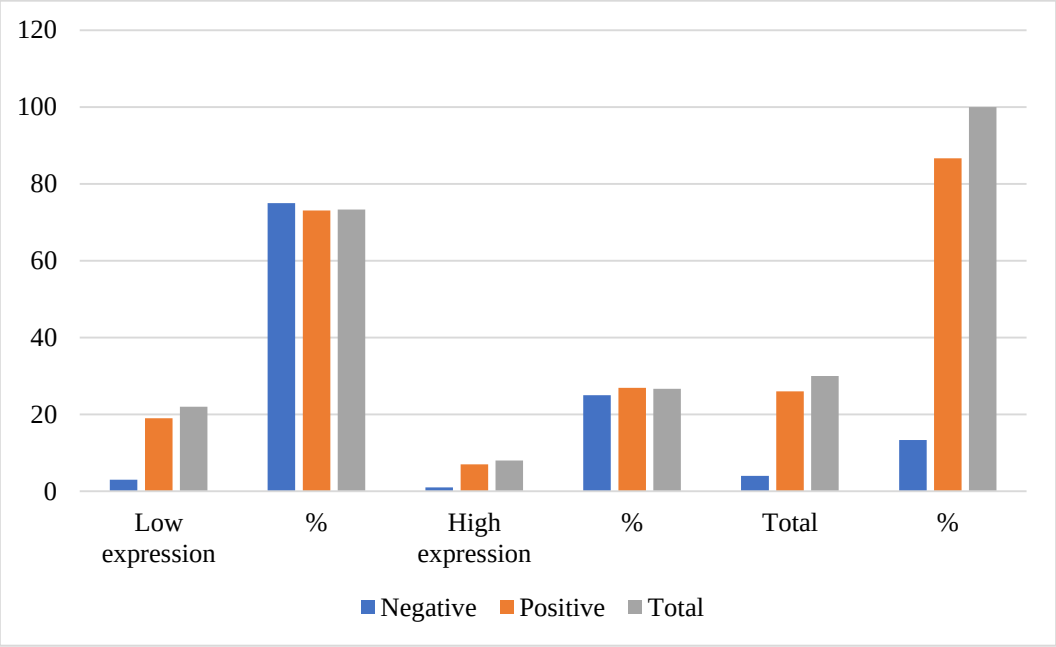
Stromal AR expression was compared with the status of PNI in all the cases. The stromal AR expression levels are similar between PNI-negative and PNI-positive cases, with low expression observed in 75% and 73.07% of cases, respectively. There was no statistically meaningful correlation. [Table 21, Graph 18]

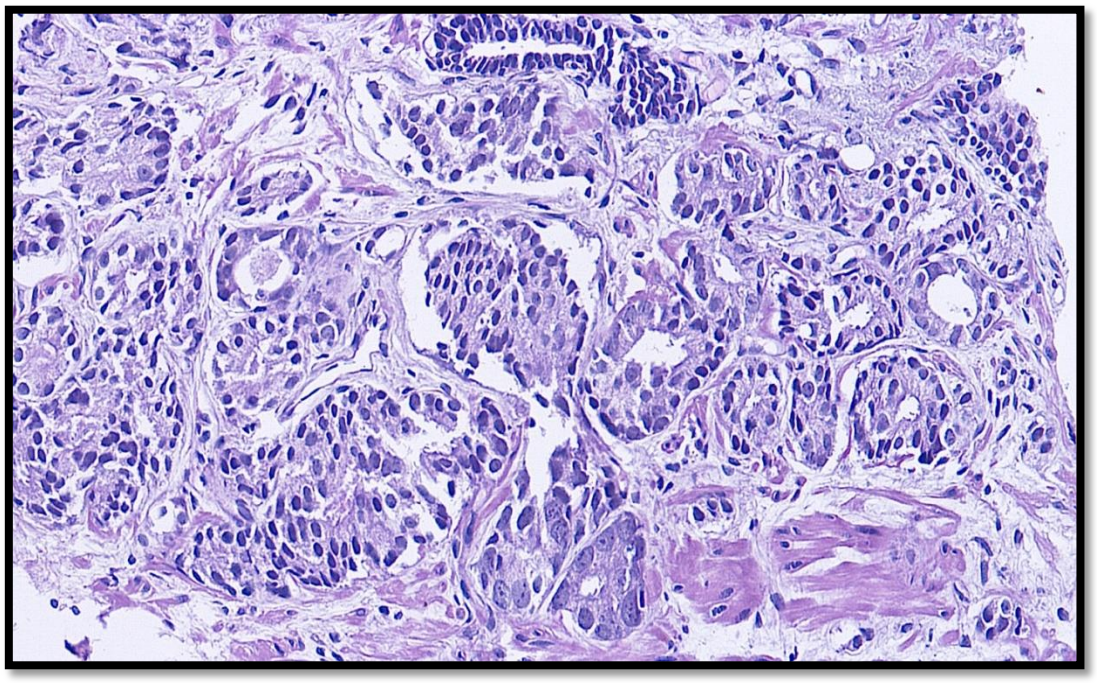
Table 21: Association between levels of Stromal AR expression with Status of PNI

Status of PNI	Stromal AR Expression					
	Low Expression	%	High Expression	%	Total	%
Negative	3	75	1	25	4	13.33
Positive	19	73.07	7	26.92	26	86.67
Total	22	73.33	8	26.66	30	100.00

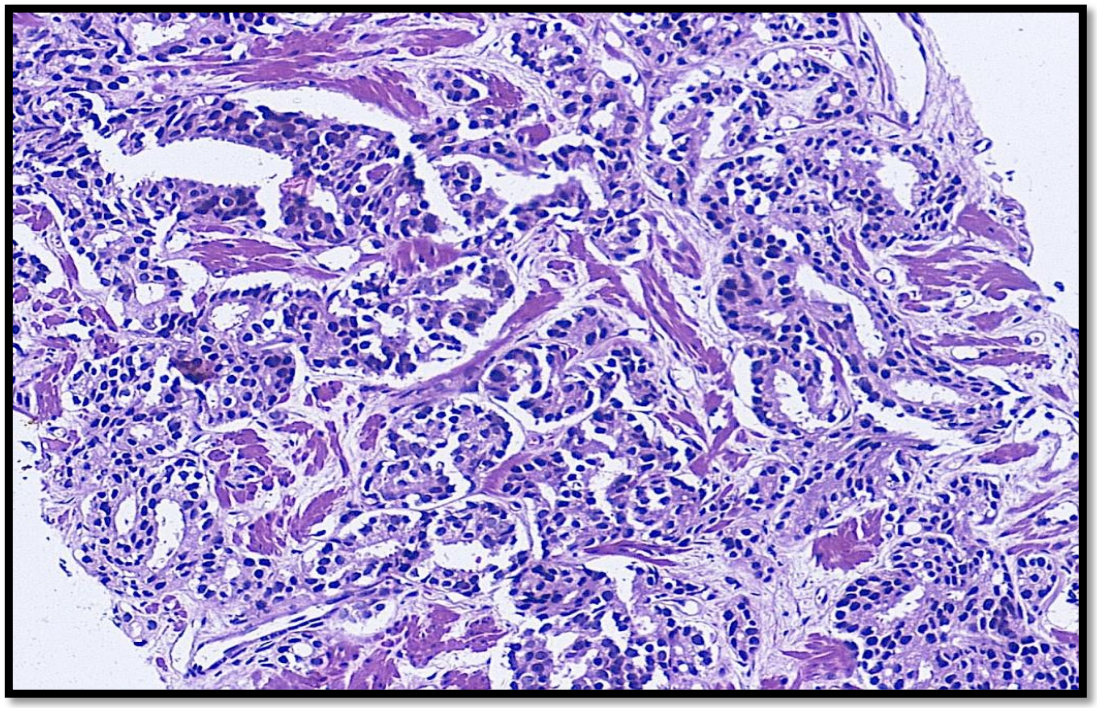
Chi-square=0.0183, p=0.892

Graph 18:Association between levels of Stromal AR expression with Status of PNI

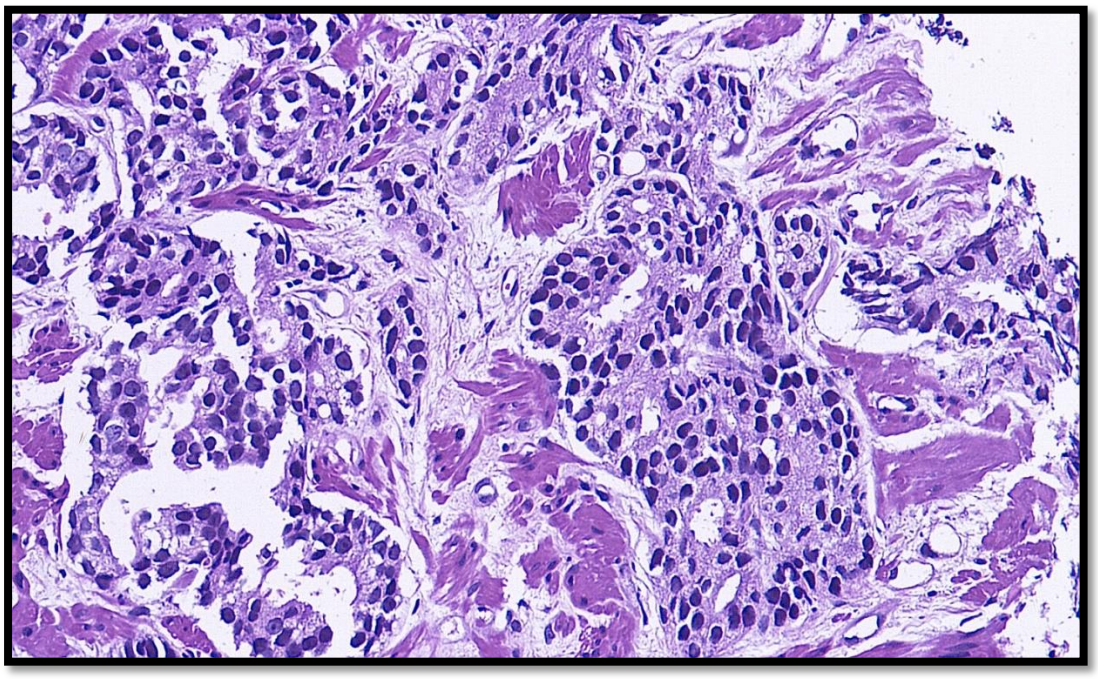




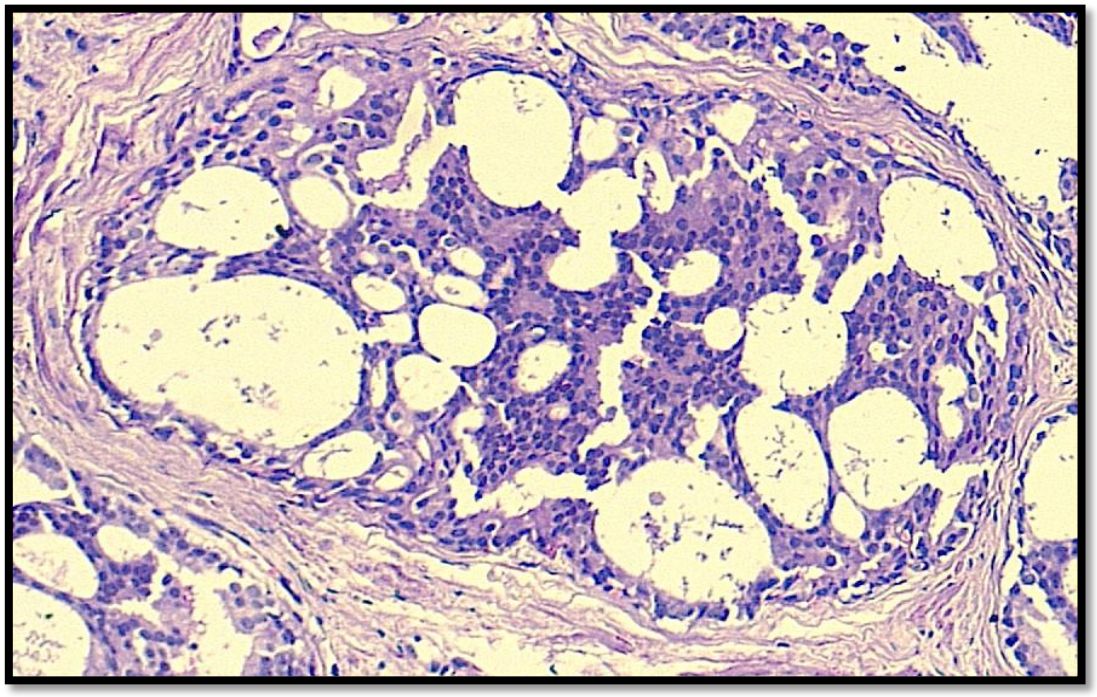
Photomicrograph 1: Shows Grade Group 2 (3+4=7) composed of discrete glands and fused glands (H&E, x100)



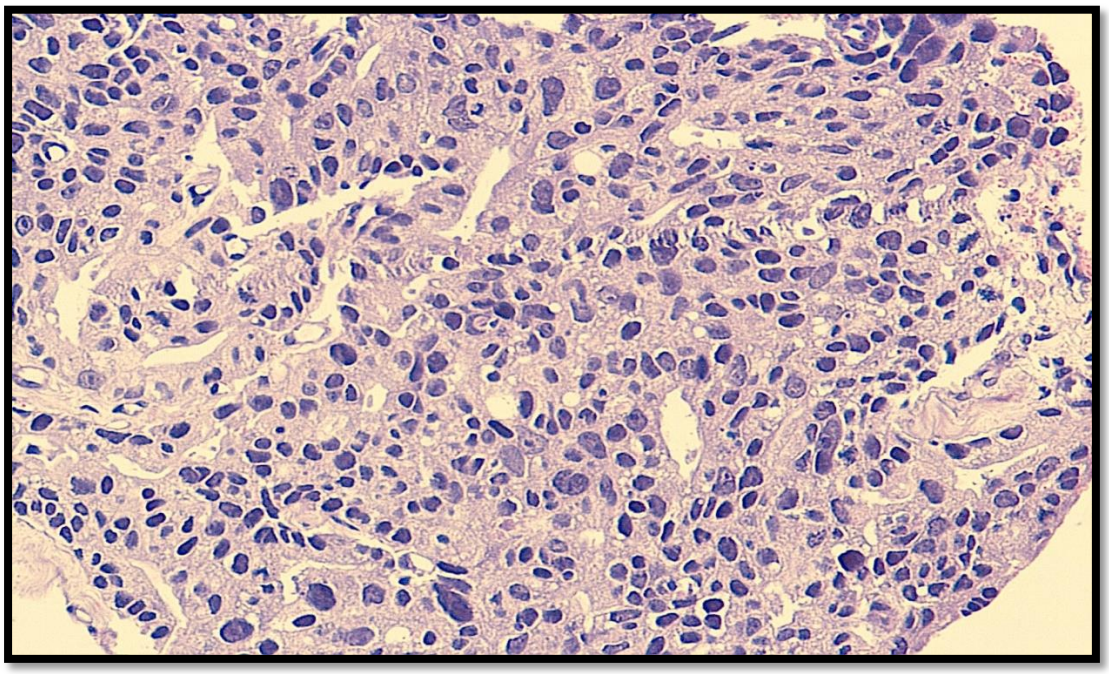
Photomicrograph 2: Shows Grade Group 3 (4+3=7) with fused glands and discrete glands (H&E, x100)



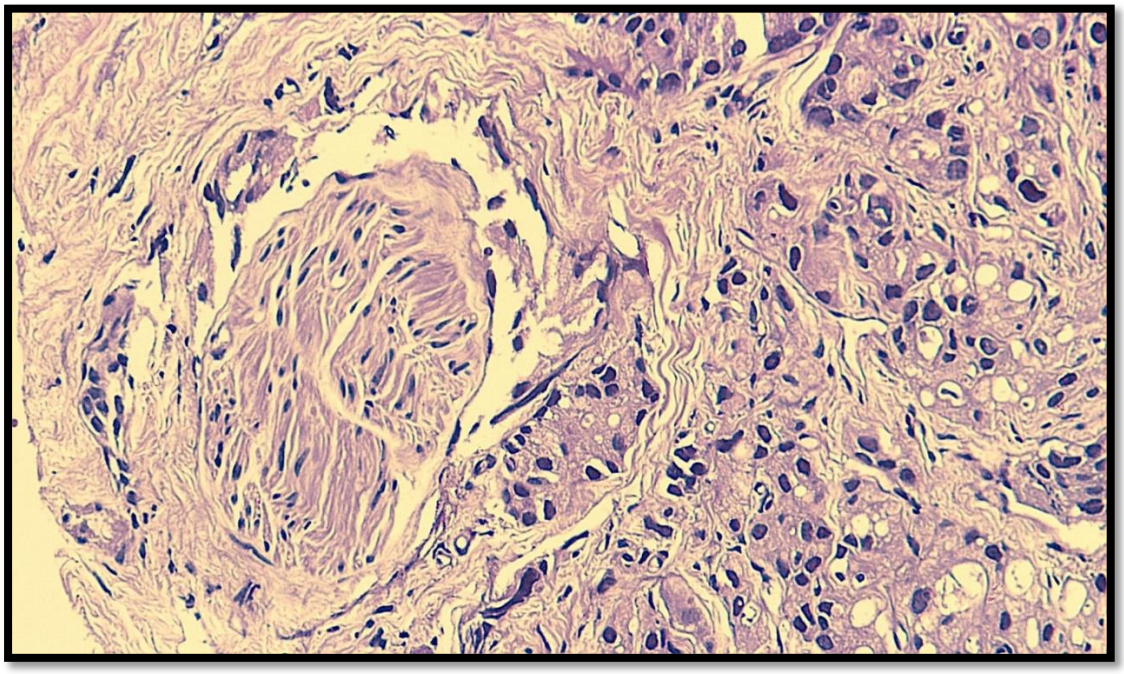
Photomicrograph 3: Shows Grade Group 3 (4+3=7) with fused glands and discrete glands (H&E, x200)



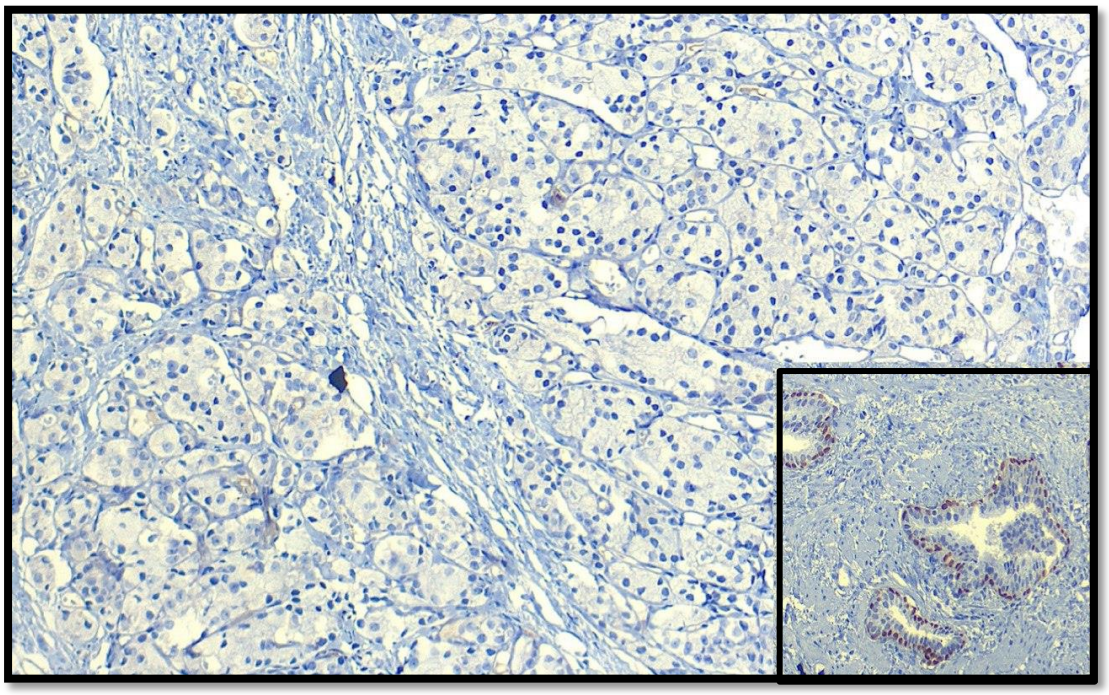
Photomicrograph 4: Shows Grade Group 4 with cribriform pattern (H&E, x200)



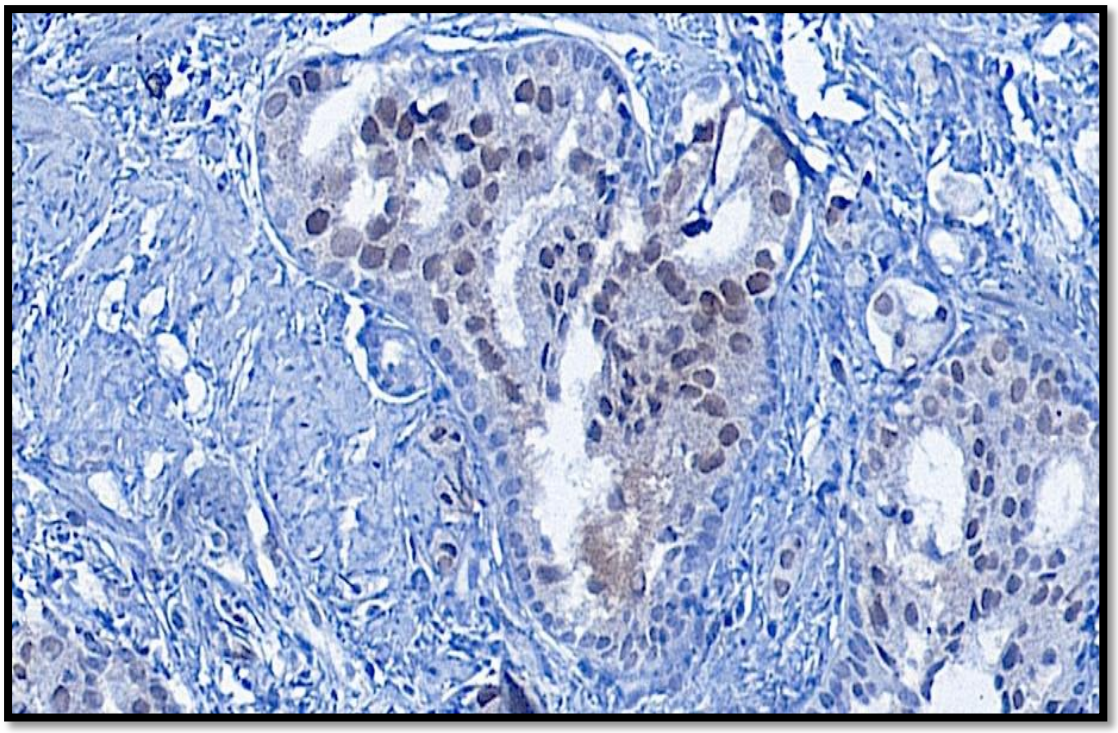
Photomicrograph 5: Shows Grade Group 5 with sheets of cells (H&E, x200)



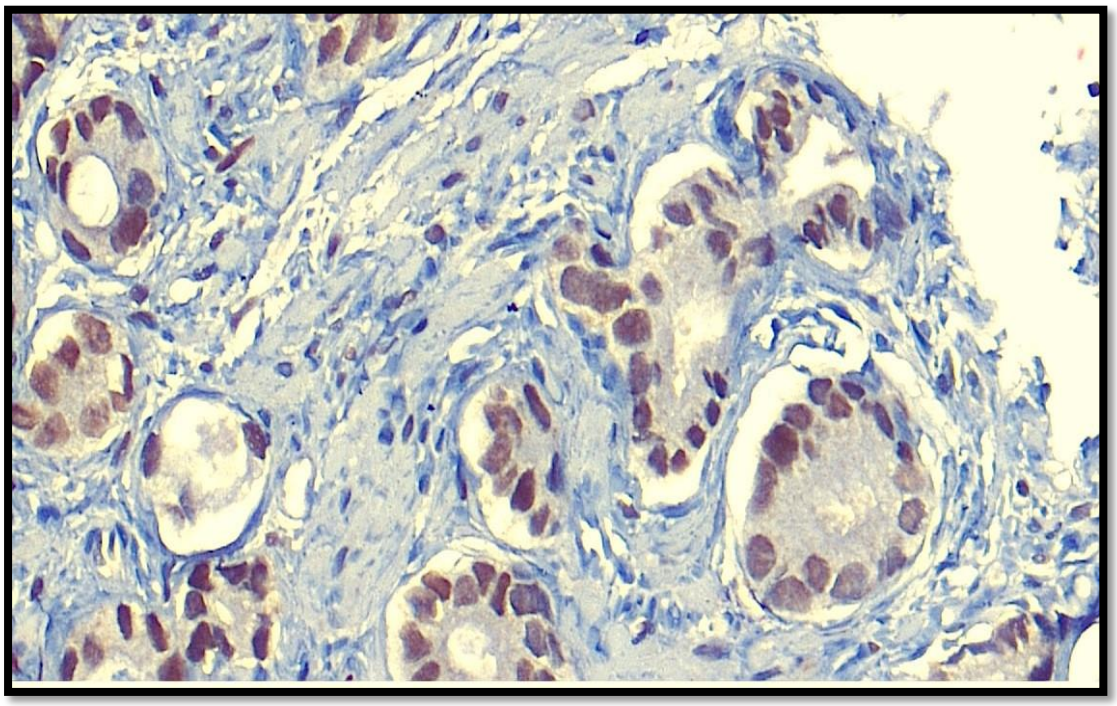
Photomicrograph 6: Shows Perineural Invasion in PCa (H&E x200)



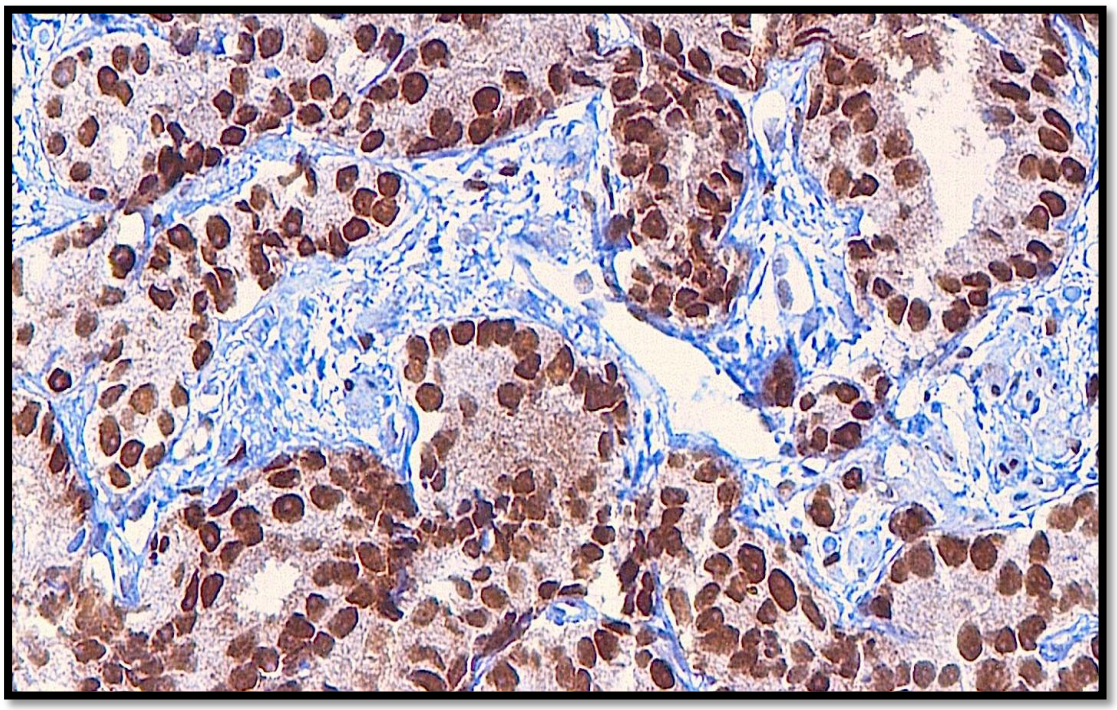
Photomicrograph 7: Shows P63 negative in PCa with inset showing positive P63 in a foci of normal area (H&E x200)



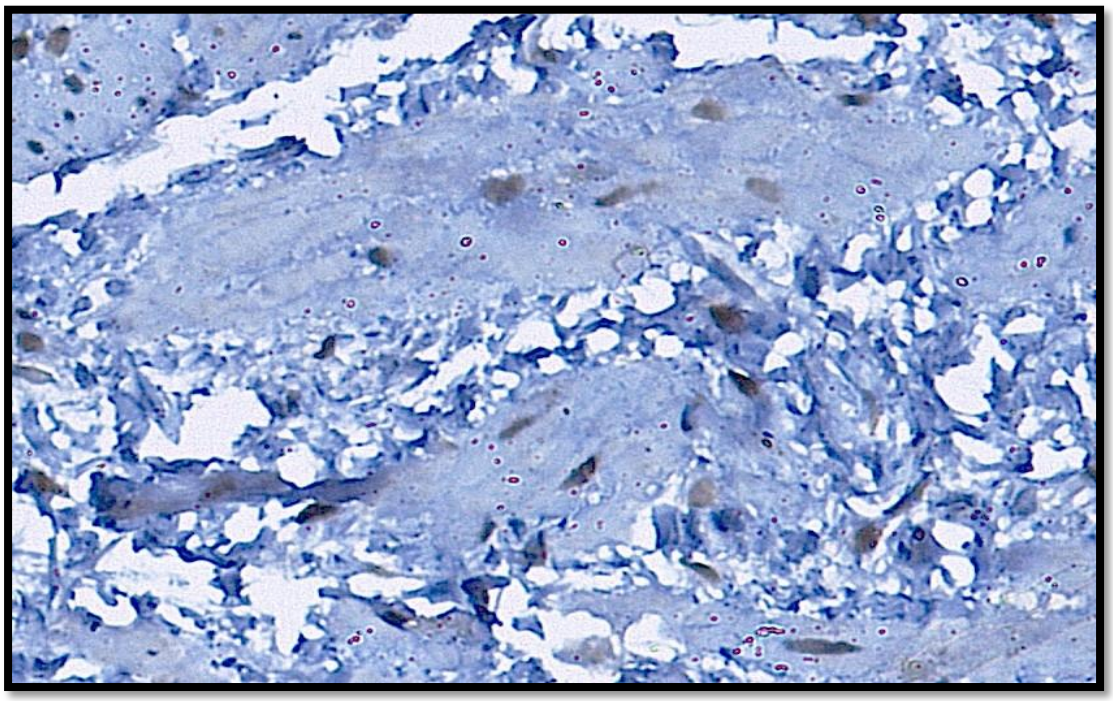
Photomicrograph 8: Shows 1+ AR staining in tumor cell (H&E x200)



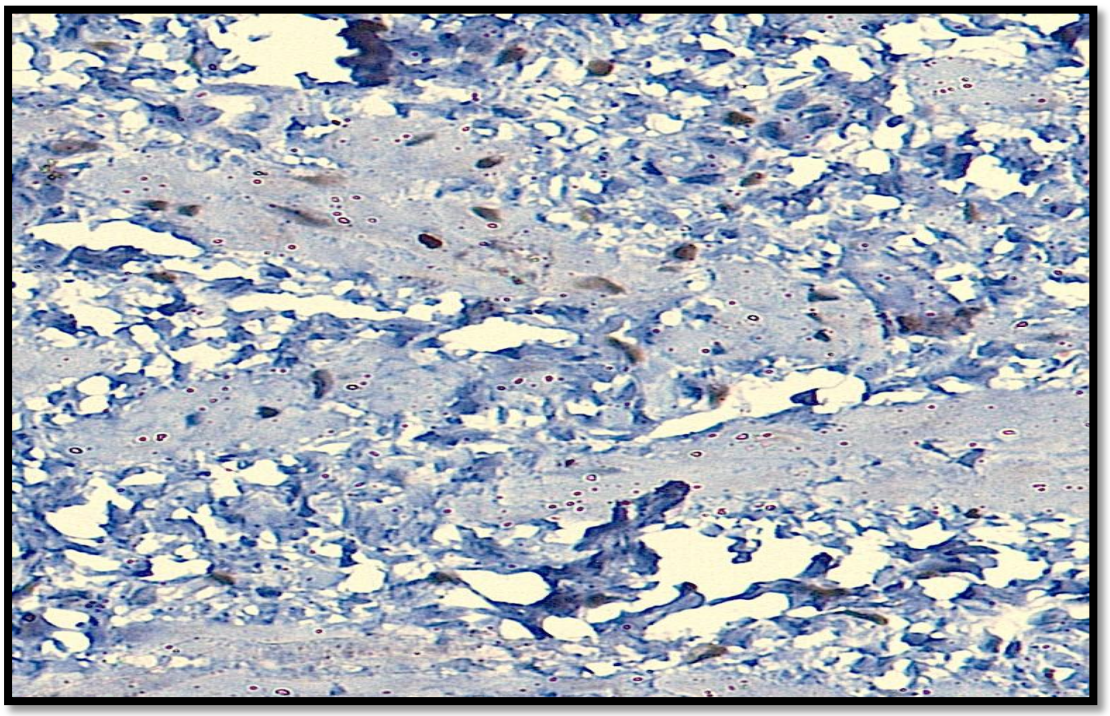
Photomicrograph 9: Shows 2+ AR staining in tumor cells (H&E x200)



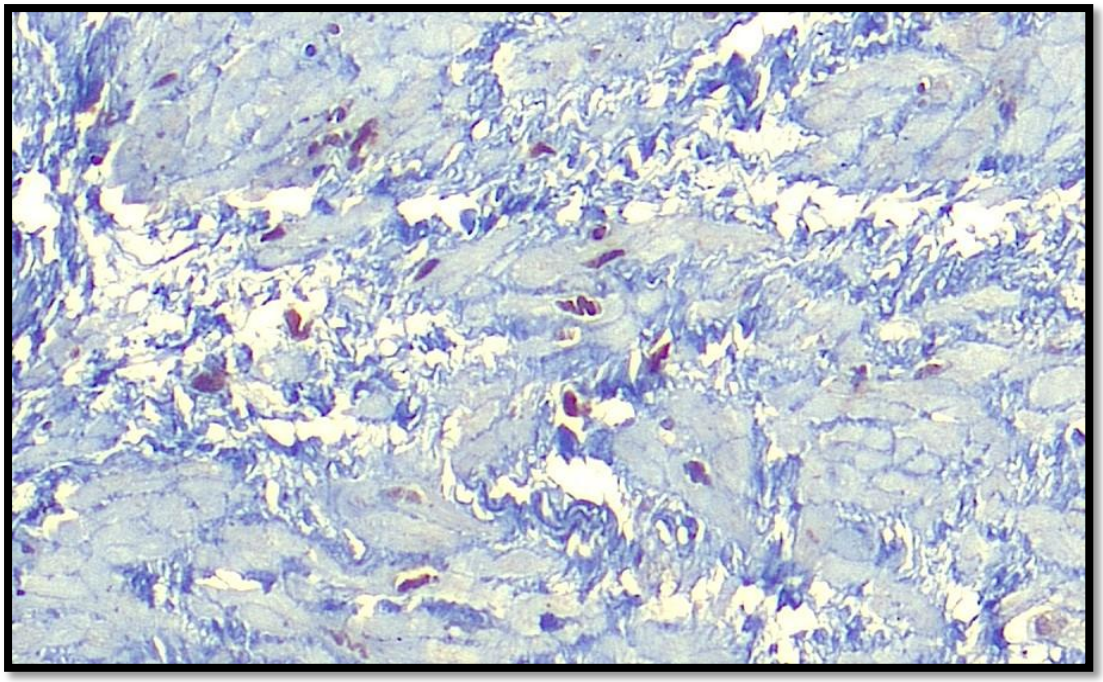
Photomicrograph 10: Shows 3+ AR staining in tumor cell (H&E x200)



Photomicrograph 11: Shows 1+ AR staining in stroma (H&E x200)



Photomicrograph 12: Shows 2+ AR staining in stroma (H&E x200)



Photomicrograph 13: Shows 3+ AR staining in stroma (H&E x200)

DISCUSSION

Prostate cancer is ranked the second highest commonly diagnosed cancer in the male gender worldwide, surpassing 1.4 million new cases; prostate cancer was widely reported in 2020.[27]

The AR plays a crucial part in the onset and advancement of prostate cancer. It controls genes responsible for cell growth and survival, making it a critical factor in the progression of the disease.[106]

p63 is a nuclear regulatory protein that belongs to the p53 family. It is typically found in the basal cells.[107]

Numerous research has been done on the significance of Tumor cell AR expression with clinicopathological parameters and as a prognostic marker. In this study, we have assessed theP63 expression and AR expression in both tumor cells and the stomal cells of Pca patients and its correlation with Clinicopathological parameters.

In the current study, the age of the patients varied between 58 to 86 years. Maximum number of cases were in the 66-75 years range, which is consistent with prostate cancer trends [109, 104] The mean age of cases was (70.87±6.31), similar to the observations of Husain et al., Hashmi et al., Putriyuni et al., and Shokeir et al.[109, 102, 105, 101] This suggests the incidence of PCa increase with increasing age.

In this study, Prostate Specific Antigen (PSA) values were available in 24 of the 30 cases where mean PSA is 124.13 and median 86.2. Shokeir et al. observed a median PSA of 10.3 [101], and Husain et al. reported a median PSA level of 39.8

ng/ml.[109] Whereas Thompson I M et al in his study reported the mean PSA value of 1.78 ng/ml among PCa cases.[110]

In the present study 60% cases showed hard nodule on DRE, whereas Shokeir et al and Irekpita et al reported hard nodule in 34.5 % and 48.85% of their cases.[101,111]

In the present study, there is a predominance of higher-grade prostate cancer, which is in concordance with the studies by Hashmi et al., Putriyuni et al., and Fatima et al.[102,105,112]

This increase in higher grades may be because the study is conducted in a tertiary care center with cancer hospital.

In the current research, no statistically meaningful correlation of Gleason grade group and presence or absence of hard nodule on DRE. In a study by Irekpita et al. stated that a hard, nodular feel during DRE had a predictive value of 73.3% for PCa diagnosis but did not independently predict higher Gleason scores. This highlights the fact that while DRE is valuable for detecting prostate abnormalities, its findings may not directly correlate with tumor aggressiveness.[111]

In the present study, majority (53.33%) had a tumor volume between 31-60%, while 36.66% had a volume of 61-90%, and only 10% had a volume of ≤30%. This indicates that most cases had moderate to high tumor volume, suggesting a relatively advanced disease. Hashmi et al had similar findings where 52.1% of patients were with >50% tumor.[102]

Shokeir et al reported 50.8%of cases of Pca having tumor volume less than 50%.[101]

In the present study highest number of cases was observed in GG4and GG5 with majority of cases having tumor volumes between 31-60% and 61-90%. This association was not statistically significant. Mayer et al. indicated that prostate tumor eccentricity was a stronger predictor of Gleason scores than tumor volume. This implies that tumor shape might be more important in deciding tumor aggressiveness than volume alone.[113] whereas Stamey et al. analyzed the impact of cancer volume and high Gleason scores on biochemical recurrence-free survival, finding that both factors were significantly related to worse outcomes.[114]

The Perineural Invasion (PNI) status among 30 cases were studied, with 86.67% (26 cases) being PNI-positive. Similar results were described by Loeb et al. and Niu et al.[115,116] Shokeir et al and Putriyuni et al reported less number of PNI-positive cases.[101,105]

PNI was seen more in GG4 AND GG5, indicating a strong association between PNI and higher Gleason grade groups. However this association is not statistically significant.

de la Calle et al. found that men with PNI were more likely to see their cancer grade change over time compared to those without PNI, which indicate that PNI could indicate a higher chance of the cancer becoming more aggressive or advancing to a more serious stage.[117]

The P63 immunohistochemistry was studies in all 30 cases and was found to be negative which supports the typical pattern seen in PCa, where the glands lose the myoepithelial cells, in concordance with Giannico et al.[118]

AR positivity was seen in all 30 cases, whereas Hermien et al reported one AR negative case[104]

In the current study, AR staining was shown in all the cases of PCa. Strong AR staining was most prevalent in higher-grade tumors, particularly in Grade Group 5 (30%). A p-value (0.0227) indicated that tumor cell AR staining intensity may be linked to tumor aggressiveness. Similar findings were observed by Fatima G. et al. and Hermien et al.[104,112]

In our study, 63.33% of cases showed low tumor cell AR expression, while 36.67% exhibited high expression. In contrast, Hashmi et al. reported 43.8% with low expression and 56.2% with high expression.[102]

Most of the cases (63.33%) had low tumor cell androgen receptor (AR) expression (H score<200), while 36.67% showed high expression (H Score >200), with both types seen in patients aged 66–70 years. But, p-value of 0.9748 indicates no statistical significance. Hashmi et al. and Hermien et al. had similar observations [102, 104] The lack of significant association between AR expression levels with age suggests that participant age alone may not be a determining factor in AR-related prostate cancer progression.

Among the 18 cases presenting with a hard nodule on DRE, 33.33% exhibited low tumor cell AR expression, while 26.66% demonstrated high tumor cell AR expression. There was no statistical correlation between the status of Hard nodule on DRE and AR expression. Henshall et al. reported that altered AR expression in PCa is related to early relapse but did not find a direct association between AR expression levels and physical examination findings such as DRE. These findings suggest that

while DRE is valuable for detecting prostate abnormalities, its findings may not directly correlate with molecular characteristics like AR expression.[91]

In the current study, Tumor cell AR expression was low in tumors with ≤30% and higher Tumor cell AR expression was seen in tumor burden of 31-60% volume group. Hashmi et al reported > 50 % volume group had 70% high AR expression in tumor cells.[102]

Sena et al. found that AR activity is pivotal in how well treatments work for prostate cancer. They observed that changes in AR activity are linked to tumor size, meaning that tumors with higher AR expression may grow larger and be more aggressive.[119]

Guo et al. identified a unique AR splice variant that is augmented during prostate cancer progression, promoting androgen-deprivation-resistant growth. This suggests that increased AR activity may be associated with tumor progression, independent of tumor volume.[120]

The study reveals a significant association (p-value of 0.019) between tumor cell androgen receptor (AR) expression levels and Gleason grade groups in prostate cancer. Specifically, higher AR expression is predominantly encountered in Grade Group 5 (81.81%), while lower AR expression is more frequent in lower grade groups. In contrast, Husain et al and Park et al found no statistical correlation between between levels of tumor cell AR expression with Gleason grades [109,121]

In the present study, 78.94% had low Tumor cell AR expression and the remaining had high tumor cell AR expression. The chi-square value of 2.681 and p-value of 0.101 indicate no statistically significant association between PNI status and

AR expression. Similarly, Hashmi et al., and Putriyuni et al. found no statistically significant association between Tumor cell AR expression and PNI. [102,105] Although PNI is a recognized pathway for prostate cancer dissemination, its occurrence does not seem to have direct correlation with the levels of AR in tumor cells.

Here in this study, stromal androgen receptor (AR) expression was evaluated in 30 cases using the Allred scoring system, which combines intensity (0–3) and proportion (0–5) scores to give a final value ranging from 0 to 8. 0–4 was categorized as low expression, while 5–8 indicated high expression. Notably, reduced stromal AR expression has been associated with higher Gleason grades and advanced prostate cancer progression.[122,97]

Our study demonstrates low stromal AR expression was seen across all age groups and found no statistically significant association. Similarly, Shokeir et al found no statistically important association linking Stromal AR expression and age. [101,122] Suggesting that stromal AR expression remains relatively consistent regardless of patient age.

The study shows lower stromal AR expression in Grade Groups 4 and 5 and higher Stromal AR expression in lower Grade Groups. The two parameters had a statistically significant relationship (p value=0.022). Shokier et al. also reported a significant correlation between lower stromal AR expression and higher GGs.[101] Another study reported that stromal AR loss increases as prostate cancer becomes less differentiated, correlating with a higher Gleason grade. This decline in stromal AR also aligns with the cancer's progression.[123]

In our study, Stromal AR expression did not vary with the status of PNI. These findings concord with the analysis by Ricciardelli et al., who reported no significant

link between the expression of stromal AR and PNI status in prostate cancer specimens.[92] Similarly, Leach et al. found that variations in stromal AR levels did not significantly impact PNI presence.[124] In contrast to this, Shokeir et al reported the statistically important correlation linking stromal AR expression levels and PNI.[101]

SUMMARY

- This study employed a cross-sectional design and was carried out at KAHER'S JNMC and KLE'S Dr. Prabhakar Kore Hospital and Medical Research Center, Belagavi, utilizing data and core biopsy specimens of prostatic adenocarcinoma for comprehensive analysis.
- A total of 30 core biopsy specimens of prostate cancer (PCa) were analyzed over a 2-year duration, from January 2023 to December 2024.
- All cases underwent immunohistochemical (IHC) analysis for AR and P63 expression, where both tumor cell and stromal cell AR expression were carefully examined and correlated with clinicopathological parameters and histological grade groupings.
- PCa was most commonly found in individuals aged 66 to 75 years, with a mean age of 70.87 ± 6.31 years.
- DRE revealed hard nodules in 18 cases (60%) out of 30 cases.
- In the study, the highest percentage of patients fell in the GG4, followed by GG5.
- There was no significant association between the presence or absence of hard nodules in DRE and Gleason grade groups.
- In the study, 53.33% of cases had a volume of 31-60%, followed by 61-90 % and < 30 %.
- The study evaluated the association between approximate tumor volume and Gleason grade groups and found no statistically significant association.
- Among the 30 cases, 26 were PNI positive and the remaining were negative. PNI was seen more in GG4 and GG5, however no significant correlation was seen.

- P63 expression (basal cell marker) was absent in all cases.
- AR expression was studied in all cases and was entirely positive.
- In this study, Grade Groups 4 and 5 were the most commonly observed, with strong AR staining intensity in tumor cells being most prevalent in higher-grade tumors and also showed significant P value (0.0227).
- The Hscore utilized to estimate expression in tumor cell across all cases showed that 63.33% exhibited low expression, while 36.67% showed high expression.
- The study estimated the relationship between tumor cell AR expression and Gleason grade groups, revealing that higher AR expression was predominantly observed in higher-grade groups, while lower expression was found in lower-grade groups. A significant correlation was identified between these variables (p-value = 0.019).
- No meaningful link was observed with tumor cell AR expression and factors such as age, digital rectal examination -DRE findings, approximate tumor volume, or the presence of perineural invasion PNI.
- Stromal AR expression was assessed in all 30 cases using the Allred score for semi-quantitative evaluation [101]. The analysis demonstrated that 19 cases exhibited low expression, while the remaining cases showed high expression.
- Higher Allred score was observed in GG 2, while lower scores were seen in GG 4 and 5, showing a statistically significant correlation (P value:0.022).
- No significant association between the expression of stromal AR and factors such as age, approximate tumor volume, and the presence of PNI.

CONCLUSION

This study highlights the significance of the expression of AR in prostate cancer (PCa). P63 expression was negative in all cases. AR was strongly expressed in malignant cells and was more common on higher-grade tumors, showing a significant correlation (P=0.0227). Stromal AR expression, assessed using the Allred score, was higher in Grade Group 2 and lower in Grade Groups 4 and 5, also showing a meaningful association (P-0.022). Nevertheless, no notable association linking AR expression (tumor or stromal) and factors like age, tumor volume, DRE findings, or PNI status was identified. These findings highlight AR's potential role as a key predictive marker in PCa.

LIMITATIONS OF THE STUDY

- In this study, staging was not executed.
- Limited sample size due to time constraints.

FUTURE PERSPECTIVES

Future research incorporating genomic, transcriptomic, and proteomic methodologies will be crucial in fully understanding the clinical relevance of AR and p63 in prostate cancer. Advancements in this field may contribute to the development of more accurate diagnostic techniques, enhanced treatment options, and improved patient prognoses.

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ANNEXURES

ANNEXURES I - PROFORMA

PATIENT HISTORY

Name: Age: IP. No:

History:

Clinical Diagnosis:

Nature of Specimen:

Surgery Done:

DRE Findings:

USG/CT/MRI Findings:

Microscopy:

1. Histopathological Diagnosis and Gleason Grade Group:
2. P63 Staining:
    - Positive
    - Negative
3. AR Staining:
    - AR Intensity of Staining in Tumor Cells
    - Percentage positivity in Tumor Cells
    - AR Intensity of Staining in Stromal Cells
    - Percentage positivity in Stromal Cells

ANNEXURES II – INFORMED CONSENT FORM

"ANDROGEN RECEPTOR AND P63 EXPRESSION IN PROSTATIC ADENOCARCINOMA – A HOSPITAL-BASED CROSS-SECTIONAL STUDY"

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Introduction: The purpose of this study is to evaluate association of AR & p63 expression with prognostic marker like Gleason score.

Explanation of procedure: During this study, block will be collected from the pathology department and would be used for study purpose for the general benefit.

Possible benefits from participating in the study: This study may give opportunities for novel targeted therapies.

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 from identifying 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 purposes 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:
Principal Investigator, Department of Pathology, J.N Medical College, Belagavi
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.

ANNEXURES III – CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"ANDROGEN RECEPTOR AND P63 EXPRESSION IN PROSTATIC ADENOCARCINOMA – A HOSPITAL-BASED CROSS-SECTIONAL STUDY"**. 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:**BN0122012**

Signature of the investigator:

ANNEXURES IV - STAINING PROTOCOL H&E

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).

ANNEXURES V -TYPES OF PROSTATITIS[28]

- Chronic prostatitis or chronic pelvic pain syndrome.
- Acute bacterial prostatitis.
- Chronic bacterial prostatitis.

Asymptomatic inflammatory prostatitis.

ANNEXURES VI- WHO HISTOLOGIC CLASSIFICATION OF TUMOR OF THE PROSTATE[36,37]

Epithelial tumors

- Glandular neoplasms
- Adenocarcinoma (acinar)

  Atrophic

  Pseudohyperplastic:

  Foamy gland

  Mucinous (colloid)

  Signet ring-like cell

  Pleomorphic giant cell

  Sarcomatoid
- Prostatic intraepithelial neoplasia (PIN), high-Grade
- Intraductal carcinoma
- Ductal adenocarcinoma

  Cribriform

  Papillary

  Solid
- Urothelial carcinoma
- Squamous tumors

  Adenosquamous carcinoma

  Squamous cell carcinoma
- Basal cell carcinoma

Neuroendocrine tumors

- Adenocarcinoma with neuroendocrine differentiation
- Well-differentiated neuroendocrine tumor
- Small cell neuroendocrine carcinoma
- Large cell neuroendocrine carcinoma

Mesenchymal tumors

- Stromal tumor of uncertain malignant potential
- Stromal sarcoma
- Leiomyosarcoma
- Rhabdomyosarcoma
- Leiomyoma
- Angiosarcoma
- Synovial sarcoma
- Inflammatory myofibroblastic tumor
- Osteosarcoma
- Undifferentiated pleomorphic sarcoma
- Solitary fibrous tumor
- Solitary fibrous tumor malignant
- Hemangioma
- Granular cell tumor

Hematolymphoid tumors

- Diffuse large B-cell lymphoma
- chronic lymphocytic leukemia/small lymphocyte motors,
- Follicular lymphoma
- Mantle cell lymphoma

- Acute myeloid leukemia
- B lymphoblastic leukemia/lymphoma

Miscellaneous tumors

- Cystadenoma
- Nephroblastoma
- Rhabdoid tumor
- Germ cell tumors
- Clear cell adenocarcinoma
- Melanoma
- Paraganglioma
- Neuroblastoma

Metastatic tumors

- Tumors of the seminal vesicles

Epithelial tumors

- Adenocarcinoma
- Squamous cell carcinoma

Mixed epithelial and stromal tumors

- Cystadenoma

Mesenchymal tumors

- Leiomyoma
- Schwannoma
- Mammary-type myofibroblastoma
- Gastrointestinal stromal tumor
- Leiomyosarcoma
- Angiosarcoma

- Liposarcoma
- Solitary fibrous tumor
- Hemangiopericytoma

Miscellaneous tumors

- Choriocarcinoma
- Seminoma
- Well-differentiated neuroendocrine tumor
- Lymphomas
- Ewing sarcoma

Metastatic tumors

ANNEXURES VII - CRITERIA FOR DIAGNOSIS OF PROSTATIC ADENOCARCINOMA[37]

MAJOR CRITERIA
Architectural: infiltrative small glands or cribriform glands too
large or irregular to represent high-grade PIN
Single cell layer (absence of basal cells)
Nuclear atypia: nuclear and nucleolar enlargement
MINOR CRITERIA
Intraluminal wispy blue mucin (blue-tinged or basophilic mucinous secretions)
Pink amorphous secretions
Mitotic figures
Intraluminal crystalloids
Adjacent high-grade PIN
Amphophilic cytoplasm

ANNEXURES VIII - THE NEW GRADE GROUPING SYSTEM[49,50]

Gleason Grade Group (GG)	Description
GG-1 **(Gleason SCORE≤6)**	Only individual discrete well-formed glands
GG-2 **(Gleason SCORE 3+4=7)**	Predominantly well-formed glands with lesser components of poorly formed/fused/cribriform glands
GG-3 **(Gleason SCORE 4+3=7)**	Predominantly poorly formed/fused/cribriform glands with lesser component of well-formed glands*
GG-4 **(Gleason SCORE 4+4=8; 3+5=8; 5+3=8)**	Only poorly formed /fused/cribriform glands** OR Predominantly well-formed glands and lesser component lacking glands** OR Predominantly lacking glands and lesser component of well-formed glands**
GG-5 **(Gleason SCORE 9-10)**	Lacks gland formation (or with necrosis) with or without poorly formed /fused/cribriform glands*
*For case with > 95% poorly formed /fused/cribriform glands or lack of glands on a core or at resection specimen, the component of < 5% well formed glands is not factored in to the grade ** Poorly formed/fused/cribriform glands can be a minor component.	

ANNEXURES IX- TNM STAGING[51]

Primary tumour

- TX Primary tumour evaluation is impossible;
- TO there is no sign of a primary tumour;
- T1 Clinically inapparent tumour that cannot be felt or shown on imaging
- T1a tumour (non-palpable) discovered histologically in 5% of the prostate tissue removed after transurethral resection,
- T1b Tumour (non-palpable) discovered in >5% of the tissue removed after transurethral resection of the prostate,
- T1c Tumour (non-palpable) Found on Needle Biopsy (for Elevated Serum PSA): Includes Bilateral Non-Palpable Tumour on Needle Biopsy;
- T2 tumour restricted to the prostate (including the prostatic apex and prostate capsule), palpable, detectable on imaging, or shown in a specimen from radical prostatectomy (with the p-prefix);
- T2a tumour that only affects one-half of a lobe;
- T2b tumour that affects more than half of one lobe but not both;
- T2c tumour that affects both lobes;
- prostatic capsule via T3 tumour extension;
- Extracapsular extension (ECE) in T3a;
- T3b Seminal vesicle invasion;
- T4 tumour permanent or invading nearby tissues, including the pelvic wall, bladder neck, external sphincter, rectum, and levator muscles;

Regional lymph nodes

- Pelvic lymph nodes: Below the point where the common iliac arteries bifurcate are referred to as
- NX: It is impossible to examine regional lymph nodes;
- N0: No localized metastases to lymph nodes;
- N1: Unilateral or bilateral regional lymph node metastases below the common iliac artery bifurcation in the true pelvis;

Metastasis

- MX It is impossible to evaluate distant metastases;
- No distant metastases, or M0
- M1a Metastasis to non-regional lymph nodes;
- M1b Bone metastatic disease;

M1c Additional metastatic site(s)

ANNEXURES X- AGE- SPECIFIC VALUES OF PSA[64]

AGE (Years)	PSA (ng/ml)
≤ 40	1.40
41-50	1.70
51-60	3.10
61-70	5.80
71-80	8.82
>80	11.31

ANNEXURES XI - STAINING PROTOCOL OF IHC

Staining protocol of AR IHC:

About 3-4-micro, thick sections were cut from the formalin fixed paraffin embedded block from each case and placed on the coated slides.

Slides are immersed in xylene for deparaffinization, followed by rehydration where they pass through graded concentration of alcohol 100%,80%,50% each for 3 minutes followed by water wash 3 times with distilled water.

Antigen retrieval: Tris-EDTA buffer is used as antigen retrieval solution (ARS) for heat induced epitope retrieval method (HIER). The slides are steam pressure heated with ARS for about 20-30 minutes at 95-100 degree Celsius. The slides are cooled at room temperature to avoid any tissue damage.

Peroxidase Block: Incubate the slides with 3% hydrogen peroxide (H_20_2) in methanol for 10-15 minutes to block the endogenous peroxidase activity. This step prevents background staining caused by endogenous hydrogen peroxidase activity in tissues.

Primary antibody incubation: Based on manufacturers recommendation primary antibody is diluted and slides are incubated with AR EP120 for 1 hour at room temperature.

Wash the slides and incubated with secondary antibody for 30-60 minutes at room temperature.

Water wash followed by application of DAB (3,3'-Diaminobenzidinechromogen) substrate. Horseradish peroxidase (HRP) enzyme catalyses the reaction and generated brown precipitate at site of AR expression which is the nucleus.

Counterstain with hematoxylin to visualize tissue structure (2-5 minutes), bluing in warm water bath for 1 minute.

Clearing with xylene for 2 minutes, dry the slides and the mount them with DPX.

STAINING PROTOCOL OF IHC

Staining protocol of P63 IHC:

About 3-4-micro, thick sections were cut from the formalin fixed paraffin embedded block from each case and placed on the coated slides.

Slides are immersed in xylene for deparaffinization, followed by rehydration where they pass through graded concentration of alcohol 100%,80%,50% each for 3 minutes followed by water wash 3 times with distilled water.

Antigen retrieval: Tris-EDTA buffer is used as antigen retrieval solution (ARS) for heat induced epitope retrieval method (HIER). The slides are steam pressure heated with ARS for about 20-30 minutes at 95-100 degree Celsius. The slides are cooled at room temperature to avoid any tissue damage.

Peroxidase Block: Incubate the slides with 3% hydrogen peroxide (H_20_2) in methanol for 10-15 minutes to block the endogenous peroxidase activity. This step prevents background staining caused by endogenous hydrogen peroxidase activity in tissues.

Primary antibody incubation: Based on manufacturers recommendation primary antibody is diluted (1:50) and slides are incubated with P63(Clone:4A4) for 30-60 minutes at room temperature.

Wash the slides and incubated with secondary antibody for 30-60 minutes at room temperature.

Wash slides with buffered saline and apply DAB chromogen (3–10 min) until color develops.

Rinse in distilled water and counterstain with Hematoxylin for 30 seconds to 1 min

Counterstain with hematoxylin to visualize tissue structure (2-5 minutes), bluing in warm water bath for 1 minute.

Clearing with xylene for 2 minutes, dry the slides and the mount them with DPX.

ANNEXURES XII-KEY TO MASTER CHART

BX.No	**Biopsy Number**
GS	**Gleason Score**
GG	**Gleason Grade Group**
PNI	**Perineural Invasion**
PSA	**Prostate Specific Antigen**
HN on DRE	**Hard Nodule on Digital Rectal Examination**
Wt	**Weight of Prostate**
ATV	**Average Tumor Volume**
H Score	**Histological Score**

ANNEXURES XIII - MASTER CHART

Sl.no	Age	BX No	GS	GG	PNI	PSA(ng/ml)	HN on DRE	Wt	ATV	P63	Stromal AR (Allred)	H SCORE
1	60	3196/23e	4+5=9	5	(+)	100	(+)	38cc	60	(-)	1	210
2	65	2930/23	4+5=9	5	(+)	-	(-)	-	60	(-)	1	230
3	67	1801/23	3+4=7	2	(+)	-	(+)	41.3cc	70	(-)	5	140
4	78	986/23	5+5=10	5	(+)	8.21	(+)	42.2cc	80	(-)	1	240
5	75	4584/23	3+5=8	4	(-)	100	(+)	-	50	(-)	2	160
6	65	2457/24	3+4=7	2	(+)	97.4	(+)	48.7cc	50	(-)	5	80
7	66	4787/23	4+4=8	4	(+)	22	(+)	-	30	(-)	2	60
8	70	1103/24	4+4=8	4	(-)	-	(-)	-	50	(-)	2	25
9	75	5434/23	4+4=8	4	(+)	47	(+)	-	40	(-)	2	240
10	70	3121/23	4+4=8	4	(+)	3.3	(-)	-	40	(-)	2	50
11	67	4999/23b	4+5=9	5	(+)	19.7	(-)	-	70	(-)	1	80
12	76	5000/23a	4+5=9	5	(+)	100	(+)	45cc	70	(-)	1	40
13	76	5002/23	4+5=9	5	(+)	100	(+)	37cc	60	(-)	1	240
14	63	5007/23	5+4=9	5	(+)	500	(+)	102cc	80	(-)	1	210
15	69	4584/24L	3+4=7	2	(-)	100	(+)	-	40	(-)	6	120
16	73	2843/23	4+4=8	4	(+)	-	(-)	-	50	(-)	2	160
17	68	1779/24	5+4=9	5	(+)	100	(+)	50cc	90	(-)	1	270
18	69	5287/24	3+4=7	2	(+)	605	(-)	40.2cc	25	(-)	7	75
19	75	5364/23	4+3=7	3	(+)	70.7	(+)	-	80	(-)	6	160
20	81	3884/24	4+4=8	4	(+)	-	(-)	-	50	(-)	5	270
21	63	4724/23	4+4=8	4	(+)	550	(-)	-	70	(-)	2	80
22	58	4618/23	3+4=7	2	(+)	100	(+)	45cc	25	(-)	6	50
23	80	370/23	4+4=8	4	(+)	75	(+)	-	40	(-)	2	60
24	72	567/24	4+4=8	4	(-)	5.1	(+)	56.7cc	50	(-)	2	90
25	86	4809/23	4+4=8	4	(+)	29.6	(-)	-	70	(-)	5	160
26	70	4640/23	4+5=9	5	(+)	-	(-)	-	50	(-)	1	210
27	76	2564/23	4+4=8	4	(+)	58.3	(-)	-	60	(-)	1	140
28	72	5008/23	4+5=9	5	(+)	75	(+)	45cc	50	(-)	1	210
29	69	3197/23f	4+5=9	5	(+)	97.4	(+)	49cc	90	(-)	1	270
30	72	4991/23c	3+4=7	2	(+)	16	(-)	50cc	90	(-)	4	180