"Utility of Estrogen Receptor Beta and ki67 expression in Benign and Malignant Prostatic Lesions"

By

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

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ABSTRACT

Introduction –

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

The gold standard for the diagnosis of prostatic carcinoma is light microscopic findings, although there are complicated cases which would benefit from immunohistochemical studies. Recently studies have revealed the role of estrogen signaling pathways in the carcinogenesis of prostate. It is noted that there is wide variation in results of various studies regarding the expression of ER β with respect to grade of tumor in prostate carcinoma. There is need of additional research to standardize distribution of this receptor in human prostatic tissue. This will be useful in synthesizing the understanding of its role in regulation of prostate epithelial cell proliferation at various stages in lifecycle of prostate carcinoma.

Aims and Objectives– To find out the pattern of expression of ER β and ki-67 in nodular hyperplasia and prostatic carcinoma by immunohistochemistry.

Materials and methods –

A hospital-based Retroprospective study was conducted. Paraffin blocks of BPH and carcinoma prostatewere retrieved from Histopathology Section, Department of Pathology, BLDE (Deemed to be University), Shri B.M Patil Medical College, Hospital and Research Centre, Vijayapura. IHC for ER-β and ki67 was performed in all these cases.

Study period: 1st August, 2016 to 31st July, 2021.

Results –

40 cases of BPH and 40 cases of Adenocarcinoma of prostatewere included in the study.Comparison of the Immunohistochemistry marker Estrogen receptor beta and ki67 was done between the two study groups.Ki67 showed high sensitivity of 100% and specificity of 75% between the study groups.The Area Under Curve for ki67 (0.972, 95% CI 0.944-0.999) indicates that ki67 is the best discriminator of malignant cases with a p value <0.001. %ER-β showed sensitivity and specificity of 95% and 45% respectively and was statistically significant.

Conclusion-

There is reduced ER β expression in adenocarcinoma prostate when compared to benign hyperplasia. Considering Ki 67, expression was higher in carcinoma prostate compared to benign hyperplasia. All of the cases with carcinoma had proliferation index >10 %. So, these markers can be used as a distinctive marker in diagnosis of benign and malignant cases of prostate.

Keywords – ER-β, ki67, BPH, Adenocarcinoma of prostate.

LIST OF ABBREVATIONS USED

BPH-	Benign Hyperplasia of Prostate
AR-	Androgen Receptor
ER-β	Estrogen Receptor Beta
DHT-	Dihydrotestosterone
TURP-	Transurethral Resection of Prostate
IHC-	Immunohistochemistry
KLK3-	Kallikrein 3
KDa-	KiloDalton
TGF-	Transforming Growth Factor
TRUS-	Transrectal Ultrasonography
PAA-	Prostate Adenocarcinoma
AAH-	Atypical Adenomatous Hyperplasia
CCCH-	Clear Cell Cribriform Hyperplasia
SA-	Sclerosing Adenosis
HMWCK-	High Molecular Weight Cytokeratin
CK-	Cytokeratin
SMA-	Smooth Muscle Actin
AFIP-	Armed Force Institute of Pathology
CAP-	College of American Pathologist
ADASP-	Association of Directors of Anatomic and Surgical Pathology
GG-	Gleason Grade
IDC-	Intraductal Carcinoma of Prostate
GS-	Gleason Score

PIRADS- Prostate Imaging Reporting and Data System

HGPIN- High Grade Prostatic Intraepithelial Neoplasia

BCH- Basal Cell Hyperplasia

AMACR- Alpha-Methylacyl-CoA Racemase

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INTRODUCTION

Prostate cancer accounted about 1,276,106 new cases and 358,989 deaths which is around 3.8% of deaths in men with cancer during the year 2018. The numbers however varies world wide. Incidence shows a surge with age. Incidence of prostate cancer in men in the age group < 50 years is under the ratio of 1:350 and gradually this increases to 1:52 in the age group of 50-59 years and this further increases to 60% in the age group > 65 years. Based on the current research it is noted that the highest incidence of prostate cancer is widely seen in African – American population and higher probability of developing disease early in life as compared to other racial and ethnic groups.[1]

Initially it was assumed that in India the number of prostate cancer was very low as compared to the western globe but with increasing population in urban areas due to migration which led to change in lifestyles, easy access to medical facility & increased awareness the number of cases increased drastically. This clearly indicated that we would soon reach the numbers of western countries. Based on the cancer registries reports it is expected that we might face surge in cancer incidence in upcoming years.[2]

The prostate gland weighs about 20 gram and is comprised of a base, an apex, anterior, posterior, and inferior lateral surfaces. The human prostate is composed of glandular and stromal elements, tightly fused within a pseudocapsule. Prostate gland is comprised of small branched tubuloacinar prostatic glands embedded in fibromuscular stroma having smooth muscle bundles, mixed with collagen and elastic

fibres surrounding the prostatic glands.[3]

BPH and prostate adenocarcinoma are the leading causes of morbidity and mortality in the male population over 65 years of age. The growth, differentiation, and maintenance of prostatic gland activity are mainly controlled by androgens. The mediation of physiological effects of androgens and estrogens are mainly due to AR and ER receptors. Specifically the receptor present in the cytoplasm binds to dihydrotestosterone or testosterone, dissociates Heat Shock Protein, dimerizes it and, then translocated to the nucleus leading to activation or inactivation of different genes.[4]

One of the key cause of prostate enlargement in men (> 40 years) is BPH which is a very common urologic issue. However it is benign in nature and there is an increase in the size of the prostate gland along with hyperplasia of the glandular and stromal component. Lower Uterine Tract Symptom is due to hormonal changes which raises with age and resulting in abnormal proliferation of epithelial and stromal cell.[4]

Circulating testosterone is converted to DHT, which is the main androgen in the prostate and this step occurs in the presence of 5α-reductase, which is expressed mainly in stromal cells but is not expressed in prostatic epithelial cells. DHT binds to and activates androgen receptors (ARs) found in both stromal and epithelial prostate cells. Binding of DHT stimulates ARs to translocate from the cytoplasm to the nucleus and activate the transcription of androgen-dependent genes, which encode several growth factors and their receptors.[5]

Prostatic Adenocarcinoma is a predominant malignant disorder occuring in males and topmost cause of mortality in the same gender worldwide.[6] Coming to etiopathogenesis, there is an interplay of both environmental exposures and inherited genetic factors which contribute to the incidence of prostate carcinoma across geographic locales.[5]

The dietary and nutritional impact on prostate cancer can be high intake of saturated fat and low level of vitamin D, E and selenium. However, beyond this, radiation as well might have a significant impact. Prostate cancer is understood to be arising after at least eight events of mutation. Early events may be due to loss of tumor suppressive genes such as p53 which is mutated in up to 64% of tumors and p21 in up to 55%. P10 is widely mutated tumor suppressor gene in prostate cancer leading to acquisition of the metastatic phenotype.[7]

Histopathological microscopic findings carries the definitive diagnosis of prostatic malignancy. Clinical history and findings are significant but it should not impact the histological interpretation and findings of prostatic core biopsy and TURP specimen. Although there are multiple supportive features and diagnostic criteria specified only couple of them are absolutely specific. The pathologist should observe even minute differences between benign glands and atypical glands while assessing small focus of atypical glands under microscopy. The pathologist should look for nuclear features, cytoplasmic features and intraluminal content. One of the important criteria for prostate cancer is enlargement of nuclei, prominent nucleoli along with infiltration

like growth pattern, nonappearance of basal cells and nuclear atypia. Major challenges for a pathologist are interpretation of prostate needle biopsy and TURP Chips & the reason for this is bigger number of specimens and focus or small foci of suspicious glands and minimal nuclear atypia. Therefore, when seeing into these morphological features, it is important to have a systematic approach for the pathologist.[8]

The gold standard for the diagnosis of prostatic carcinoma is light microscopic findings , although there are complicated cases which would benefit from immunohistochemical studies. There can be significant variation in the morphology of basal cells like Secretory cells which are cut tangentially, stromal fibroblasts, tumor cells showing distortion and crushing artefacts in a small focus of cancer which resembles basal cells.[8]

Immunohistochemistry (IHC) is a widely used ancillary testing method in anatomic surgical pathology for cell classification and diagnosis and utilizes antibodies targeted against certain antigens in specific tissues and cells to facilitate determination of cell type and organ of origin.[9]

Recently studies have revealed the role of estrogen signaling pathways in the carcinogenesis of prostate. Several phytoestrogens in the diet bind to estrogen receptors and activate detoxification enzymes such as glutathione-S-transferase in prostatic epithelium highlighting the chemo-preventive role of estrogen. The opposing effects exerted by estrogens on prostatic epithelium are proposed to be mediated by two types of receptors: ER α and ER β .Studies performed on benign human prostatic tissue have shown that ER α expression is limited to prostatic stromal cells and the

basal cell layer, while secretory luminal cell types of the prostatic epithelium lack ER α at the mRNA and protein level. Considering estrogen receptor- β, there is no well recognition regarding the localization in human prostate tissue.[10]

In various studies there is extensive differences in the results regarding the expression of ER β with respect to grade of tumor in prostate carcinoma. So, there is need of additional research to standardize distribution of this receptor in human prostatic tissue. This will be useful in synthesizing the understanding of its role in regulation of prostate epithelial cell proliferation at various stages in lifecycle of prostate carcinoma. ER β is antiproliferative marker whereas Ki 67 is a marker of proliferation.[10]

As this receptor is proposed to be antiproliferative, a better understanding of the function of ER β in the evolution of prostate carcinoma could strongly impact on the therapeutic options for patients who have ER β expressing tumors. We conducted this study to find out the pattern of expression of ER β in prostaticcarcinoma and prostatic nodular hyperplasia by immunohistochemistry and compare the results between the two groups. We also determined the proliferation index by Ki 67 immunoexpression in benign and malignant prostate lesions. As ER β is proposed to be antiproliferative and Ki 67 a marker of proliferation, we tried to find out the correlation between ER β and Ki 67 expression and ER β with the grade of the tumor, if any.[10]

AIMS AND OBJECTIVES

- To find out the pattern of expression of ER β and ki-67 in nodular hyperplasia and prostatic carcinoma by immunohistochemistry.

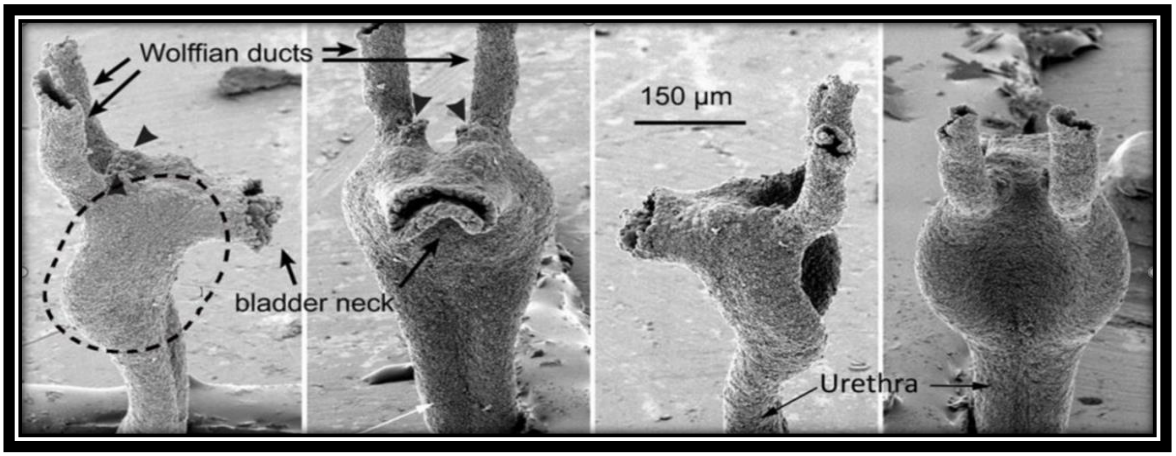
REVIEW OF LITERATURE

DEVELOPMENT OF PROSTATE

Epithelial bud is the root of prostate which arises out from embryonic urogenital sinus(UGS)**(Fig 1)**.Development of the human prostate is divided into the following stages: Pre-bud stage, Initial budding, Bud elongation and branching, and Ductal canalization.**(Table 1)**[11]

Table 1: Time line of prostatic development in humans shows: [11]

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



A B C D

Fig 1: Electron microscopyphotograph showing 90° rotations of the sample, starting with a lateral view (A) which indicates portion of the UGS from which prostatic buds will emerge. Arrowheads show entry of Mullerian ducts into the UGS epithelium.[11]

ANATOMY AND HISTOLOGY

In the subperitoneal compartment, between the pelvic diaphragm and the peritoneal cavity, lies the prostate gland. It is positioned posterior to the pubic symphysis, anterior to the rectum, and inferior to the urinary bladder, thus permitting digital palpation for examination. It is classically described as “walnut-shaped,” it is conical in shape and environs the proximal urethra as it exits from the bladder.

The prostate gland has five surfaces, comprising of a base, an apex, anterior, posterior, and inferolateral surfaces. The base is attached to the neck of the bladder and the prostatic urethra enters the middle of it, which is near the convex anterior surface. The apex rests on the superior surface of the urogenital diaphragm and links the medial surface of the levator ani muscle. The posterior surface is triangular and flat, and respites on the anterior wall of the rectum. The inferolateral surface joins the anterior surface and rests on the levator ani fascia which is situated above the urogenital diaphragm.

The prostate has been divided into 3 zones: the central zone (CZ), transition zone (TZ), and peripheral zone (PZ). These three zones have different embryologic origins and can be notable by their features like histology, anatomic landmarks, biological functions, and susceptibility to pathological disorders. Peripheral zone is the most common site from where prostate carcinoma arises and this zone is primarily derived from the urogenital sinus. By contrast, a very low incidence of prostate cancer has been seen arising from the central zone, which is derived from the Wolffian duct, which is also similar to another Wolffian-derived structure, the seminal vesicles.[3]

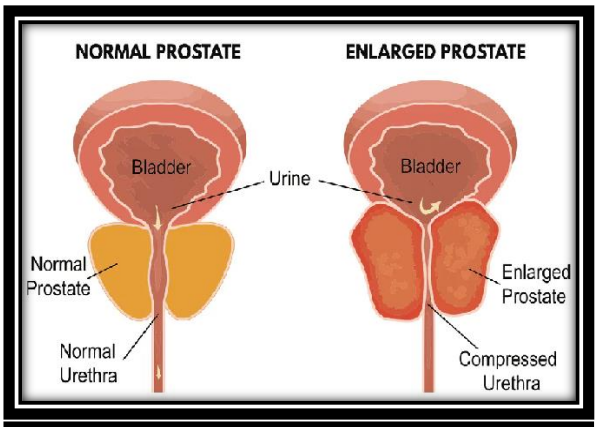


Fig 2: Normal and Enlarged Prostate

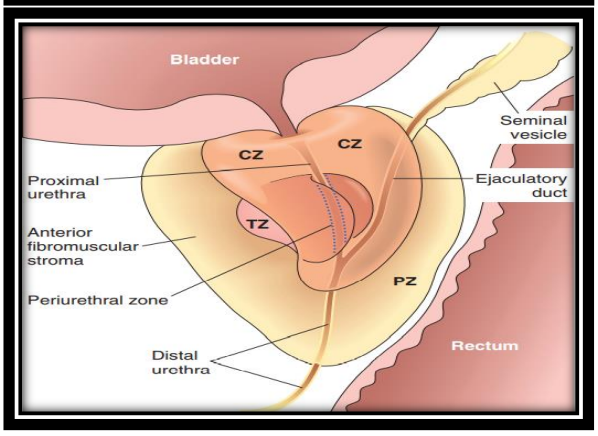


Fig 3: Zones of Prostate

At microscopic level, the prostate gland is comprised of tubuloacinar glands embedded in fibromuscular stroma. There is a complex architectural arrangement of the duct which is comprised of tubule like structure and glandular system.[12] Lining of the glands is by two layered epithelium, the inner tall columnar secretory epithelium have prominent round basal nuclei and pale cytoplasm and outer flattened basal epithelium.[13] Lumen of few glands contain proteinaceous prostatic secretions whereas few other lumen of glandular acini contain luminal spherical prostatic concretions suggestive of corpora amylacea.

Papillary infolding& undulating contours are often found on luminal surface of benign prostate glands. The supporting fibromuscular stroma is comprised of collagenous fibrous tissue and smooth muscle fibres.[13]**(Fig 3)**. There are three distinct

epithelial cell compartments lining the glands, the major being the basal and secretory cells and minor component being the neuroendocrine cells.[12] The fluid in the semen constitutes various products which are secreted by the luminal secretory cells of the gland. They produce prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) which carries significance in diagnosis due to their organ related specificity and these can be identified easily using immunohistochemistry.[14]

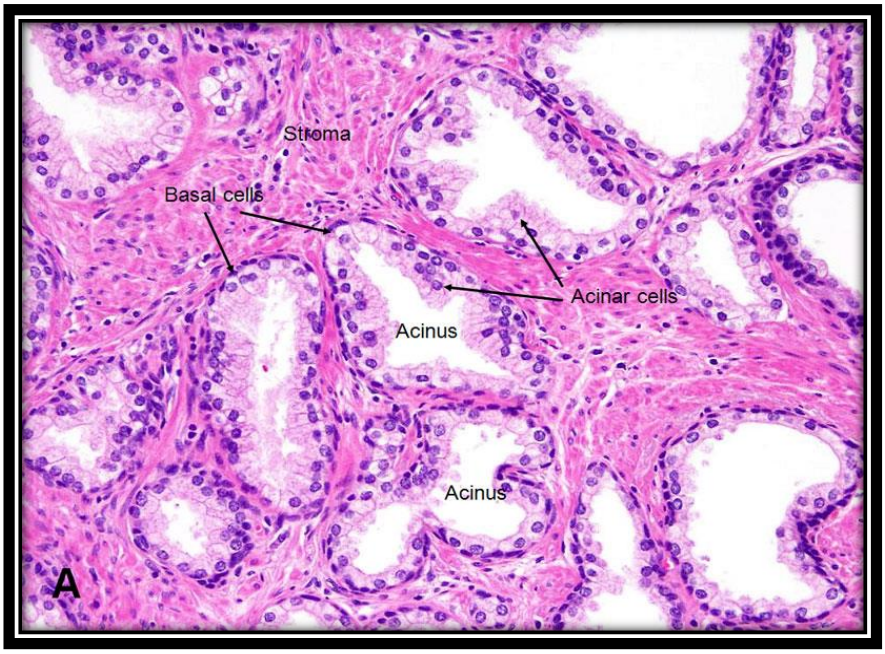


Fig 4: Normal histology of Prostate Gland

PSA

PSA is a glycoproteinenzyme set by the gene named KLK3 present in humans, the normal level being <4 ng/ml. Serum PSA levels of 4-10ng/ml raises the suspicion of malignancy, whereas levels >10ng/ml recommends a strong preference for malignancy.[14] However, recent studies have shown that some men with PSA levels below 4.0 ng/mL have been diagnosed with prostate malignancy and that many men with higher levels have been diagnosed with benign disorders of prostate.[15]In

addition, various factors can cause fluctuation in PSA level. For example, a man's PSA level often increases beyond normal level if he has prostatitis or a urinary tract infection. Increase in PSA level is also seen in prostate biopsies and post- prostate surgery. Conversely, some drugs including finasteride and dutasteride, which are used for the treatment of BPH lowers PSA level. PSA level also varies somewhat across different testing laboratories.[16]

PAP

Human PAP, also known as Acid phosphatase, prostate (ACPP) or prostatic specific acid phosphatase (PSAP), is a secreted glycoprotein enzyme having molecular weight of 100 KDa that is synthesized in the epithelial cells of prostate gland. In human, following puberty, PAP is one of the major proteins that is secreted by luminal tall columnar cells. PAP protein has been determined to be about 0.5 mg/gm weight of prostate tissue and around 1 mg/ml in seminal fluid. PAP expression is associated with testosterone, which is a sex hormone and it governs secondary sexual characteristics. PAP may be increased in men having prostate malignancy. PAP can also be detected in other tissues like brain, kidney, liver, lung, placenta, salivary gland, spleen, thyroid and thymus cells. PAP is absent in carcinoma breast tissue in contrast to normal breast tissue. Recently, however, it was revealed that PAP can also be present in large quantities in breast cyst fluid (BCF), particularly in metaplastic epithelium signifying the role of PAP in protecting several carcinomas by activating TGF-beta which is a similar molecule to PSA.[17]

BENIGN HYPERPLASIA PROSTATE

The three common pathological processes which affect the prostate gland having

increased frequency are inflammatory disorders, BPH, and tumors. BPH is the most common among all three pathological processes and occurs often in older males that it can almost be viewed as a "normal" part of aging.[5]

The common benign disorder of prostate found in older age group is BPH.Nodular hyperplasia of stromal and epithelial component of prostate is noted, often leading to urinary obstruction.[5]

Histologic evidence of BPH can be seen over 40 years of age in approximately 20% of males, it increases to 70% and 90% by 60 and 80 years respectively. Out of these, only 50% of males develop clinical symptoms. BPH has become a problem of enormous extent to white Americans where approximately 30% of males older than 50 years of age have moderate to severe symptoms.[5]

Chief androgen in the prostate which is formed from testosterone by the presence of an enzyme type 2 5α-reductase is DHT. This enzyme is expressed mainly in stromal cells. DHT production from testosterone is mediated by another enzyme named Type 1 5α-reductase which is seen in extraprostatic locations (e.g., liver and skin) and it provides an additional source of DHT that reaches the prostate through the blood. [5]

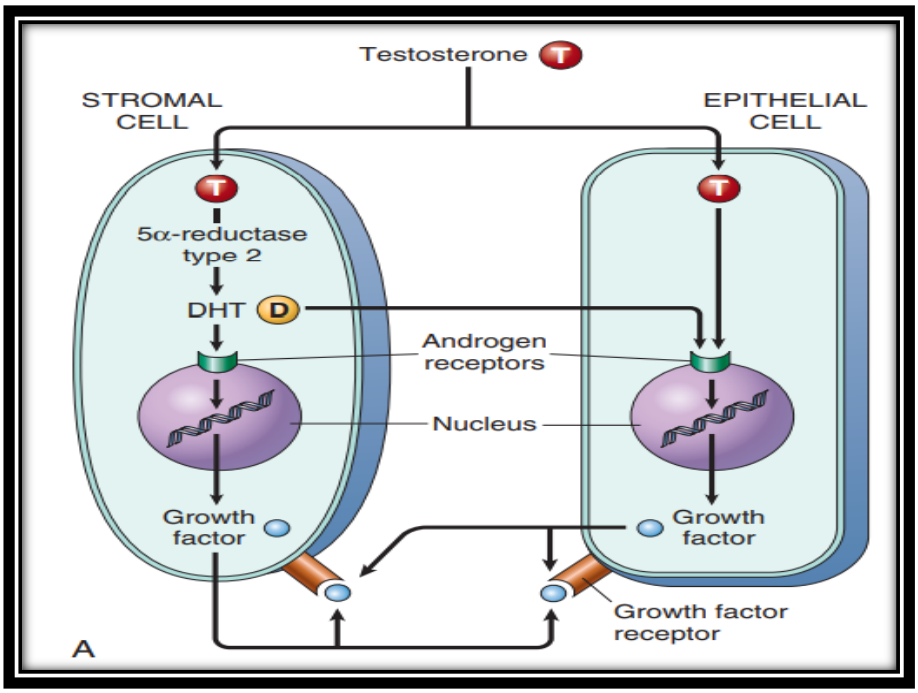


Fig 5:The central role of the stromal cells in generating dihydrotestosterone[5]

Androgen receptors (ARs) are found in both stromal and epithelial prostatic cells and DHT binds to and activates androgen receptors, thereby, stimulating ARs to translocate from the cytoplasm to the nucleus thus activating the transcription factor of androgen-dependent genes, which encode several growth factors and their receptors.[5]

In addition to recognition, that androgens play a permissive role in BPH pathogenesis, there are numerous lines of evidence that support a role for estrogens as well. Two different forms of estrogen receptor (ER), specifically ERα and ERβ, have opposing proliferative and antiproliferative effects on prostate cells respectively. There are few effects of estrogens on the prostate gland which are associated with various mechanisms including apoptosis, aromatase expression, and paracrine regulation via

prostaglandin E2, thus contributing to pathogenesis of BPH by leaning the balance toward proliferation.[5]

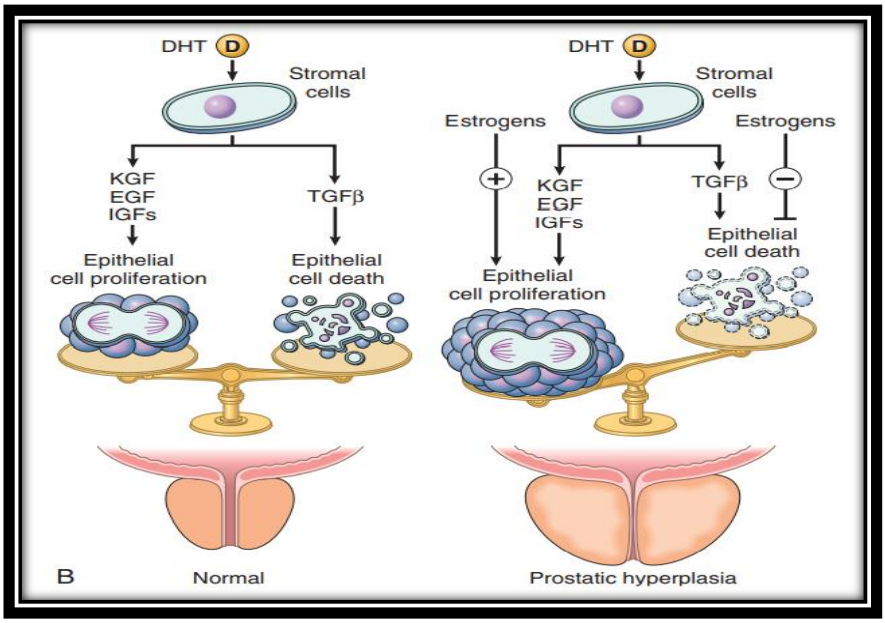


Fig 6:The contribution of estrogen in tipping the balance of cell proliferation and cell death toward the BPH[5]

PATHOLOGIC FEATURES OF BPH

The average weight of a prostate gland affected by BPH is 33 g ± 16 g at autopsy. Average weight of the specimen obtained post operatively is around 100 g and rarely weighs more than 800 g. Grossly, varying sizes of nodules having tan gray to yellow color are noted and above the surface, granular appearance is seen bulging out.[14]Microscopically, stromal proliferation about small sinusoidal spaces is the earliest change seen most commonly around the urethral regions and, to a lesser degree in areas surrounding the ducts and intralobular areas. More of smooth muscle and less of elastic tissue is seen in stromal proliferation as compared to the normal stroma which is followed by hyperplastic glands. There is dilatation of glands, resembling a cyst. Lumen of the cystic glands contain concentric calcified

eosinophilic secretions suggestive of corpora amylacea. The epithelium is lined by flat to columnar cells showing pale cytoplasm, having regular centrally located nuclei and inconspicuous nucleoli.[14]

In BPH, a variety of metaplastic changes can also be seen which includes transitional cell metaplasia, mucinous metaplasia, squamous metaplasia, and eosinophilic metaplasia. In around 0.6% of Hyperplastic prostate gland, metaplastic changes are encountered; most common being squamous and transitional metaplasia.

Coming to squamous metaplasia, which occurs in normal prostatic glands, as well as seen in a variety of reactive situations such as in prostatic infarcts and in males who are hormonally treated including estrogen therapy. Squamous metaplasia should always be distinguished from squamous differentiation of residual or recurrent prostatic adenocarcinoma, a finding that can be also encountered post radiation or hormonal therapy as squamous metaplasia can show reactive atypia, mainly if present adjacent to prostatic infarcts, it usually lacks obvious ‘‘malignant’’ atypia. Another metaplasia namely Transitional metaplasia which can be a pitfall for high-grade prostatic intraepithelial neoplasia and it should be differentiated by the presence of nuclear elongation and grooves, as well as inconspicuous nucleoli. Likewise in squamous metaplasia, in transitional metaplasia, there is also absence of cytologic atypia, and when present, it raises the likelihood of extension of urothelial carcinoma within prostatic glands and ducts.[18]

Mucous gland metaplasia, which is uncommon and noted approximately in 1% of prostate, consists of tall, mucin-filled goblet cells with small hyperchromatic basal nuclei. Mucous gland metaplasia may be found in many conditions like normal glands, hyperplastic prostate glands and in areas of urothelial metaplasia, basal cell hyperplasia, or atrophy.[12]Lastly, Eosinophilic Metaplasia is characterized by the

presence of varying sizes of intensely eosinophilic cytoplasmic granules filling the apical cytoplasm of the benign prostatic secretory epithelium and this metaplasia is found in close association with chronic inflammation[19]

There are various other changes in addition to the above findings seen in BPH. Prostatic infarcts can be found in roughly 20% to 25% of specimens removed for BPH. In the acute phase, the epithelium and connective tissue shows discrete foci of coagulative necrosis. Immediately adjacent to the infarcts, there may be reactive epithelial nests showing mild degree of nuclear pleomorphism having prominent nucleoli, and even atypical mitotic figures, which mimic urothelial or squamous cell carcinoma. Secondly, there can be epithelial nodules which appear crowded, which have been categorized as adenosis (atypical adenomatous hyperplasia). In this, both fibrous and smooth muscle elements are present in the stromal component. Vascular amyloid deposits are seen in around 2% to 10% of benign or malignant prostatic disorders. A strong association is seen between prostatic amyloidosis and patients having plasma cell myeloma, primary amyloidosis or anychronic disease.[12]

ADENOCARCINOMA OF PROSTATE

Most common form of cancer in men is Adenocarcinoma of the prostate, constituting 29% of cancer in the United States in 2012.[5]Majority of male population has been diagnosed with prostate cancer after lung cancer and the latter is the leading cause of cancer-related deaths in men. 5–10 percent of prostatic carcinomas seems to have a genetic factor. There is also correlation with diet, venereal disease, sexual habits, smoking, or occupational exposure which acts as a triggering factor for cancer development.[16]It has been noted that majority of male population who have been

diagnosed with prostatic carcinoma are more than equal to 65 years, but this tumor can also be seen in children and adolescents.[14]

These locally advanced tumors cause debilitating symptoms and serious complications, such as intractable pelvic pain, recurrent urinary tract infections, hematuria and urinary and rectal bleeding or obstruction in long haul.[20]Coming to investigations, serum determination of PSA has a high sensitivity, is rapid and inexpensive, and is minimally invasive. Mild elevations of serum PSA level can be seen with nodular hyperplasia, but levels above 4 ng/ml indicates serial determination, with the performance of a biopsy if they continue to rise. Approximately half of patients with prostatic carcinomas have levels over 10 ng/ml. Elevations of serum PSA also be seen in prostatitis, prostatic infarct, and prostatic trauma, such as needle biopsy or TUR, but these elevations should be temporary and gets resolved with proper treatment. Skillful rectal examination is done which remains a practical and efficient method for the detection of prostatic carcinoma. The triad of digital rectal examination, transrectal ultrasonography, and serum PSA represents a powerful diagnostic tool for the early detection of prostatic carcinoma.[14]

Prostatic malignancy can be alienated into two classes: (1) malignancy of prostatic periphery ducts and acini, and (2) carcinoma of large ducts. Peripheral zone of the prostate, whether anterior, posterior or lateral surface is the most common site for prostate malignancy but it spares the periurethral region except for the late stages of the malignancy.[14]

Prostate cancers typically contain crowded glands as compared to benign prostatic tissue, although there is an intersection with certain benign mimickers of prostate

cancer. Neoplastic glands shows haphazard arrangement of glands. Few of the benign glands are perpendicular to each other. Presence of small foci of atypical glands in between larger benign glands is also characteristic of an infiltrative process. In undifferentiated prostate cancer, tumor tissue is comprised of solid sheets, cords or isolated cells.[16]

Nuclear features in prostate cancer includes nuclear enlargement with prominent nucleoli. Some neoplastic nuclei are enlarged and hyperchromatic but lack prominent nucleoli. Nuclei in prostate cancer show mild nuclear anisopoikilocytosis. Rarely, in high grade prostate cancer, marked nuclear pleomorphism is typically seen in the late advanced stage of the disease. Mitotic figures may be observed in high-grade malignancy.[16] Glands of prostatic malignancy have sharp luminal borders without cytoplasmic undulations or ruffling. Neoplastic glands may have amphophilic cytoplasm, which is a valuable diagnostic criterion of malignancy.[16]

Generally, Prostate malignancy is not a challenging diagnosis to give based on histological and cytological features. But sometimes the diagnosis of Adenocarcinoma prostate becomes a task as there are many mimickers of PAA, especially when it comes in a small piece of tissue from TRUS or TURP specimen, which may mislead the diagnosis leading in inappropriate treatment.[21]

Among the mimickers of Prostate Adenocarcinoma, Prostatic /Lobular atrophy is the most common lesion among the elderly population. The atrophic glands are usually arranged in multiple lobules, separated by fibrotic stroma and acini appear normal. Growth pattern is lobular, well-circumscribed, usually admixed with atrophic glands having pale to clear cytoplasm, separating bland nuclei and having inconspicuous

nucleoli, basal cells are small, inconspicuous and may be focally discontinuous.[21]

In needle core biopsy, Basal cell hyperplasia(BCH) is seen in about 23 percent of whole prostatic tissues. BCH usually co-occurs with BPH and grossly it has well circumscribed lobules having smooth borders. Microscopy of BCH shows glands of different sizes and shapes and the cells are arranged in solid sheets. Individual cells are basaloid, and have multilayered basal cells having basophilic cytoplasm in the absence of nucleoli or nuclear pleomorphism. The rare florid and atypical BCH, may mimic PAA which can be differentiated from PAA, as the latter shows nuclear atypia showing prominent nucleoli, eosinophilic secretions inside the lumen, hyaline globules, or occasional mitotic figures.[22]

Atypical Adenomatous Hyperplasia(AAH) having an incidence of 1.5 to 19.6 percent in all prostate specimensis a benign lesion, which shows small multiple acinus proliferation.. AAH is a well circumscribed lesion having lobular appearance. Microscopic features show small, round, and densely packed glands showing an expandible or minimally infiltrative margin, overcrowding of glands having medium-sized nucleoli, mimics low-grade PAA. In contrast to above features, there is absence of macro nucleoli (>3 micron), mucin and straight luminal borders in AAH, which are usually the features of PAA.[21]

Clear Cell Cribriform Hyperplasia(CCCH) belongs to a morphological variant of BPH. It is comprised of glands which are enlarged, filled with clear cells, thus giving it cribriform pattern, which mimics adenocarcinoma of higher grade showing

cribriform pattern. The differentiation points are proliferation of nodules, fibrotic stroma, and the presence of basal cell layer which confirms the diagnosis of CCCH. Clue to the differential diagnosis with carcinoma is the presence of basal cells at the lesion periphery, which are highlighted by the 34βE12 keratin stain.[23]

Sclerosing Adenosis(SA) of prostate is very analogous to that of breast. It belongs to benign lesion with both small acini proliferation and fibrotic stroma. Gross feature of the lesion shows nodules having well defined border, but there is absence of capsule. Microscopy shows glandular shape and size variability, disorderly embedded into sclerotic stroma. Hyperplastic glands lined by inner clear cells and outer flattened basal cells. In addition, some glands are seen surrounded by cells showing prominent nucleoli and intraluminal acid mucin but cytologic atypia is absent. Sclerosing adenosis should be diffrentiated from small acinar adenocarcinoma. On IHC, the basal cells shows positivity for HMWCK, CK 5/6, p63, SMA and S-100, thereby suggesting that those cells have myoepithelial differentiation.[21]

Few other uncommon mimickers of prostate Carcinoma include Florid hyperplasia of mesonephric remnants, Cholesterol-laden macrophages, Signet-ring-like changes, melanosis and extramedullary hematopoiesis. There can also be rectal tissuein the prostate in the course of a needle biopsy; when these glands are distorted, they can also mimic prostatic cancer.[14]

Multiple benign lesions and normal structures can also mimic PAA. It is, therefore, important for the pathologist to be aware of these microscopic features and to recognize the same. However, it is a task for the pathologists to make a correct

diagnosis when there is a small suspicious area in prostatic specimens. Morphologically, PAA and its benign mimickers are very different. However, features like perineural invasion, presence of micronodules confirms the diagnosis of PAA without any doubts. In accumulation to the above findings, the presence of complex pattern, nuclear pleomorphism and hyperchromatic nuclei showing prominent nucleoli, few mitotic figures and lumen of the glands showing eosinophilic secretions favors the diagnosis of PAA.Ancillary techniques including IHC staining and careful analysis of clinical history and investigations, if done, can be useful in making correct diagnosis leading to appropriate treatment.[14]

GRADING SYSTEMS FOR PROSTATE CARCINOMA

In more than 80 years since the original grading system described by Broders, more than 40 grading systems for prostatic adenocarcinoma have been proposed. These schemes are created depending on a variety of morphologic features, including architectural pattern, degree of differentiation, mitotic activity, and various nuclear factors. [24]

Numerous grading systems have been proposed for histopathological grading of prostate adenocarcinoma. The main controversies in grading system was whether grading should be based on glandular differentiation alone or a mixture of glandular differentiation and nuclear atypia, and also whether prostate cancer should be graded according to its least differentiated or dominant pattern. [16]

Table 2: NUMEROUS GRADING SCHEMES FOR PROSTATIC ADENOCARCINOMA USING ROUTINE MICROSCOPY[25,26,27,28,29,30]

Study	Year	Descriptions
Jewett et al.[25]	1968	Grades I-III, three grades of well, moderately and poorly differentiated cancer.
Mobley & Frank[26]	1968	Grades I-III, using gland-forming ability and nuclear features.
Utz & Farrow[27]	1969	Grades I-IV, based on architecture, cytology, and mitotic figures.
Hohbach& Dhom[28]	1972, 1977	Four groups based on histologic pattern, in order from best to worst prognosis: well differentiated, poorly differentiated, cribriform, solid-anaplastic
Gaeta et al.[29]	1980	Grades I-IV, defined by gland formation and organization,

		amount of stroma, nuclear atypia, and mitotic count
Uchida et al.[30]	1988	Japanese General Rules of Prostatic Cancer: well, moderately, and poorly differentiated

The Gleason grading system was termed after Donald F. Gleason is now the main Gleason grading system, and in 1993, it was recommended by WHO consensus conference(WHO). The Gleason grading system (1966) is one of the few number of systems that employs a low-power architectural approach [24]. At present time, the Gleason Grading system is the most commonly used grading system followed worldwide.[31]. Both the AJCC TNM manual (2002 edition) and the WHO classification (2003) have endorsed the Gleason system for the first time and in contrast to the previous recommendations, undoubtedly making it more consistently practiced worldwide. There are few other influential recommendations or guidelines, including those written by the AFIP, CAP, and ADASP that have also endorsed the Gleason Grading system.[32]

The Gleason grading system is predominantly based on architecture of glands; nuclear atypia is not evaluated. The Gleason grading system is differentiated into five histological patterns or grades based on decreasing differentiation. **(Fig 6).** In Gleason

pattern 1 to 3, there is retained epithelial polarity with luminal differentiation in virtually all glands. In Gleason pattern 4, there is partial loss of normal polarity and in Gleason pattern 5, there is an almost total loss of polarity with only occasional luminal differentiation.[16]

Fig 7: Modified Gleason grading schematic diagram

Coming to the details of Gleason Grading, Gleason grade should be reported for prostatic carcinoma in all tissue samples of the prostate from patients with no prior radiation or hormonal therapy. [24]

Prostate cancer has a pronounced morphological heterogeneity and usually more than one histological pattern is present. The primary and secondary pattern, i.e. the most prevalent and the second most prevalent pattern are added to obtain a Gleason score

or sum. It is recommended that the primary and secondary pattern as well as the score be reported, e.g. Gleason score 3+4=7. If the tumor only has one pattern, Gleason score is obtained by doubling that pattern, e.g. Gleason score 3+3=6. Gleason scores 2 and 3 are only exceptionally assigned, because Gleason pattern 1 is unusual. [16]

Minor/Tertiary Patterns

The presence of minor/tertiary patternsis considered separately for biopsy and radical prostatectomy specimens. For biopsies, the ISUP has recommended the inclusion of tertiary higher grade patterns in the GS, irrespective of extent, since the 2005 consensus meeting. Thus, a needle biopsy with 60% Gleason pattern 4, 36% pattern 3, and 4% pattern 5 would be reported as Gleason Score 4+5=9 (Gleason Grade 5). It has since been suggested that use of the term "minor" rather than "tertiary" is preferable because the primary and secondary grades may be identical, with a very small second higher grade cancer component. But in 2019 International Society of Urological Pathology(ISUP) Consensus Conference, few modifications have been made to Prostate Cancer Grading i.e., For radical prostatectomies, include the presence of tertiary/minor Gleason patterns 4 and 5 in the GS, if constituting >5% of the tumor volume.[33]

Below is the Summary of the Modification made By ISUP in the year 2019[33]

- Report in biopsies the percentage Gleason pattern 4 for all GS 7 (ISUP GG 2 and 3)
- For radical prostatectomies, include the presence of tertiary/minor Gleason patterns 4 and 5 in the GS, if constituting >5% of the tumor volume.

- Report in radical prostatectomies presence of tertiary/minor Gleason patterns 4 and 5
- Do not grade IDC without invasive cancer
- Incorporate the grade of IDC into the GS when invasive cancer is present
- Comment on the presence and significance of IDC in biopsies and radical prostatectomy specimens
- Comment on the presence and significance of invasive cribriform cancer in biopsies and radical prostatectomy specimens
- Report in systematic biopsies a separate GS (ISUP GG) for each individual biopsy site
- Report in multiparametric- Magnetic Resonance Imaging-targeted biopsies a global (aggregate) GS (ISUP GG) for each suspicious MRI lesion
- Report specific benign histologic findings in suspicious (PIRADS 4-5) MRI-targeted biopsies without cancer

RECOMMENDATIONS FOR REPORTING GLEASON HISTOLOGICGRADE[24]

- Report Gleason grade of carcinoma for all prostate tissue samples.

- Report primary Gleason pattern and secondary pattern = score.

- If one pattern present, double to yield Gleason score.

- Provide grade for each separately submitted sample (container).

- For needle biopsy cases, provide composite Gleason score for all cores.

- For needle biopsy cases, note Gleason score of core with highest score

- For needle biopsy cases : If >= two patterns are present and the worst grade neither belongs to the primary pattern nor to the secondary pattern, the predominant grade should be selected to give Gleason score.

- For radical prostatectomy cases in which more than two patterns are present and the highest grade neither belongs to primary pattern nor to secondary pattern, a note can be added to the comment about the tertiary grade.

- Provide Gleason grade for adenocarcinoma histologic variants—ductal, signet ring, and mucinous.

- Do not provide Gleason grade for small cell carcinoma of the prostate

- Provide Gleason grade for adenocarcinoma growth/cytologic variants, such as hypernephroid, atrophic, and pseudohyperplastic patterns.

- Do not report Gleason grade in metastatic deposits.

- Do not report Gleason grade after hormonal therapy.

- Report Gleason grade after radiation therapy of carcinoma that shows no treatment effect.

Table 3: CRITERIA FOR DIAGNOSIS OF EACH GLEASON GRADE [16]

GG 1	GS <=6	Only individual discrete well-formed glands.
GG 2	GS 3+4=7	Predominantly well-formed glands with lesser component of poor formed/fused/cribriform glands.
GG 3	GS 4+3=7	Predominantly poorly formed/ fused/ cribriform glands with lesser component of well-formed glands
GG 4	GS 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 Predominantly lacking glands and lesser component of well- formed glands
GG 5	GS 9-10	Lack gland formation (or with necrosis) with or without poorly formed/ fused/ cribriform glands

IMMUNOHISTOCHEMISRY

GENERAL PRINCIPLE

Immunohistochemistry (IHC) is a widely used ancillary testing method in anatomic surgical pathology for cell classification and diagnosis and utilizes antibodies targeted against certain antigens in specific tissues and cells to facilitate determination of cell type and organ of origin. The method is most commonly performed on formalin fixed paraffin embedded (FFPE) tissue which has the advantage of being amenable to easy

storage, although it was first developed on frozen sections and can also be done on plastic embedded tissue.[34]The use of IHC has recently further expanded to assess predictive and prognostic biomarkers in many malignancies including those of the breast, gastrointestinal tract, lung, hematolymphoid and central nervous systems.[35,36]

The sequential steps in IHC can be summarized as follows: antigen retrieval, addition of primary antibody, application of a secondary antibody that binds the primary antibody, and addition of a detection reagent to localize the primary antibody.**(Fig 7)**[37]The first step in IHC is usually antigen retrieval, which involves the pretreatment of tissue to retrieve antigens masked by fixation and make them more accessible to antibody binding.[38]Currently, the most popular method is heat induced antigen retrieval (HIAR) using microwave ovens most commonly, as well as pressure cookers, autoclaves and water baths.[39] To visualize the antigen antibody interaction under light microscopy, either the primary antibody or secondary antibody, which is targeted against the immunoglobulin of the species in which the primary antibody was produced, must be labeled. In the direct method, the primary antibody is labeled and applied to the tissue in a quick one step process; however, this method is not commonly used due to lack of signal amplification and thus the requirement for a higher concentration of antibody as well as the need to label each primary antibody. In the indirect method, the secondary antibody is labeled, allowing for signal amplification and use with many different primary antibodies. There are various labels that can be used, such as fluorescent molecules and enzymes such as horseradish peroxidase or alkaline phosphatase which produce a colored product after incubation with a chromogenic substrate such as diaminobenzidine (DAB)[40,41]

ROLE OF IMMUNOHISTOCHEMISTRY IN PROSTATE LESIONS

Needle biopsies of the prostate are typically performed either because of an abnormal rectal exam or elevated serum prostate-specific antigen (PSA) level; some men are screened because of a strong family history of prostate cancer. Asymmetry, induration, and discrete hard nodules are findings on digital rectal examination (DRE) that are suspicious for cancer.

In the past, the standard method used to diagnose prostate cancer was that of ultrasound-guided systematic sextant biopsy. Routine sextant biopsies sample the parasagittal midlobe region of the prostate despite the recognition that many prostate cancers arise posterolaterally. In recent years, studies have suggested alternative needle biopsy sampling techniques to increase prostate cancer detection. Three general modifications of the sextant biopsy technique have been proposed: (a) addition of transition zone biopsies, (b) addition of biopsies for enlarged prostates, and (c) modifying the location of the nontransition zone biopsies.[42]

One of the most common difficulties on needle biopsies of prostate pathology is a single focus/ limited adenocarcinoma on the slide. Most adenocarcinoma prostate shows benign glands of variable size and shape along with malignant glands infiltration in between larger benign glands. So we can take several needle biopsy cores of prostatic tissue where there are few malignant glands. The significance of identifying limited adenocarcinoma of the prostate is that there is no association between the amount of neoplasm seen on the core biopsy of prostatic tissue and actual tumor within the same organ. It is however possible to get a significant tumor within the prostate gland even though few neoplastic glands are seen in the core biopsy.

Evaluating an atypical focus in a needle biopsy of the prostate should be a methodical process. When reviewing needle biopsies, one should develop a mental balance sheet where on one side of the column are features favoring the diagnosis of carcinoma and on the other side of the column features against the diagnosis of carcinoma. At low magnification, the limited adenocarcinoma of prostate should be recognized. At scanner view, one should raise a suspicion of carcinoma if there is a presence of overcrowding glands. Secondly, we should notice the presence of small glands located between larger benign glands as in most adenocarcinomas of prostate, the neoplastic glands are smaller than adjacent benign glands. Indication of their infiltrative nature includes the presence of focus of malignant glands in between benign glands of varying sizes.[42]Coming to the microscopic findings, the first important step is to scan sections at scanner and 40x power magnification and confirmmorphological features of benign glands present in the tissue. As procedure like fixation of the tissue, processing, thickness of the section and staining procedure differs from institutions to institutions, it affects nuclear features like variability in size, appearance of prominent nucleoli due to understaining, and variable staining of the cytoplasm.

Even if small focus of atypical glands are present on the slide, pathologists have to pay added attention, even to the subtle differences between benign and malignant glands. Major points to confirm prostatic malignancy includes prominence of nucleoli, features showing infiltration, absence of basal layer and malignant nuclear features. However, there is no single criterion to diagnose malignancy.[8] On needle core biopsy, prominent nucleoli is an important feature in diagnosing malignancy but it should not be the onlycriteria to come to the diagnsois. Sole dependency of feature

like prominence of nucleoli can lead to both over and underdiagnosis of prostate cancer. Other features like cytological features and architecture in addition to prominent nucleoli should also be considered within the case. In few cases, nuclear enlargement is noted without the presence of prominent nucleoli. Another cytological feature is nuclear hyperchromasia, which helps to distinguish cancerous from benign glands. Mitotic figures are not always seen in adenocarcinoma prostate but it differentiates malignant from benign glands.[42]

It should always be kept in mind that rare subtypes of prostate cancer, such as foamy carcinoma is characterized by bland nuclear features, absence of prominent nucleoli and the diagnosis of HGPIN in many cases depends on the presence of prominent nucleoli. On the contrary, inflammatory disorders of prostate, prostatic atrophy and atypical BCH can occasionally display prominent nucleoli.[42]

Perineural Invasion(PNI) on prostate specimens was independently associated with adverse pathologic features and worse disease-free and overall survival after radical prostatectomy. These findings persisted after controlling for established pretreatment predictors of biochemical recurrence and adverse pathologic features at radical prostatectomy. PNI should likely be discussed as a possible predictor of worse oncologic outcomes when counseling prostate cancer patients about their treatment options. The most common approach to prostate cancer risk stratification uses pretreatment PSA, biopsy Gleason score, and clinical stage to classify patients into low-risk, intermediate-risk, and high-risk groups by their risk of disease recurrence after treatment. PNI could also be a valuable parameter to consider when making treatment decisions.PNI on the prostate specimen in men with low-risk or low-volume

intermediate-risk disease may indicate the need for definitive treatment instead of pursuing active surveillance. The worse oncologic outcomes seen with PNI may be because the perineural space provides a path of decreased resistance to tumor spread.[43]

In addition, tumor cells in the perineural space have been found to show increased proliferation and decreased apoptosis. A recent review of this issue identified several studies that support the finding that PNI increases the risk of biochemical recurrence after prostatectomy. Moreover, patients with PNI treated with external-beam radiotherapy may also experience worse clinical outcomes.[43]

Circumferential perineural invasion by glandular epithelium is one of the diagnostic features for adenocarcinoma, although perineural indentation or cuffing by benign or atrophic prostatic glands can be seen. The glands in the latter cases appear totally benign and are present at only one edge of the nerve. It has been postulated that neural cell adhesion molecule (N-CAM), one member of the immunoglobulin superfamily of adhesion molecules, may play an important role in perineural invasion and metastasis in prostate cancer.[44]

Routine microscopic features remains the gold standard for the diagnosis although ancillary studies like Immunohistochemistry may get benefitted in difficult cases. Sometimes, it becomes a challenge to identify basal cells in routine H&E stains as the absence of the latter is the major criteria for diagnosis of malignancy and needs to be confirmed by IHC markers, particularly when focus of atypical glands are present on the slide. Mimickers of basal cells includes when secretory cells cut tangentially and

fibroblasts in stroma and to some extent, distortion and crushing of tumor cells in small foci.

Even though basal cell IHC markers like CK5/6, p63 are helpful in establishing diagnosis in malignant cases, it should always be kept in mind, benign glands doesn't always contain basal cells. Therefore, one should see other features apart from absence of basal cells to confirm the diagnosis. Most specific among all basal cell marker is p63 but with diffuse p63 expression one should make definitive diagnosis only after looking into the morphological features and confirmation is done by IHC markers like HMWCK and AMACR. False negative staining for basal cell markers are also shown by few of the benign mimickers. In addition to the morphology on routine stain and use of basal cell marker, markers like AMACR is also commonly used for confirmation of diagnosis. In about 60-70% cases, AMACR helps to recognize few rare mimickers of prostate cancer. In addition, AMACR is not specific for malignancy as many other premalignant condition and benign glands also stains positive for AMACR. Double staining or triple staining of the tissue helps in making the correct diagnosis and has the advantage to conserve tissue.[42]

As there are many controversies regarding the diagnosis of benign and malignant prostatic lesions, so a cocktail of immunohistochemical markers should be used to diagnose these lesions.

The role of androgens in pathogenesis of prostate carcinoma is well known. Recently studies have revealed the role of estrogen signaling pathways in the carcinogenesis of prostate. The function of estrogen in regulation of normal and abnormal growth of

prostate is an area of imminent research. Many authors have attempted but left conflicting results.[10]

ROLE OF ESTROGEN IN PROSTATIC LESIONS

Etiology of prostate cancer is mainly unclear but seems to be multifactorial in origin such as alteration of genes, diet, race and hormones. Increasing age, weight, and a fat-rich diet as potential risk factors for prostate cancer may be associated with an increase in estrogen levels or high estrogen/androgen levels in circulating blood. Both epidemiological and experimental data suggest that estrogens are involved in prostatic cancer carcinogenesis and tumor progression.[45]

The role of estrogen in prostate cancer progression is not well understood. It is generally accepted that estrogens have influence on prostatic growth indirectly through effects at the hypothalamic and pituitary levels, reducing gonadotrophin secretion and hence the synthesis of testicular testosterone, but the effects of estrogens currently known to be intervened by ligand-specific receptor proteins termed estrogen receptor-a and -b (ER-a and ER-b). ER-a and ER-b belong to the nuclear hormone receptor family, many of whose members are ligand-activated transcription factors that regulate gene expression in a cell- and promoter-specific manner. In many other hormone dependant tumors like uterus, breast, lung cancer and few brain tumors, an expression of estrogen receptors have been found.[45]

Although ER-α and ER-β have similar ligand-binding domains and both bind estrogen

receptor, there are evidences that ER-α and ER-β demonstrate distinct and sometimes opposing transcriptional activities.[45] In prostate epithelium, ER-β is preferentially expressed in epithelial cells whereas ER- α is found in stromal and basal cells. The presence of ER-α in epithelial cells is controversial. Both types exhibit variable degrees of homology to one another, bind estrogen with similar affinity, and regulate some estrogen-dependent promoters.[46] ERβ is encoded by chromosome locus 14q22-24.[47]ERβ may play a significant role in human Prostate Carcinoma affecting progression as indicated by the distinct expression of its spliced variants during the phases of progression.[48]

In humans, there are at least five identified isoforms of ERβ. ERβ1, ERβ2, ERβ4, and ERβ5 isoforms can be found in various cell types in the normal prostate and are differentially expressed during the prostate cell cycle.[49]Recent studies suggested that ERβ1 is the only fully functional isoform of the ERβ family. Other ERβ isoforms have no intrinsic activity, since they neither form homodimers or recruit coregulator proteins and are characterized as variable dimer partners of the ERβ complex altering its activity.[50]

Several phytoestrogens in the diet bind to estrogen receptors and activate detoxification enzymes such as glutathione-S-transferase in prostatic epithelium highlighting the chemopreventive role of estrogen. Considering expression of ER β , its localization in human prostate tissue is not well recognized. There are conflicting results by different authors on its location in prostatic tissue. According to some authors ER β is highly expressed in rat prostate epithelial cells and in the secretory epithelium of normal human prostate, where the levels of ER β mRNA are higher than

the levels of ER α mRNA. As this receptor is proposed to be anti- proliferative a better understanding of the function of ER β in the evolution of prostate carcinoma could strongly impact on the therapeutic options for patients who have ER β expressing tumors. [10]

ROLE OF KI-67 IN PROSTATIC LESIONS

Ki67 is a nuclear protein that is expressed in all phases of cell cycle(G1,S,G2 and mitosis)but is not expressed in the G0 phase, this property has made it a reliable marker for estimating the growth fraction of a determined cell population normal or tumoral.[51] This nuclear protein is best detected during the interphase of the cell cycle within the nucleus of the actively dividing cells. During mitosis, Ki67 migrates to the surface of the chromosome and the cellular content peaks during the synthetic phase. The analysis of the IHC stained cells involves detection of the cells with high Ki67 content as they are positively stained (dark brown).[52]

Glandular proliferation plays significant role in the clinical behavior of prostate cancer. Increased proliferation is strongly associated with aggressiveness of tumor and poor prognosis. Counting of mitotic figures has been of an independent prognostic value in prostate adenocarcinoma. Ki-67 binding is an important marker to establish cell proliferation in the management of the prostate cancer. Ki-67 index acts as prognostic indicator of disease as index is high in carcinoma as compared to hyperplasia and is higher in metastatic rather than non-metastatic cases.[53]

The increasing number of biopsies, time constraints and the demands of quality

management and legal issues have prompted pathologists to adapt their workflow accordingly and to increase their diagnostic efficiency. Over the past 20 years, immunohistochemistry has become an indispensible tool in surgical pathology and some areas, like lymphoma classification, even depend strictly on immunophenotyping. In the evolution of current concepts of prostate pathology, immunohistochemistry has also become increasingly important. No single marker can establish a diagnosis on its own, but has to be used in close conjunction and with a thorough assessment of the individual cases, morphological as well as the clinical context, lead to correct conclusions for improved patient care. Every tool has pros and cons. The generally increased diagnostic certainty achieved with immunohistochemistry also opens up the possibility of new pitfalls that the pathologist must be aware of.[54]

MATERIALS AND METHODS

Study Design: Retroprospective study.

Source of Data: Transurethral Resection Of Prostate specimen received in the Department Of Pathology, Shri B. M. Patil Medical College, BLDE (Deemed to be University), Vijayapura.

Study period:5-year study (1st August, 2016 to 31st July, 2021)

Inclusion criteria:

- Clinically suspected and histologically diagnosed cases of benign and malignant lesions of prostate.

Exclusion criteria:

- . Cases where tissue obtained was scant for immunostaining were excluded from the study.

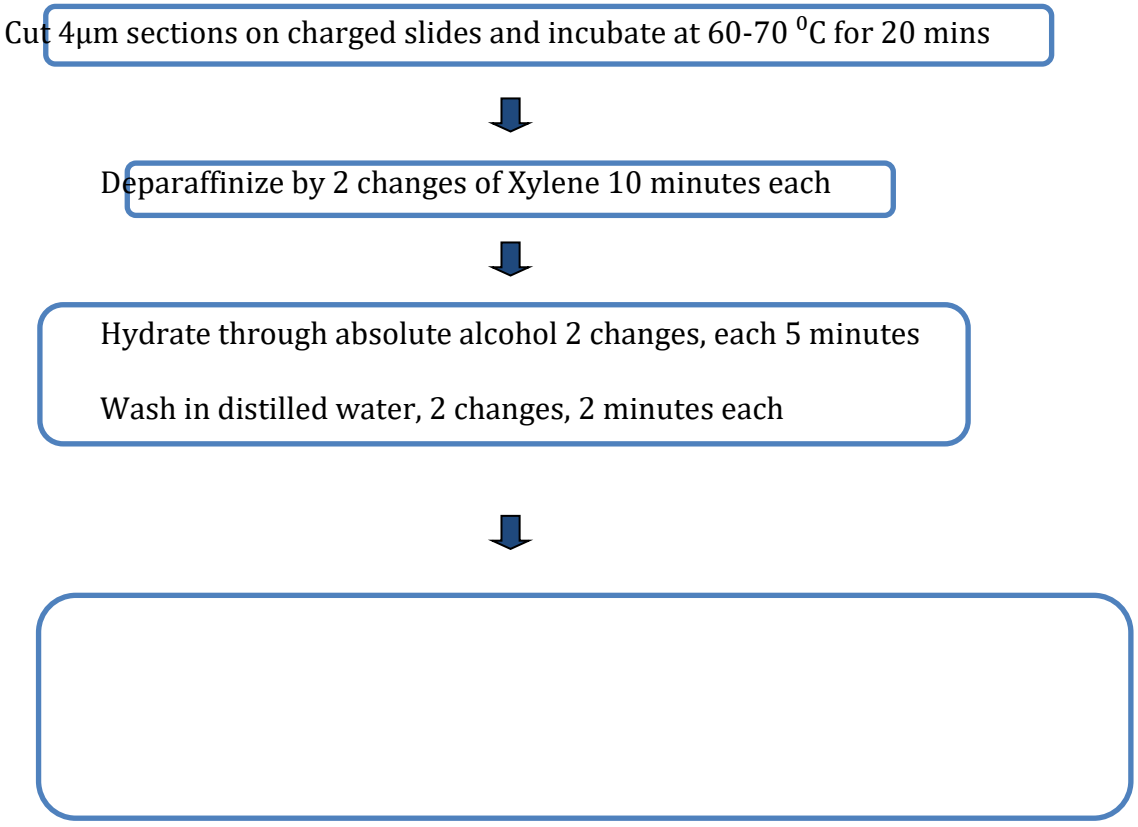
METHODS OF COLLECTION OF DATA

Paraffin blocks of all the cases which were reported as Benign Hyperplasia Of Prostate or Adenocarcinoma Prostatewere retrieved and collected from Histopathology Section, Department of Pathology, BLDE (Deemed to be University),

Shri B.M Patil Medical College, Hospital and Research Centre, Vijayapura from 1st August, 2016 to 31st July, 2021. All the clinical details, PSA levels and findings on imaging if any, were recorded. 40 cases each of Adenocarcinoma prostate and nodular hyperplasia prostate confirmed on histopathological examination were included in the study

Tissue for study included Transurethral Resection Of Prostate chips. The tissue were preserved in 10% buffered formalin and processed routinely. Three 4µ-thick sections were prepared from each tissue paraffin block. One section was stained with Haematoxylin and Eosin (H & E) for morphologic diagnosis and Gleason's score and grade. Rest two sections were mounted on poly L lysine coated slides, which were subjected to ER β and Ki 67 immunohistochemical staining and interpretation of each marker was done.

IMMUNOHISTOCHEMICAL STAINING PROTOCOL[55]



Antigen retrieval for 15 minutes in Pressure cooker 2 whistles with retrieval buffer on TRIS EDTA, maintain pH between 8.5-9.0(preparation- 1 part of concentrated buffer+49 parts of distilled water)

Wash in distilled water, 2 changes, 2 minutes each

Wash in phosphate buffer solution(PBS)/ tris buffer solution(TBS) for 2 minutes

Add primary antibody and keep for 45 minutes in moist chamber. Then wash in wash buffer two changes, 3 minutes each

Add Polyexcel Target binder reagent and keep for 12 minutes. Wash in wash buffer two changes, 3 minutes each

Add polyexcel Horse Radish Peroxidase(HRP)and incubate for 12 minutes. Wash with buffer, 2 changes, 3 minutes

Add working Diaminobenzidine(DAB) chromogen(1 ml DAB buffer+1 drop DAB chromogen, mix well) and keep it for 2- 5 minutes

Wash it with distilled water 2 changes 2 minutes each

Counterstain with Hematoxylin for 30 seconds, wash with water

Dehydrate, clear and mount the slide

EVALUATION OF ESRTOGEN RECEPTOR BETA EXPRESSION

Nuclear staining within the cell, whether strong or weak, were considered positive. A section of breast cancer was stained as a positive control. Immunostained slides were scored as for the routine evaluation of the ER status in breast cancer.

First, a proportion score (PS) was assigned, which represents the estimated proportion of positive cells in each individual lesion present in the entire slide. The PS included six categories ranging from 0 to 5 was done as follows: **0: none; 1: 1%; 2: >1–10%; 3: >10–33%; 4: >33–66%; 5: >66%.**[56]

Next, an intensity score (IS) was assigned which represents the average intensity of positive cells **(0: none; 1: weak; 2: intermediate; 3: strong)** in a particular lesion when compared with the ER β immunoreactivity of host cells.

The PS and IS were then added to obtain a total score (TS) (range, 0–8).

For statistical analysis, the TS was subdivided in four categories including **negative (TS, 0–2), weak (TS, 3–4), moderate (TS, 5–6), and strong (TS, 7–8).**[56]

IMMUNOHISTOCHEMICAL STAINING FOR KI-67

Similar procedure was followed for evaluating proliferative index by Ki 67 immunostaining. Antibody used for Ki 67: rabbit monoclonal prediluted antibody. A section of lymph node was also stained as a positive control. The percentage of immunostained nuclei across the cancer areas was calculated and grade as follows: **<1 % (1+), 1–5 % (2+), ≥5–10 %(3+), ≥10–20 %(4+), ≥20 % (5+).**[10]

STATISTICAL ANALYSIS

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean±standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square (χ^2) test was used for association between two categorical variables. The formula for the chi-square statistic used in the chi square test is:

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

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

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

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

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

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

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

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

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

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

ROCanalysis for Sensitivity- specificity was done to check relative efficiency.

sensitivity or true positive rate (TPR)

eqv. with hit rate, recall

$$TPR = TP/P = TP/(TP + FN)$$

specificity (SPC) or true negative rate

$$SPC = TN/N = TN/(FP + TN)$$

precision or positive predictive value (PPV)

$$PPV = TP/(TP + FP)$$

negative predictive value (NPV)

$$NPV = TN/(TN + FN)$$

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

RESULTS

Total number of cases included in the present study was 80 histologically diagnosed cases of Benign and malignant lesions of prostate.

TABLE 4: DISTRIBUTION OF AGE BETWEEN STUDY GROUPS

AGE(years)	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
41-50	0	0	5	12.5	0.004*
51-60	3	7.5	11	27.5	
61-70	19	47.5	17	42.5	
71-80	11	27.5	6	15	
>80	7	17.5	1	2.5	
Total	40	100	40	100	

Note: p value* significant at 5% level of significance ($p<0.05$)

In the present study, majority of the cases wereof the age group of 61-70yrs in both prostate hyperplasia and prostate carcinomas amounting to 19 (42.5%) and 17(47.5%) respectively.Malignant cases in the age group of 71-80yrs and benign cases in the age group of 51-60yrs amounted to 11 cases each (27.5%). There were 5 benign cases belonging to age group of 41-50yrs (12.5%) whereas there was not a single malignant case belonging to this same age group.

FIGURE 8: DISTRIBUTION OF AGE BETWEEN STUDY GROUPS

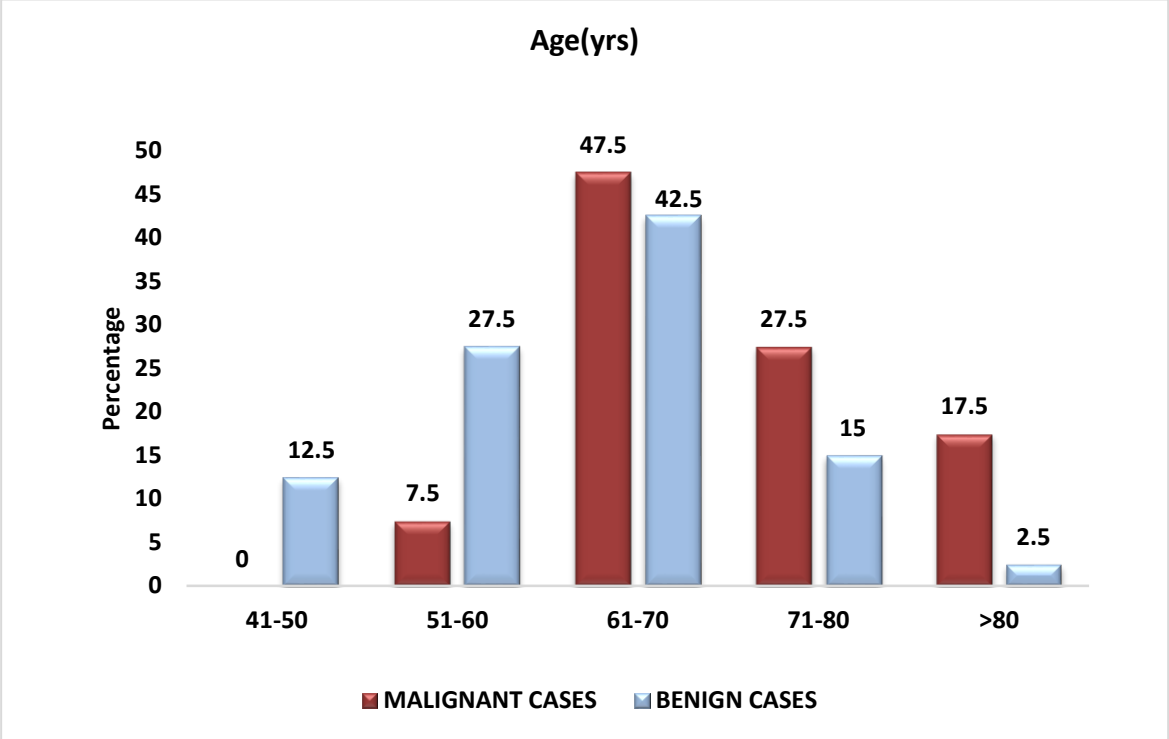


TABLE 5: DISTRIBUTION OF GLEASON GRADE IN MALIGNANT CASES

GLEASON GRADE	Malignant		Benign	
	No. of patients	Percentage	No. of patients	Percentage
1	0	0	NA	NA
2	7	17.5	NA	NA
3	13	32.5	NA	NA
4	9	22.5	NA	NA
5	11	27.5	NA	NA
Total	40	100	NA	NA

Out of 40 Prostate carcinomas , 13 cases were seen belonging to Gleason Grade 3 (32.5%) followed by 11 patients belonging to Gleason Grade 5 (27.5%). 9 cases (22.5%.) were seen belonging to Gleason Grade 4. Only 7(17.5%) out of 40 patients belonging to Gleason Grade 2. Gleason Grade is not applicable to Benign Hyperplasia.

FIGURE 9: DISTRIBUTION OF GLEASON GRADE IN MALIGNANT CASES

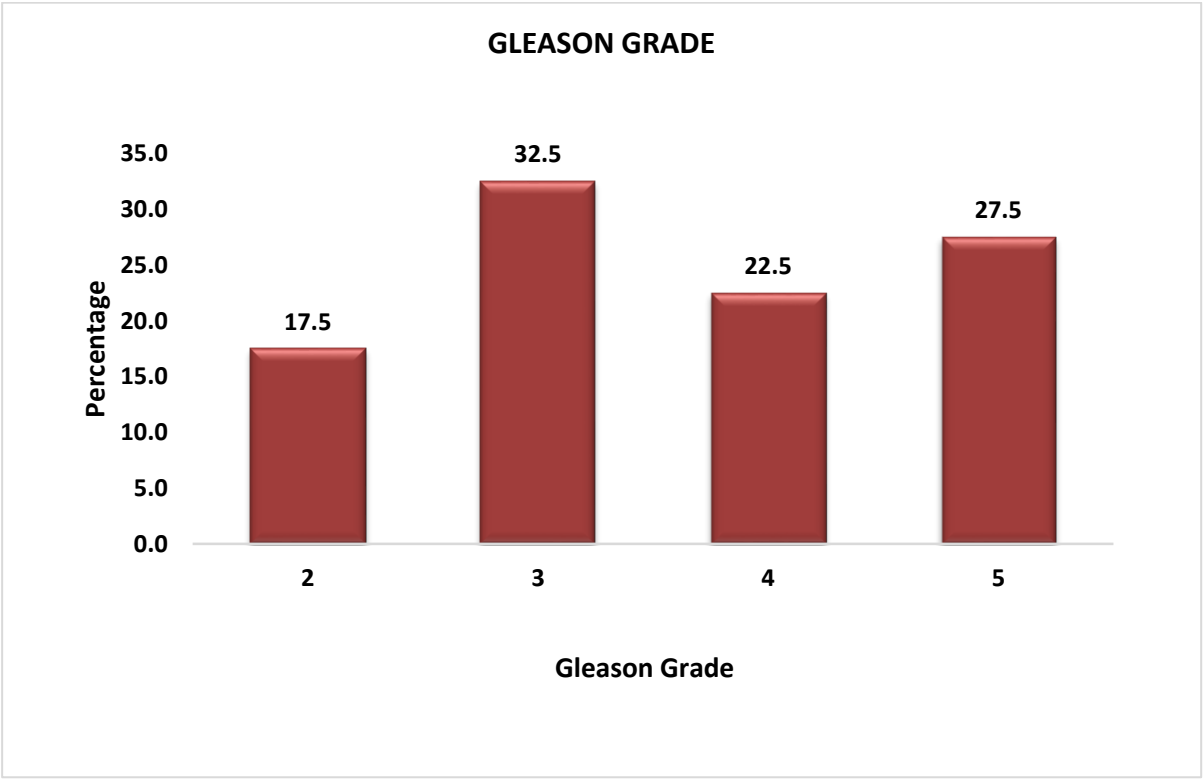


TABLE 6:DISTRIBUTION OF PATIENTS ACCORDING TO GLEASON SCORE

GLEASON SCORE	Malignant		Benign	
	No. of patients	Percentage	No. of patients	Percentage
3+4	9	22.5	NA	NA
3+5	4	10.0	NA	NA
4+3	10	25.0	NA	NA
4+4	3	7.5	NA	NA
4+5	4	10.0	NA	NA
5+3	2	5.0	NA	NA
5+4	5	12.5	NA	NA
5+5	3	7.5	NA	NA
Total	40	100.0		

Out of 40 Prostate carcinomas, 10 cases(25%) belonged to Gleason score 4+3 followed by 9 cases(22.5%.)belonging to Gleason score 3+4. 5 cases(12.5%) fall under the category of Gleason score 5+4 followed by 4 casesof Gleason score 3+5 and 4+5 category amounting for 10% each. Only 2 cases(5%) belonged to Gleason score 5+3. Gleason score is not applicable for Benign Hyperplasia.

FIGURE 10: DISTRIBUTION OF GLEASON SCORE IN MALIGNANT CASES

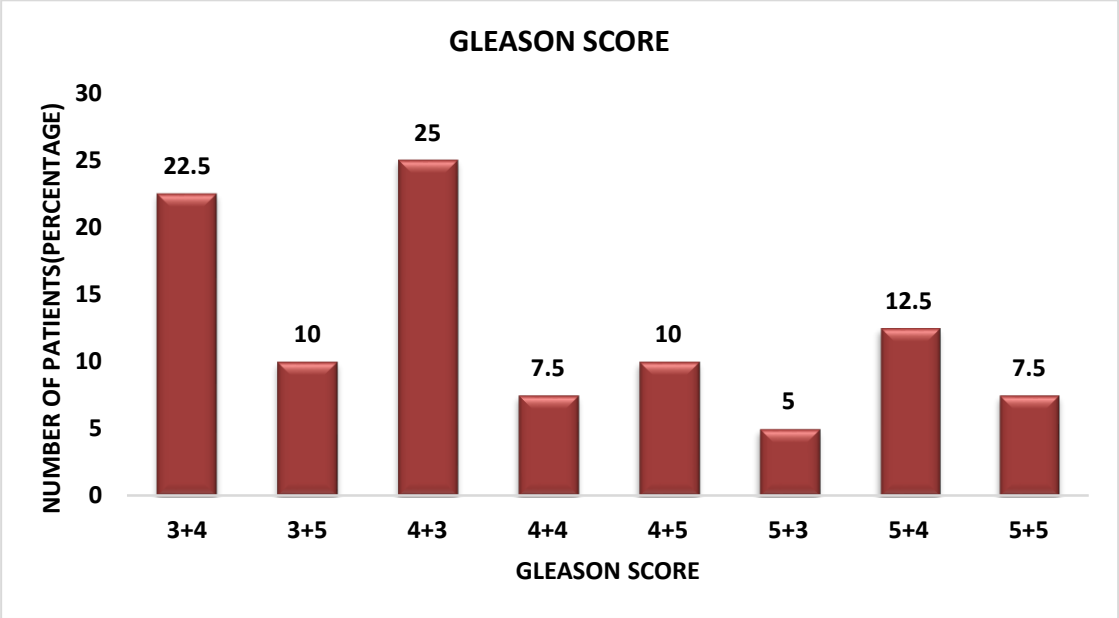


TABLE 7: DISTRIBUTION OF ER BETABETWEEN STUDY GROUPS

ER BETA	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
NEGATIVE	28	70	9	22.5	<0.001*
POSITIVE	12	30	31	77.5	
Total	40	100	40	100	

Note: p value* significant at 5% level of significance (p<0.05)

Out of 40 prostatic Carcinomas, ER β positivity was noted in only 12 cases(30%) and it was negative for rest 28 cases(70%). Out of 40 benign Hyperplasia, ER βpositivity was noted in 31 cases (77.5%).

FIGURE 11: PERCENTAGE DISTRIBUTION OF ER BETABETWEEN STUDY GROUPS

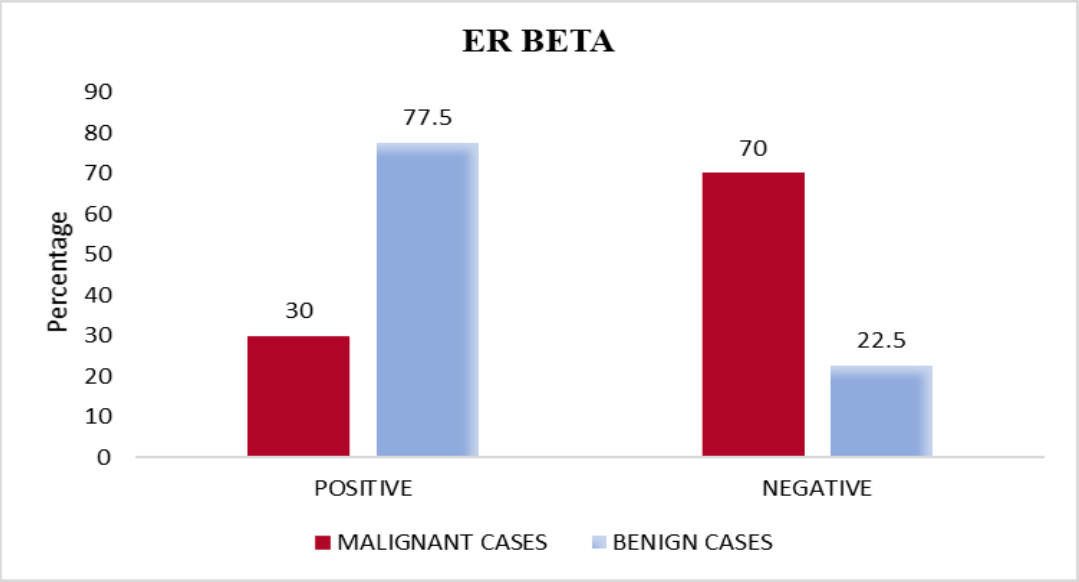


TABLE 8: ER BETA PROPORTION SCORE BETWEEN STUDY GROUPS

ER BETA PROPORTION SCORE	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
0	28	70.0%	9	22.5%	
1	9	22.5%	4	10.0%	
2	2	5.0%	22	55.0%	
3	1	2.5%	3	7.5%	<0.001
4	0	0.0%	0	0%	*
5	0	0.0%	2	5.0%	
Total	40	100.0%	40	100.0 %	

Note: p value* significant at 5% level of significance (p<0.05)

Out of 40 Prostatic carcinomas, 12 cases(30%) showed ER-β expression. 9 cases(22.5%) showed positive expression with proportion score of 1. 2 cases(5%) showed proportion score of 2. Only 1(2.5%) case showed proportion score of 3. Out of 40 Benign Hyperplasia, 9 cases(22.5%)did not show immunoexpression. 22 cases(55%) showed proportion score of 2 Followed by 4 cases(10%) with score of 1 and 3 cases(7.5%) with a score of 3. Only 2 cases (5%) show proportion score of 5. There was not a single case with proportion score of 4 in either of the study groups.

FIGURE 12: ER BETA PROPORTION SCORE BETWEEN STUDY GROUPS

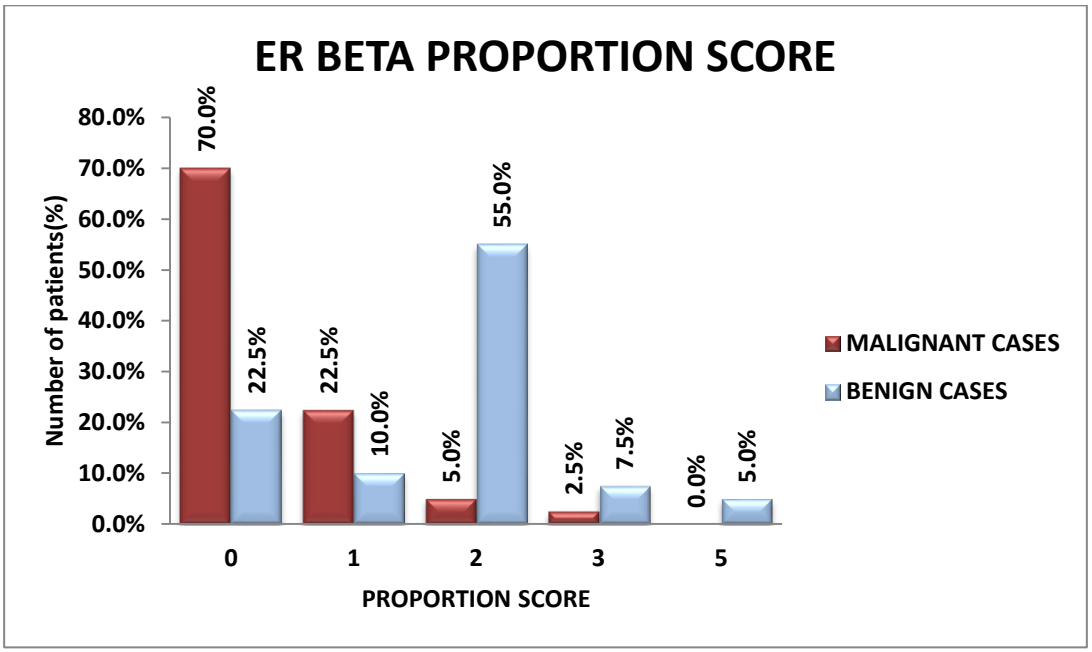


TABLE 9: ER BETA INTENSITY SCORE BETWEEN STUDY GROUPS

ER BETA INTENSITY SCORE	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
0	28	70.0	9	22.5	<0.001 *
1	9	22.5	15	37.5	
2	2	5.0	13	32.5	
3	1	2.5	3	7.5	
Total	40	100.0	40	100.0	

Note: p value* significant at 5% level of significance (p<0.05)

Out of 40 Prostatic Carcinomas, 12 cases(30%) showed ER-β expression. 9 cases(22.5%), 2 cases (5%) and 1 case (2.5%) showed positive expression with intensity score of 1,2 and 3 respectively. Out of 40 cases of Benign Hyperplasia, 15(37.5%) cases showed intensity score of 1 followed by 13(32.5%) cases with intensity score of 2 and 3 cases (7.5%)with intensity score of 3.

FIGURE 13: ER BETA INTENSITY SCORE BETWEEN STUDY GROUPS

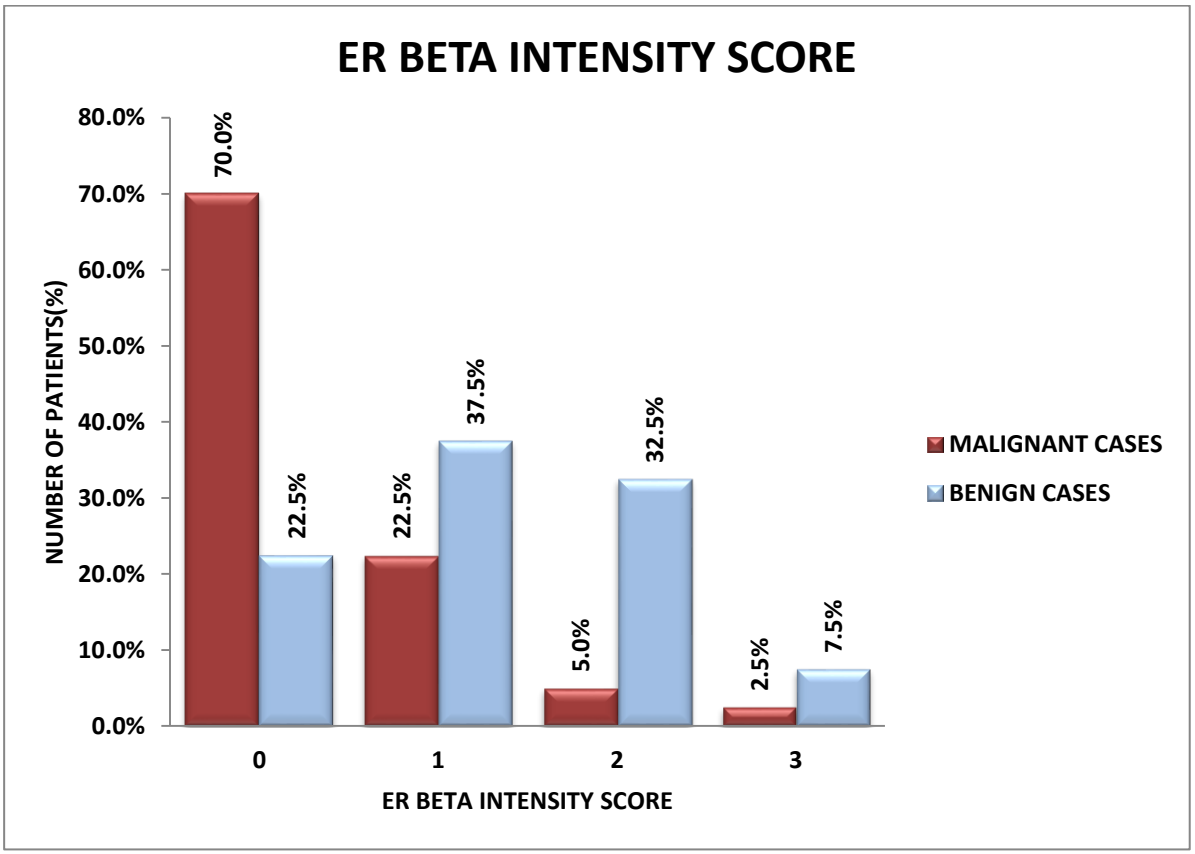


TABLE 10: ER BETA TOTAL SCORE BETWEEN STUDY GROUPS

ER BETA TOTAL SCORE	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
0	28	70.0	9	22.5	<0.001 *
2	7	17.5	4	10.0	
3	3	7.5	9	22.5	
4	1	2.5	15	37.5	
5	1	2.5	0	0.0	
6	0	0.0	1	2.5	
7	0	0.0	0	0.0	
8	0	0.0	2	5.0	
Total	40	100.0	40	100.0	

Note: p value* significant at 5% level of significance ($p<0.05$)

Out of 40 Prostate carcinomas, 12 cases showed expression of ER-β. 7 cases (17.5%),3 cases (7.5%)showed ER-β score of 2 and 3 respectively. 1 case each with a score of 4 and score 5 were noted. Out of 40 Benign Hyperplasia, 15 cases (37.5%) showed ER-β score of 4 followed by 9 cases(22.5%) with a score of 3. 2 cases (5%) showed total score of 8.

FIGURE 14: ER BETA TOTAL SCORE BETWEEN STUDY GROUPS

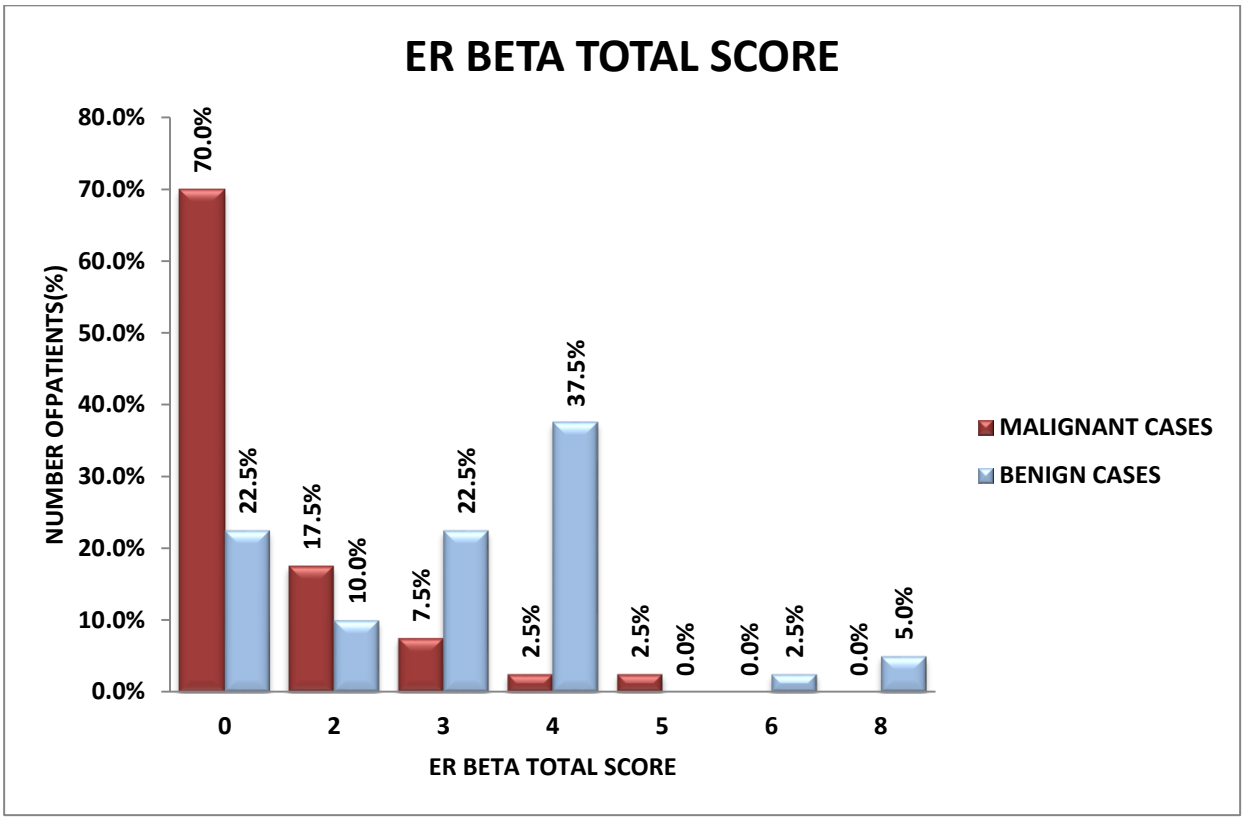


TABLE 11: DISTRIBUTION OF MEAN ER BETA PARAMETERS BETWEEN STUDY GROUPS

Parameters	MALIGNANT CASES		BENIGN CASES		p value
	Mean	SD	Mean	SD	
ER BETA PROPORTION SCORE	0.40	0.71	1.68	1.21	<0.001*
ER BETA INTENSITY SCORE	0.40	0.71	1.25	0.90	<0.001*
ER BETA TOTAL SCORE	0.80	1.34	2.93	2.02	<0.001*

Note: p value* significant at 5% level of significance (p<0.05)

The mean ± SD values of ER β Proportion score showed statistically significant variation between the two groups, i.e., in malignant cases it was 0.40 ±0.71 whereas in benign cases, it was 1.68 ±1.21. ER β intensity score showed mean ± SD values of 0.40 ± 0.71 and 1.25 ± 0.90 in prostatic carcinoma and benign Hyperplasia respectively with p value< 0.001. Similarly, ER β total score showed mean ± SD values of 0.80± 1.34 in the prostatic carcinomaand 2.93± 2.02 in the benign Hyperplasia (p < 0.001).

FIGURE 15: DISTRIBUTION OF MEAN ER BETA PARAMETERS BETWEEN STUDY GROUPS

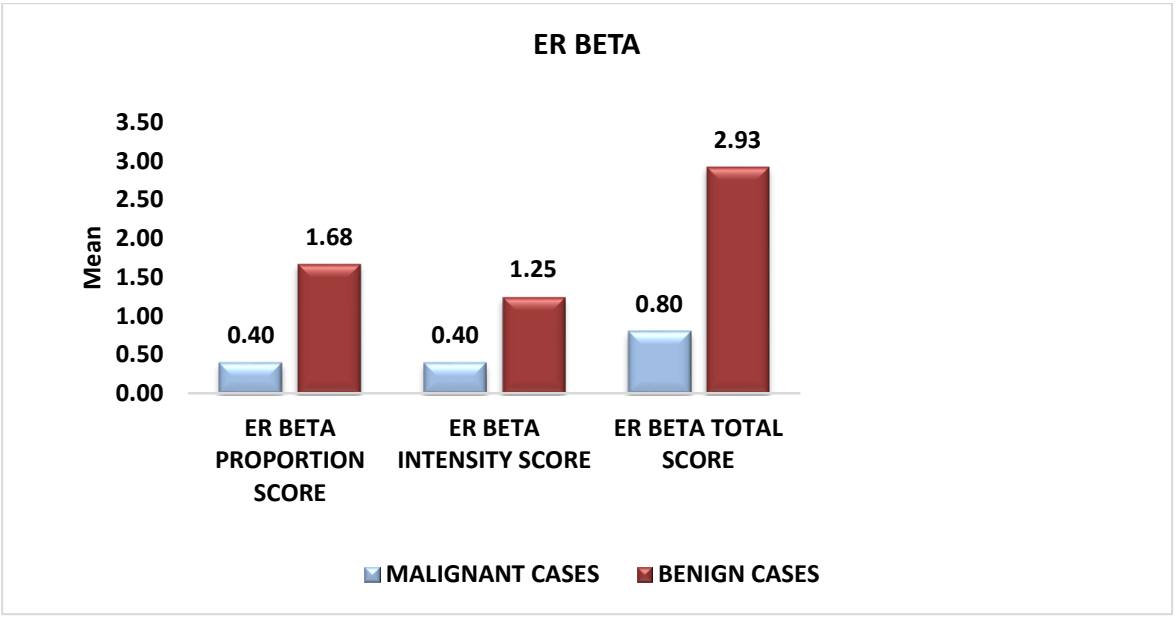


TABLE 12: DISTRIBUTION OF KI-67 (PERCENTAGE) AND KI67 SCORE BETWEEN STUDY GROUPS

KI-67 % (Score)	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
<5 (2)	0	0	30	75	0.001*
>5-10 (3)	9	22.5	10	25	
>10-20 (4)	16	40	0	0	
>20 (5)	15	37.5	0	0	
Total	40	100	40	100	

Note: p value* significant at 5% level of significance ($p<0.05$)

Out of 40 prostate carcinoma, ki67 expression was noted in>10-20% tumor cells (Score 4) in 16 cases (40%) . 15 (37.5%) cases showed ki67 expression in>20% tumor cells(Score 5). Only 9 cases (22.5%) with tumor cell expression of >5-10% (Score 3) was observed. On the contrary, proliferative index was noted in<5% tumor cells (Score 2) in 30(75%)benign hyperplasia cases. 10 cases(25%) of Benign hyperplasia showed proliferative index of 10% (Score 3) in tumor cells.

FIGURE 16:DISTRIBUTION OF KI-67 (PERCENTAGE) AND KI67 SCORE BETWEEN STUDY GROUPS

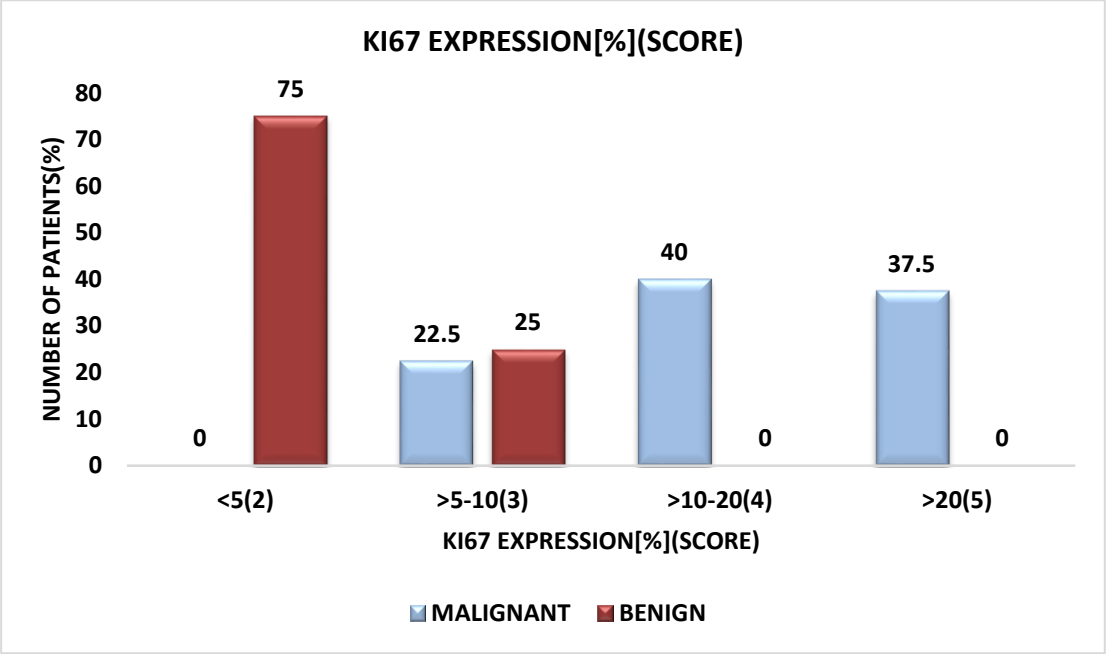


TABLE 13: ROC ANALYSIS OF ER BETA AND KI67 SCORES IN PREDICTING MALIGNANT CASES

Parameters	AUC	St. Error	p value	95% CI	
				Lower	Upper
ER BETA PROPORTION SCORE	0.807	0.051	<0.001*	0.706	0.907
ER BETA INTENSITY SCORE	0.766	0.054	<0.001*	0.66	0.873
ER BETA TOTAL SCORE	0.799	0.052	<0.001*	0.697	0.9
KI67 SCORE	0.972	0.014	<0.001*	0.944	0.999

Note: p value* significant at 5% level of significance (p<0.05)

Parameters	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
ER Beta proportion score	1.5	92.5%	67.5%	74.0%	90.0%	80.0%
ER Beta Intensity score	1.5	97.5%	57.50%	69.6%	95.8%	77.5%
ER Beta Total Score	3.5	95.0%	45.0%	63.3%	90.0%	70.0%
KI67 SCORE	2.5	100.0%	75.0%	80.0%	100%	87.5%

FIGURE 17: ROC CURVE OF ER BETA SCORES IN PREDICTING MALIGNANT CASES

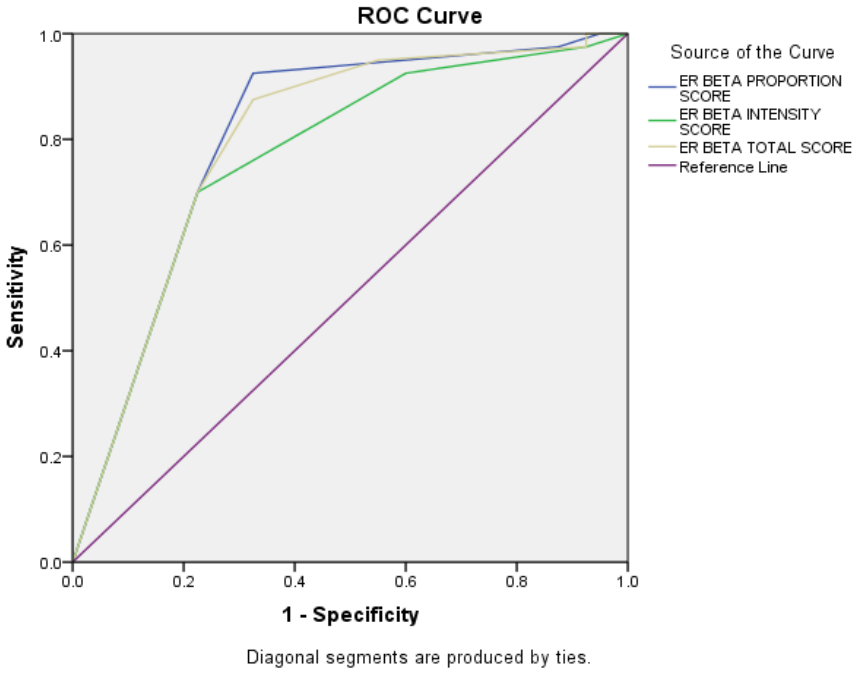
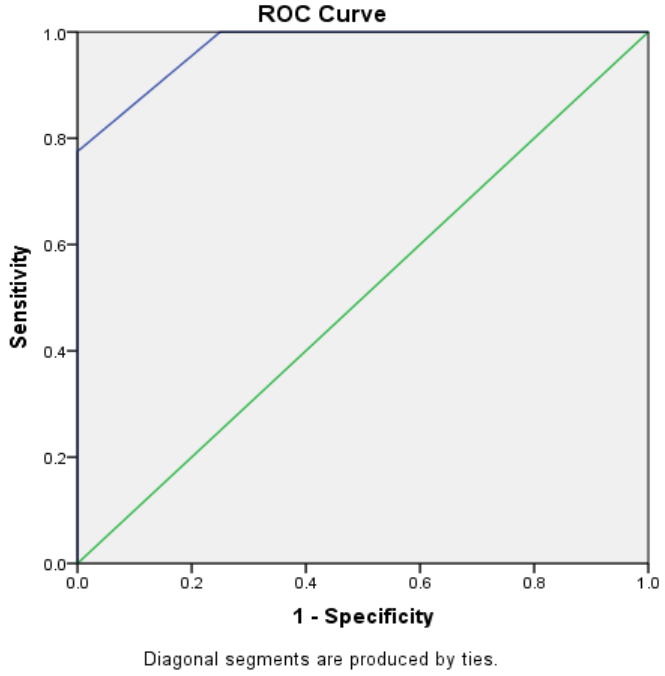


FIGURE 18: ROC CURVE OF KI67 SCORES IN PREDICTING MALIGNANT CASES



The Receiver operating characteristic (ROC) analysis of the parameters showed cutoff values for predicting malignant cases as mentioned in the Table 13. Amongst the parameters ER β total score showed sensitivity of 95% and specificity of 45% for a cut off value of 3.5 for identifying malignant cases. ($P < 0.001$). Comparison of the parameter ki67 score with ER β total score showed that the sensitivity and specificity of ki67 was more than ER Beta total score. At the optimal cut-off value of 2.5, the sensitivity and specificity was 100% and 75.0% respectively. The Area Under Curve for ki67 (0.972, 95% CI 0.944-0.999) indicates that ki67 is the best discriminator of malignant cases with a p value <0.001.

MICROPHOTOGRAPHS

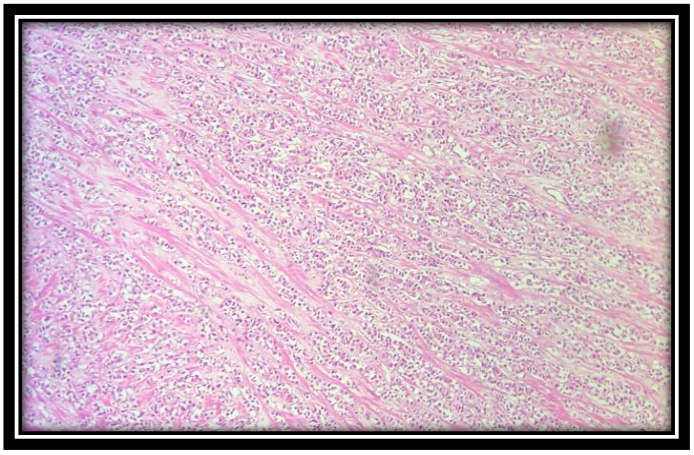


Figure 19: Microphotograph of Prostate Adenocarcinoma showing characteristic morphological features of trabecular pattern (H&E,100X) (Gleason score=5+4)

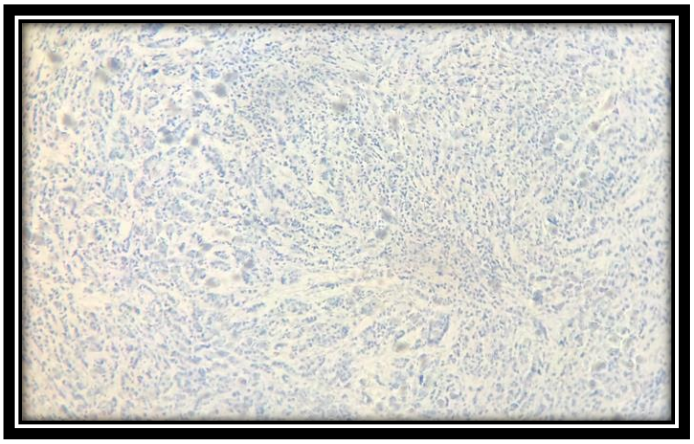


Figure 20: Microphotograph of Adenocarcinoma Prostate showing IHC staining of ER-Beta in Prostate cancer showing negative expression. (100X)

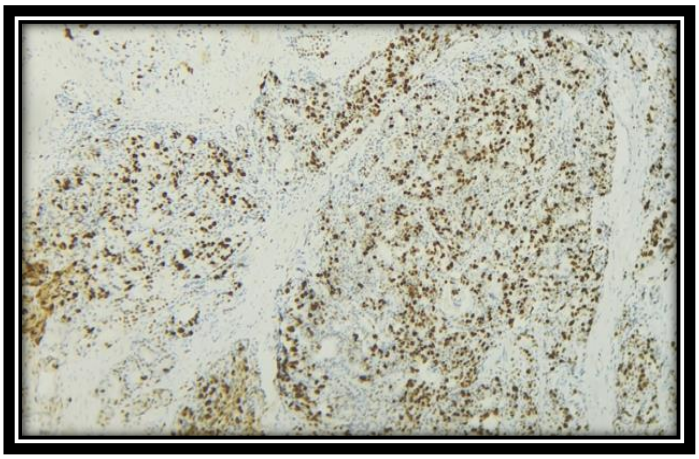


Figure21: Microphotograph of Adenocarcinoma prostate showing IHC staining of ki67 in Prostate cancer showing >20% proliferative index. (100X)

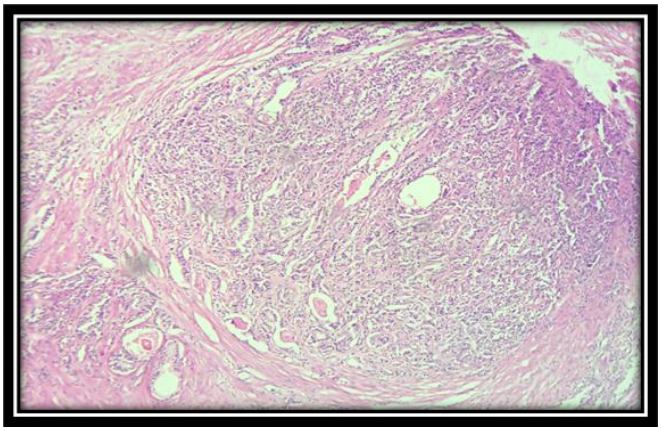


Figure 22: Microphotograph showing H&E stain of tumor in lobules in prostatic adenocarcinoma(100X) (Gleason score=4+5)

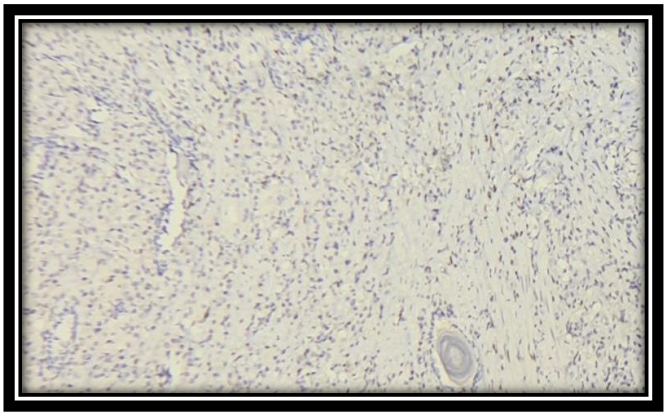


Figure 23: Microphotograph of a prostate Adenocarcinoma showing positive expression of ER- Betabut weak intensity in stromal cells. (100X)

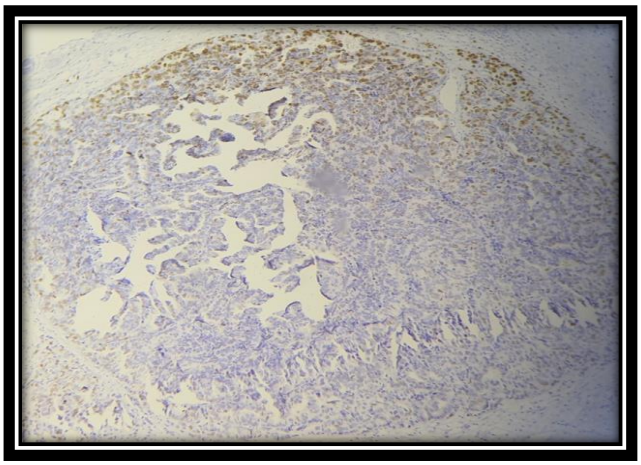


Figure24: Microphotograph of a prostate Adenocarcinoma showing Ki67 >10% proliferative index. (100X)

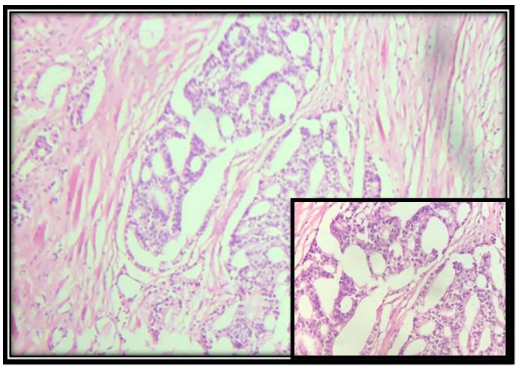
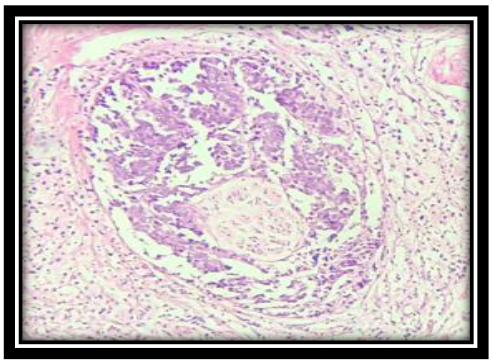


Figure 25: Microphotograph showing H&E Stain showing tumor arranged in cribriform pattern(100X) and image at the bottom right corner showing closer view of tumor cells (400X) in prostate adenocarcinoma.Gleason score=5+5

Figure 26: Microphotograph showing H&E Stain showing perineural invasion. (200X)



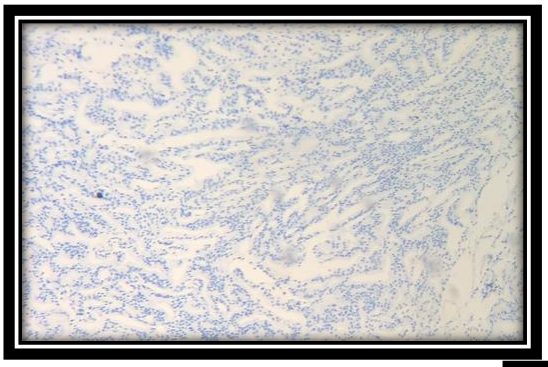


Figure 27: Microphotograph of a carcinoma prostate showing Negative expression of ER- Beta. (100X)

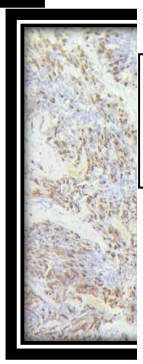


Figure 28: Microphotograph of carcinoma prostate showing ki67 >20% proliferative index. (100X)

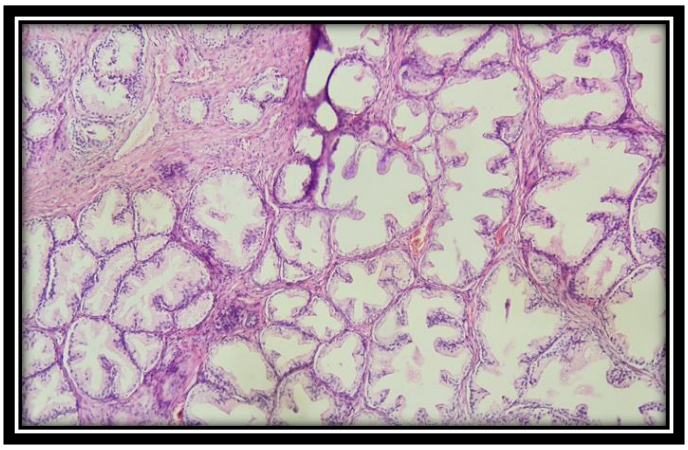


Figure 29: Microphotograph of H&E Stain showing back-to-back arranged and fibromuscular stroma glands in BPH. (100X)

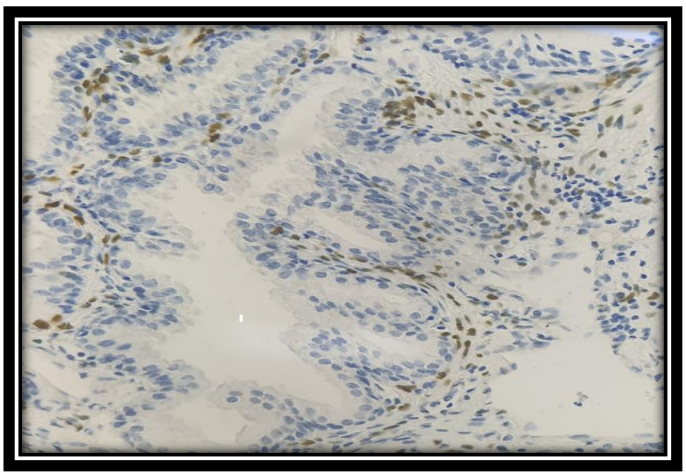


Figure30: Microphotograph of BPH showing positive ER-Beta immunoexpression in myoepithelial cells and few stromal cells. (100X)

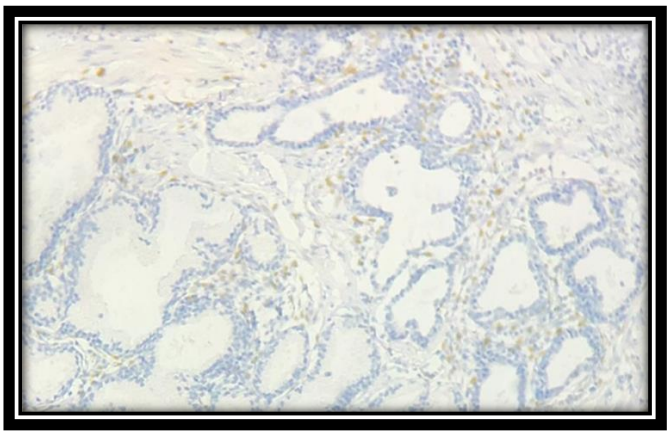


Figure 31: Microphotograph of BPH showing weakscattered immunoexpression of ki67 of <5% proliferative index. (100X)

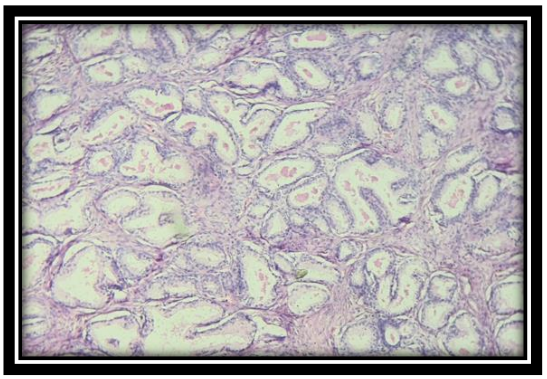
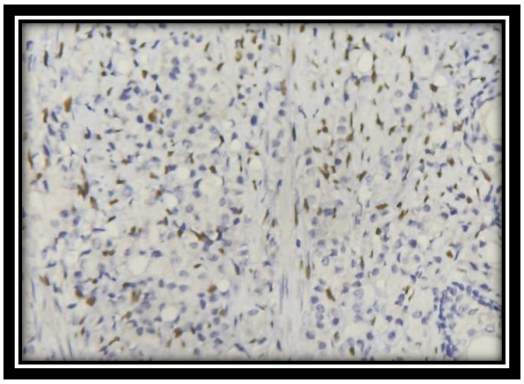


Figure 33: Microphotograph of BPH showing positive ER-Beta immunoexpression in stromal cells. (100X)

Figure 32: Microphotograph of H&E Stain showing Glands with eosinophilic secretions in the lumen. (100X)



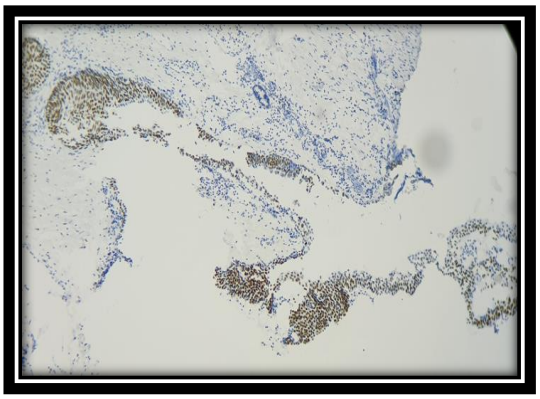
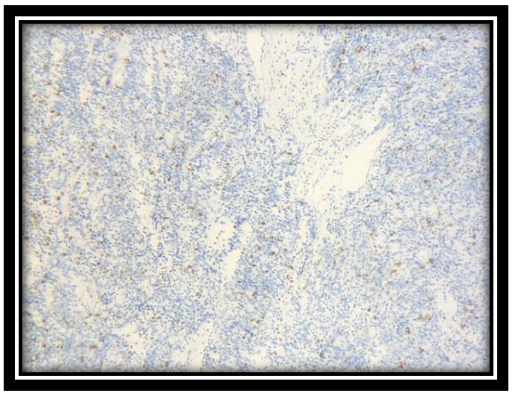


Figure 34: Microphotograph of BPH showing positive ER-Beta immunoexpression in transitional epithelium. (100X)

Figure 35: Microphotograph of BPH showing ki67 proliferative index >5-10%. (100X)



DISCUSSION

Prostate cancer is primarily a disease of the elderly with more than three quarter of the cases occurring in men above 65 years of age. This disease has become a major health problem globally during the last few decades. Prostate cancer is the second most frequently diagnosed cancer in men worldwide and the fifth most common cancer overall. Prostate cancer accounted to about 1,276,106 new cases and

358,989 deaths which is around 3.8% of deaths in men with cancer during the year 2018.[1] The numbers however varies world wide. In the Asian countries, prostate cancer incidence has been reported to vary from 3.0/100,000 in Iran to the highest of 20.3/100,000 in Phillipines. There has been a consistent increase in the age-standardized incidence rates (ASIRs) of prostate cancer in Asian countries over the last few decades. It has been reported that although the cancer rates in India are lower than those seen in Western countries, increase in life expectancy and changes in lifestyles increase the rates of cancers in this country, particularly prostate cancer.[73]

Of cancers that involve the prostate gland, the typical acinar pattern adenocarcinoma is by far the most prevalent, constituting more than 95% of all prostate malignancies. An estimated 9% of prostate malignancies are due to inherited predisposition. Among environmental factors, diet is one of the most commonly cited reasons for increased risk of prostate cancer. In particular, a high amount of dietary fat is the most commonly cited risk factor for progression of disease, with red meat being the most strongly associated risk factor among high-fat items. [74]

Diagnosis of prostate carcinoma has become a challenge for the pathologists due to various architectural patterns and as there are many mimickers of prostate adenocarcinoma but one of the gold standard for the diagnosis of prostatic carcinoma is light microscopic findings. But there can be significant variation in the morphology of basal cells like secretory cells which are cut tangentially, stromal fibroblasts, tumor cells showing distortion and crushing artefacts in a small focus of cancer which resembles basal cells.In cases where there are a large number of atypical glands present for evaluation, the use of antibodies that label basal cells of the prostate may resolve the diagnosis.[42] However, not all benign glands label uniformly with basal cell markers like High molecular weight Cytokeratin. The use of high molecular weight

cytokeratin when presented with only a few atypical glands is not as diagnostic, since benign glands may not show uniform positivity with this marker. More recently, antibodies to P63, which is a nuclear stain, has also been shown to label basal cells of the prostate, but this is also not diagnostic as uniform positivity with these marker is not seen. AMACR, a cytoplasmic protein also known as P504S, has recently been recognized as a tumor marker for several cancers but its role in prostatic carcinogenesis is unclear. Recent studies have shown that AMACR expression is significantly upregulated in prostate cancer. By IHC, the majority of prostate cancers (80–100%) are positive for AMACR, although a high proportion of high-grade prostatic intraepithelial neoplasia (PIN), some foci of adenosis, and also some entirely benign glands have also been reported positive for this marker.[57] Certain mimickers of adenocarcinoma of the prostate are even less frequently labeled uniformly with these stains. Consequently, negative staining in a small focus of atypical glands for basal cell markers is not diagnostic of adenocarcinoma of the prostate.[42] So there is a need of series of Immunhistochemical studies to reach to a definitive diagnosis of malignancy.[57]Markers like Estrogen receptor beta have been recently studied on prostate epithelium and are still under debate. Regarding this ER-Beta expression on benign and malignant prostatic lesions, contradictory results are existing.[58]

Coming to immunoexpression of ER-Beta in benign prostatic lesions, it is detected in the nuclei of some, but not all, epithelial and stromal cells, whereas it is strong in the nuclei of basal cells in normal and hyperplastic tissues and more importantly, the proportion of ER-Beta positive cells is significantly higher in the stroma of peripheral zone than that of Transitional zone. Considering that BPH nodules develop from Transitional zone, these results seem to indicate that ER-Beta may be involved in the pathogenesis of BPH.[65]On the other hand, it is noted that there is wide variation in

results of various studies regarding the expression of ER β with respect to grade of tumor in prostate carcinoma.[10]The data in the current study supports that rate of ER-beta expression is significantly lower in tumors belonging to Gleason grade4/5 as compared to the low and intermediate grade tumors and these findings are in concordance to most of the studies. Also it has been demonstrated and supported by few studies that ER β is reduced in carcinoma prostate compared to nodular hyperplasia as it is anti proliferative.[59]

Table 14 : Immunoexpression ofER-β in Benign cases

Studies	Benign Cases	Immunoexpression of Estrogen Receptor-β(%)
Grover SK *et al*[10]	30	22(73.3)
Gabal SM *et al*[59]	10	9(90)
Present study	40	31(77.5)

A study done by Grover SK et al[10] concluded that ER- β immunoexpression was seen in 22/30 cases of Nodular Hyperplasia amounting to 73.3%. In the present study, 31 out of 40 cases showed positive immunoexpression amounting to 77.5%. Another study conducted by Gabal SM *et al*[59] found that 90% of the studied BPH cases showed ER-β positive expression, which is in concordance with the present study.

In a study done by Bera KN *et al*[62], they found ER-β expression score was >3 in the majority of cases in the benign group which is in concordance to the present study, where Estrogen receptor-β immunoexpression is seen in 31 cases amounting to 77.5%. Contrary to the above findings, study done by Horvath *et al*[60]found that there is progressive loss of ER β in prostatic hyperplasia.

Table15 : Immunoexpression of ER-β in Malignant cases

Studies	Malignant cases	Immunoexpression of Estrogen Receptor - β (%)
Grover SK *et al*[10]	30	9(30)
Gabal SM *et al*[59]	35	6(17.1)
Horvarth*et al*[60]	100	11(11)
Fixemer*et al*[56]	60	52(87)
AsgariM *et al*[45]	52	48(92.3)
Present study	40	12(30)

A study done by Grover SK *et al*[10] concluded that out of 30 malignant cases, ER-β showed immunoexpression in 9 cases amounting to 30%. Similarly, study done by Gabal SM *et al*[59] and Horvarth*et al*[60] concluded that 17.1% and 11% cases respectively showed ER-β expression in malignant cases. Gabal SM *et al*[59] also concluded that twenty-nine(82.8%) of the studied adenocarcinoma cases were negative for ER-β expression, p<0 .0001 suggesting the loss of ER-β expression is associated with progression from hyperplastic prostate epithelium to Prostate Carcinoma. The above findings are in concordance to the current study, where we found 12 out of 40 cases showed positivity for estrogen receptor β.

On the other hand, study done by Fixemer*et al*[56] showed that 52 out of 60 cases accounting to 87% shows retention of ER-β immunoexpression, therefore, concluding that ER β expression are retained in all primary adenocarcinomas and metastatic at high levels.

Another study done by AsgariM *et al*[45] found that 48 out of 52 cases constituting 92.3% showed positive immunoreactivity for ER-β expression thus concluding that ER-β expression was present in all low and intermediate grade cancers and 17/23 cases were positive for ER-β among high grade cancers. These findings did not show similar results as the present study.

Growth fractions were assessed immunohistochemically in prostatic tissues with benign glandular hyperplasia (BPH) and in specimens of prostatic cancer using the monoclonal antibody Ki-67. This antibody is specific for a proliferation-associated nuclear antigen. In BPH tissues about 0.3% of nuclei of epithelial cells was reactive with Ki-67. The Ki-67 positive nuclei were distributed equally among the basal and luminal cells of the hyperplastic prostatic acini[66] whereas in malignant prostatic lesions high immunoreactivity is seen, which is used to assess the growth fraction of neoplastic cell populations.[67]

Table 16 : Immunoexpression of Ki67 in Benign cases

Studies	Benign cases	Immunoexpression of Ki67(%)
Mohammed AA *et al*[64]	11	2(19)
Verma R*et al*[63]	10	1(10)
Grover SK *et al*[10]	30	26(86.3)

Present study	40	30(75)

In present study, out of 40 malignant cases, 30 cases amounting to 75% showed immunoexpression of ki67 having nuclear positivity of <5% (score 2) which showed similar result in a study done by Grover SK et al[10]where they concluded that Ki67 expression was low (<5%) in most of the benign cases(86.3%).The ki67 scoring system was similar to the current study. A study done by Mohammed AA et al[64]concluded that Ki-67 expression was significantly is low in benign prostatic hyperplasia (19%) with p value <0.05, but the ki67 scoring system mentioned in this study and sample size was very less in their studies, which could be the reason of the discordance of the results as compared to the current study.

A study done by Verma R et al[63]found thatKi-67 was expressed in only 1 of 10 (10%) BPH cases but the ki67 semiquantitative scoring system was different which is as follows: percentage of stained cells was ≤2% were considered negative.[68]

Table 17: Immunoexpression of ki67 in Malignant cases

Studies	Malignant cases	Ki67 Immunoexpression(%)
Grover SK *et al*[10]	30	19(90)
Mohamed AA *et al*[64]	47	38(81)
GangwarS *et al*[69]	27	18(66.6)
Azizan N *et al*[70]	101	98(97)

Madani SH *et al*[71]	49	35(71.4)
Rasheed IA *et al*[72]	30	30(100)
Present study	40	40(100)

A study done by Grover SK *et al*[10] found that Ki 67 expression was higher in carcinoma prostate which was 19 out of 30 cases constituting 90% with proliferation index >5% which was similar to the present study which showed ki67 immunoexpression in all cases with proliferation index more than 5%.

A study done by Gangwar S *et al* [69] concluded that Ki-67 was expressed in majority of the moderately and poorly differentiated tumors with 18 cases (66.6%) showing strong positivity which is in concordance with the current study.

Mohamed AA *et al*[64] studied that the expression of Ki-67 in 47 cases of prostatic adenocarcinoma was evaluated and scored according to KI Score system. In this study found that Ki-67 expression was significantly higher in prostatic carcinoma (81%), ($P < 0.05$). This result is similar to that of the study done done by Azizan N *et al*[70] who concluded that out of 101 cases, 98 cases (97.0%) were positive for Ki67 and 3 cases (3.0%) were negative for Ki67. In their study, 50 of 98 cases showed a low Ki67 proliferation rate (less than 10%) and 48 of 98 cases showed a high Ki67 proliferation rate (10% or more). They also concluded that high Ki67 expression was observed in the higher prognostic group, whereas low Ki67 or negative expression was found in the lower prognostic group ($p<0.001$).

Study done by Madani SH *et al*[71] stated that 24 (49%) were reported as 1+ with equal

frequency in both poorly and moderately differentiated tumors. In the current study, we did not get any cases with score 1+.Madani SH *et al*[71]also concluded that six cases (12.2%) as 2+, and two cases (4.1%) were 3+ and three cases (6.1%) were 4+ which when compared to the current study, we got only 9 cases(22.5%) belonging to score 3+ and 16 cases(40%) belonging to score 4+.

A study done by Rasheed IA *et al*[72] concluded that 60% Prostate Carcinoma casesshowed moderate positivity (++), while 16.7% showed intense positivity (+++) and 23.3% showed weak positivity (+) which when compared to the present study 15 cases(37.5%) showed >20%(5+) positivity, 16 cases(40%) showed positivity in 10-20%(4+) followed by 9 cases(22.5%) showed positivity in >5-10% (3+) tumor cells . But the ki67 scoring system which was followed by Rasheed IA *et al*[72] differs from the present study.

SUMMARY

- A hospital-based retroprospective cross-sectional study was conducted. The study included specimens of benign Hyperplasia and prostate carcinoma received for the first time in Department of Pathology from 1stAugust, 2016 to 31stJuly, 2021(Five year study).

- The diagnosis of all cases included in this study were based on routine microscopic examination on H&E stain.
- The IHC marker, Estrogen receptor β and ki67 were detected for all benign and malignant cases and these markers were compared between the two groups.
- A total of 80 cases were included in the study, out of which 40 cases were benign Hyperplasia and 40 cases were prostate carcinoma. Majority of the cases in the study were between age group of 60-95yrs. The youngest patient was 42yrs and the oldest was 94yrs.
- ER- β and Ki67 showed statistically significant difference between Benign and Malignant groups. There was increased ER-βimmunoexpression in BPH as compared to Adenocarcinoma of Prostate and increased ki67 immunoexpression in carcinoma prostate as compared to BPH.
- Ki67 showed sensitivity and specificity of 100% and 75% respectively in Benign hyperplasia and Malignant lesions of prostate.
- ER-β proportion score showed a sensitivity and specificity of 92.5% and 67.5% respectively, intensity score showed sensitivity of 97.5% and specificity of 57.5% and ER-β total score showed 95%(sensitivity) and 45%(specificity) in both the study groups.

CONCLUSION

- This study adds to our understanding of the efficacy of the Immunohistochemical markers that is ER-β and Ki67 in Benign and Malignant prostatic lesions. The results obtained were comparable within the

two study groups taken in this study for confirmatory diagnosis.

- In this study, there was reduced ER-β immunoexpression in prostatic Adenocarcinoma as compared to BPH and increased ki67 immunoexpression in carcinoma prostate as compared to BPH. Therefore, use of these markers will help in early diagnosis, which will prevent the untoward complications like metastasis in prostatic carcinomas .
- The sensitivity and specificity of Ki-67 was 100% and 75% respectively in both the study groups in the present study.
- Sensitivity and specificity of ER-β immunoexpression was 95% and 45% respectively in both the study groups.
- Based on these findings we conclude that ER-β and Ki-67 can be used as animmunohistochemical marker for differentiating Benign Hyperplasia of prostate from prostatic carcinoma.
- As the sample size in present study was less and there was a discordance of ER- β in prostatic carcinomascompared to other studies, further extensive study is needed to standardize the immunoexpression of this marker.

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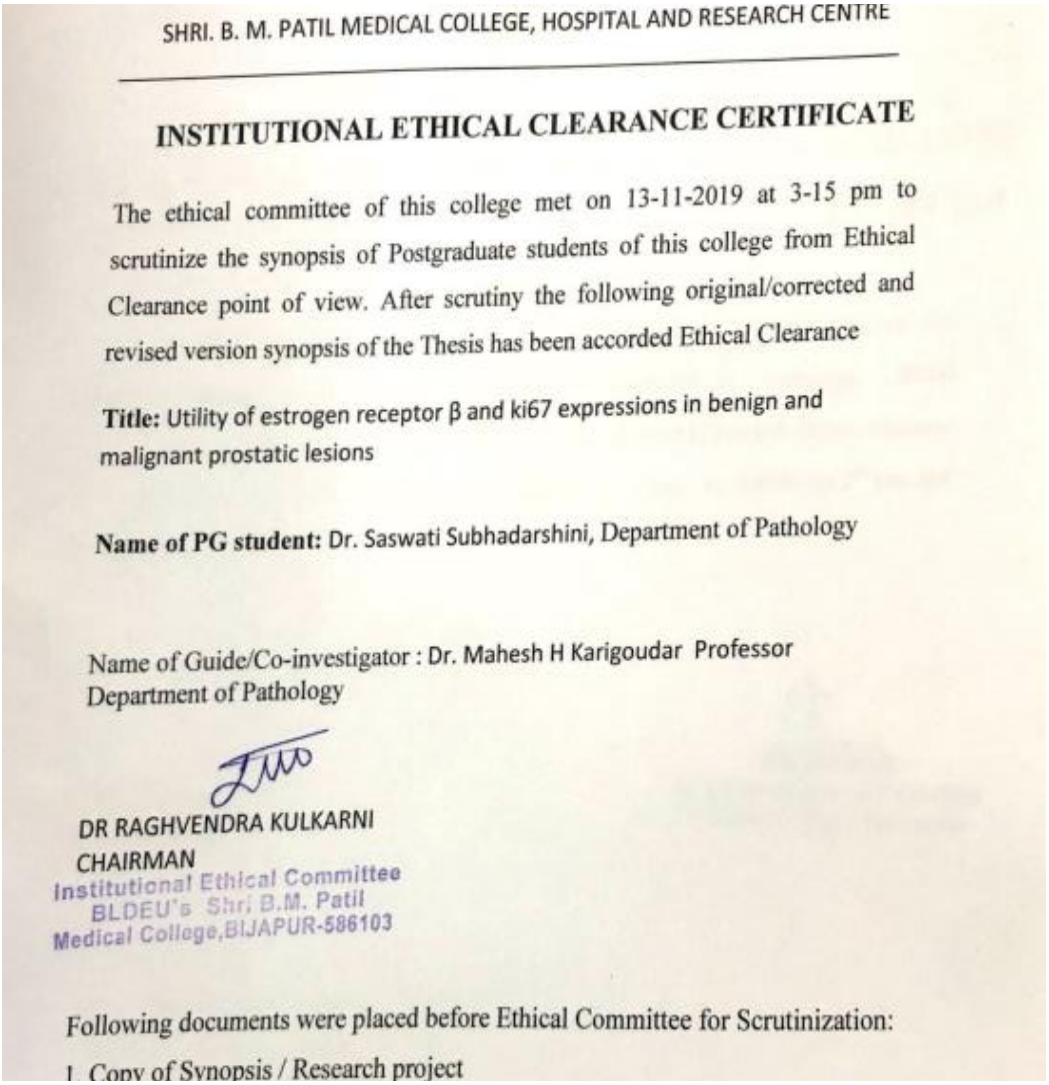
ANNEXURE-I



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

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical committee of this college met on 13-11-2019 at 3-15 pm to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Utility of estrogen receptor β and ki67 expressions in benign and malignant prostatic lesions

Name of PG student: Dr. Saswati Subhadarshini, Department of Pathology

Name of Guide/Co-investigator : Dr. Mahesh H Karigoudar Professor
Department of Pathology

DR RAGHVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project

Inward No : 23
Date : 17/6/2021

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE(DU)/REG/PG-Guide/2021-22/518 June 16, 2021

To,
The Professor and HOD
Department of Pathology,
BLDE (DU)'s Shri B. M. Patil Medical College,
Hospital and Research Centre,
Vijayapura

Sir,

Sub: Regarding change of PG Guide.
Ref: Your letter no. Path/2021/420 dated 1st June, 2021.

With reference to the subject and letter cited above, on approval of the Hon'ble Vice-Chancellor, the change of PG Guide is permitted in respect of PG Student of your department as per below:

Sl. No.	Name of the Student	Previous Guide	New Guide	Batch/ Year
1.	Dr. Sahitya H.	Dr. R. M. Potekar	Dr. S. U. Arakeri	2018 - 19
2.	Dr. Saswati Subhadarshini		Dr. Vijayalaxmi Patil	2019 - 20
3.	Dr. Sultana Shahnaz		Dr. S. B. Hipparagi	2020 - 21
4.	Dr. Anin Prakash		Dr. Savitri Nerune	2020 - 21

This is for your information and needful.

REGISTRAR
REGISTRAR
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Copy to:
- The Dean, Faculty of Medicine and Principal
- The Controller of Examinations
- The Concerned PG Teacher

Circulate to concerned PG guides & teachers.

Prof. & HOD
Dept. of Pathology
BLDE (Deemed to be University)
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VIJAYAPUR

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

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

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I,theundersigned…… S/OD/O W/O…….aged…….years, ordinarilyresident of ……..do hereby state/declare that Dr…….of Hospital has examined me thoroughlyon at (place) and it has been explained to me in my own language that I amsufferingfrom disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research titled under the guidance of Dr…….. requesting my participation in the study. Apart fromroutinetreatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

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

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

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

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

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

Signature of patient:

Signature of doctor: Witness:

1.

2.

Date:

Place:

ANNEXURE-III

PROFORMA

NAME : OP/IP No. :

AGE :

SEX :

RELIGION :

OCCUPATION :

RESIDENCE :

PresentingComplaints :

Pasthistory :

Personalhistory :

Familyhistory :

Treatmenthistory :

Per Rectum

Examination Finding :

Catheterisation : Yes/No

USG Prostatic Findings :

PSA Level :

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

Type of Specimen : Biopsy/ TURP/ Radical Prostatectomy

HPR Diagnosis :

Benign/ Malignant

If Malignant: Gleason Score:

Gleason Grade:

IHC Findings :

ER-Beta -- Intensity score :

-- Proportion score :

-- Total score :

Ki-67 -- Percentage :

--Score :

Key to Master Chart

1. Sl.No. SerialNumber
2. HPR no Histopathology Reporting number
3. Yrs Years
4. PSA Prostate Specific Antigen
5. ER-BETA Estrogen receptor Beta
6. TURP Transurethral Resection Of Prostate
7. ng/ml Nanogram/millilitre

MASTER CHART

MALIGNANT CASES

Sl.no	HPR no	Age(yrs)	Sex	PSA(ng/ml)	SPECIMEN	HPR DIAGNOSIS	GLEASON SCORE	GLEASON GRADE	ER-BETA	ER-BETA PROPORTION SCORE	ER-BETA INTENSITY SCORE	ER-BETA TOTAL SCORE	KI67 (%)	KI67 SCORE
1	3304/18	83	M	38	TURP	ADENOCARCINOMA OF PROSTATE	5+4	5	NEGATIVE	0	0	0	>10-20	4
2	5844/18	70	M	42	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	POSITIVE	1	1	2	>5-10	3
3	6095/18	85	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	5+5	5	NEGATIVE	0	0	0	>20	5
4	1205/19	60	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>5-10	3
5	1987/19	65	M	16.3	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	POSITIVE	1	1	2	>10-20	4
6	2149/19	78	M	34.9	TURP	ADENOCARCINOMA OF PROSTATE	4+4	4	NEGATIVE	0	0	0	>10-20	4
7	2351/19	60	M	14.5	TURP	ADENOCARCINOMA OF PROSTATE	3+5	4	POSITIVE	1	1	2	>5-10	3
8	4167/19	87	M	67.2	TURP	ADENOCARCINOMA OF PROSTATE	4+5	5	POSITIVE	1	1	2	>10-20	4
9	6140/19	62	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+4	4	NEGATIVE	0	0	0	>20	5
10	6647/19	76	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	POSITIVE	2	1	3	>20	5
11	7863/19	70	M	79.6	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>10-20	4
12	7864/19	65	M	89.2	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	POSITIVE	1	1	2	>20	5
13	7894/19	82	M	98.4	TURP	ADENOCARCINOMA OF PROSTATE	5+4	5	POSITIVE	3	1	4	>20	5
14	8066/19	68	M	69.6	TURP	ADENOCARCINOMA OF PROSTATE	5+4	5	POSITIVE	1	1	2	>20	5
15	1197/20	78	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>5-10	3
16	2080/20	69	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	5+4	5	NEGATIVE	0	0	0	>20	5
17	2127/20	80	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	5+4	5	NEGATIVE	0	0	0	>20	5
18	2184/20	75	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>20	5

19	2197/20	70	M	39.12	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>10-20	4
20	2283/20	65	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>5-10	3
21	2374/20	74	M	28	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	POSITIVE	2	3	5	>10-20	4
22	2416/20	94	M	7.2	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>5-10	3
23	2450/20	70	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>5-10	3
24	2560/20	68	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	5+3	4	NEGATIVE	0	0	0	>5-10	3
25	2580/20	65	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>20	5
26	2767/20	74	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>10-20	4
27	2912/20	70	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+5	5	NEGATIVE	0	0	0	>20	5
28	2922/20	75	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+5	4	POSITIVE	1	1	2	>10-20	4
29	3566/20	76	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>10-20	4
30	4219/20	70	M	38	TURP	ADENOCARCINOMA OF PROSTATE	3+5	4	POSITIVE	1	2	3	>20	5
31	4274/20	65	M	31.6	TURP	ADENOCARCINOMA OF PROSTATE	3+4	3	NEGATIVE	0	0	0	>10-20	4
32	4282/20	70	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+4	4	NEGATIVE	0	0	0	>10-20	4
33	4381/20	58	M	112	TURP	ADENOCARCINOMA OF PROSTATE	5+5	5	NEGATIVE	0	0	0	>20	5
34	863/21	61	M	100	TURP	ADENOCARCINOMA OF PROSTATE	4+5	5	NEGATIVE	0	0	0	>10-20	4
35	910/21	70	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>20	5
36	1081/21	75	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+4	4	NEGATIVE	0	0	0	>10-20	4
37	1284/21	83	M	147	TURP	ADENOCARCINOMA OF PROSTATE	3+4	2	NEGATIVE	0	0	0	>5-10	3
38	1575/21	65	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+3	3	NEGATIVE	0	0	0	>10-20	4
39	1991/21	81	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	3+5	4	NEGATIVE	0	0	0	>10-20	4
40	2018/21	72	M	Not done	TURP	ADENOCARCINOMA OF PROSTATE	4+5	5	POSITIVE	1	2	3	>20	5

BENIGN CASES

Sl.no	HPR no	Age	Sex	PSA(ng/ml)	SPECIMEN	HPR DIAGNOSIS	ER-BETA	ER-BETA PROPORTION SCORE	ER-BETA INTENSITY SCORE	ER-BETA TOTAL SCORE	KI67 (%)	KI67 SCORE
1	1626/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	1	1	2	<5	2
2	1658/20	80	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
3	1727/20	75	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	5	3	8	>5-10	3
4	1800/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
5	1840/20	62	M	7.9	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	1	1	2	>5-10	3
6	1916/20	59	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
7	1942/20	65	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
8	1955/20	65	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	3	1	4	<5	2
9	1975/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
10	1976/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
11	2012/20	55	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
12	2017/20	60	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
13	2018/20	56	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	3	3	6	<5	2
14	2019/20	52	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
15	2027/20	63	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	5	3	8	>5-10	3
16	2053/20	75	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2

17	2064/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	3	1	4	<5	2
18	2099/20	58	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
19	2217/20	42	M	35.9	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	>5-10	3
20	2224/20	46	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
21	3825/20	73	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
22	3827/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	>5-10	3
23	3830/20	55	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	>5-10	3
24	3844/20	60	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
25	3845/20	55	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
26	3903/20	78	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	>5-10	3
27	3925/20	65	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
28	3926/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
29	3953/20	45	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	1	1	2	<5	2
30	3967/20	65	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
31	3998/20	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
32	4031/20	46	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	>5-10	3
33	4070/20	60	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
34	4094/20	46	M	8.3	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	>5-10	3
35	1694/21	63	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
36	1742/21	70	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	1	3	<5	2
37	1819/21	66	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	NEGATIVE	0	0	0	<5	2
38	1901/21	60	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	3	2	5	>5-10	3
39	1902/21	72	M	Not done	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	2	2	4	<5	2
40	1948/21	84	M	2.47	TURP	BENIGN HYPERPLASIA OF PROSTATE	POSITIVE	1	1	2	<5	2