"EXPRESSION OF BETA ESTROGEN RECEPTOR IN BENIGN PROSTATIC HYPERPLASIA AND ADENOCARCINOMA OF PROSTATE"

BY

Dr.Nishit, *MBBS*

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH,

TAMAKA, KOLAR,

KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF

Dr. CSBR PRASAD, *MD*

PROFESSOR AND HEAD OF DEPARTMENT,

DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR
MAY 2017

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH,

TAMAKA, KOLAR, KARNATAKA.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation ENTITLED

"**Expression of beta estrogen receptor in benign prostatic hyperplasia and Adenocarcinoma of prostate"**

at R. L. Jalappa Hospital and research centre, kolar

is a bonafide and genuine research work carried out by me under the direct guidance of

Dr. CSBR PRASAD, *md*

Professor AND HEAD,

Department of PATHOLOGY,

Sri Devaraj Urs Medical College, Kolar

DATE: SIGNATURE OF THE CANDIDATE

PLACE: KOLAR **Dr. NISHIT**

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH,

TAMAKA, KOLAR, KARNATAKA.

CERTIFICATE BY THE GUIDE

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

"Expression of Beta estrogen receptor in benign prostatic hyperplasia and Adenocarcinoma of prostate"

AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

IS A BONAFIDE RESEARCH WORK DONE

BY

Dr. NISHIT

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

M.D IN PATHOLOGY

DATE:

PLACE: KOLAR

SIGNATURE OF THE GUIDE

Dr. CSBR PRASAD, ***MD***
PROFESSOR AND HEAD
DEPARTMENT OF PATHOLOGY

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH, TAMAKA, KOLAR, KARNATAKA.

ENDORSEMENT BY THE HOD,PRINCIPAL/HEAD OF THE INSTITUTION

THIS IS TO CERTIFY THAT THE DISSERTATION ENTITLED

"Expression of beta estrogen receptor in benign prostatic hyperplasia and Adenocarcinoma of prostate"

IS A BONAFIDE RESEARCH WORK DONE BY

Dr. NISHIT

UNDER THE GUIDANCE OF

DR. CSBR PRASAD, *MD*

PROFESSOR AND HEAD

DEPARTMENT OF PATHOLOGY

Dr. CSBR PRASAD

SEAL & SIGNATURE OF THE HOD
PRINCIPAL

DATE:
PLACE: KOLAR

Dr. HARENDRA KUMAR

SEAL & SIGNATURE OF THE

DATE:
PLACE: KOLAR

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH, TAMAKA, KOLAR, KARNATAKA.

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that Sri Devaraj Urs academy of higher education and research, Tamaka, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation, in print or electronic format, for academic / research purpose.

DATE: SIGNATURE OF THE CANDIDATE

PLACE: KOLAR **Dr. NISHIT**

SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA, KOLAR.

ETHICS COMMITTEE

CERTIFICATE

THIS IS TO CERTIFY THAT,

THE ETHICS COMMITTEE OF SRI DEVARAJ URS MEDICAL COLLEGE, TAMAKA,

KOLAR

HAS UNANIMOUSLY APPROVED

Dr. NISHIT

POST GRADUATE STUDENT

IN THE DEPARTMENT OF PATHOLOGY
OF
SRI DEVARAJ URS MEDICAL COLLEGE

TO TAKE UP THE DISSERTATION WORK ENTITLED

"EXPRESSION OF BETA ESTROGEN RECEPTOR IN BENIGN PROSTATIC

HYPERPLASIA AND ADENOCARCINOMA OF PROSTATE"

TO BE SUBMITTED TO

SRI DEVARAJ URS ACADEMY OFHIGHER EDUCATION AND RESEARCH,

TAMAKA, KOLAR

MEMBER SECRETARY **PRINCIPAL**

<u>*ACKNOWLEDGEMENT*</u>

It is most appropriate that I begin by expressing my undying gratitude to ***The ALMIGHTY LORD GANESHA, LORD SHIVA AND MY PARENTS*** *for Their blessings.*

I convey my deepest regards and earnest gratitude to my guide, Professor and H.O.D, Department of Pathology, ***Dr. CSBR PRASAD****, for his enriching words and action of support, profound wisdom, advice and constant encouragement in preparing this dissertation.*

My continued reverence and acknowledgement to my beloved teacher, ***Dr. SWAROOP RAJ BV,*** *Assistant Professor of Pathology, for his able guidance, constant support, expert advice and encouragement that helped me to complete my study. His sense of punctuality, research oriented approach, the painstaking effort to weed out errors and his affection during the entire course of study leaves me permanently indebted to him. I dedicate the good part of the work to him. I will forever be indepth to him and his family.*

I am thankful to my Principal and Professor, Department of Pathology, ***DR. M L HARENDRA KUMAR*** *for his unswerving support, words of encouragement and wisdom throughout the course of my s*tudy.

I would like to thank ***Dr. KALYANI R.*** *and* ***Dr. T.N. SURESH****, Professors of Pathology, whose constant enthusiasm, encouragement, thought provoking questions and slides, mind blowing quizzes and enriching words/actions of support and wisdom helped me complete my study. I am gratefully indebted for their support.*

I would like to extend my gratitude towards ***Dr. HEMALATHA A****, Associate Professor, Department of Pathology, for her vital help in the procedure of IHC and constant encouragement throughout this study.*

I express my deep sense of gratitude and humble thanks to ***Dr. SUBHASHISH DAS****, Professor, Department of Pathology and* ***Dr. MANJULA K****, Associate Professor, Department of Pathology, for their advice and encouragement throughout the study.*

*I thank **Dr. SUPREETHA M.S. and Dr. SHILPA M.D.**, Assistant Professors, Department of Pathology, for their constant guidance and encouragement in preparing this dissertation.*

*I wish to express my sense of gratitude to **Dr. VIDHYAVATHI K**, Associate Professor, Department of Pathology, for selecting this dissertation and having unyielding faith in it and for being a source of constant support and guidance throughout the course of this study. I wish to express my deepest sense of gratitude to my seniors **Dr. SHUBHAM AGARWAL** and **Dr. SHARITHA NAGANNA** for proof reading my dissertation and **OTHER SENIORS** for their kind help and support in preparing this dissertation.*

*I express my sincere thanks to my colleagues **Dr. ANKITA BAGHEL, Dr. SHUBHRA RAMACHANDANI** and **Dr. KARTHIK KASI REDDY** for their co-operation and wholehearted help in carrying out this study.*

*I thank my juniors **Dr. SHAH MANAN BHARATKUMAR** and **Dr. HAJRA KHURSHEED MEHDI** for their enthusiasm, persistently staying with me in the long hours at night and during holidays, unconditionally encouraging me and completing whatever task was assigned to them with precision and in a timely manner. Thanks a lot.*

*I thank my junior **Dr. CHENNA CHANDANA REDDY** for her support during the study.*

*I am infinitely obliged to my mom **Dr. NAMITA OJHA** and my dad **Dr. PURNENDU OJHA** who supported my decision to be a pathologist, helped me realize my dreams and also my brother **Dr. LAVNISH** for fulfilling all my duties as a son in my absence.*

*My special thanks to all my friends **Dr. ANUSHA RAJ, Dr. HUDA ALI SAYED** and **Dr. SHIVANI SINGH** for their constant encouragement and inspiration throughout my study.*

*I am thankful to **Dr. MAHESH VENKY** for his guidance in statistics.*

*Lastly, I am also thankful to **Mr. VEERENDRA KUMAR** and **Mr. NAZIMUDDIN** and all*

*the **Technical Staff** and **non-teaching staff** for their invaluable help without whom this study would not have been possible.*

Date: *Signature of the Candidate*

Place: Kolar ***Dr. Nishit***

LIST OF ABBREVIATIONS

AAH	**-**	**ATYPICAL ADENOMATOUS HYPERPLASIA**
AMACR	**-**	**ALPHA METHYL ACYL COA RACEMASE**
AR	**-**	**ANDROGEN RECEPTOR**
ASAP	**-**	**ATYPICAL SMALL ACINAR PROLIFERATION**
BPH	**-**	**BENIGN PROSTATIC HYPERPLASIA**
CK	**-**	**CYTOKERATIN**
DHT	**-**	**DIHYDROTESTOSTERONE**
DPX	**-**	**DISTYRENE PLASTICIZER XYLENE**
DRE	**-**	**DIGITAL RECTAL EXAMINATION**
ER	**-**	**ESTROGEN RECEPTOR**
ER α	**-**	**ESTROGEN RECEPTOR ALPHA**
ER β	**-**	**ESTROGEN RECEPTOR BETA**
HPIN	**-**	**HIGH GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA**
IHC	**-**	**IMMUNOHISTOCHEMISTRY**
NH	**-**	**NODULAR HYPERPLASIA**
PAP	**-**	**PROSTATIC ALKALINE PHOSPHATASE**
PCA	**-**	**PROSTATIC ADENOCARCINOMA**
PIN	**-**	**PROSTATIC INTRAEPITHELIAL NEOPLASIA**

PSA	**-**	**PROSTATE SPECIFIC ANTIGEN**
PSAP	**-**	**PROSTATE SPECIFIC ACID PHOSPHATASE**
PSMA	**-**	**PROSTATE SPECIFIC MEMBRANE ANTIGEN**
REDUCE	**-**	**REDUCTION BY DUTASTERIDE OF PROSTATE CANCER EVENTS**
TRUS	**-**	**TRANSRECTAL ULTRASOUND IMAGING**
TURP	**-**	**TRANSURETHRAL RESECTION OF PROSTATE**

ABSTRACT

TITLE OF THE STUDY: Expression of beta estrogen receptor in benign prostatic hyperplasia and Adenocarcinoma of prostate.

INTRODUCTION

In aging men, benign prostatic hyperplasia (BPH) and prostate adenocarcinoma (PCa) are the most common prostatic diseases. BPH commonly arises from the transitional zone where stromal and epithelial nodules are most likely to develop, whereas PCa arises in the peripheral zone of the prostate gland where mostly epithelial cells undergo malignant transformation.

These diseases are androgen-dependent and are treated by inhibiting androgens or their action. However recent studies have shown that prostate growth is also influenced by estrogen.

Estrogen induction of cell proliferation which plays a crucial role in hormone dependent tumors like breast and uterus is now also thought to play a significant role in normal and abnormal growth of the prostate gland.

The detection of two types of estrogen receptor α and β has brought new insight into the mechanism underlying estrogen signaling. Estrogen beta is found to be expressed in epithelium of the normal prostate gland. The role of estrogen receptor Beta (ER β) in the pathogenesis or prognosis of PCa is unclear, it seems to have a role in the control of proliferation and the prevention of hyperplasia.

The expression of Estrogen Receptor beta in BPH and PCa may provide an insight into the carcinogenesis of PCa. Though antiestrogen was earlier used in therapy of prostatic lesions, it was discontinued due to its side effects. The detection of a new ER beta and its expression may also help in providing newer therapeutic modalities for BPH and androgen resistant PCa with recurrence. Only few studies determining expression of ER beta has been done in prostate. Hence the study is initiated to determine the expression of ER beta in BPH and PCa.

OBJECTIVES OF THE STUDY:

1) To determine the expression of estrogen receptor beta in BPH and adenocarcinoma of prostate

2) To correlate the expression of estrogen receptor beta of BPH with different grades of prostatic adenocarcinoma.

MATERIALS AND METHODS:

TURP specimens of BPH and PCa received in the Dept. of Pathology R.L.Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka and Kolar from November 2014 to October 2016 was included in the study.

Total of 58 cases were collected .BPH -29 cases and Adenocarcinoma -29 cases.

All TURP specimens confirmed to be BPH and PCa by histopathological examination were included in the study. Data regarding the clinical detail and serum PSA levels were collected. H and E stained slides were reviewed for the grading and scoring of Gleason's score. The tumors were categorized as "low grade" if Gleason score was equal to or less than 4 and "high grade" if Gleason score was equal to or more than 8. Gleason score of 5, 6 and 7 will be considered as intermediate grade. Immunohistochemistry (IHC) staining for ER beta was performed on all cases of BPH and PCa using appropriate positive and negative controls

RESULTS: In BPH cases 93.1% of them were ER beta positive and 6.9% were ER beta negative. However in adenocarcinoma group only 3.4% were ER beta positive and 96.6% were ER beta negative. This observation was statistically significant.

CONCLUSION: Elderly age group is most commonly affected with BPH and Adenocarcinoma of prostate. Based on the results of the present study, ER beta seemed to play a definitive role in the carcinogenesis. Estrogen beta is hypothesized to be involved in the carcinogenesis neoplastic progression of adenocarcinoma of prostate and underlying pathogenesis of BPH.

KEY WORDS: ER Beta, IHC, BPH, PCa

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES (PHOTOGRAPHS)

LIST OF FIGURES (CHARTS)

INTRODUCTION

INTRODUCTION

The main pathologic processes that affect the prostatic gland are inflammation, nodular enlargement and tumors.[1] In aging men, benign prostatic hyperplasia (BPH) and prostate adenocarcinoma (PCa) are the most common prostatic diseases. BPH is more common that prostatic carcinoma. Prostatic adenocarcinoma is now the sixth most common cancer in the world and third in men in terms of incidence. The incidence varies based on geographic location, ethnic background and age. The incidence increases dramatically with advancing age.

The diagnosis of BPH and Prostatic adenocarcinoma is readily done on morphological grounds by use of various histological parameters, including architecture, nuclear features and the presence or absence of basal cell layer. However, in morphologically equivocal cases the use of immunohistochemistry helps to clinch the diagnosis.[2]

BPH commonly arises from the transitional zone where stromal and epithelial nodules are most likely to develop. Prostatic adenocarcinoma arises in the peripheral zone of the prostate gland where mostly epithelial cells undergo malignant transformation.

These diseases are androgen-dependent and are treated by inhibiting androgens or their action. However recent studies have shown that prostate growth is also influenced by estrogen.[3]

Estrogen induction of cell proliferation which plays a crucial role in hormone dependent tumors like breast and uterus is now thought to play a significant role in normal and abnormal growth of the prostate gland.[4]

The detection of two types of estrogen receptor α and β has brought new insight into the mechanism underlying estrogen signaling. Estrogen receptor beta (ER beta) is found to be expressed in epithelium of the normal prostate gland.[3]

The role of estrogen receptor beta in the pathogenesis or prognosis of PCa is unclear. It seems to have a role in the control of proliferation and prevention of hyperplasia.[5]

The expression of ER beta in BPH and PCa may provide an insight into the carcinogenesis of PCa. Though antiestrogen was earlier used in therapy of prostatic lesions, it was discontinued due to its side effects.

The detection of ER beta and its expression may also help in providing newer therapeutic modalities for BPH and androgen resistant PCa with recurrence. Extensive review of literature has shown limited Indian published literature so far. Only few studies determining expression of ER beta has been done in prostate. Hence the study is initiated to determine the expression of ER beta in BPH and PCa.

OBJECTIVES

OBJECTIVES

1) To determine the expression of estrogen receptor beta in BPH and adenocarcinoma of prostate.

2) To correlate the expression of estrogen receptor beta of BPH with different grades of prostatic adenocarcinoma.

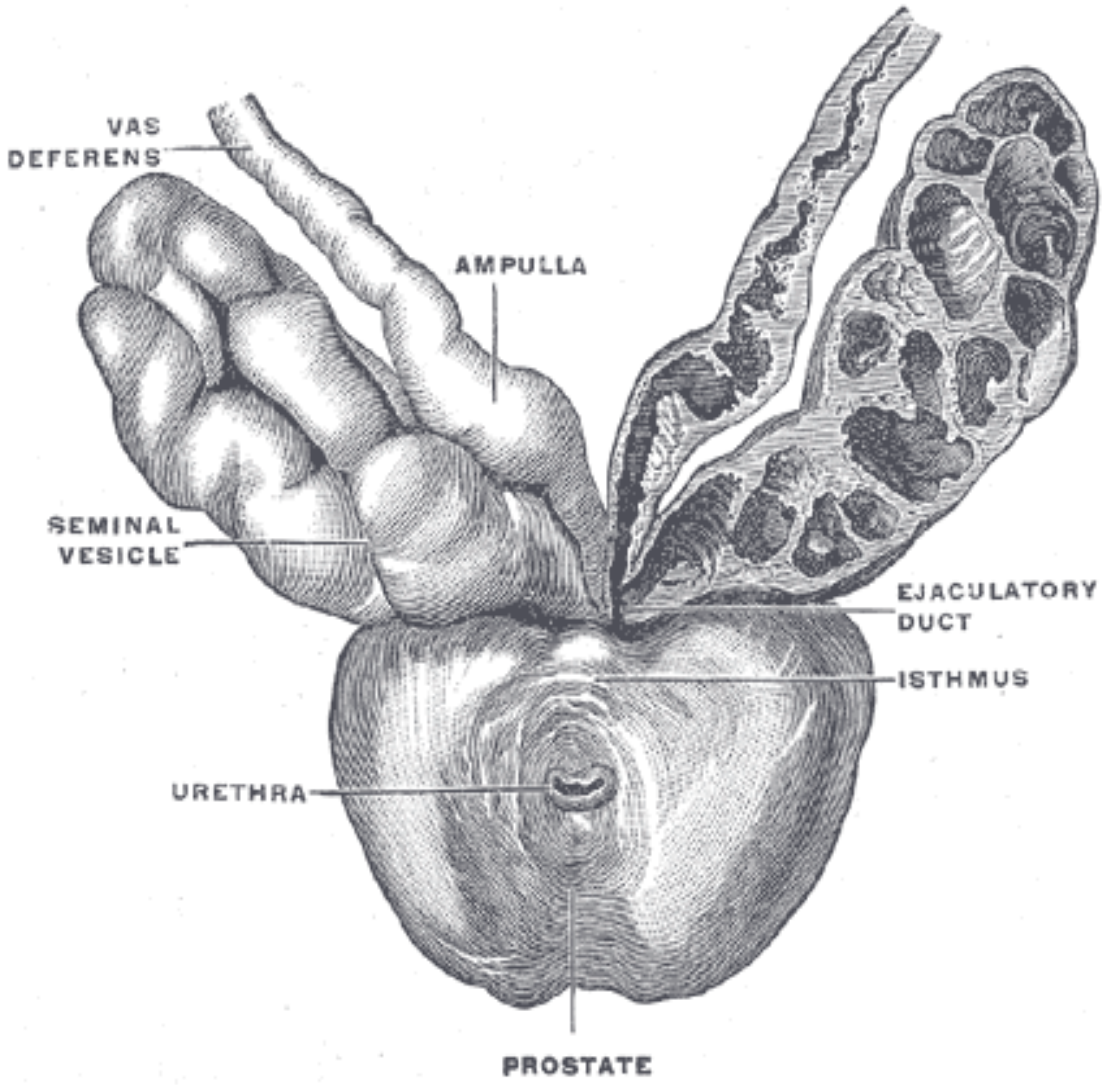


Figure: 1: Anatomy of prostate

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY

Prostate is a pear shaped glandular organ with the base located closer to the bladder neck and the apex towards the penile urethra. It weighs up to 20g in a normal adult male. Traditionally, it has been divided into anterior, middle and lateral lobes by drawing lines from the centrally located urethra. Prostatic urethra is the portion of the urethra that traverses through the prostate and exits the prostate at its apex, where it is continuous with membranous urethra. Another division that correlates better with physiological and pathological features of the organ is based on McNeal's model. Grossly, McNeal's model is often simplified such that the central inner periurethral aspect of the prostate is termed the ―transition zone, and the outer peripheral aspect is referred to as the ―peripheral zone and includes the ―central zone, which is located toward the base of the prostate.[6] (Figure.2)

Central zone – Urethra and ejaculatory ducts course through this zone

Transition zone is the most common site of Benign Prostatic Hyperplasia

Peripheral zone is the common site where most carcinoma arises.[7]

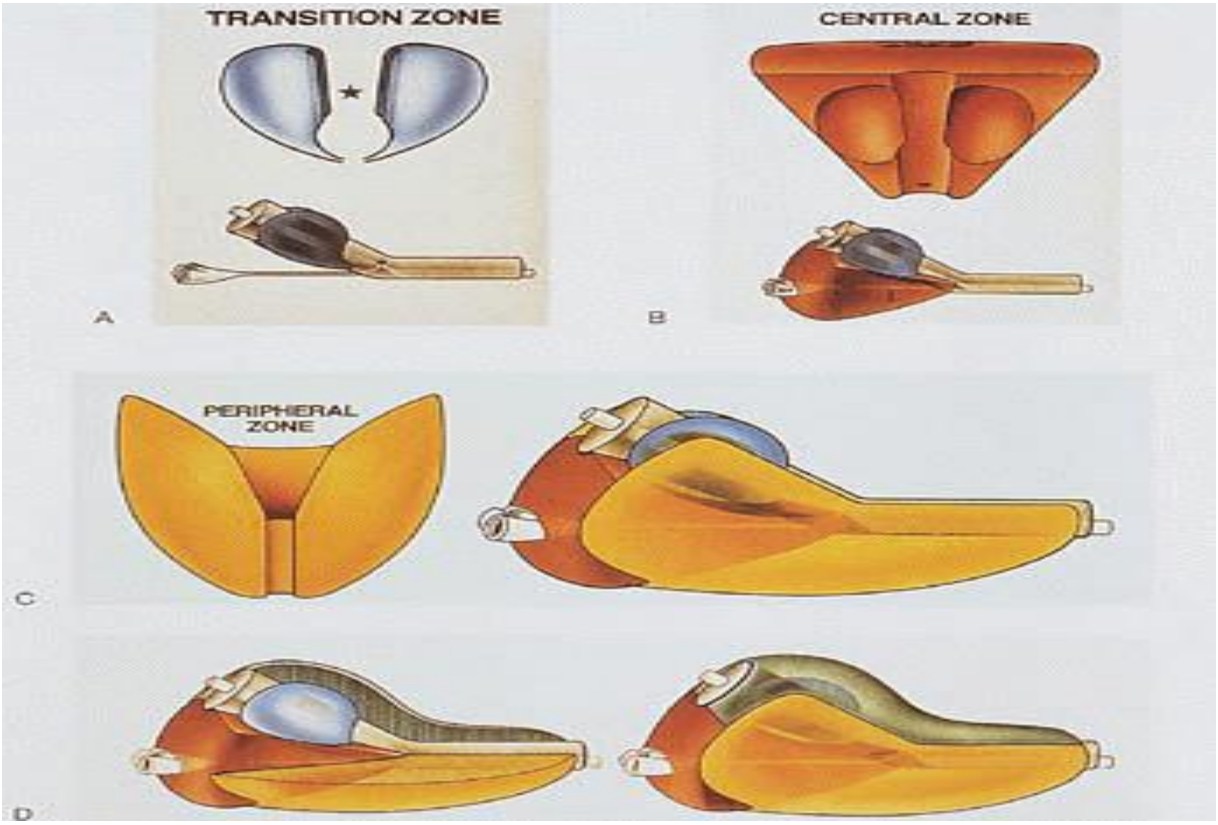


Figure-2: McNeal's model of zonal anatomy of the prostate

The prostate gland is a functional conduit that allows urine to pass from the urinary bladder to the urethra and adds nutritional secretions to the sperm to form semen during the process of ejaculation. The prostate gland secretions contains various hydrolytic enzymes (like PSA, prostate-specific acid phosphatase [PSAP]) functioning to increase sperm motility.

Age-Related Changes

After puberty there is significant growth in prostate. It achieves an average weight of 20 ±6 g at age 25 years to 30 years. Over the next 2 decades prostatic growth is minimal and around the fifth decade of age, the prostate undergoes progressive atrophy.

HISTOLOGY

Microscopically, the prostate is composed of glandular epithelium and fibromuscular stroma. In the central zone, the glandular epithelial cells are appear dense with an epithelial stromal ratio of 2:1 compared to 1:1 at the transitional and peripheral zone. The duct and glandular system is arranged in a very complex architectural pattern. These glands are lined by four distinct epithelial populations of cells:

a) The luminal secretory cells stain positively with PSA and prostatic acid phosphatase. These cells are columnar with pale to clear cytoplasm. Strong α-methyl acyl CoA racemase (AMACR) positivity indicates malignancy.

b) Basal cells which are cigar shaped and resemble those of fibroblasts are peripherally located in the gland between the secretory cells and basement membrane and are oriented parallel to the basement membrane. Presence of basal cells confirms the diagnosis of benign condition and absence of basal cell marker in the glands is one of the hallmark feature of adenocarcinoma. Basal cells are thought to represent the stem cell component within the prostate.[8,9] These basal cells are flat that stain positively for high molecular weight cytokeratin,CK5/6,and p63.These basal cells do not show myoepithelial feature like those of breast.

c) Neuroendocrine cells are irregularly distributed throughout the ducts and acini and stain positively with neuron-specific enolase (NSE), chromogranin and synaptophysin is observed. They regulate adjacent cells by paracrine action.

d) Urothelial cells: Presence of these cells in the peripheral prostatic ducts and acini is referred to as urothelial metaplasia. These are composed of spindle shaped epithelial cells with occasional nuclear grooves, oriented parallel to the basement membrane.[6]

BENIGN PROSTATIC HYPERPLASIA

INCIDENCE AND EPIDEMIOLOGY

Three factors such as geography, race and age appear to be related to the incidence of clinical prostatic hyperplasia. Clinically hyperplasia is classified into lateral, middle, posterior enlargement. In recent years, the influence of family history on the risk of development of this disorder has been recognized.[10, 11]

AGE

The peak age of patients with clinical prostatism is seen in the seventh decade. It shows progressive increase in the frequency with age. Most reports indicate 75% of men aged 80 years or older have prostatic hyperplasia while Berry et al studied concluded than younger men are also affected.[12]

FAMILIAL BENIGN PROSTATIC HYPERPLASIA

Sanda et al suggested that a gene with Mendelian-dominant transmission is responsible for familial benign prostatic hyperplasia and there age of presentation is earlier than those of sporadic cases.[13]

PATHOGENESIS

Although the pathogenesis is not yet completely understood, it has been established Benign Prostatic Hyperplasia occurs in people with intact testis and it is an androgen dependent disorder. The factors could be inflammation induced release of platelet derived factor and human papilloma virus. The role of chronic inflammation is emerging as an important factor

in Benign Prostatic Hyperplasia development and progression.[14] Benign prostatic hyperplasia is frequently associated with inflammatory infiltrates mainly composed of T and B lymphoid cells and macrophages. The Medical Therapy of Prostate Symptoms (MTOPS) trial showed that about 40% of baseline biopsy specimens had chronic inflammatory infiltrates in particular, in men with higher PSA values and larger prostate volumes.[15] Furthermore, patients with inflammation were at a greater risk of benign prostatic hyperplasia progression and acute urinary retention when compared with patients without inflammatory infiltrates at baseline. The REDUCE (Reduction by Dutasteride of Prostatic Carcinoma Events) trial confirmed this hypothesis.[16] In prevent of benign prostatic hyperplasia and adenocarcinoma of prostate and inflammation is a possible target anti-inflammatory agents have been tested for management of both these conditions.

Earlier studies outlined, prostatic enlargement had been variously interpreted to reflect a neoplastic process, compensatory hypertrophy, a response to inflammation or arteriosclerosis.[19]

Hyperplasia of the periurethral glands is also to be considered the primary underlying cause. Pure stromal hyperplasia with nodule production was first reported by Resichauer in the year 1925. Deming and associates, Newmann, and Moore confirmed this particular observation. Investigators regarded the glandular component of prostatic nodules as an event secondary to a stromal stimulus to epithelial proliferation within adjacent ducts, which then will infiltrate the hyperplastic nidus. Evidence of prostatic stromal–epithelial interaction (first suggested by Deming and Newmann in 1939) is supported by the in vitro experiments of Cunha and his associates, who demonstrated an inducing effect of urogenital mesenchyme on epithelial gland formation. The role of testosterone in the hormonal regulation of prostatic growth is

very well documented. However, the role of testosterone in the pathogenesis of prostatic hyperplasia is less clearly understood. Castration before puberty seems to prevent it. In addition, patients with inherited deficiency of 5α-reductase do not develop benign prostatic hyperplasia. The efficacy of castration in treating established prostatic hyperplasia has produced inconclusive results.[19] Noteworthy is the patient reported by Marinello et al—A 69-year-old phenotypical male was reported with an XX karyotype, and essentially no circulating testosterone, who nonetheless had severe prostatic hyperplasia.[20] Exogenous testosterone has no observable effect on the histology of hyperplastic prostatic nodules and in areas of the prostate evidencing senile atrophy. Advancing age is associated with a reduction of circulating testosterone in both normal controls and men with prostatic hyperplasia. However, no reduction in serum DHT (Dihydrotestosterone) level is noted.

Serum levels of estrogen increases with increasing age in men is said to be elevated with advancing age in men. Exogenous estrogens have been reported to have a role in squamous metaplasia of the urethra and distal prostatic ducts but not associated with any significant histologic change in nodules of prostatic hyperplasia. The role of estrogens in the production of prostatic hyperplasia is not clearly understood. These observations suggest that testosterone and its more active metabolite, DHT, is responsible for the origin of benign prostatic hyperplasia.[19]

CLINICAL FEATURES

The clinical manifestations of benign prostatic hyperplasia reflect a range of severity and duration of urinary bladder outlet obstruction. Most patients are usually asymptomatic or having mild symptoms. In symptomatic patients, the initial symptom is reflected in diminished urinary stream. Progressive obstruction leads to hypertrophy and decrease in muscular strength of the urinary bladder wall. If this progresses untreated, it will lead to the formation of bilateral hydronephrosis and renal failure, a complication rarely observed currently except in developing countries. The enlarging gland leads to increase in the Serum PSA. The PSA elevation resulting from inflammation is transient, temporary and amenable to appropriate antibiotic therapy.[21]

PATHOLOGIC FEATURES:

The nodular expansion of the transition zone is clearly evident in fully mounted prostatectomy specimens. Medially, the nodules expand, distort and compress the urethral lumen adjacent to it. Laterally and posteriorly, the nodules expansion eventually compress the non-hyperplastic prostate tissue into an attenuated rim of tissue beneath the prostatic capsule. The nodules may expand distally toward the apex again leading to compression of the peripheral zone prostate glands in this region. In rare instances, benign prostatic hyperplasia may be identified in the peripheral zone. Grossly, benign prostatic hyperplasia appears as well-defined clustered nodules typically with variable solid and cystic composition. Focal hemorrhage, calcification and macrocytic change may also be present.

HISTOPATHOLOGY

Microscopically, benign prostatic hyperplasia is the result of proliferation of epithelial cells in acini, smooth muscle cells, and fibroblasts of variable proportions. On this basis, Franks has described five types of nodules 1) stromal (fibrous)2) fibromuscular; 3)muscular; 4) fibroadenomatous; and the most common type, 5) fibromyoadenomatous.

The epithelium lining the ducts and acini within the fibromyoadenomatous nodules generally comprises tall columnar cells over underlying basal cell layer. Characteristic of the epithelium and stroma of the nodules is the absence of any cytologic atypia. This epithelium lines the acini of varying sizes, some of microcystic proportions. Intraglandular papillary hyperplasia is characteristic feature. The epithelium of the fibroadenomatous nodules comprises of low cuboidal cells with frequent foci of urothelial or squamous cell metaplasia of the intranodular ducts. The ultrastructural features of the acinar epithelium of hyperplastic nodules and normal prostate are not significantly different.

The stroma of each type of nodule differs in composition, as suggested in their various descriptive names. Common to all types of nodules is the absence of elastic tissue in the stroma. Franks describes wide stromal septa separating lobules of large hyperplastic nodules in contrast to the more common interacinar stromal septa within the lobules. Some nodules are composed exclusively of stromal elements.[19]

ADENOCARCINOMA

An invasive malignant epithelial tumor consisting mainly of luminal cells.

EPIDEMIOLOGY

PCa is now the sixth most common cancer in the world (in terms of number of new cases), and third in in men. The estimated number of cases was 513,000 in the year 2000. This represents 9.7% of cancers in men.[22]

WHO HISTOLOGICAL CLASSIFICATION OF TUMORS OF THE PROSTATE:[22]

Epithelial tumors

Glandular neoplasms

Adenocarcinoma (acinar)

Atrophic

Pseudohyperplastic

Foamy

Colloid

Signet ring

Oncocytic

Lymphoepithelioma-like

Carcinoma with spindle cell differentiation

(carcinosarcoma, sarcomatoid carcinoma)

Prostatic intraepithelial neoplasia (PIN)

Prostatic intraepithelial neoplasia, grade III (PIN III)

Ductal adenocarcinoma

Cribriform

Papillary

Solid

Urothelial tumors

Urothelial carcinoma

Squamous tumors

Adenosquamous carcinoma

Squamous cell carcinoma

Basal cell tumors

Basal cell adenoma

Basal cell carcinoma

Neuroendocrine tumors

Endocrine differentiation within adenocarcinoma

Carcinoid tumor

Small cell carcinoma

Paraganglioma

Neuroblastoma

Prostatic stromal tumors

Stromal tumor of uncertain malignant potential

Stromal sarcoma

Mesenchymal tumors

Leiomyosarcoma

Rhabdomyosarcoma

Chondrosarcoma

Angiosarcoma

Malignant fibrous histiocytoma

Malignant peripheral nerve sheath tumor

Haemangioma

Chondroma

Leiomyoma

Granular cell tumor

Haemangiopericytoma

Solitary fibrous tumor

Hematolymphoid tumors

Lymphoma

Leukaemia

Miscellaneous tumors

Cystadenoma

Nephroblastoma (Wilms tumor)

Rhabdoid tumor

Germ cell tumors

Yolk sac tumor

Seminoma

Embryonal carcinoma & teratoma

Choriocarcinoma

Clear cell adenocarcinoma

Melanoma

Metastatic tumors

AGE DISTRIBUTION

The risk of PCa rises with age. Worldwide, about three-quarters of all cases occur in men aged 65 or more

ETIOLOGY

The marked differences in risk by ethnicity suggest that genetic factors are responsible for pathogenesis. The change in rates with time and on migration, implies that differences in environment or lifestyle are also very important. There is a strong positive association with intake of various animal products, especially red meat and fats.[23]

There is an about 5-11 fold increased risk among men with two or more affected first-degree relatives. It is clear that male sex hormones play an important role in the development and growth of prostate cancers. Testosterone diffuses into the gland, where it is converted by the enzyme steroid 5-alpha reductase type II (SRD5A2) to the more metabolically active form of testosterone, dihydrotestosterone (DHT). DHT and testosterone bind to the androgen receptors, and the receptor/ligand complex translocate to the nucleus for DNA binding and transactivation of genes which have androgen-responsive elements, including those controlling cell division. Role of polymorphisms of SRD5A2 and AR genes in regulating this process and inter-ethnic variations in such polymorphisms might explain the higher risk of Prostatic adenocarcinoma in men of African descent. Studies suggest that men with a lower number of ARCAG repeat lengths are at higher risk of Prostatic adenocarcinoma.

GENETIC ASSOCIATION

Genetic changes show differences between the sporadic and hereditary type of PCas, but there is significant overlap and differences within cases of both types such that the two cannot be distinguished just on the basis of genetic analysis alone. In inherited type of prostate cancer, chromosomal gains at 7q, 8q and 19q are common and chromosomal losses involving 5q, 7q, 8p, 10q and 16q are the most common. Petrovics et al (2005) had described frequent overexpression (~70%) of the ETS related gene (ERG), a proto-oncogene in an analysis of 110 types of prostate cancers. This discovery was followed by the identification of recurrent gene fusions of the 5' untranslated region of TMPRSS2 to ERG or ETV1 in 23 of 29 prostate cancers. TMPRSS2-ERG fusions are predominant while other studies have described rare TMPRSS2-ETV4 fusions. In numerous recent studies, 50–70% frequency of this fusion has been confirmed. The presence of this fusion could be correlated with histological features of PCa, and to date is one of the most common genetic rearrangements present in human cancer. In multifocal disease, the fusion status and type of fusion can vary between various tumors. Recently, the TMPRSS2-ERG fusion associated with duplication of the fusion and interstitial deletion of sequences 5' to ERG has been identified a subset of prostate cancers associated with poor prognosis.[24, 25]

ADENOCARCINOMA OF PERIPHERAL DUCTS AND ACINI

Majority of prostatic adenocarcinoma as arise in the peripheral zone, whether posteriorly, laterally, or anteriorly, with sparing of the periurethral region except for the late stages of the disease. However, a small percentage of tumors do actually arise in the prostatic transition zone. Grossly, the tumor can be identified as a gray or yellowish, poorly delineated and firm area.

Microscopically, Prostatic adenocarcinoma exhibit a wide spectrum of appearances, ranging from anaplastic tumors to highly differentiated neoplasms that are distinguished from the non-neoplastic gland with great difficulty. Four major cytoarchitectural patterns are; medium-sized glands, small glands, diffuse individual cell infiltration, and cribriform. Carcinomas composed of medium-sized glands are detected on low-power examination by virtue of the closely spaced arrangement of those glands, irregular outline, smooth inner surface, and intervening scanty stroma. Tumors made up of small glands appear as expansive nodules on low power, the individual glands having a regular round configuration small size. Both of these architectural patterns (but particularly the latter) are associated with cytologic abnormalities in the form of nuclear enlargement, irregularity of contour, hyperchromasia, and most important prominent nucleoli (macronucleoli, defined as measuring >1 μ in diameter). These nucleoli often tend to be marginated and are often multiple. Mitoses are also of significance, but they are rarely found in well- differentiated tumors composed of either medium-sized or small glands. The pattern of diffuse cell infiltration is similar to that of invasive lobular carcinoma of the breast, whereas the cribriform pattern represents intraductal carcinoma, as evidenced by the presence of the epithelial basal layer. The gland-forming types of PCa are usually lined by a single cell layer but occasionally they exhibit a stratified epithelium that may mimic prostatic intraepithelial neoplasia (PIN).

Another pattern of growth that has recently been described is that referred to as glomeruloid. It is characterized by the presence of intraluminal ball-like clusters of tumor cells and is regarded by many as a pathognomonic sign of malignancy. [22, 27]

Squamous metaplasia may also be associated with Adenocarcinoma of prostate (especially of the high-grade type). It is often seen with hormonal or radiation therapy, and is associated with a poor prognosis. The presence of prostatic glands within perineural spaces is common in these tumors. This finding is a strong indicator suggestive of malignancy but is not pathognomonic.

The stroma surrounding the neoplastic glands show a combination of hypercellularity and deposition of a basophilic ground substance (mucinous fibroplasia or collagenous micronodules). Both intraluminal and stromal calcification is seen in association with adenocarcinoma of prostate but the incidence of the latter is much lower than in benign prostates. Protein crystalloid structures are morphologically and immunocytochemically similar to Bence Jones crystals are seen in the glandular lumina and are particularly very common in tumors composed of medium-sized glands.

Their presence usually an indicator of malignancy. In benign cases, it is a significant factor for the subsequent development of cancer. Electron probe x-ray microanalytic studies shows that they are predominantly composed of inorganic sulfur. Exceptionally, these crystalloids are also found in other metastatic foci. The intraluminal secretion of malignant glands often has a bluish hue (wispy blue mucin), an indicator of a mucinous composition.[28]

THE VARIATIONS OF PCa INCLUDE:

a) Foamy gland carcinoma. The cytoplasm of these carcinoma cells have fine granular appearance, but on occasionally it is clear or foamy ("xanthomatous") because of the large accumulation of lipids. Grossly, it appears bright yellow and soft in consistency. The majority tumor cells are cuboidal to columnar, and the nuclei are small and hyperchromatic. The nucleoli are not conspicuous. Foamy gland carcinomas is often aggressive, even in the presence of deceptively bland microscopic features.[29]

b) Prostatic adenocarcinoma with atrophic features. It is composed of tumor cells with an attenuated cytoplasm such that the nuclei occupy almost the entire cell height. They have an infiltrative pattern of growth, nuclear enlargement, macronucleoli, and sometimes the presence of adjacent carcinoma of the ordinary type.[28]

c) Pseudo hyperplastic prostatic adenocarcinoma: It resembles hyperplastic glands at the architectural level, including papillary infoldings, branching and corpora amylase. On low power, the tumor has a microcystic appearance. There is nuclear enlargement, macronucleoli, mitoses, intraluminal crystalloids and sometimes the presence of adjacent PIN.[30]

d) Colloid & signet ring variant: Some of these tumors have a signet-ring-cell appearance, yet the vacuoles do not contain any intracytoplasmic mucin. These vacuolated cells may present as singly invasive cells, in single glands and in sheets of cells. It has lakes of mucin lined by tall columnar epithelium with goblet cells showing varying degrees of nuclear atypia. These tumors have been negative stained for PSA and PAP.

e) Mucinous Prostatic adenocarcinoma behave very aggressively. Although the tumors are not as hormonally responsive as their nonmucinous counterpart tumors, some respond to androgen withdrawal. Mucinous PCa have a tendency to develop bone metastases and increased Serum PSA levels with advanced disease.[31]

f) Oncocytic variant: Tumor cells have round to ovoid hyperchromatic nuclei, and stain strongly positive for PSA. Numerous mitochondria are present on ultrastructural examination. A high Gleason grade, elevated serum PSA and metastasis of similar morphology have been reported.[22]

g) Lymphoepithelioma-like variant: This undifferentiated carcinoma is characterized by presence of asyncytial pattern of malignant cells associated with a heavy lymphocytic infiltrate.

h) Sarcomatoid carcinoma of the prostate: It is a rare neoplasm composed of both malignant epithelial and malignant spindle-cell and mesenchymal elements. The gross appearance of these tumour often resembles sarcomas. Microscopically, sarcomatoid carcinoma is composed of a glandular component having variable Gleason score. The sarcomatoid component consists of a nonspecific malignant spindle-cell proliferation. Amongst the various mesenchymal elements are osteosarcomas, chondrosarcoma, rhabdomyosarcoma, leiomyosarcoma, liposarcoma, angiosarcoma or multiple types of heterologous differentiation are specific. Serum PSA levels are within normal limits in most cases. Nodal and distant organs metastases at diagnosis are common. There is less than a 40% five-year survival period .[22]

Tumor multicentricity: Multiple tumor foci have been demonstrated in 75–85% of radical prostatectomy specimens studied by step-section or whole-mount techniques.

‘MINIMAL ADENOCARCINOMA’ AND ATYPICAL SMALL ACINAR PROLIFERATION (ASAP)

These are a foci of small atypical glands that are suspicious but not diagnostic of carcinoma. Grignon has proposed minimal criteria for the diagnosis of malignancy. For the cases in which the recommended threshold is not reached, terms such as atypical gland suspicious of malignancy and ASAP have been proposed. It is used for certain prostatic biopsies (about 4–6%) that cannot be confidently placed into a benign or malignant category, either with plain morphology or after immunostaining with 34ßE12 keratin and/or racemase. A patient with such a diagnosis usually warrants a second biopsy.[32, 33]

CARCINOMA OF LARGE (‘PRIMARY’) DUCTS

The other major, but numerically less significant types of adenocarcinoma of prostate originates from the large primary ducts that are normally found in a periurethral location. Cystoscopy examination often shows a polypoidal villous or an infiltrative urethral component. Microscopically, the following types have been recognized;

1) Large (prostatic) duct adenocarcinoma: These tumor are characterized by malignant changes in large dilated ducts, with a cribriform and papillary architecture lined by columnar pseudostratified malignant epithelium, occasionally with a clear cell (mesonephroid) type. Sometimes these tumor are accompanied by pagetoid spread in the prostatic urethra. Positivity for PSA and PAP is the definitive rule. These tumors tend to have a more advanced stage at presentation and a higher short-term survival rate than peripheral duct–acinar carcinomas. It is distinguished from HPIN, by the presence of cystically dilated glands of varying sizes, a greater predominance of flat architecture, a lesser frequency of

macronucleoli, absence of basal cells on high molecular weight keratin immunostain and higher Ki-67 index.

Endometrial-type (endometrioid) adenocarcinoma regarded as a variant of large duct PCa Microscopically, glands and papillae are seen, lined by tall, pseudostratified columnar epithelium.

2) Primary urothelial (transitional cell) carcinoma of the prostate: The existence of these tumor type are because the outer portion of the prostatic (periurethral) ducts emptying into the urethra is lined by urothelium. This variant comprises less than 2% of the total tumours. The microscopic appearance of this tumor is identical to that of the homonymous bladder tumor.

3) Mixed adenocarcinoma – urothelial (transitional cell) carcinoma, exhibiting features of both types 1 and 2.[34]

GLEASON'S GRADING

The most commonly used pathologic grading system for PCa was first described in 1966 by Donald F. Gleason, a pathologist. Gleason's system is based entirely on the architectural pattern of the tumor, without taking cytologic features into account. Additionally, it takes into account two most common architectural patterns. The original five Gleason pattern as follows: (Figure-2)[35]

Pattern 1. A well-circumscribed, tight cluster of uniform, separate, medium-sized glands with round or oval shape.

Pattern 2. The tumor acini are present in a circumscribed nodule, with less uniformity of size and shape, and more loosely packed than observed in pattern 1. Additionally, the peripheral leading edge of the tumor focus is more irregular, and may suggest minimal stromal invasion.

Pattern 3. Tumor acini are generally smaller, with variation of shape and size. Importantly, the tumor acini are discrete and separate, and they infiltrate the stroma accompanying benign prostatic acini. A cribriform pattern may be present, and show a smooth, round configuration.

Pattern 4. There is fusion of tumor acini, which are poorly defined glands with equally poorly delineated gland lumina. The ―hypernephroid form of PCa, originally described by the Gleason protocol, and resembling renal cell carcinoma, is uncommonly observed and designated pattern 4

Pattern 5. The neoplastic proliferation comprises sheets, solid cords, or single cells devoid of gland formation, infiltrating the prostatic stroma. Alternatively, papillary, cribriform, or solid masses with true comedonecrosis is regarded as Gleason pattern 5. [36, 37]

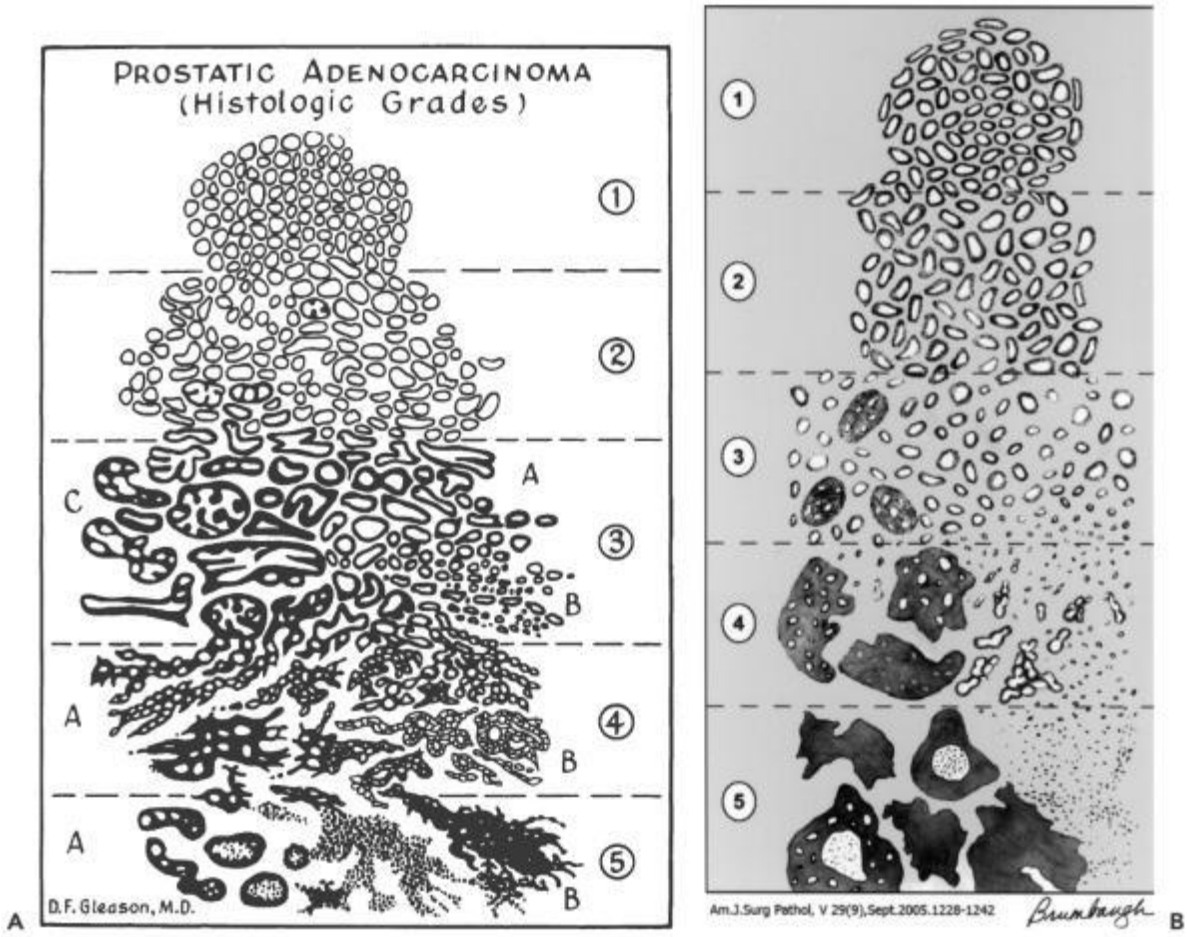


Figure-3: Gleason's grading system

Thus, under this schema the most well-differentiated tumors have a Gleason score of 2 (1 + 1), and the least-differentiated tumors merit a score of 10 (5 + 5). Gleason scores are often combined into groups with similar biologic behavior, with grades 2 through 4 representing well-differentiated cancer, 5 and 6 intermediate-grade tumor, 7 moderate to poorly differentiated cancer, and 8 through 10 high-grade tumor.

Tertiary Gleason pattern: On needle biopsies with patterns 3, 4, and 5, both the primary pattern and the highest grade should be recorded. Consequently, tumors with Gleason score 3 + 4 and a tertiary pattern 5 would be recorded as Gleason score 3 + 5 = 8. In cases where there are three patterns consisting of patterns 2, 3, and 4, it was the consensus of the group that one would ignore the pattern 2 and the biopsy would be called Gleason score 3 + 4 = 7 or Gleason score 4 + 3 = 7, depending on whether pattern 3 or pattern 4 was more prevalent. For a radical prostatectomy specimen one assigns the Gleason score based on the primary and secondary patterns with a comment as to the tertiary pattern.[38]

REPORTING SECONDARY PATTERNS OF LOWER GRADE WHEN PRESENT TO A LIMITED EXTENT

It was the consensus of the group that in the setting of high-grade cancer one should ignore lower-grade patterns if they occupy less than 5% of the area of the tumor. For example, a needle biopsy core that is 100% involved by cancer, with 98% Gleason pattern 4 and 2% Gleason pattern 3, would be diagnosed as Gleason score 4 + 4 = 8.

IMMUNOPROFILE

Prostate specific antigen (PSA): It is a useful IHC marker of prostatic differentiation with both polyclonal and monoclonal antibodies available. PSA is localized to cytoplasm of non-neoplastic prostatic glandular cells in all prostatic zones because of its relatively high specificity for prostatic glandular cells, PSA is a useful tissue marker expressed by most adenocarcinoma of prostate. A minority of higher grade adenocarcinoma of prostate are PSA negative, although some of these tumors have been shown to express PSA mRNA.[39]

Prostate specific membrane antigen (PSMA) (membrane bound antigen expressed in benign and malignant prostatic acinar cells) and androgen receptor may be immunoreactive in some high grade, PSA immunonegative PCas. Extraprostatic tissues which are variably immunoreactive for PSA, include urethral and periurethral glands (male and female), urothelial glandular metaplasia (cystitis cystitica and glandularis), anal glands (male), urachal remnants and neutrophils.

Prostate specific acid phosphatase (PAP):

The polyclonal antibody is more sensitive, but less specific than the monoclonal antibody. PAP and PSA have similar diagnostic utility; since a small number of PCa are immunoreactive for only one of the two markers.

High molecular weight cytokeratins detected by 34βE12 (Cytokeratin-903):

Only basal cells express high molecular weight cytokeratins. An immunoperoxidase cocktail containing monoclonal antibodies to cytokeratins 5 and 6 is also an effective basal cell stain. Absence of a basal cell layer is suggestive of invasive carcinoma. P63, a nuclear protein encoded by a gene on chromosome 3q27-29. p63 like high molecular weight cytokeratins helps in the diagnosis of PCa.[40]

Methyl-CoA racemase (AMACR):

AMACR mRNA was recently identified as being overexpressed in PCa by cDNA library subtraction utilizing high throughput RNA microarray analysis. However, AMACR is also present in BPH (12%), atrophic glands, HPIN (>90%), and Atypical adenomatous hyperplasia (17.5%).[41]

Androgen receptor (AR) is a nuclear localized, androgen binding protein complex occurring in prostatic glandular, basal and stromal cells. AR immunoreactivity was demonstrated in HPIN and invasive PCas.[42]

HISTOGENESIS

Clinical Features:

The patients have been classified based on ethnical presentation, incidental cancers, clinically symptomatic cancers, and occult cancers, respectively.

The detection of incidental PCa in TURP specimens obtained for benign disorders range from 3 to 24% with an average of 9.2% in 16 studies. The "eight-block protocol" has been recommended to identify virtually all types of T1b and importantly, the smaller T1a cancers.[26]

Local and Systemic Symptoms of PCa:

Serum PSA helps in early diagnosis of prostatic adenocarcinoma. Hence, reducing the frequency of patients who present with symptoms of urinary bladder outlet obstruction, rectal bleeding and pelvic pain. Systemic manifestations of PCa reported in few cases include hypercalcemia, leukemoid reaction, thromboembolism, and Eaton-Lambert syndrome.

Clinical Presentation with Distant Metastases:

The early clinical manifestations in PCa are rarely associated with symptoms of distant metastases and were called occult carcinomas. There has been a decline in frequency of clinical presentation from 4.2% to 1.6% during the years 1990 to 2003.[43] Examples of patients initially presenting with metastases to supraclavicular lymph nodes, lung, brain, infraorbital, skin abdominal and retroperitoneal sites, pleura and esophagus have been recorded in literature. The application of traid of immunostains comprising of PSA, PSAP,

and AMACR, and a high index of clinical suspicion, will help in establishing the definitive diagnosis.[19]

Diagnostic Procedures

Digital Rectal Examination (DRE): It is an important tool in diagnosing prostatic carcinoma When DRE is correlated with serum PSA levels by ultrasound. It plays an intergnal role in diagnosing early PCa .The low sensitivity of DRE may be due to smaller size of the tumor which cannot be palpated clinically .Smallest size of the tumour that can be detected on palpation ranges from 0.3 to 1.3 cm as reported by a study Stamey et al.[44]

Imaging:

TRUS is used to evaluate gland volume, measures focal lesion specially to take image biopsies but, the low specificity of TRUS in detecting PCa and its extraglandular limits its utility in diagnosis PCa.[45] Computed tomography (CT) and magnetic resonance imaging (MRI). MRI is reserved for staging of patients with biopsy proven PCa while CT has low sensitivity for detection.[46]

Monoclonal antibody radioimmunoscintigraphy (Prostate specific membrane antigen-PMSA) is chelated to Indium111(Prostacint®, Cytogen Corporation, Princeton, N.J.) even detects microscopic metastatic deposits in regional and distant sites. Positron emission tomography (PET), which allows in vivo-characterization of tumors, may have implications for the evaluation of patients with PCa in the future

LABORATORY TEST

Prostate specific antigen (PSA) is produced by the epithelial cells lining the prostatic ducts and acini and is secreted directly into the prostatic ducts. The PSA gene is located on chromosome 19. Androgen-regulated transcription results in the synthesis of a 261 amino acid PSA precursors that is activated by the proteolysis of a small amino-terminal fragment. Different types of PSA exist in serum. These result from complex formation between free PSA and two major extracellular protease that are synthesized in the liver. As PSA is a serine protease, its mode of existence in the serum is in a complex with α-1-anti-chymotrypsin (ACT), a 67 kDa single chain glycoprotein, and α-2-macroglobulin (AMG), a 720 kDa glycoprotein. Only a small percentage of the PSA found free in the serum. Subtypes of free PSA include: mature single-chain, and multichain, nicked free PSA forms.[47]

Serum total PSA and age specific reference ranges:

Serum PSA level is determined with immunoassay techniques. Monoclonal antibodies have been designed to detect the free form of PSA (29kDa), the complex of PSA and the total PSA. Total PSA correlates very well with advancing age.

Based on the 95th percentile values of the regression model, white men under the age of 50 have PSA values <2.5 ng/ml, under age 60 have PSA values <3.5 ng/ml, under age 70 have PSA values <4.5 ng/ml, while under age 80 PSA levels were <6.5 ng/ml. It has been suggested that these age-related values be used as the upper limit of normal in PSA-related diagnostic strategies in clinical practices. PSA is raised beyond the arbitrary cutoff point of 4.0 ng/ml in the majority of patients with PCa. It may also be greater than 4.0 ng/ml in some benign conditions, including benign prostatic hyperplasia. PCa may also be present in men with Serum PSA values lower than the above quoted cutoff points. This may be specifically

true for men considered at higher risk (like family history; men with faster doubling time; and in the United States African American men). Therefore serum PSA lacks high sensitivity and specificity for prostate cancer.

This problem has been partially overcome by calculating several PSA-related indices and/or evaluating other serum markers. PSA tests are useful to detect recurrence and response of cancer following therapy. The exact value used to define recurrence varies depending on the treatment modality. Free form of PSA occurs to a greater proportion in men without cancer and in contrast, the α-1-chymotrypsin complex PSA comprises a greater proportion of the total PSA in men with malignancy. The median values of total PSA and of the free-to-total PSA ratio are 7.8 ng/ml and 10.5% in PCa patients, 4.3 ng/ml and 20.8% in patients with benign prostatic hyperplasia, and 1.4 ng/ml and 23.6% in a control group of men without benign prostatic hyperplasia. Complex PSA value may offer better specificity than total and free-total PSA ratio. PSA velocity: It is the rate of change in total PSA levels over time period. Rate of increase is greater in patients with adenocarcinoma of prostate. This is linked to the fact that the doubling time of PCa is estimated to be 100 times faster than that of benign prostatic hyperplasia. Given the short term variability of serum PSA values, Serum PSA velocity should be calculated over an 18-month period with at least three measurements. PSA doubling time (PSA DT) is closely related to PSA velocity. Patients with benign prostatic hyperplasia have PSA doubling times of 12± 5 and 17 ± 5 years at years 60 and 85, respectively. In patients with prostate cancer, PSA change has both a linear and exponential phase.

Prostatic acid phosphatase (PAP) is produced by epithelial cells lining the prostatic ducts and acini, is secreted directly into the prostatic ductal system. Serum PAP may be significantly

increased in patients with benign prostatic hyperplasia, prostatitis, prostatic infarction and adenocarcinoma of prostate. The sensitivity and specificity of PAP is very low.

Human glandular kallikrein 2 (hK2) and PSA exhibit different proteolytic specificities, but show similar patterns of complex formation with serum protease inhibitors. The serum level of hK2 is relatively high, especially in men with diagnosed PCa and not proportional to total PSA or free PSA concentrations.

Prostate specific membrane antigen (PSMA) is a membrane bound glycoprotein with high specificity for benign or malignant prostatic epithelial cells. This is a novel prognostic marker that is present in the serum of healthy men, according to studies. An elevated concentration is associated with the presence of prostate cancer. PSMA levels correlate best with advanced stage or with a hormone refractory state.[48]

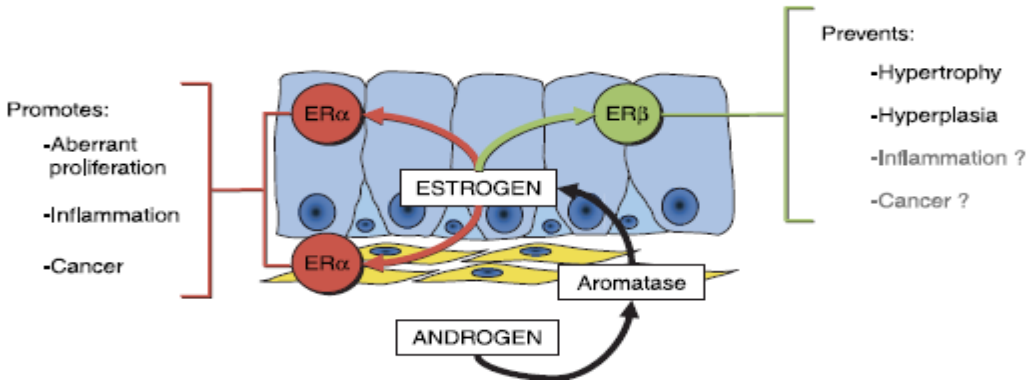
Reverse transcriptase-polymerase chain reaction (RT-PCR) is an extremely sensitive assay, capable of detecting one prostate cell diluted in 10^8 non-prostate cells because of the high sensitivity of RT-PCR, low-level basal transcriptions of prostate-specific genes from non-prostate cells will also result in a positive RT-PCR signal.[49]

ROLE OF HORMONES IN PROSTATIC LESIONS:

In men, unlike androgens which are responsible for proliferation, estrogens affects the proliferation as well as differention of the epithelial cells. Estrogen also plays a role in indirectly suppressing the release of luteinizing hormone from the pituitary, decreasing the synthesis of testicular androgens as well as systemic androgens, and induction of apoptosis and atrophy in prostate epithelium.[50] Simultaneously, estrogen causes a local direct affect in the prostatic stroma via ER alpha which stimulates aberrant epithelial differention and basal layer proliferation, thus developing squamous metaplasia.[51] The proliferation caused by ER alpha differs from that caused by androgenic hormones that is it can progress to inflammation and malignancy. Due to the presence of ER alpha in the stroma, its effect on the epithelium is mediated by paracrine mechanisms.[52] ER beta has an anti-proliferative action on the prostatic epithelium, while action of estrogen through its ER alpha receptors lead to hyperplasia, dysplasia, and carcinogenesis. Estrogen receptor have lesser affinity for 3 beta –adiol when compared to E2 but the amount of 3 beta –adiol is 100 times more than that of E2 ,hence making 3 beta –Adiol endogenous ligand for estrogen. DHT is a synthetic precursor of 3 beta –adiol. The affinity of 3 beta –adiol is greater for ER beta than ER alpha and does not bind to androgens.[53] Thus, it can be concluded that growth and differention of prostatic epithelium is regulated by ER beta.

As, men age the level of circulating estrogen increases the body due to the increase in body fat which is a source of estrogen. This leads to the decline of testosterone, hence increasing the action of estrogen through its receptors leading to BPH, dysplasia and adenocarcinoma of prostate.[50,52,54] ER beta exerts its anti-proliferative effect not only by direct action but also by inhibiting activity of ER alpha which in turn further inhibits epithelial proliferation and

assists differention in prostate .Hence ER beta plays a protective role by in habiting epithelial proliferation and in turn carcinogenesis.



Local estrogen signaling mechanisms in the prostate.[51] Testosterone is metabolized to estrogen by aromatase and acts *via* estrogen receptor (ER) alpha or ER-beta. Adverse effects via ER-alpha in stroma and epithelia include aberrant proliferation, inflammation and cancer. In contrast, estrogen also exerts beneficial effects *via* ER-beta in epithelia in preventing hyperplasia and hypertrophy, being antiproliferative and anticarcinogenic. Figure: 4

IMMUNOHISTOCHEMISTRY (IHC):

Immunohistochemistry is diagnostic tool used by the pathologist. Despite the presence of various diagnostic criteria of lesions, there is always some grey zone among different entities and dissimilarities among the same diagnosis, also the fact due to subjective dissimilarities between two pathologists, a definitive diagnosis is difficult to reach.[55] This lead to the development of special staining techniques that stained cells of particular lineage, brought about the origin of histochemistry in the mid-19th century. Francis Vincent Raspard, was the first to establish the use of immunohistochemistry. He was a botanist by profession. Advent of aniline dyes revolutionized histochemistry between 1862 to 1929.[56] The origin of Immunohistochemistry techniques lies in the pioneering work of Albert Coons, started in 1941. He described his first attempts to label antibodies directly with fluorescent isocyanate. Later the indirect technique was introduced by Nakane and Pierce in 1966, in that they took an unlabeled antibody is followed by second antibody or substrate. Various stages of

development of Immunohistochemistry from peroxidase antiperoxidase (1970), alkaline phosphatase labeling (1971), biotin technique (1977, 1979) and to two layer dextrin polymer technique (1993) have both advantages and disadvantages for each techniques.[57]

DETECTION SYSTEM:

Subsequent to the development of specific antibodies to the antigens, next step for the immunochemist was to develop techniques to visualize the antigen-antibody reaction complex.

Two methods employed for this purpose:

l) Direct method

2) Indirect method

DIRECT METHOD:

In this method the primary antibody is conjugated directly to the label. Most popular direct conjugates are those which are labelled with a fluorochrome, horse radish peroxidase and an alkaline phosphatase. The advantage of this method over others is that it is simple to use as it requires one application of reagent followed by an appropriate chromogen substrate solution.[57]

INDIRECT METHOD:

This is a two-step technique in which labeled secondary antibody reacts with the antigen bound to primary antibody. Furthermore increase in sensitivity was achieved with the introduction of peroxidase enzyme complex. Subsequent development resulted in Avidin-Biotin Complex method's. [57] Most widely used method.

A dextran polymer conjugate -two step visualization systems is a new indirect system based on dextran technology employed in the Epos system. This offers greater sensitivity than the traditional indirect system. It is less time consuming than the three stage Avidin -Biotin system and does not read with endogenous biotin.[57]

HORMONE RECEPTORS:

ESTROGEN RECEPTORS:

In 1950s Elwood V Jensen was first to locate Estrogen Receptor, further in 1996 Kuiper identified its gene. [58,59]

Walker D (1999) described that Estrogen receptors are regarded as unligand cytoplasmic receptors. Being steroid receptors, membrane bound receptors are not required for their activation. Estrogen receptor, during activation diffuses into the cytoplasm, from the cytoplasm migrates to the nucleus, causing dimerization of the receptor followed by binding with the HREs (Hormone Response Elements.) This DNA receptor complex initiates the cell proliferation by activating the P13K pathway .This action is backed by two hypothesis .One of these hypothesis states that the ER induced transcription activity is brought about by alternate RNA splicing of the ER alpha subunit ,therefore promoting rapid uncontrolled proliferation, and the genetic mutations accumulate over time .The second hypothesis holds the genomic waste production responsible for same.[59,60] In approximately 70% of the epithelial cells of normal breast tissue the ER and PR are nuclear in location. Lobular cells show the highest proportion of these receptors.[59]Shoker et al described a contiguous pattern of ER positivity of varying sizes in the lobular units.[61] Estrogen receptor positive pattern of valuable size in lobular unit. Variation in expression during menstrual period was also reported.[61]

Estrogen and Progesterone receptors are members of the super family protein. Being nuclear transcription factors, they are involved the breast development, growth, differentiation and tumorigenesis in the breast tissue. Estrogen receptor medicates the regulation of the expression of other genes like Progesterone & bcl2. Thus Progesterone receptor indicates the intactness of the Estrogen receptor functioning. There are two forms of Estrogen receptor named as Estrogen receptor alpha & Estrogen receptor beta encoded by 6p25.1 and 14q respectively. Estrogen receptor alpha is present in endometrium, breast cancer cells, ovarian stroma and hypothalamus while Estrogen receptor beta distribution is seen in kidney, brain, bone, heart and lungs.[60]

ESTROGEN RECEPTOR BETA

In the year 1996, a new class of estrogen receptor was discovered, and was named as ER beta.[62]

Estrogen receptor beta has five isoforms, which seem to have different functions and have different tissue distributions.[63, 64] The significance of ER beta expression in tumors was first demonstrated in breast cancer, with several studies demonstrating that women with ER beta-positive breast cancers treated with adjuvant tamoxifen have better survival, and was independent of estrogen receptor α expression. Estrogen receptor beta and its isoforms have a wider tissue distribution (gastrointestinal tract, lung, and brain) than the traditional estrogen receptor now called as estrogen receptor α.[65] Estrogen receptor beta expression in breast cancer is associated with a favorable outcome in women treated with adjuvant tamoxifen, even in tumors negative for estrogen receptor α.[65] Since, the role of ER beta was seen in the pathogenesis of carcinoma of hormone dependent organs like uterus and breast, studies into other hormone dependent organs like prostate were done.

Prostatic lesions like BPH and adenocarcinoma are androgen-dependent and are treated by inhibiting androgens or their action. However recent studies have shown that prostate growth is also influenced by estrogen.

The detection of two types of estrogen receptor α and β has brought new insight into the mechanism underlying estrogen signaling. The steroid hormone receptor, estrogen receptor (ER)-a, was thought to mediate all estrogen actions. However in 1996, a second ER, ER-ß, was identified and found to differ significantly from ER-a. Unlike ER-a, which is the predominant ER in female reproductive organs, the ß-isotype is highly expressed in the male reproductive tract, including the prostate. Although the precise biological function of ERß is incompletely defined, it has been suggested that the receptor, acting through estrogens, may protect the normal prostate epithelium from undergoing unscheduled cell proliferation, neoplastic transformation and from oxidative injuries.Estrogen beta is nuclear stain found to be expressed in epithelium of the normal prostate gland. The role of estrogen receptor Beta in the pathogenesis or prognosis of PCA is unclear, it seems to have a role in the control of proliferation and prevention of hyperplasia. In mice, the absence of ER-ß leads to failure of the prostatic epithelium to differentiate fully. Whereas, the epithelial cells continue to proliferate. If the ER-ß is included as a factor in the endocrine control of prostate growth, we have androgen receptors causing proliferation and secretion and ER-ß suppressing proliferation and promoting differentiation.[67] Since proliferation and differentiation oppose each other, cellular homeostasis must be the result of a dynamic balance. ER β is preferentially expressed in prostatic epithelial cells where as ER α is expressed in stromal cells and not in epithelial cells. In another study Estrogen beta receptor agonist has been proved to have pro-apoptotic an activity and the expression is found to be decreased in BPH and in severe grades of PCa. [66]

Prasenjit Dey et al studied estrogen-sensitive malignancies and concluded that ER beta usually is a tumor suppressor and ERa is an oncogene. ER beta regulates genes in several key pathways including tumor suppression (p53,PTEN); metabolism (PI3K); survival (Akt); proliferation pathways (p45Skp2, cMyc, and cyclin E);cell-cycle arresting factors (p21WAF1, cyclin-dependent kinase inhibitor 1 (CDKN1A)),p27Kip1, and cyclin-dependent kinases (CDKs); protection from reactive oxygen species, glutathione peroxidase.[68]

Paul Mak et al found that ERβ is targeted for repression in prostate cancer caused by PTEN deletion and that loss of ERβ is important for tumor formation. [69]ER alpha has a proliferative role where as ER beta has an antiproliferative role on prostatic epithelium. Based on these finding, hypothesis for the present study was ER beta will be present in non neoplastic and benign lesions compared to neoplastic lesion. Therefore explaining the protective role of ER beta in prostate.[70]

MATERIALS & METHODS

MATERIAL AND METHODS

STUDY DESIGN – Cross sectional study.

SOURCE OF DATA – TURP specimens of BPH and PCa received in the Dept. of Pathology, R. L. Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, Kolar from November 2014 to October 2016 was included in the study.

DURATION OF STUDY – Two years

Inclusion criteria: BPH and adenocarcinoma

Exclusion criteria: Previous radiotherapy and chemotherapy

Sample Size: Sample size was estimated for difference in proportions using Al Maghrabi et al.[3]

29 (BPH) + 29 (Ca Prostate) = 58 subjects at 5% α error and 90% power was calculated.

Material: Total of 58 cases were collected .BPH -29 cases, Adenocarcinoma -29 cases

METHOD OF COLLECTION:

All TURP specimens confirmed to be BPH and PCa by histopathological examination were included in the study. Data regarding the clinical detail and serum PSA levels were collected. H and E stained slides were reviewed for the grading and scoring of Gleason's score. The tumors were categorized as "low grade" if Gleason score was equal to or less than 4 and "high grade" if Gleason score was equal to or more than 8. Gleason score of 5, 6 and 7 will be considered as intermediate. Immunostaining for ER beta was performed on all cases of BPH and PCa using appropriate positive and negative controls

Immunohistochemistry (IHC):

It was done using primary antibody ER beta (Biogenex,) to know the activity in all the cases. Sections are cut at approximately 3-4μm thickness, floated on 4% organosialine coated slides and incubated at 58°C over night.

Deparaffinization using Xylene I and II for 15 minutes each.

Dexylenisation using absolute alcohol I and II for 1 minute each.

Dealcoholisation using 90% and 70% alcohol for 1 minute each.

Tap water wash for 10 minutes followed by distilled water wash for 5 minutes.

Antigen Retrieval technique: Microwave at power 10 for 6 minutes in EDTA TRIS buffer pH 9.0 for 3 cycles. Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH-7.6) for 5 minutes washing Peroxidase block: 10-15 min to block endogenous Peroxidase enzyme using 3% Hydrogen peroxide. TBS buffer wash thrice for 5 minutes.

Power block: 10-15 mins to block non-specific reaction with the other tissue antigen.

Primary stain: Drain and cover the sections with ready to use Biogenex primary antibody ER beta antibody for 2 hours, followed by TBS buffer wash for 5 minutes twice to wash unbound antibodies.

Super sensitive poly–horse radish peroxidase (HRP) for 30 minutes– to elongate chain and also to label the enzyme. Followed by TBS buffer wash for 5 minutes thrice to wash unbound antibodies. Color development with working DAB solution for 5-8 minutes, which imparts color to the antigens. TBS buffer wash for 5 minutes thrice then tap water wash for 5 minutes.

Counter stain Hematoxylin for 2 seconds followed by tap water wash for 5 minutes to wash out the excess stain. Dehydration and clearing by Alcohol: Xylene for 2 minutes. Then the slides were mounted with DPX.

Immunohistochemical evaluation:

The immunostained sections were examined using light microscopy Estrogen receptor beta expression was thoroughly sought for in tumoral cells for nuclear staining.

Slide was examined under Low power (40 x) for area of maximum positivity.

Areas showing maximum positivity were chosen, and 200 cells were counted under High Power (400x).

Estrogen receptor-beta expression was assigned as positive when more than 10% of tumoral nuclei are stained, according to Agsari et al.[71] Rate of ER-β expression was defined as percentage of positive nuclei per 200 cells counted.[71] Intensity of staining was not taken into consideration, weak and strong staining was considered positive.[72,73]

ANALYSIS AND RESULTS

Statistical analysis:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test of Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data. **Yates correction** was applied were ever chi-square rules were not fulfilled (for 2x2 tables only).

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram and Scatter plots.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data. EPI Info (CDC Atlanta), Open Epi, Med calc and Medley's desktop were used to estimate sample size, odds ratio and reference management in the study.

RESULTS

RESULTS

Study group consisted of a total of 58 cases, comprising of BPH -29 cases and Adenocarcinoma -29 cases.

Table 1: Age distribution of subjects in the study

		Group					
		BPH		Adenocarcinoma		Total	
		Count	%	Count	%	Count	%
Age	<50 years	6	20.7%	1	3.4%	7	12.1%
	51 to 70 years	23	79.3%	23	79.3%	46	79.3%
	> 70 years	0	0.0%	5	17.2%	5	8.6%

$\chi 2 = 8.571$, df = 2, p = 0.014*

Mean age of subjects in BPH group was 54.1 ± 5.3 years in Adenocarcinoma subjects was 63.9 ± 6.5 years.

Significant difference in age distribution was observed between BPH and Adenocarcinoma subjects. All the Subjects >70% in the study had Adenocarcinoma.

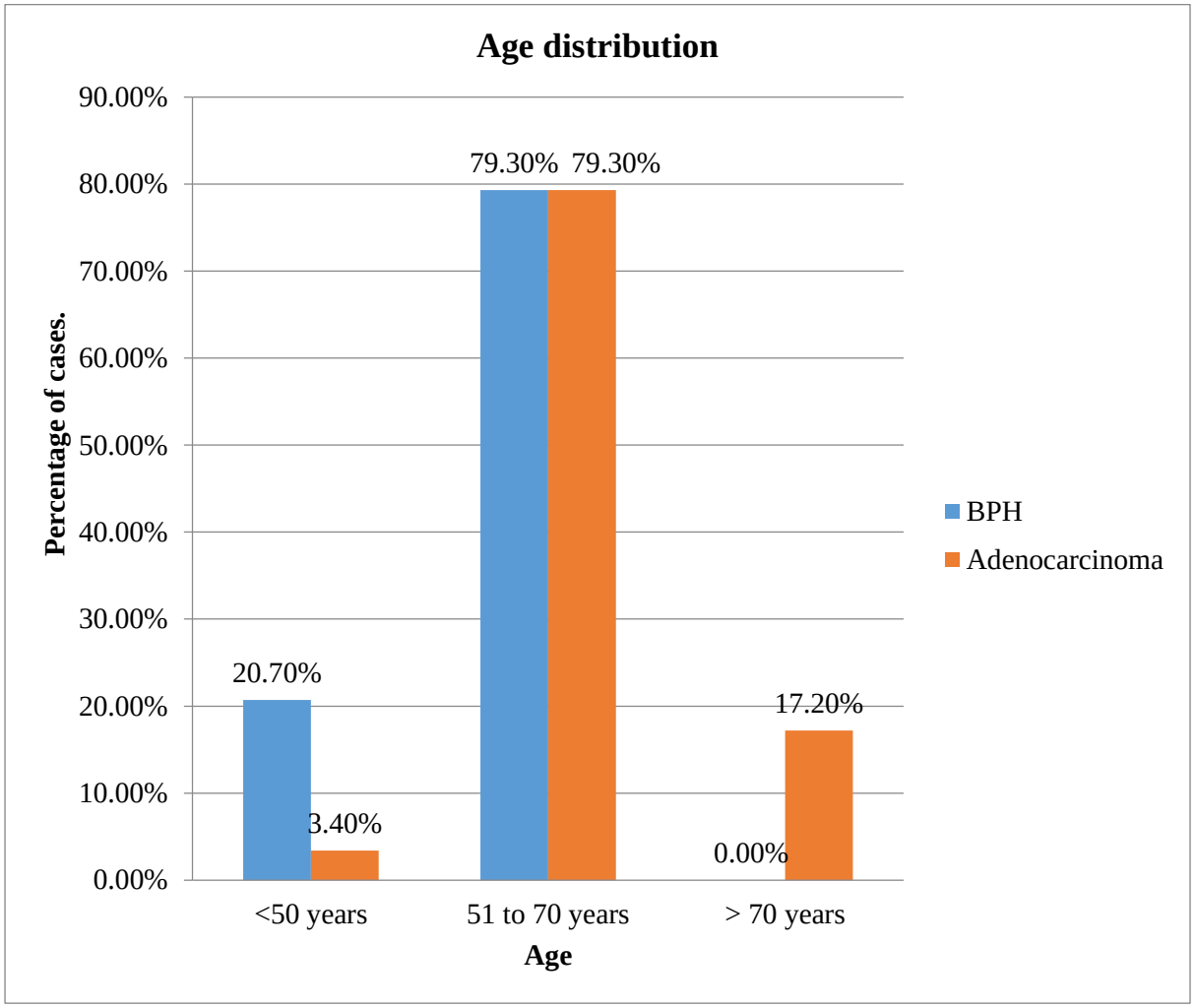


Figure 5: Bar diagram showing Age distribution of subjects in the study

Table 2: Comparison of PSA levels between two groups

		Group					
		BPH		Adenocarcinoma		Total	
		Count	%	Count	%	Count	%
PSA	≤10ng/ml	26	89.7%	0	0.0%	26	44.8%
	>10ng/ml	3	10.3%	29	100.0%	32	55.2%

$\chi 2 = 47.12$, df = 1, p <0.001*

In BPH subjects 89.7%of them had PSA ≤10ng/ml and 10.3% had PSA > 10ng/ml. were as in Adenocarcinoma subjects 100% of them had PSA > 10ng/ml. This observation was statistically significant.

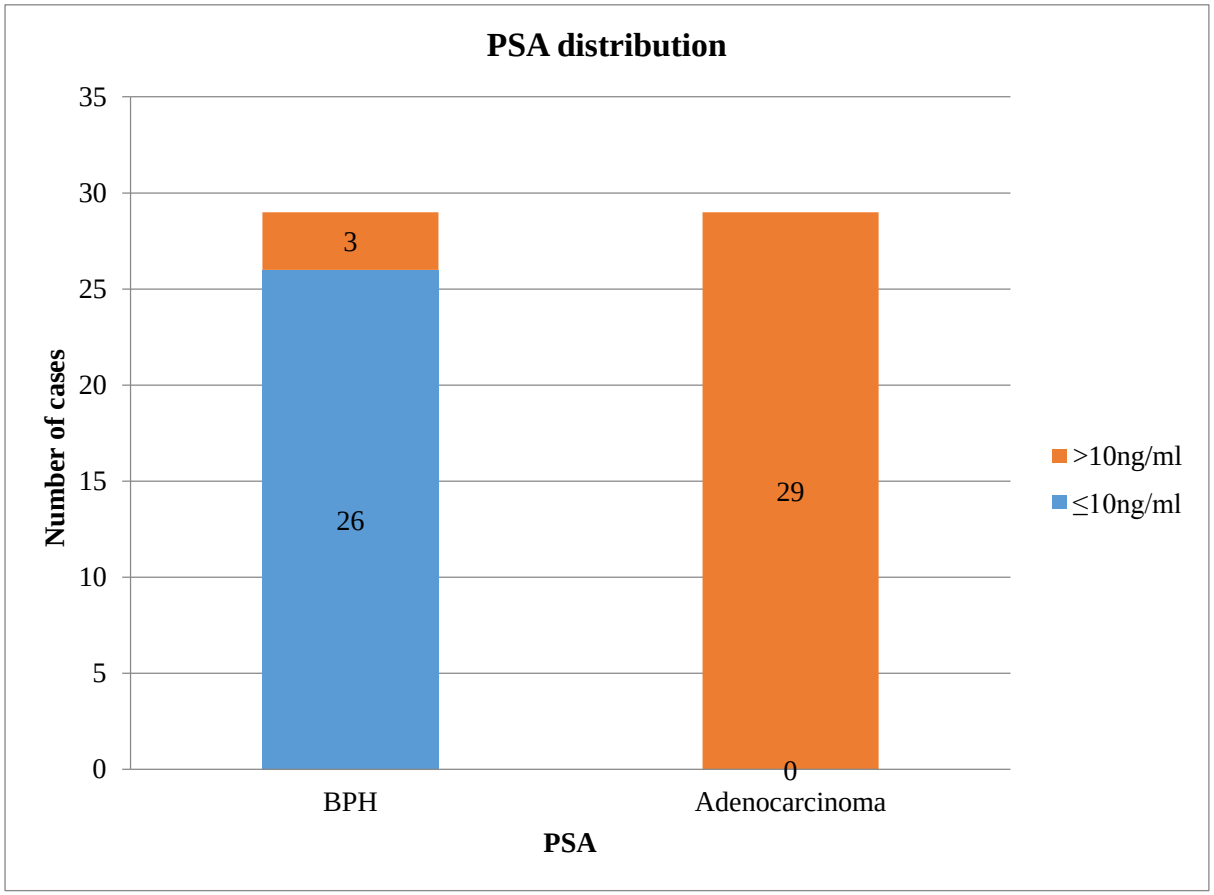


Figure 6: Bar diagram showing Comparison of PSA levels between two groups

Table 3: Gleason grade in Adenocarcinoma subjects

		Adenocarcinoma	
		Count	%
Gleason Grade	High	11	37.9%
	Intermediate	17	58.6%
	Low	1	3.4%

In Adenocarcinoma subjects, 37.9% had high, 58.6% had intermediate and 3.4% had low Gleason grade.

Table 4: Stage of Adenocarcinoma

		Group	
		Adenocarcinoma	
		Count	%
Stage	Stage I	1	3.4%
	Stage II	28	96.7%

Among Adenocarcinoma subjects 3.4% were in stage I and 96.7% were in stage II.

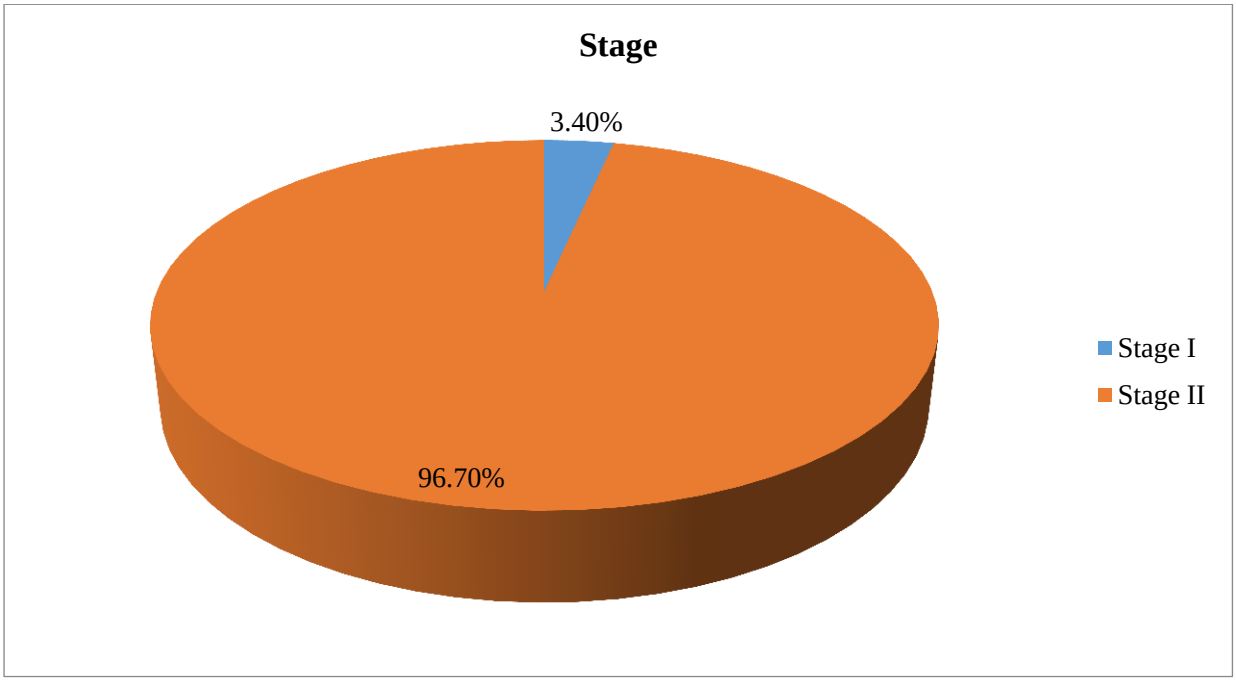


Figure 7: Pie diagram showing Stage of Adenocarcinoma

Table 5: ER Beta findings comparison between two groups

		Group			
		BPH		Adenocarcinoma	
		Count	%	Count	%
ER Beta staining	Negative (≤10)	2	6.9%	28	96.6%
	Positive (>10)	27	93.1%	1	3.4%

$\chi 2 = 46.67$, df = 1, p <0.001*

In BPH subjects 93.1% of them were ER beta positive and 6.9% were ER beta negative. In Adenocarcinoma group only 3.4% were ER beta positive and 96.6% were ER beta negative. This observation was statistically significant.

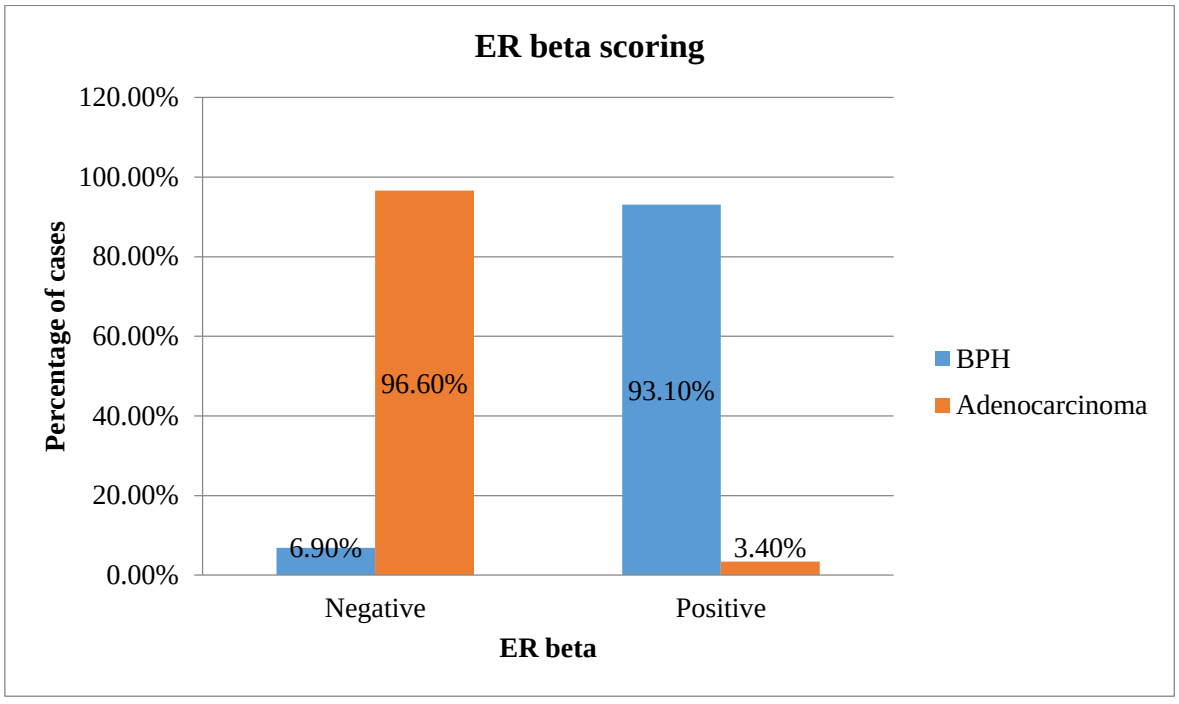


Figure 8: Bar diagram showing ER Beta findings comparison between two groups

Table 6: ER beta scoring comparison between two groups

		Group			
		BPH		Adenocarcinoma	
		Count	%	Count	%
ER beta staining	1 to 4%	0	0.0%	28	96.6%
	5 to 10%	2	6.9%	0	0.0%
	11 to 20 %	7	24.1%	0	0.0%
	> 20 %	20	69.0%	1	3.4%

$\chi 2 = 54.19$, df = 3, p <0.001*

In BPH group 6.9% had ER score of 5 to 10%, 24.1% had ER score of 11 to 20% and 69% had ER score of >20%. Were as in Adenocarcinoma group 96.6% had ER score of 1 to 4% and 3.4% had ER score of >20%. This observation was statistically significant.

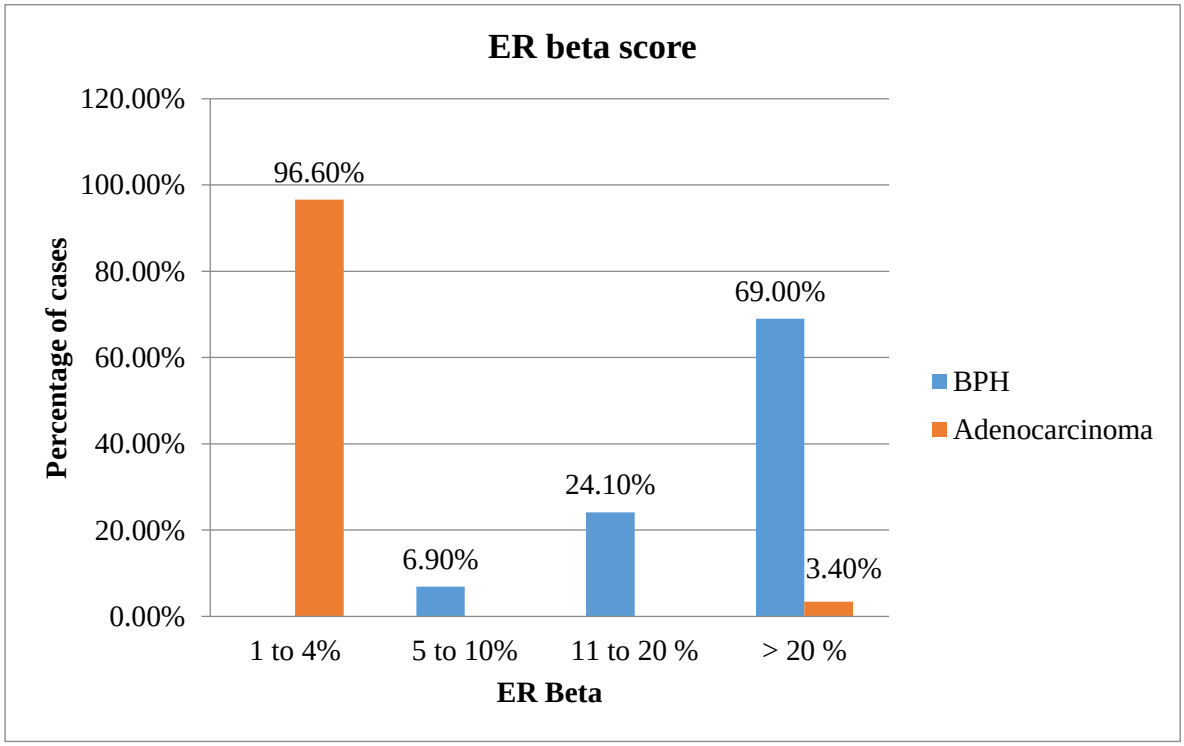


Figure 9: Bar diagram showing ER beta score comparison between two groups

Table 7: ER beta findings comparison with PSA values in BPH group

		PSA			
		≤10ng/ml		>10ng/ml	
		Count	%	Count	%
ER Beta staining	Negative (≤10)	0	0.0%	2	66.7%
	Positive (>10)	26	100.0%	1	33.3%

$\chi 2 = 18.61$, df = 1, p <0.001*

In BPH group among subjects with PSA≤ 10ng/ml, 100% of them were positive for ER beta and among subjects with PSA >10ng/ml, 66.7% were negative and 33.3% were positive for ER beta. This observation was statistically significant.

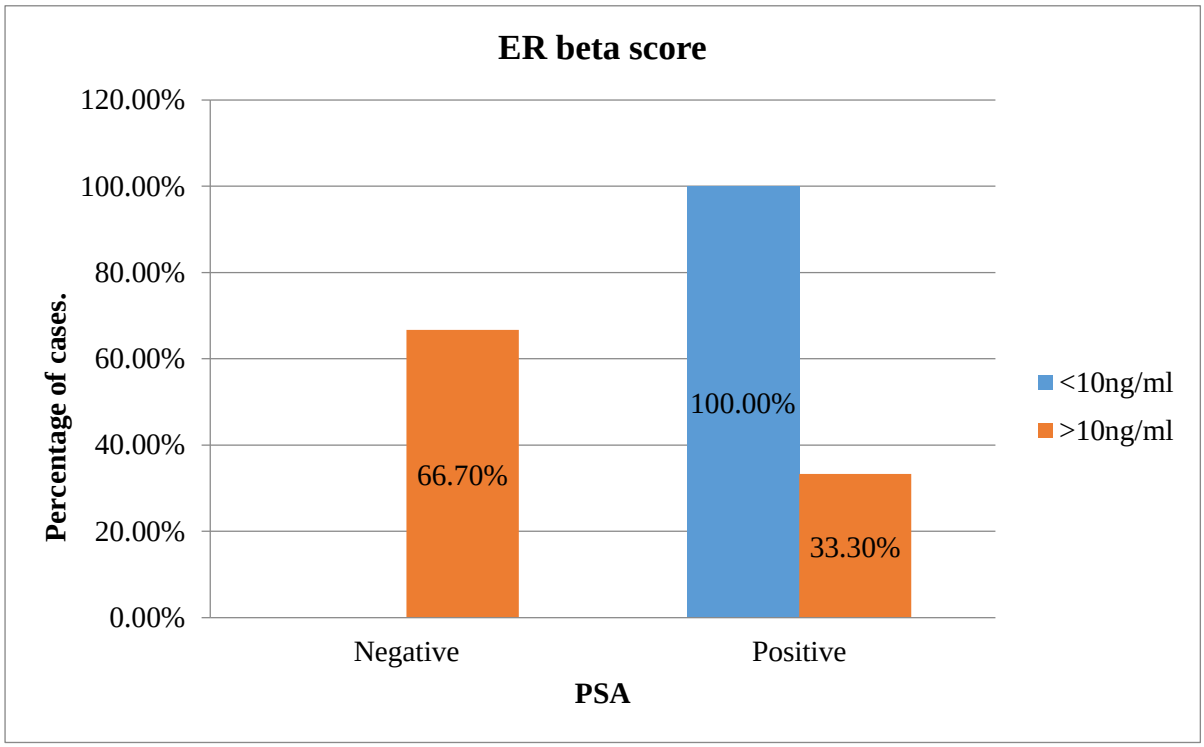


Figure 10: Bar diagram showing ER beta findings comparison with PSA values in BPH group

Table 8: ER beta findings comparison with PSA values in Adenocarcinoma group

		PSA			
		≤10ng/ml		>10ng/ml	
		Count	%	Count	%
ER Beta staining	Negative (≤10)	0	0.0%	28	96.6%
	Positive (>10)	0	0.0%	1	3.4%

P<0.001*

Significant association was observed between ER beta staining and PSA levels in Adenocarcinoma group. None of the subjects had PSA≤ 10ng/ml and among subjects with PSA >10ng/ml, 96.6% were ER beta negative and only 3.4% were ER beta positive.

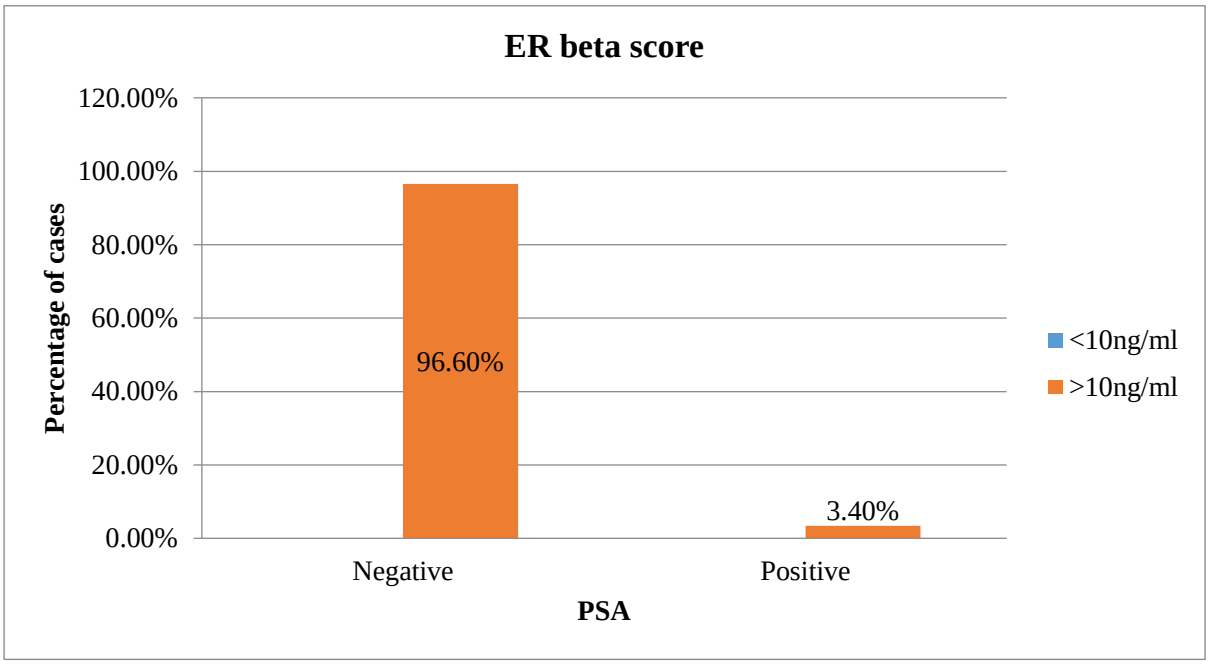


Figure 11: Bar diagram showing ER beta findings comparison with PSA values in Adenocarcinoma group

Table 9: ER beta findings comparison with Gleason Grade in Adenocarcinoma group

		Gleason Grade					
		High		Intermediate		Low	
		Count	%	Count	%	Count	%
ER Beta staining	Negative (≤10)	11	100.0%	17	100.0%	0	0.0%
	Positive (>10)	0	0.0%	0	0.0%	1	100.0%

$\chi 2 = 29$, df = 2, p <0.001*

In Adenocarcinoma subjects with high and intermediate Gleason grade being 11 and 17 cases respectively, 100% were negative for ER beta, and 100% of subjects with low grading were positive for ER beta. This observation was statistically significant.

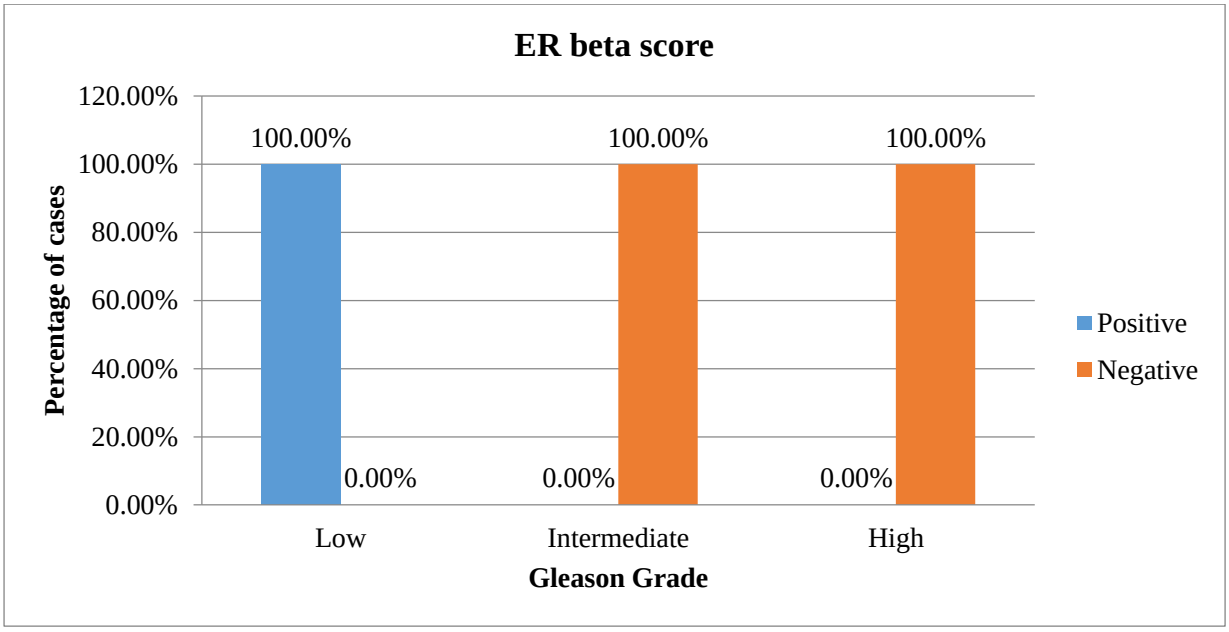


Figure 12: Bar diagram showing ER beta findings comparison with Gleason Grade in Adenocarcinoma group

Table 10: ER beta score comparison with Gleason Grade in Adenocarcinoma group

		Gleason Grade					
		High		Intermediate		Low	
		Count	%	Count	%	Count	%
ER beta grading	0%	0	0.0%	0	0.0%	0	
	1 to 4%	11	100.0%	17	100.0%	0	0.0%
	5 to 10%	0	0.0%	0	0.0%	0	0.0%
	11-20%	0	0.0%	0	0.0%	0	0.0%
	> 20 %	0	0.0%	0	0.0%	1	100.0%

$\chi 2 = 29$, df = 2, p <0.001*

In Adenocarcinoma subjects out of 11 and 17 subjects with high and intermediate Gleason grade, 100% had ER beta score of 1 to 4% and 100% of subjects with low grading had ER beta score of >20%. This observation was statistically significant.

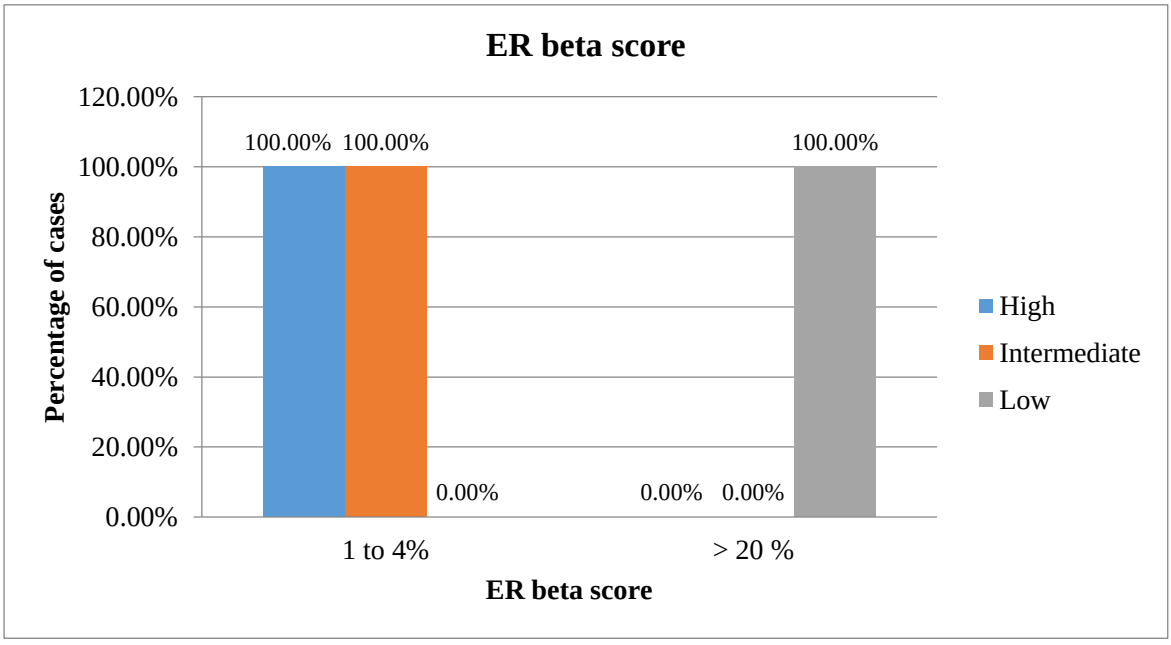


Figure 13: Bar diagram showing ER beta scoring comparison with Gleason Grade in Adenocarcinoma group

Table 11: ER beta findings comparison with Stage of Adenocarcinoma

		ER Beta staining			
		Negative		Positive	
		Count	%	Count	%
Stage	Stage I	0	0.0%	1	100.0%
	Stage II	28	100.0%	0	0.0%

$\chi 2 = 29$, df = 1, p <0.001*

Among Adenocarcinoma subjects with ER beta negative, 100% were in stage II and in ER beta positive subjects 100% were in stage I. This observation was statistically significant in Adenocarcinoma group.

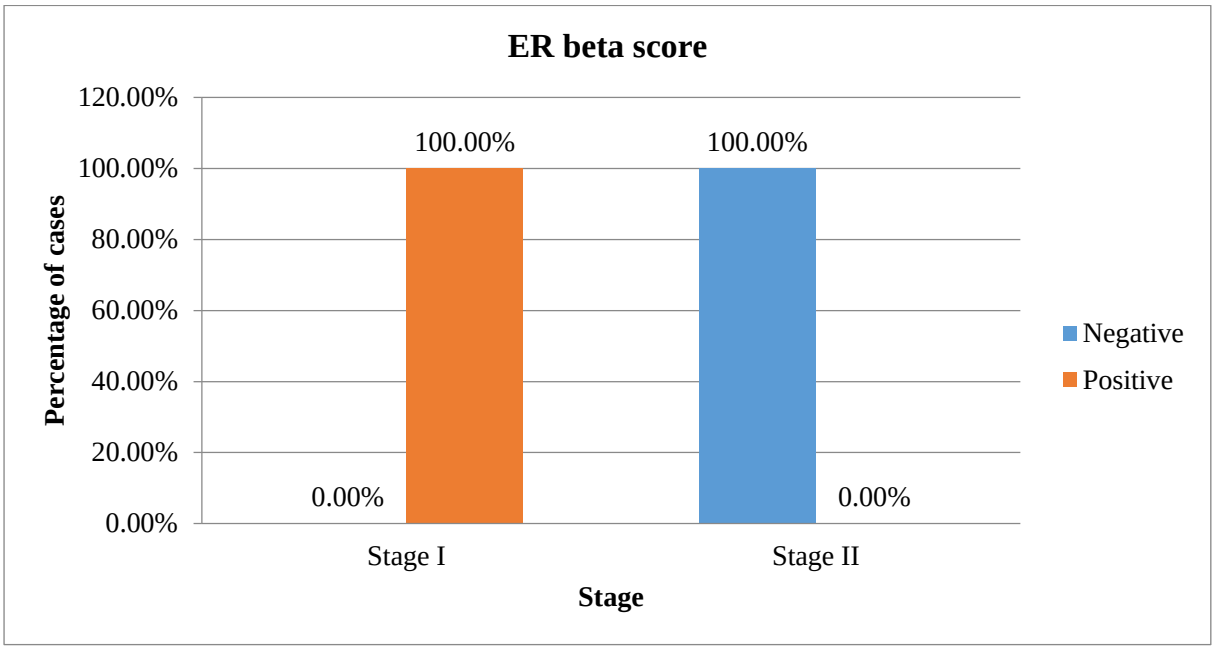


Figure 14: Bar diagram showing ER beta findings comparison with Stage of Adenocarcinoma

Table 12: ER beta scoring comparison with Stage of Adenocarcinoma

		ER beta grading			
		1 to 4%		> 20 %	
		Count	%	Count	%
Stage	Stage I	0	0.0%	1	100.0%
	Stage II	28	100.0%	0	0.0%

$\chi 2 = 29$, df = 1, p <0.001*

In Adenocarcinoma subjects with ER beta grade of 1 to 4%, 100% were in stage II and in subjects with ER beta grading >20%, 100% were in Stage I. This observation was statistically significant.

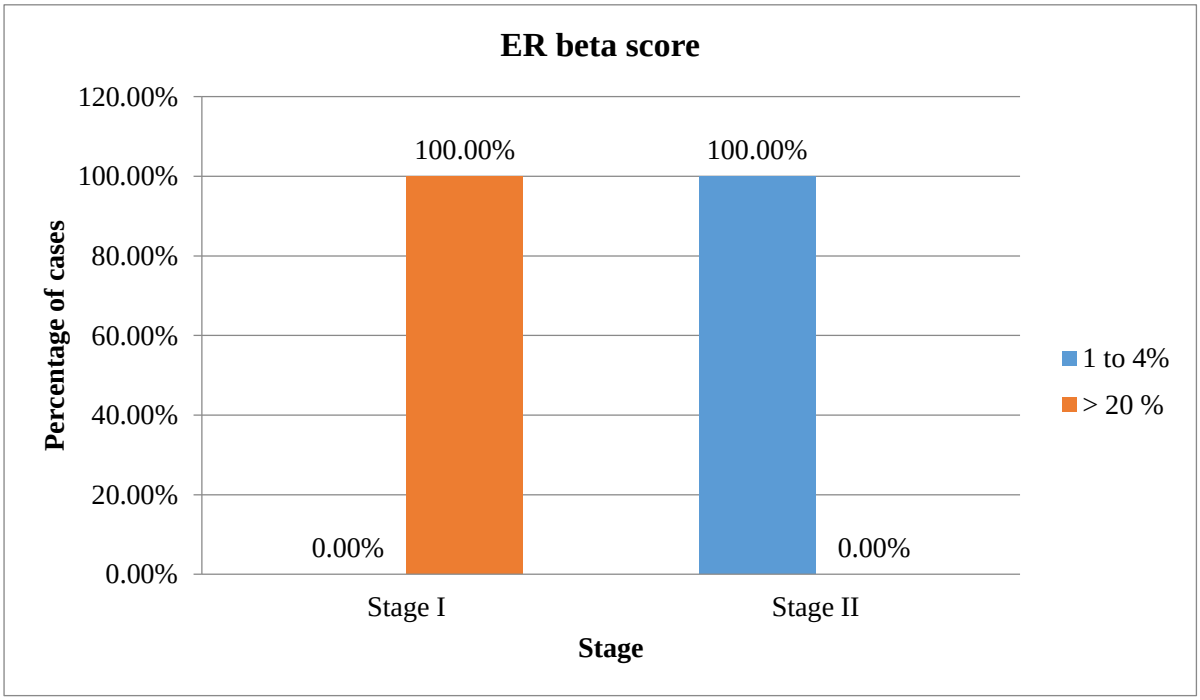


Figure 15: Bar diagram showing ER beta scoring comparison with Stage of Adenocarcinoma

PHOTOGRAPHS

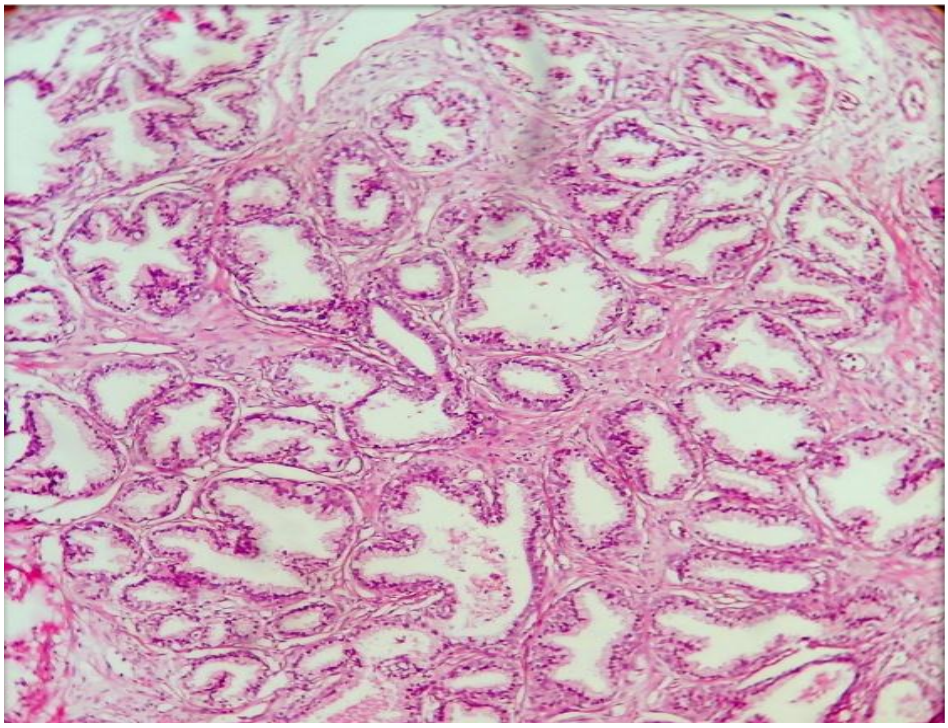


Figure-16: BPH (H&E, x400)

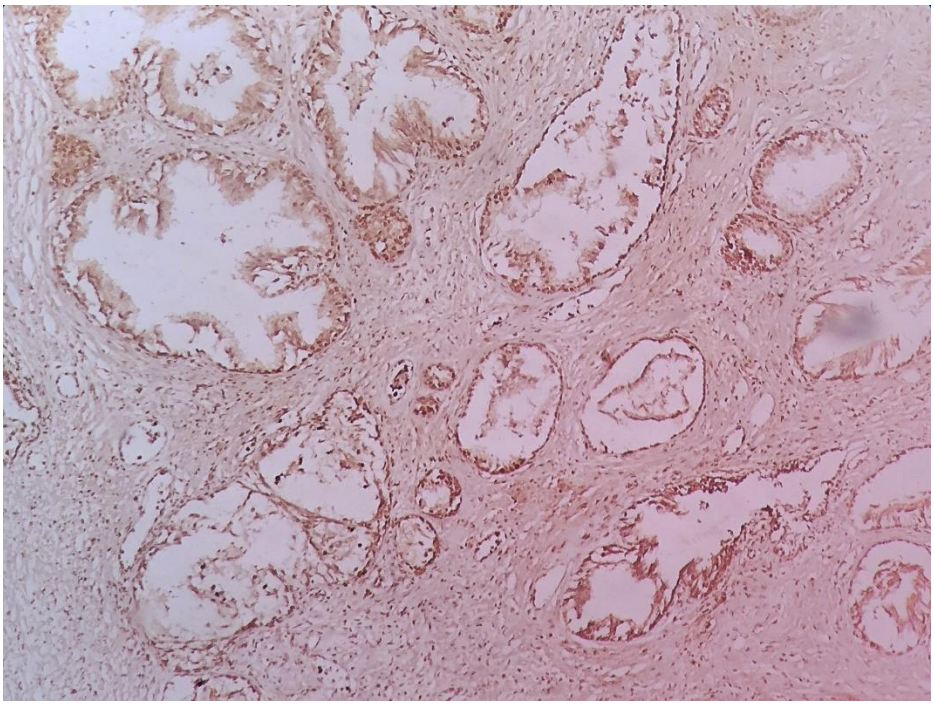


Figure-17: BPH, ER beta Positive (IHC ER beta, x400)

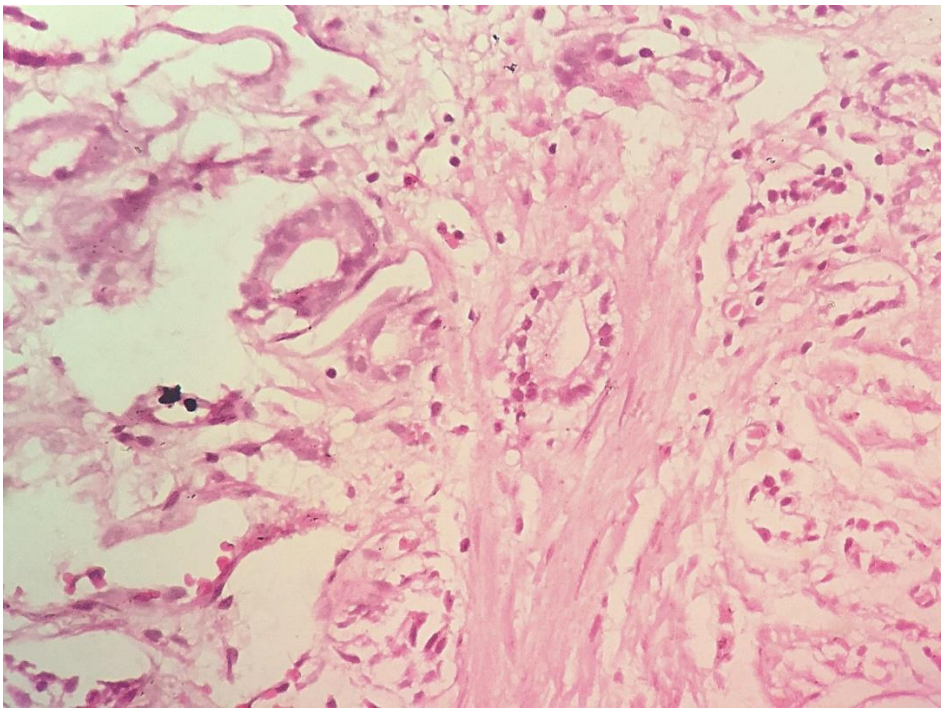


Figure-18: Prostatic Adenocarcinoma Gleason Score - 4 (H&E, x400)

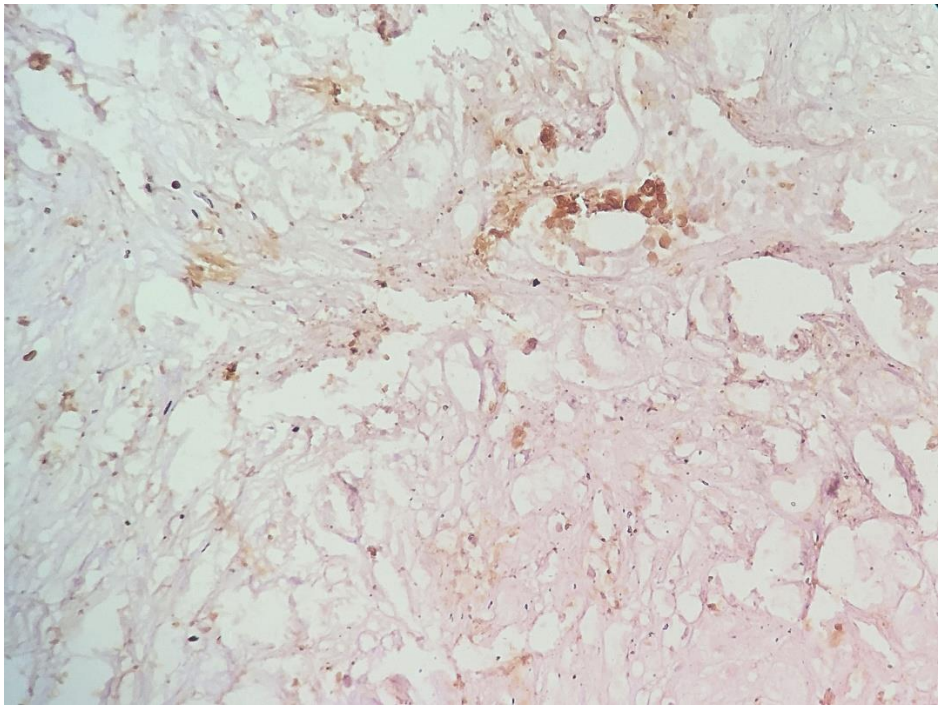


Figure-19: Prostatic Adenocarcinoma Gleason Score - 4, ER beta Positive (IHC ER beta, x400)

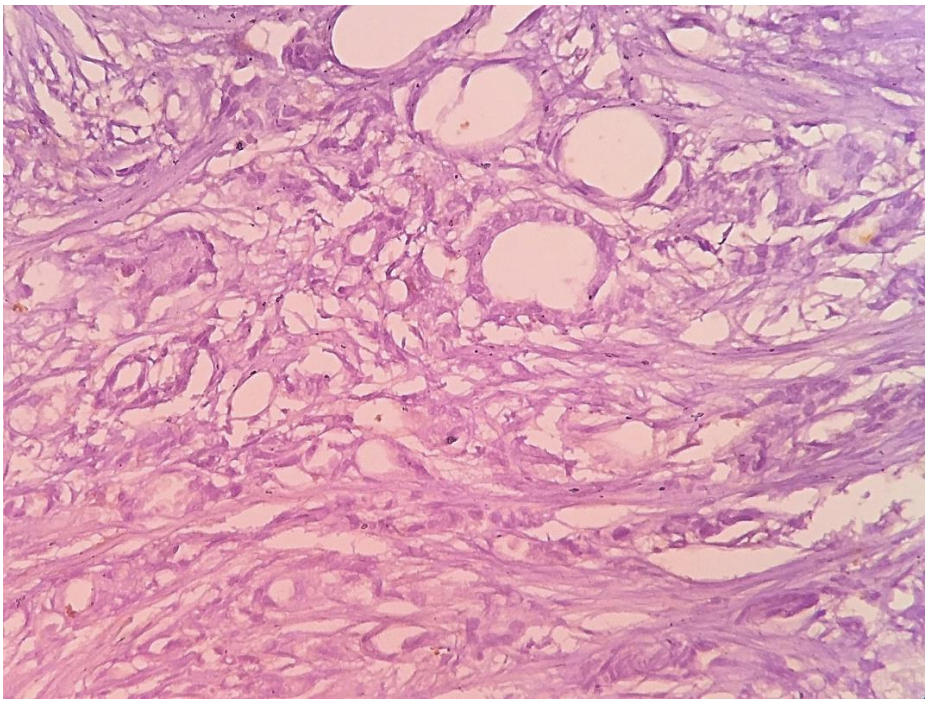


Figure-20: Prostatic Adenocarcinoma Gleason Score - 7 (H&E, x400)

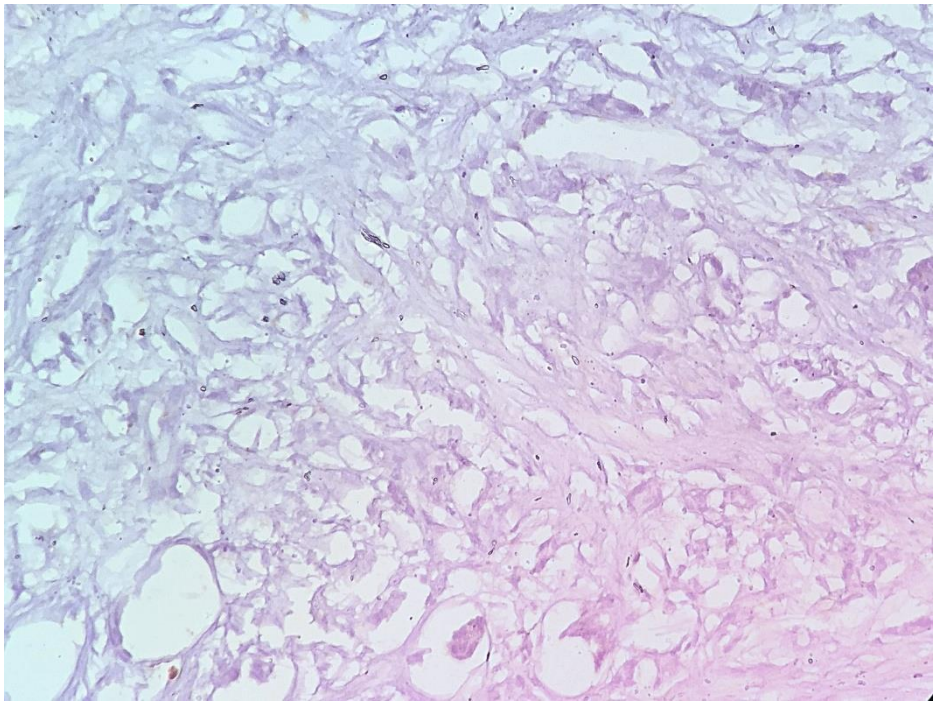


Figure-21: Prostatic Adenocarcinoma Gleason Score - 7, ER beta Negative (IHC ER beta, x400)

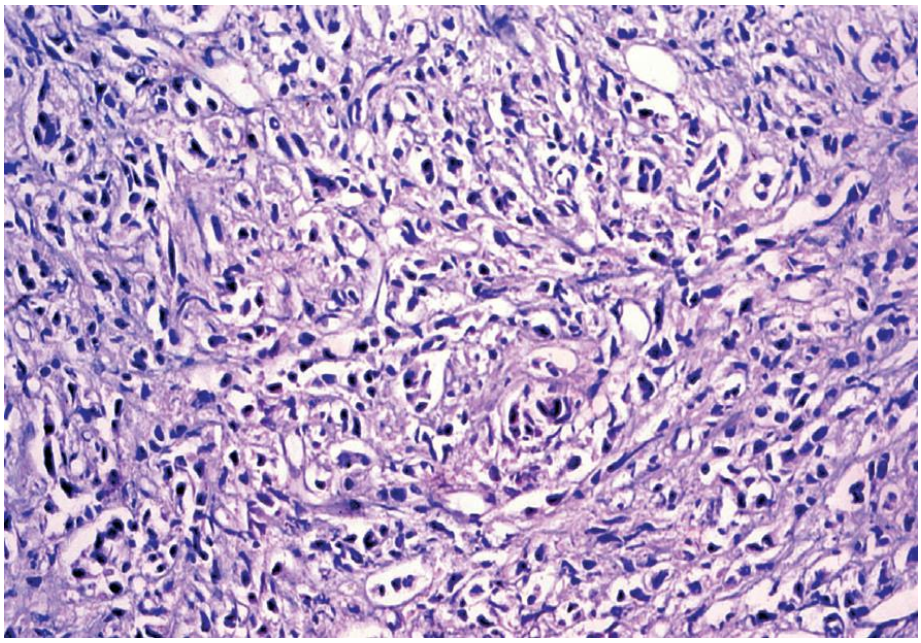


Figure- 22: Prostatic Adenocarcinoma Gleason Score - 10 (H&E, x400)

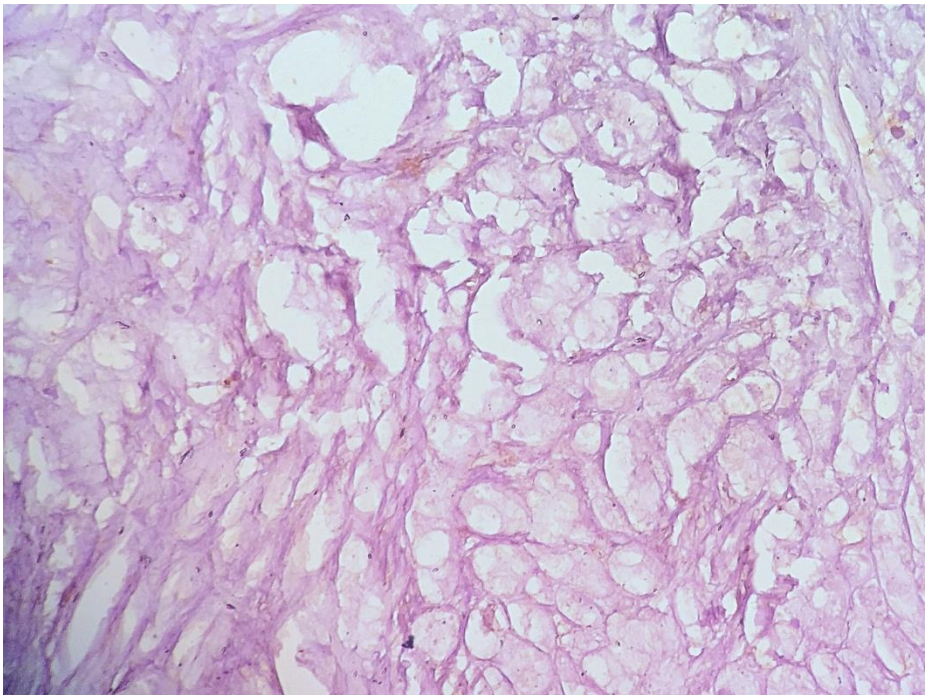


Figure-23: Prostatic Adenocarcinoma Gleason Score - 10, ER beta Negative (IHC ER beta, x400)

DISCUSSION

DISCUSSIONS

BPH

Table 13: COMPARISON OF AGE DISTRIBUTION OF BPH IN DIFFERENT STUDIES

AGE DISTRIBUTION IN YEARS (BPH)	<50		51-70		>70		TOTAL	
	NO.	%	NO.	%	NO.	%	NO.	%
PRESENT STUDY	6	20.7	23	79.3	0	0	29	100
GABAL ET AL (2007)[74]	2	20	4	40	4	40	10	100
ASLAM ET AL (2013)[75]	3	7.9	10	23.8	29	69.1	42	100
GHARTIMAGAR ET AL (2012)[76]	23	52.3	13	29.5	8	18.2	44	100

ADENOCARCINOMA

Table 14: COMPARISON OF AGE DISTRIBUTION OF PCa IN DIFFERENT STUDIES

AGE DISTRIBUTION IN YEARS (ADENOCARCINOMA OF PROSTATE)	<50		51-70		>70		TOTAL	
	NO.	%	NO.	%	NO.	%	NO.	%
PRESENT STUDY	1	3.4	23	79.3	5	17.3	29	100
GABAL ET AL (2007)[74]	0	0	14	40	21	60	35	100
ASLAM ET AL (2013)[75]	1	16.7	5	83.3	0	0	6	100
GHARTIMAGAR ET AL (2012) [76]	2	66.7	0	0	1	33.3	3	100

Around the world, diseases of Prostate gland can lead to significant morbidity and mortality among adult males. It is basically a geriatric disease increasing in incidence with advancing age of men. Most of the patients (72.0%) affected in our study group were in fifth decade for BPH sixth and seventh decade (62%) for adenocarcinoma, with a mean age of 54.1 years and 63.9 years in BPH and adenocarcinoma of prostate respectively. This finding was consistent with the results of various other studies (Table-14) in the Indian subcontinent and outside the Indian subcontinent, except the finding of Ghartimagar et al, whose findings relied on autopsy samples, hence the majority of cases were seen in the fourth decade.[76] Horvath et al, study showed the mean age of patients with Adenocarcinoma was 63.0 years (range, 44.1 – 75.9 years). [66]In Grover et al, patient age varied from 45 to 80 years in adenocarcinoma of prostate patients.[77]

PSA

Table 15: Comparison of PSA level in studied BPH, and prostatic adenocarcinoma cases.

PSA		≤10 ng/ml NO (%) CASES	>10 ng/ml NO (%) CASES	TOTAL CASES
PRESENT STUDY	**BPH**	26 (89.6%)	3(10.4%)	29
	PCa	0	29(100%)	29
GABAL ET AL[74] STUDY (2007)	**BPH**	6(60%)	4(40%)	10
	PCa	4(12%)	31(88%)	35

In the present study, there was high expression of ER beta in cases of BPH with ≤10 ng/ml PSA level, and low expression in adenocarcinoma cases with >10 ng/ml PSA level.

In our study, taking 10ng/ml as the cutoff the specificity becomes 100 % and diagnostic accuracy was 82.2 %. On increasing the cutoff as 20ng/ml the diagnostic accuracy and sensitivity rises to 47.6% and 86.7% respectively, but specificity falls to 98.6%.

Gabal et al also showed similar finding to the present study while Grover et al studied stated that adenocarcinoma of prostate had elevated PSA levels ranging from 10 to 649 ng/ml with all these patients having value more than 10 ng/ml.[74,77]

Similar results were given by Chang et al who also mentioned that the underlying mechanism for these findings in dysplasia awaits further investigation, based on the proposed anti-proliferative function of the receptor.[78]

These finding in present study was similar to that of Gabal et al were majority of the BPH cases had only had PSA level < 10 ng/ml , however the low percentage in Gabal et al compared to present study can be due to the bias to fewer BPH cases.[74]

While the present study was similar to Gabal et al in terms of adenocarcinoma of prostate were majority the cases had PSA level greater than 10 ng/ml with 100 % and 89.7% in present study and Gabal et al respectively.[74]

Table 16: Relation of ER-*ß* scores to PSA level in studied **BPH cases**

PSA		**≤10 ng/ml NO (%)**	**>10ng/ml NO (%)**	**TOTAL NO (%)**
PRESENT STUDY	**ER-*ß(+)***	26 (100%)	1(33.3%)	27(93.10%)
	ER-*ß (-)*	0	2(66.6%)	2(6.89%)
GABAL ET AL STUDY (2007)[73]	**ER-*ß (+)***	8(100%)	1(50%)	9(90%)
	ER-*ß (-)*	0	1(50%)	1(10%)

These finding was similar to that Gabal et al were majority of the BPH cases with PSA value less than < 10 ng/ml showed ER beta positivity.[74] .Even Leav I et al ,concluded that 90% cases had high ER beta expression with PSA levels ≤ 10 ng/ml.[79]

Table 17: Relation of ER-*ß* scores to PSA level in studied in adenocarcinoma

PSA		≤ 10 ng/ml NO (%)	>10ng/ml NO (%)	TOTAL NO
PRESENT STUDY	ER-*ß(+)*	0	1(3.4%)	1
	ER-*ß (-)*	0	28(96.6%)	28
GABAL ET AL STUDY (2007)[73]	ER-*ß(+)*	6(40%)	0	6
	ER-*ß (-)*	9(60%)	20(100%)	29

These finding was similar to that Gabal et al were majority of the Adenocarcinoma of prostate cases with PSA value less than > 10 ng/ml ,showed loss of ER beta expression .[74]

However the absence of PCa with ≤ 10ng/ml PSA values can be attributed to the late presentation of PCa cases with increased tumour bulk and increased PSA.

BPH

Table 18: The ER-ß expression in the studied BPH cases.

	PRESENT STUDY	HORVATH ET AL (2001) [66]	GABAL ET AL (2007)[74]	AL – MAGHRABHI ET AL (2010)[3]	GROVER ET AL (2014)[77]
ER BETA (+) (>10)	93.1%	24.2%	70%	94.3%	100%
ER BETA(-) (≤10)	6.9%	75.8%	30%	5.7%	0%

In the present study, ER beta positivity was seen in 93.1% of the cases, while loss of expression in only 6.9% of the BPH cases. These finding were similar to studies by Gabal et al, which showed ER beta positivity in majority cases of BPH.[74] and Al –Maghrabhi et al which showed ER beta positivity in 94.3% of the BPH cases,[3] and Grover et al, showed ER beta positivity in 100% of the BPH cases.[77]Fixenar et al study concluded that ER beta levels were high in all BPH.[80]

However few studies showed contradictory findings .Hovarath et al, have stated the progressive loss of ER beta in BPH with ER beta positivity seen only in 24.2% of BPH cases.[66]

Table 19: Distribution of ER beta expression in BPH cases with different scores.

ER BETA SCORE	PRESENT STUDY	GABAL ET AL (2007)[74]
0	**0**	**0**
1-4	**0**	**10%**
5-10	**6.9%**	**20%**
11-20	**24.1%**	**20%**
>20	**69.0%**	**50%**

In the present study, nearly 69.0% of the cases of BPH showed ER beta Scores >20 while 24.1% scored between 11-20 and 6.9% of the cases scored between 5 -10 of ER-ß .This finding was similar to Gabal et al in which more than half of BPH cases showed ER beta positivity with score above 20 .[74]

ADENOCARINOMA

Table 20: The ER-ß expression in the studied Adenocarcinoma cases.

	ER-ß(+) (>10)	ER-ß(-) (≤10)
PRESENT STUDY	**3.4%**	**96.6%**
GABAL ET AL (2007)[74]	**17.2%**	**82.8%**
HORVATH ET AL (2001)[66]	**11.3%**	**88.7%**
GROVER ET AL (2014)[77]	**96.6%**	**3.4%**
AL – MAGHRABHI ET AL (2010)[3]	**93.8%**	**6.2%**
ASGARI M ET AL (2011)[71]	**92.1%**	**7.9%**
FIXEMER ET AL (2003)[80]	**87%**	**13%**
TORLAKOVIC ET AL (2002)[5]	**93%**	**7%**

Estrogens are believed to play a vital role in the pathogenesis of prostate adenocarcinoma cancer (PCa).

Although the precise biological function of ER-*ß* is incompletely understood, it has been suggested that the receptor, acting through estrogens, may protect the normal prostate epithelium from undergoing cell proliferation, neoplastic transformation, and from oxidative injuries.

In present study, majority of the adenocarcinoma cases 96.6% were negative for ER beta expression. These finding were similar to the findings of Gabal et al, which concluded that 82.8% of adenocarcinoma cases showed loss of ER beta expression. [74] while Horvath et al stated 88.7% of the adenocarcinoma of prostate cases showed loss of ER beta expression.[66] Also in the present study, there is progressive loss of ER beta expression in different grades (Gleason) of adenocarcinoma of prostate, with low grades (2 -4) showing ER beta positivity in 100% cases and loss of ER beta expression in intermediate (5-7) and high grades (8-10) of adenocarcinoma of prostate .This ER beta is inversely proportional to grades of adenocarcinoma of prostate.[71]

Leavakov et al study concluded that that 87% of low grade adenocarcinoma of prostate showed ER beta positivity while only 20% of the intermediate grade adenocarcinoma of prostate showed ER beta positivity. These finding were similar to the present study.[70]

However many studies discussed below had findings different from that of our study.

Though Grover et al, study concluded that majority of the PCa cases were positive for ERβ expression, all his BPH cases were not only positive but also had higher level of ERβ expression concluding that in PCa ERβ though positive shows lower scores than BPH .[77]

Asgari M et al, study stated 92.1% cases of adenocarcinoma of prostate showed ER beta positivity which was different from the present study.[71]

AL-Maghrabhi et al, study concluded that ER beta positivity was seen in 93.8% of adenocarcinoma of prostate and did not show any correlation between Gleason score and ER Beta expression which was different the present study .[3]

Fixemer et al study concluded that ER beta levels were high in all Adenocarcinoma of Prostate, with majority showing high level expression that is 87% of the adenocarcinoma cases were as ER beta was reduced significantly only in recurrent carcinoma.[80]

Torlakovic et al, stated that ER beta was expressed in 93% of adenocarcinoma of prostate and was associated positively with primary Gleason grade.[5]

Reason for contradicting results in the above studies can be due to the method used for ER β detection, clone of antibody used, the different processing techniques and in some studies different cut off used for positivity and negativity of the marker.

Table 21: Distribution of ER beta expression in Adenocarcinoma cases.

ER BETA SCORE	PRESENT STUDY	GABAL ET AL (2007)[74]
0	**0**	**71.4%**
1-4	**96.6%**	**11.4%**
5-10	**0**	**14.2%**
11-20	**0**	**3%**
>20	**3.4%**	**0**

These finding in the present study were similar to Gabal et al were majority of adenocarcinoma of prostate ER beta positivity was less than and equal to four and since the cutoff of positivity was taken as score of 10 or above, it was considered as negative. [74]

Table 22: Relation of ER beta scores to Gleason score in prostatic adenocarcinoma cases.

Present study		Gleason Grade					
		High		Intermediate		Low	
		Count	%	Count	%	Count	%
ER Beta staining	Negative	11	100.0%	17	100.0%	0	0.0%
	Positive	0	0.0%	0	0.0%	1	100.0%
Gabal et al (2007)[74]		Gleason Grade					
		High		Intermediate		Low	
		Count	%	Count	%	Count	%
ERBeta staining	Negative	15	83.3%	10	100.0%	4	57.1%
	Positive	3	16.7%	0	0.0%	3	42.9%
Asgari et al (2011)[71]		Gleason Grade					
		High		Intermediate		Low	
		Count	%	Count	%	Count	%
ERBeta staining	Negative	4	17.40%	0	0%	0	0.0%
	Positive	19	82.60.%	29	100.0%	0	0.0%

These findings in the present study were similar to that of Gabal et al were majority of the High grade adenocarcinoma of prostate was negative for ER beta expression, while both the study had 100% loss of ER beta expression in Intermediate cases while in cases of low grade adenocarcinoma there was variation in ER beta expression because in a rural center like ours

patients usually present late and hence the present study had only one case of adenocarcinoma.[74]

However Asgari et al, study concluded that majority of the high and intermediate cases showed ER beta positivity, but all the cases that were negative for ER beta expression had a High Gleason score.[71]

Similar to Grover et al, Asgar et al though his study had majority of the adenocarcinoma cases were positive for ER beta .However on careful examination of the data, it revealed mean ER beta score in low and intermediate grade PCa being 68% compared to the 49% in high grade suggesting reduced ER beta expression as grade of PCa increases.[71, 77]

As we had hypothesized our finding strongly suggest that ER beta has antiproliferative role in carcinogenesis of prostate where as ER alpha has a proliferative role on prostatic epithelium. Based on these finding, of the present study was ER beta will be present in non neoplastic and benign lesions compared to neoplastic lesion. Therefore explaining the protective role of ER beta in prostate.[70]

CONCLUSION

CONCLUSION

Elderly age group is most commonly is affected with BPH and Adenocarcinoma of prostate. Our result shows majority of the BPH and all low grade PCa positive for ER beta and all the intermediate and high Gleason grade PCa negative for ER beta. Based on the results of the present study, ER beta seem to play a definitive role in the carcinogenesis. Estrogen beta, is hypothesized involved in the carcinogenesis and neoplastic progression of adenocarcinoma of prostate and underlying pathogenesis of BPH.

Futher scope of the study:

However, multicentric and further genetic work up needs to be done, with larger sample size, to confirm the hypothesis so that it can be used for therapeutic management by antiestrogen beta treatment as a part of personalized treatment.

SUMMARY

SUMMARY

A cross-sectional study was done to determine the expression of beta estrogen receptor in BPH and adenocarcinoma of prostate and correlate the expression of beta estrogen receptor in BPH and different grades of adenocarcinoma of prostate. The following are the salient observations noted in this study.

1) Benign Prostatic Hyperplasia and adenocarcinoma of prostate were common in age group of 51 – 70 years with mean age of BPH and adenocarcinoma of prostate are 54.1 years and 63.9 years respectively.

2) Majority of BPH cases had PSA level less than 10 ng/ml, while all the cases of Adenocarcinoma had PSA level more than 10 ng/ml.

3) Majority of the adenocarcinoma of prostate case had intermediate and high Gleason score because in our set up, patients usually present very late.

4) In BPH cases 93.1% of them were ER beta positive and 6.9% were ER beta negative. However in adenocarcinoma group only 3.4% were ER beta positive and 96.6% were ER beta negative. This observation was statistically significant.

5) In BPH group 6.9% cases had 5 to 10%, 24.1% cases had 11 to 20%, and 69% cases had >20% cells positive for ER beta. This observation was statistically significant.

6) In Adenocarcinoma group 96.6% had loss of ER beta expression. This observation was statistically significant.

7) In BPH group among subjects with PSA ≤10ng/ml, 100% of them were positive for ER beta and among subjects with PSA >10ng/ml, 66.7% were negative and 33.3% were positive for ER beta. This observation was statistically significant.

8) Significant association was observed between ER beta staining and PSA levels in Adenocarcinoma group. None of the subjects had PSA ≤10ng/ml and among subjects with PSA >10ng/ml, 96.6% were ER beta negative and 3.4% were ER beta positive.

9) In Adenocarcinoma, 100% cases with high and intermediate Gleason grade, were negative for ER beta, and 100% of subjects with low grading were positive for ER beta. This observation was statistically significant.

10) In Adenocarcinoma, 100% cases with high and intermediate Gleason grade, 100% had ER beta grade of 1 to 4% and 100% of subjects with low grading had ER beta grading of >20%. This observation was statistically significant

11) Among Adenocarcinoma subjects cases ER beta negative, 100% were in stage II and in ER beta positive subjects 100% were in stage I. This observation was statistically significant in Adenocarcinoma group.

12) In Adenocarcinoma subjects with ER beta grade of 1 to 4%, 100% were in stage II and in subjects with ER beta grading >20%, 100% were in Stage I. This observation was statistically significant.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Garraway WM, Collins GN, Lee RJ. High prevalence of benign prostatic hypertrophy in the community. Lancet 1991; 338:469 -71.

2. Yu CC, Filipe MI. Update on proliferation-associated antibodies applicable to formalin-fixed, paraffin embedded tissue and their clinical applications. J Histochem 1993; 25:843-53.

3. Al Maghrabi JA, Hassan TM, Abdel Meguid TA, Mosli HA. Expression of Estrogen Alpha and Beta Receptors in Prostate Cancer and Hyperplasia: Immunohistochemical Analysis. Afr J Urol 2010;16:79-87.

4. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 1996;93:5925-30.

5. Torlakovic E, Lilleby W, Tarlokovic G, Fossa SD, Chibbar R. Prostate carcinoma expression of estrogen receptor-b as detected by PPG5/10 antibody has positive association with primary Gleason grade and Gleason score. Hum Pathol 2002; 33:646-51.

6. McNeal JE: Prostate. In: Mills SE, ed. Histology for pathologists, ed. 3. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 923-42.

7. Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. Identification of a cell of origin for human prostate cancer. Science 2010;329:568-71

8. Signoretti S, Waltregny D, Dilks J. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000; 157:1769-75.

9. Shah RB, Zhou M, LeBlaanc M. Comparison of the basal cell-specific markers, 34betaE12 and p63, in the diagnosis of prostate cancer. Am J Surg Pathol. 2002; 26:1161-69.

10. Lepor H. Pathophysiology, epidemiology, and natural history of benign prostatic hyperplasia. Rev Urol 2004; 6:53-55.

11. Kristal AR, Arnold KB, Shenk JM. Race/ethnicity, obesity, health related behaviors and the risk of symptomatic benign prostatic hyperplasia: Results from the prostate cancer prevention trial. J Urol 2007; 177:1395-1400.

12. Berry SJ, Coffey DS, Walsh PC. The development of human benign prostatic hyperplasia with age. J Urol 1984; 132:474-79.

13. Sanda MG, Doehring CB, Binkowitz B. Clinical and biological characterization of familial benign prostatic hyperplasia. J Urol 1997; 157:876-79.

14. Briganti A, Capitanio U, Suardi N. Benign prostatic hyperplasia and its aetiologies. Eur Urol Suppl 2009; 8:865–71.

15. Roehrborn CG, Kaplan SA, Noble WD. The impact of acute or chronic inflammation in baseline biopsy on the risk of clinical progression of BPH: results from the MTOPS study. J Urol 2005; 173:346.

16. Nickel JC, Roehrborn CG, O'Leary MP, Bostwick DG, Somerville MC, Rittmaster RS. Examination of the relationship between symptoms of prostatitis and histological inflammation: baseline data from the REDUCE chemoprevention trial. J Urol 2007; 178:896–900.

17. Di Silverio F, Bosman C, Salvatori M. Combination therapy with rofecoxib and finasteride in the treatment of men with lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH). Eur Urol 2005; 47:72–79.

18. Adorini L, Penna G, Amuchastegui S. Inhibition of prostate growth and inflammation by the vitamin D receptor agonist BXL-628 (elocalcitol). J Steroid Biochem Mol Biol 2007; 103:689–693.

19. 3. Petersen RO, Sesterhenn IA, Davis CJ. Urinary bladder. In: Petersen RO, Sesterhenn IA, Davis CJ, editors. Urologic Pathology. 3rd ed. Philadelphia: Lippincott Williams and Wilkins, Wolters Kluwer Health; 2009. p. 195–6.

20. Marinello MJ, Montes W, Farnsworth WE,Hare D ,Fisher B, Bannerman RM. Benign prostatic hyperplasia in an XX man. Urology 1979; 13:640-45.

21. Irani J, Levillain P, Goujon J-M, et al. Inflammation in benign prostatic hyperplasia: Correlation with prostate specific antigen value. J Urol 1997; 157:1301-03.

22. Eble J, Sauter G, Epstein JI, et al. In World Health Organization classification of tumours. Pathology& genetics. Tumours of urinary system and male genital organs. Lyon: IARC Press; 2004:176p.

23. Rohrmann S, Platz EA, Kavanaugh CJ. Meat and dairy consumption and subsequent risk of prostate cancer in a US cohort study. Cancer Causes Control 2007; 18:41-50.

24. Roehl KA, Loeb S, Antenor JA. Characteristics of patients with familial versus sporadic prostate cancer. J Urol 2006; 176:2438-42.

25. Attard G, Clark J, Ambroisine L. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene. 2008; 27:253-63.

26. Thompson IM. Latent carcinoma of the prostate. Eur Urol 2001;39:41-42.

27. Varma M, Lin MW, Tamboli P. Morphologic criteria for the diagnosis of prostatic adenocarcinoma in needle biopsy specimens: A study of 250 consecutive cases in routine surgical pathology practice. Arch Pathol Lab Med 2002;126:554-61.

28. Grignon DJ. Unusual subtypes of prostate cancer. Mod Pathol 2004;17:316-27.

29. Llarena Ibarguren R, Lecumberri Castanos D, Padilla Nieva J. Foamy carcinoma of the prostate. Arch Esp Urol 2003;56:833-35.

30. Arista-Nasr J, Martinez-Benitez B, Valdes S. Pseudohyperplasitic prostatic adenocarcinoma in transurethral resections of the prostate. Pathol Oncol Res 2003;9:232-35.

31. Curtis MW, Evans AJ, Srigley JR. Mucin-producing urothelial-type adenocarcinoma of prostate: Report of two cases of a rare and diagnostically challenging entity. Mod Pathol 2005;18:585-90.

32. Epstein JI. Diagnosis and reporting of limited adenocarcinoma of the prostate on needle biopsy. Mod Pathol. 2004;17:307-15.

33. Samaratunga H, Gardner RA, Yaxley J. Atypical prostatic glandular proliferations on needle biopsy: Diagnostic implications, use of immunohistochemistry, and clinical significance. Anal Quant Cytol Histol 2006;28:104-10.

34. Maufid K, Joual A, Bennani S. Ductal adenocarcinoma of the prostate: Case report and review of the literature. Prog Urol 2004;14:414-16.

35. Rosai J. Rosai and Ackerman's surgical pathology. 9th ed. Philadelphia:Mosby, Elsevier Inc; 2004. p. 1287 -313

36. Egevad L, Allsbrook WC, Epstein JI Jr. Current practice of Gleason grading among genitourinary pathologists. Hum Pathol 2005;36:5-9.

37. Epstein JI, Allsbrook WC, Amin MB. The 2005 International society of Urological Pathology (ISUP) Consessus Conference on Gleason grading of prostatic carcinoma. Am J Surg Pathol 2005;29:1228-42.

38. Epstein JI, Netto GJ. Biopsy interpretation of the prostate. 4th ed. China:Lippincott Williams & Wilkins;2008:192-93.

39. Goldstein NS. Immunophenotypic characterization of 225 prostate adenocarcinomas with intermediate and high Gleason scores. Am J Clin Pathol 2002;117:471-77.

40. Weinstein MH, Signoretti S, Loda M. Diagnostic utility of immunohistochemical staining for p63, a sensitive marker of prostatic basal cells. Mod Pathol 2002;15:1302-08.

41. Adley BP, Yang XJ. Application of alpha-methylacyl coenzyme A racemase immunohistochemistry in the diagnosis of prostate cancer: A review. Anal Quant Cytol Histol 2006;28:1-13.

42. Magi-Galluzi C, Xu X, Hlatky L. Heterogeneity of androgen receptor content in advanced prostate cancer. Mod Pathol 1997;10:839-45.

43. Ryan CJ, Elkin EP, Small EJ. Reduced incidence of bony metastasis at initial prostate cancer diagnosis: Data from CAPSURE. Urol Oncol 2006;24:396-402.

44. Carter HB, Partin AW. Diagnosis and staging of prostate cancer. In: Walsh PC, Retik AB, Vaughn ED Jr, et al. eds. Campbell's Urology, 8th ed. Philadelphia: WB Saunders; 2002. p. 3055-79.

45. Terris MK. Ultrasonography and biopsy of the prostate. In: Walsh PC, Retik AB, Vaughn ED Jr, et al. eds. Campbell's urology, 8th ed. Philadelphia: WB Saunders; 2002. 3038 p.

46. Rasch C, Barillot I, Remeijer P. Definition of the prostate in CT and MRI: a multi-observer study. Int J Radiat Oncol Biol Phys 1999;43:57-66.

47. Stamey TA, Caldwell M, McNeal JE. The prostate specific antigen era in the United States is over for prostate cancer: What happened in the last 20 years. J Urol 2004; 172:1297-1301.

48. Kinoshita Y, Kuratsukuri K, Landas S. Expression of prostate-specific membrane antigen in normal and malignant human tissues. World J Surg 2006; 30:628-36.

49. Scattoni V, Sangalli M, Roscigno M. Detection and diagnosis of prostate cancer: What's new. Arch Ital Urol Androl 2005; 77:173-79.

50. McPherson SJ, Ellem SJ, Simpson ER, Patchev V, Fritzemeier K, Risbridger GP. Essential role for estrogen receptor beta in stromal epithelial regulation of prostatic hyperplasia. Endocrinology 2007 ; 148:566−74.

51. Risbridger GP, Ellem SJ, McPherson SJ. Estrogen action on the prostate gland: a critical mix of endocrine and paracrine signaling. J Mol Endocrinol 2007;39:183−8.

52. Prins GS, Korach KS. The role of estrogens and estrogen receptors in normal prostate growth and disease. Steroids 2008;73:233−44.

53. Weihua Z, Warner M, Gustafsson J. Estrogen receptor beta in the prostate. Mol Cell Endocrinol 2002;193:1−5.

54. Steiner MS, Raghow S. Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk. World J Urol 2003;21:31−6.

55. Mary US, Wenaejusz, Domagula (EDs In Bibbo M(ed). Comprehensive Cytopathology. Philadelphia: W.B. Saunders Company, 1997. p 1043-1071.

56. Jay H.B Histochemistry in Ivan D James L, (EDs) Anderson pathology, Mosby St Louis, 1996.p 136-189.

57. Bancroft JD, Marilyn Gamble (ED) Theory and practice of histological techniques .Churchill Livingstone 2002.p 433-515.

58. Jensen EV,Jacobobson HI. Basic guides to mechanism of action of hormone receptors.Recent Prog Horm Res 1962; 18:387-414.

59. Suzanne A.W Fuqua Rachel Schiff. Disease of breast ed: The biology of ERs-585-590.

60. Li X, Huang J, Bambara RA. Single chain estrogen (ERs) reveal that the ER alpha /Beta hetrodimer emulates functions of ER alpha dimer in genomic Estrogen signaling pathways. Mol Cell Biol 2004; 7681-91.

61. Shoker Jarvis C, Sibso n DR , Wa lke r C, Sloane JP. Oestrogen receptor expression in the normal and pre-cancerous breast. J Pathol 1999; 188:237-44.

62. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996 ; 392:49-53.

63. Gronemeyer H, Laudet V. Transcription factors 3: nuclear receptors. Protein Profile 1995;2:1173-1308.

64. Moore JT, McKee DD, Slentz-Kesler K. Cloning and characterization of human estrogen receptor beta isoforms. Biochem Biophys Res Commun 1998;247:75-78.

65. Chi A, Chen X, Chirala M, Younes M. Differential expression of estrogen receptor beta isoforms in human breast cancer tissue . Anticancer Res 2003;23:211-16.

66. Horvath LG, Henshall SM, Lee C, Head D, Quinn D, Makela S. Frequent loss of Estrogen Receptor-b expression in Prostate Cancer. Cancer Res 2001;61:5331-35.

67. Jason L Nelles, Wen-Yang Hu,Gall S Prins. Estrogen action and prostate cancer. Expert Rev Endocrinol Metab 2011;6:437-51.

68. Prasenjit D, Rodrigo PA. Insight into the mechanisms of action of estrogen receptor b in the breast, prostate, colon, and CNS. J Mol Endocrinol 2013;51:61-74.

69. Paul M, Jiarong Li, Sanjoy S, Cheng C. Prostate Tumorigenesis Induced by PTEN Deletion Involves Estrogen Receptor β Repression. Cell Rep 2015;10:1982–91.

70. Levakov AF, Kacanski MM, Vuckovic N, Zivojinov M, Amidzic J, Sabo JI. The expression and localization of estrogen receptor beta in hyperplastic and neoplastic prostate lesions. Vojnosanit Pregl 2015;72:906–13.

71. Asgari M, Morakabati A. Estrogen Receptor beta expression in prostate adenocarcinoma. Diagn Pathol 2011;6:61.

72. Mann S, Laucirica R, Carlson N, Carlson N, Younes PS, Ali N, Younes A, Li Y, Younes M.. Estrogen receptor beta expression in invasive breast cancer. Hum Pathol 2001;32:113-18.

73. Honma N, Horii R, Iwase T, Saji S, Younes M, Takubo K, Matsuura M, Ito Y, Akiyama F, Sakamoto G.. Clinical importance of estrogen receptor - beta evaluation

in breast cancer patients treated with adjuvant tamoxifen therapy. J Clin Oncol 2008;26:3727-34.

74. Gabal SM, Habib FM, Helmy DO, Ibrahim MF. Expression of Estrogen Receptor-ß (ER-ß) in Bening and Malignant Prostatic Epithelial Cells and its Correlation with the Clinico-Pathological Features. Journal of the Egyptian Nat Cancer Inst 2007;19:239-48.

75. Aslam HM, Shahid N, Shaikh NA, Shaikh HA, Saleem S, Mughal A. Spectrum of prostatic lesions. Int Arch 2013;6:36.

76. .Ghartimagar D, Naik R, Gupta A, Ghosh A. Histopathology Of Prostatic Lesions – An Autopsy Study Of 100 Cases The Internet Journal of Forensic Science 2012;5:1.

77. Grover SK, Agarwal S, Gupta S, Wadhwa N, Sharma N. Expression of Estrogen Receptor β and Ki 67 in Benign & Malignant human prostate lesion by immunohistochemistry. Pathol Oncol Res 2015;21:651-57.

78. Chang WY, Prins GS. Estrogen receptor-beta: implications for the prostate gland. Prostate 1999;40:115–24.

79. Leav I, Ho SM, Ofner P, Merk FB, Kwan PW, Damassa D. Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of Noble rats. J Natl Cancer 1988;80:1045–53.

80. Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ERb) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. The Prostate 2003;54:79–87.

ANNEXURE

ANNEXURE: I

Patient Performa

Serial number: CASE NO:

Hospital number

Name: Age:

PSA Levels:

Histopathology Diagnosis:

BPH

Prostatic adenocarcinoma

Gleason score

Immunohistochemistry:

MARKER	TOTAL NUMBER OF POSITIVE CELLS	TOTAL NUMBER OFCELLS COUNTED	PERCENTAGE OF CELLS POSITIVE	RESULT
ER BETA				

Final impression:

Positive (≤10)

Negative (>10)

ANNEXURE II

KEY TO MASTER CHART

BPH: Benign Prostatic Hyperplasia.

PSA: Prostate specific antigen

ER beta: Estrogen receptor beta

ER grade:

1 to 4%	1
5 to 10%	2
11-20%	3
> 20 %	4

Sl.No	BiopsyNo	Age	Diagnosis	Group	PSALevels	GleasonScore	GleasonGrade	ERBetastaining	ERBeta	ER Grade	Stage
1	B/543/15	59	BPH	1	3			Positive	>20%	4	
2	B/1921/15	54	BPH	1	3			Positive	>20%	4	
3	B/114/15	65	BPH	1	4			Positive	>20%	4	
4	B/1890/15	54	BPH	1	4			Positive	>20%	4	
5	B/230/15	55	BPH	1	5			Positive	>20%	4	
6	B/525/15	63	BPH	1	5			Positive	>20%	4	
7	B/1446/15	51	BPH	1	5			Positive	>20%	4	
8	B/160/16	54	BPH	1	6			Positive	>20%	4	
9	B/1285/15	48	BPH	1	6			Positive	>20%	4	
10	B/77/15	54	BPH	1	7			Positive	>20%	4	
11	B/185/15	56	BPH	1	7			Positive	>20%	4	
12	B/1426/15	58	BPH	1	7			Positive	>20%	4	
13	B/40/15	49	BPH	1	8			Positive	>20%	4	
14	B/222/15	54	BPH	1	8			Positive	>20%	4	
15	B/428/15	58	BPH	1	8			Positive	>20%	4	
16	B/1359/15	55	BPH	1	8			Positive	>20%	4	
17	B/1620/15	47	BPH	1	8			Positive	>20%	4	
18	B/103/15	55	BPH	1	9			Positive	>20%	4	
19	B/512/15	53	BPH	1	9			Positive	>20%	4	
20	B/1555/15	56	BPH	1	14			Positive	>20%	4	
21	B/1123/15	45	BPH	1	4			Positive	12%	3	
22	B/909/15	60	BPH	1	6			Positive	12%	3	
23	B/1198/15	42	BPH	1	9			Positive	12%	3	
24	B/689/15	50	BPH	1	4			Positive	14%	3	
25	B/1496/15	62	BPH	1	4			Positive	14%	3	
26	B/803/15	51	BPH	1	5			Positive	14%	3	
27	B/633/15	57	BPH	1	6			Positive	14%	3	

28	B/1547/15	48	BPH	1	15			Negative	6%	2	
29	B/1831/15	56	BPH	1	15			Negative	6%	2	
1	B/84/15	66	Adenocarcinoma	2	18.57	5+4= 9	High	Negative	3%	1	stage II
2	B/475/15	56	Adenocarcinoma	2	17	3+4= 7	Intermediate	Negative	4%	1	stage II
3	B/1468/14	59	Adenocarcinoma	2	17	3+4= 7	Intermediate	Negative	4%	1	stage II
4	B/1318/15	62	Adenocarcinoma	2	17	3+4= 7	Intermediate	Negative	4%	1	stage II
5	B/688/15	59	Adenocarcinoma	2	20	3+4= 7	Intermediate	Negative	4%	1	stage II
6	B/911/15	62	Adenocarcinoma	2	32.42	3+4= 7	Intermediate	Negative	4%	1	stage II
7	B/2059/15	49	Adenocarcinoma	2	16	2+4= 6	Intermediate	Negative	4%	1	stage II
8	B/1149/16	68	Adenocarcinoma	2	28	5+5= 10	High	Negative	3%	1	stage II
9	B/3056/15	72	Adenocarcinoma	2	34	5+4=9	High	Negative	3%	1	stage II
10	B/2062/15	63	Adenocarcinoma	2	12.25	5+4= 9	High	Negative	3%	1	stage II
11	B/1114/16	65	Adenocarcinoma	2	28	5+3= 8	High	Negative	3%	1	stage II
12	B/1426/16	58	Adenocarcinoma	2	28	5+3= 8	High	Negative	3%	1	stage II
13	B/1306/14	72	Adenocarcinoma	2	29	5+3= 8	High	Negative	3%	1	stage II
14	B/1675/16	62	Adenocarcinoma	2	32	5+3= 8	High	Negative	3%	1	stage II
15	B/530/16	66	Adenocarcinoma	2	26	4+4= 8	High	Negative	3%	1	stage II

16	B/2104/14	69	Adenocarcinoma	2	18	3+5= 8	High	Negative	3%	1	stage II
17	B/1426/16	78	Adenocarcinoma	2	24	3+5= 8	High	Negative	3%	1	stage II
18	B/1240/16	60	Adenocarcinoma	2	32	4+3=8	Intermediate	Negative	4%	1	stage II
19	B/2604/15	60	Adenocarcinoma	2	17	4+3=7	Intermediate	Negative	4%	1	stage II
20	B/1203/16	71	Adenocarcinoma	2	17	4+3=7	Intermediate	Negative	4%	1	stage II
21	B/1823/14	65	Adenocarcinoma	2	17	4+3= 7	Intermediate	Negative	4%	1	stage II
22	B/15/15	69	Adenocarcinoma	2	17	3+4= 7	Intermediate	Negative	4%	1	stage II
23	B/930/16	75	Adenocarcinoma	2	26	3+4= 7	Intermediate	Negative	4%	1	stage II
24	B/978/16	59	Adenocarcinoma	2	16	3+3= 6	Intermediate	Negative	4%	1	stage II
25	B/2808/15	63	Adenocarcinoma	2	16	3+3= 6	Intermediate	Negative	4%	1	stage II
26	B/472/14	70	Adenocarcinoma	2	15	3+2= 5	Intermediate	Negative	4%	1	stage II
27	B/2235/15	54	Adenocarcinoma	2	15	2+3= 5	Intermediate	Negative	4%	1	stage II
28	B/1202/15	61	Adenocarcinoma	2	15	2+3= 5	Intermediate	Negative	4%	1	stage II
29	B/2640/15	61	Adenocarcinoma	2	17.06	2+2= 4	Low	Positive	>20%	4	stage I