ANALYSIS OF IMMUNOHISTOCHEMICAL EXPRESSION OF CD10 IN THE LESIONS OF PROSTATE

Dissertation submitted in
Partial fulfillment of the requirements for the award of

M.D. DEGREE

in

PATHOLOGY – BRANCH III

THE TAMILNADU
DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI - 32

APRIL 2017

DECLARATION

I hereby declare that the dissertation entitled "**Analysis of Immunohistochemical Expression of CD10 in the Lesions of Prostate**" was done by me in the Department of Pathology, Chengalpattu Medical College from June 2012 to May 2016 under the guidance and supervision of **Dr. S. Sasikala M.D**., Associate Professor, Department of Pathology, Chengalpattu Medical College.

This dissertation is submitted to the Tamil Nadu Dr.MGR Medical University, Chennai towards the partial fulfillment of the requirements for the award of M.D. Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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This is to certify that the dissertation entitled "**Analysis of Immunohistochemical Expression of CD10 in the Lesions of Prostate**" is a record of bonafide work done by **Dr. D. Saranya** in the Department of Pathology, Chengalpattu Medical College, Chengalpattu under the supervision of **Dr. S. Ravi M.D.,** Professor and Head, Department of Pathology and submitted in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai. This work has not previously formed the basis for the award of a degree or diploma.

Dr. N. Gunasekaran M.D.,D.T.C.D.,
Dean,
Chengalpattu Medical College,
Chengalpattu

Dr. S. Ravi M.D.
Professor and Head,
Department of pathology,
Chengalpattu medical college
Chengalpattu

CERTIFICATE FROM THE GUIDE

This is to certify that the dissertation entitled, "**Analysis of Immunohistochemical Expression of CD10 in the Lesions of Prostate**" submitted by the candidate **Dr. D. Saranya** in partial fulfillment of the requirements for the award of M.D. Degree in Pathology by The Tamil Nadu Dr.M.G.R. Medical University, Chennai is a bonafide research work done by her under my direct guidance and supervision, in the Department of Pathology, Chengalpattu Medical College, Chengalpattu. This work has not previously formed the basis for the award of any degree or diploma.

Dr. S.Sasikala.,MD.
Associate Professor
Department of Pathology,
Chengalpattu Medical College,
Chengalpattu

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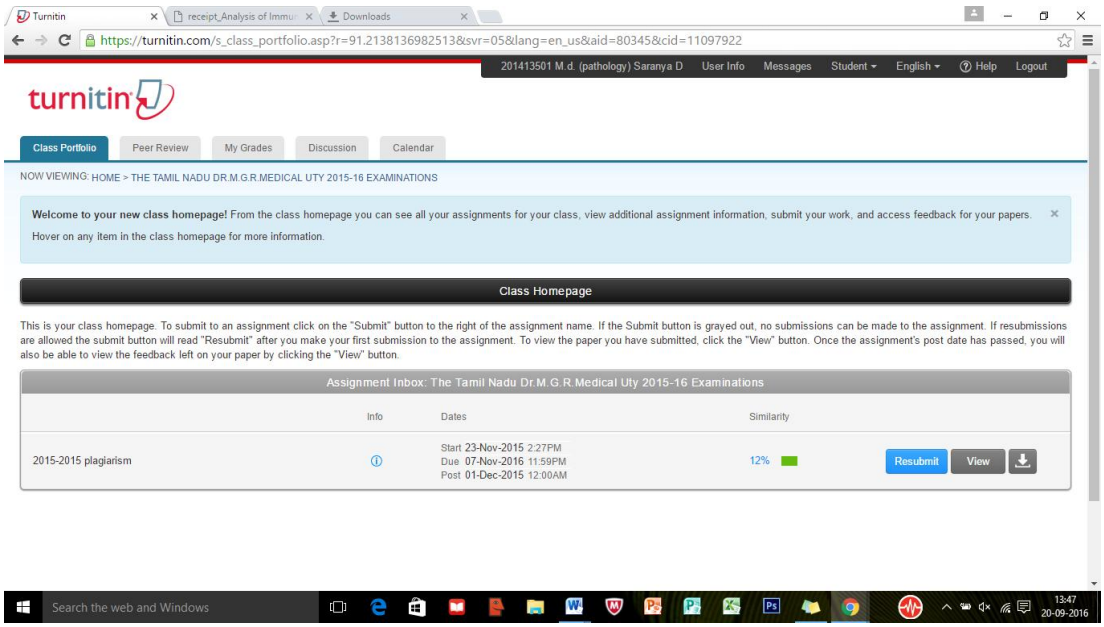
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CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF CHARTS

LIST OF COLOUR PLATES

Fig. no	TITLE
1.	H & E, Benign Prostatic Hyperplasia (BPH), 10x.
2.	H & E, Benign Prostatic Hyperplasia with prostatitis, 10x.
3.	H & E, Prostatic Intraepithelial Neoplasia, 10x.
4.	H & E, Gleason grade 3, 10x
5.	H & E, Gleason grade 4, 10x
6.	H & E, Gleason grade 5, 40x
7.	CD10 positive, BPH,10x.
8.	CD10 positive, Apical membranous staining in benign glands, 40x
9.	CD10 positive, Diffuse membranous staining in benign glands, 40x
10.	CD10 negative, Neoplastic acinar glands, 10x
11.	CD10 negative, Gleason grade 3, 40x
12.	CD10 positive, Gleason grade 4, 10x.
13	CD10 positive, Gleason grade 4, cytoplasmic positivity, 40x.

INTRODUCTION

The lesions of prostate are responsible for significant morbidity and mortality among the males worldwide (1).The age range of males presenting with symptoms due to prostatic lesions is 40- 90 years, with majority of the cases were in the age group of 60 – 70 years (1).

Prostatic lesions are broadly categorized as inflammatory and neoplastic lesions. The neoplastic lesions are inturn subclassified as benign, in situ and malignant lesions .

Prostate cancer is the most aggressive malignant neoplasm with varied clinical presentations. This tumor does not show any warning signs in its early course of development.

The most widely used screening test for detecting prostatic cancer is the measurement of serum Prostate specific antigen (PSA) level , in conjunction with digital rectal examination for all the suspected cases.

Prostate Specific antigen is secreted by normal and malignant prostatic epithelial cells. Therefore their level in the serum increases significantly in men with prostate cancer. Though it gives the suspicion for the underlying tumor, it isnot specific.There are many benign conditions like benign prostatic hyperplasia and prostatitis which increases the serum PSA levels.Therefore it is of at most significance to use a newer marker to identify the prostatic cancer at an early stage.

Cluster of Differentiation (CD) 10, also known as Common Acute Lymphoblastic Leukemia Antigen (CALLA) was first described on human leucocytes (20). Several studies on CD10 revealed that it is not only seen in lymphocytes, but also found to be expressed in other human cells both in normal and in pathological states.

Regarding the prostate gland, CD10 is expressed constantly in the apical luminal surface of the normal prostatic epithelial cells. In various lesions of prostate the pattern of expression varies ranging from altered expression to loss of expression.

In prostatic cells CD 10 acts as a transmembrane peptidase .It plays an important role in the pathogenesis of prostatic cancer. Generally it cleaves the excessive growth factor from the stroma thereby it prevents the continuous and unwanted growth in the luminal epithelial cells.

Literature review shows that loss of CD10 expression is seen in lower Gleason score prostatic tumors whereas increased and altered expression in high Gleason score tumors, lymph node metastasis and in bone metastatic prostatic carcinoma. This concept signifies the use of CD10 as a diagnostic and prognostic marker in prostatic carcinoma.

Based on this we are analysing the expression of CD 10 in a various pathological conditions of prostate.

AIMS AND OBJECTIVES

1. To identify the expression of CD 10 in the lesions of prostate.
2. To analyse the pattern of expression (membranous, cytoplasm, both).
3. To correlate the expression of CD10 with the age of the prostatic carcinoma patients.
4. To correlate the expression of CD10 with histopathological grading and serum PSA level of prostatic carcinoma.

REVIEW OF LITERATURE

ANATOMY:

The prostate is an exocrine gland constituting an important organ of male reproductive system.

It is located in the pelvis just below the urinary bladder encompasses the urethra and in front of the rectum.

It is a walnut shaped organ and its average weight is around 11-16 grams.

Anatomically it is divided into 5 lobes namely anterior, posterior, 2 lateral lobes and one median lobe but widely used terms are three zones namely peripheral zone, central zone, transitional zone.

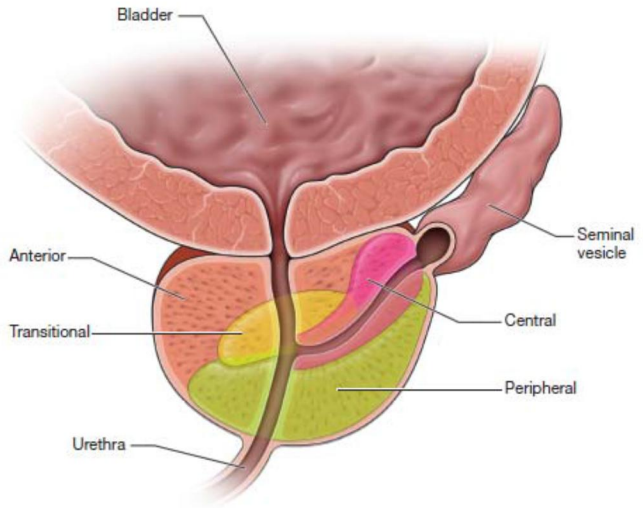


Figure 1: Zones of prostate

The transition zone surrounds the prostatic urethra; central zone that lies posterior to the transition zone encircles the ejaculatory duct. Peripheral zone forms the main bulk of the gland.

Each zone has got its own significance. Prostatic cancer usually arises from the peripheral zone and prostatic hyperplasia from the transition zone.

This anatomical knowledge is important because any lesion in a particular zone can give a clue to the underlying pathology.

VASCULAR AND NERVE SUPPLY:

The arterial supply of prostate gland is through the Internal pudendal artery, inferior vesical artery and branches of middle rectal artery.

The blood from the prostatic gland drains via the vesico prostatic plexsus to the internal iliac veins. These plexus are particularly strong under the puboprostatic ligaments

The autonomic innervation reaches the prostate gland together with the arterial branches and perforates the capsule of the prostate. Parasympathetic signals stimulate glandular activity and the sympathetic innervation of α1-receptors mediates smooth muscle contraction.

The lymphatic vessels of the prostate gland drains to external iliac lymph nodes and internal iliac lymph nodes.

HISTOLOGY:

Prostatic gland is mainly composed of branching duct, acinar glands embedded in dense fibromuscular stroma.

Prostatic glands show mild convolutions. It is lined by 2 layers of epithelial cells. Inner tall columnar cells with basally located nucleus that performs the secretory function and the outer layer of flattened basal cells.

Functions:

The gland secretes milky white colour fluid that constitutes around 30% of volume of the semen.It is alkaline in nature. Its function is to preserve the sperm and maintain its motility, and also neutralizes the acidity of the vagina.

EPIDEMIOLOGY OF PROSTATIC LESIONS:

Benign prostatic hyperplasia is the most common benign neoplastic lesion of prostate of aging men. It poses major public health problem causing significant morbidity thereby affecting quality of life of aging men.

The prevalence of BPH increases with increasing age.It is about 8% in the age group of 30 to40 years and 50% and 80% in the 8^{th} and 9^{th} decade respectively. The risk of developing BPH in men aged 70 -79 years are 4.6 times higher than those of 40 – 49 of age. In India the incidence of BPH is around 92.97%. It has been estimated that the doubling time of BPH growth is

4.5 years around the age group of 35- 50 and 10 years for the age range of 51 – 70(4)

Prostate cancer is the second most common cause of cancer (1) and the sixth leading cause of cancer death among men worldwide (2). The worldwide prostate cancer burden is expected to grow to 1.7 million new cases and 499 000 new deaths by 2030 simply due to the growth and aging of the global population.

The incidence rates of prostate cancer are considered low in Asian and North African countries, ranging from 1 to 9/100,000 persons.The prevalence of prostate cancer in India is far lower as compared to the western countries but with the increased migration of rural population to the urban areas, changing life styles, increased awareness, and easy access to medical facility, more cases of prostate cancer are being picked up. The data regarding the exact incidence of prostate cancer in India is limited mainly because of the fact that it is not a notifiable disease and only limited population based cancer registries are available in India.

In India, when comparing the incidence rates of different cancers, there is a highest incidence of oral cancers and lowest incidence and prostate cancers (3). The estimated incidence of prostate cancer in India is around 3.75/100,000 persons. The incidence rate varies among the major cities in India. It is highest in the metrocities like in Delhi, the rate being 10.9%, in

Chennai it is 4.2% and lowest in northeast India like in Manipur the rate being 0.8%.(4)

ETIOPATHOGENESIS FOR PROSTATIC LESIONS:

The major proven risk factors for prostatic lesions are age, hormonal factors and family history. Considering the importance of prostatic malignancy detailed analysis of risk factors of prostatic carcinoma are as follows.

AGE:

As the age advances the risk for prostate cancer also increases. The risk for prostate cancer begins to rise after 55 years and it peaks at around 70 and declines thereafter (6). According to statistics of United States of America it was estimated that every one in 10,000 men in their 40s and one in 15 men in their 60s will be affected by prostate cancer.

FAMILY HISTORY:

An individual with a positive family history has a significant risk in developing prostate cancer. In a family if there is a first degree relative (brother or father) with prostatic cancer then there is 2 to 3 fold risk for the individual to develop the same. It is further increased by the early age at onsetin relative or multiple relatives with the disease (5).Whole genome or partial genome analysis by linkage mapping studies among the high risk pedigrees revealed many prostatic cancer specific foci.Several studies onfamilial prostate cancer describes the pattern of inheritance of high risk

genes. It states that those high risk genes responsible for cancer follows Mendelian Autosomal dominant expression thereby results in early age of onset of the disease (12)

RACE:

Incidence rate for African American is much higher, around 60 fold when compared to men in Asian countries. This variations are mainly due to the screening programmes, diagnostic advancement, increase accessibility to total health care services etc. Migrants from Asian countries also shows similar incidence rate of prostatic cancer to that of Americans. It is the environmental factors prevailing in that region contributing to the development of cancer (13)

DIET:

Diet constituting large amounts of fat and increased intake of total calories is associated with the increased risk. Reduced intake of antioxidant like selenium, vitamin C, plays a significant role in the development of cancer.

Vitamin D deficiency has been identified as one of the possible risk factors for prostatic carcinoma. Increased age, black race and northern latitudes which was proved as an independent risk factors, are all associated with Vitamin D deficiency.

Possible explanations are increased age is associated with decreased synthesis of Vitamin D and also partly due to decreased exposure to sunlight.

In black race there is more melanin pigments which can directly inhibits the synthesis of vitamin D. When compared to Americans, Asian men have decreased incidence rate of prostatic carcinoma again can be associated with their dietary habits. Asian diet constitute rich in fish that has higher amount of Vitamin D (66).

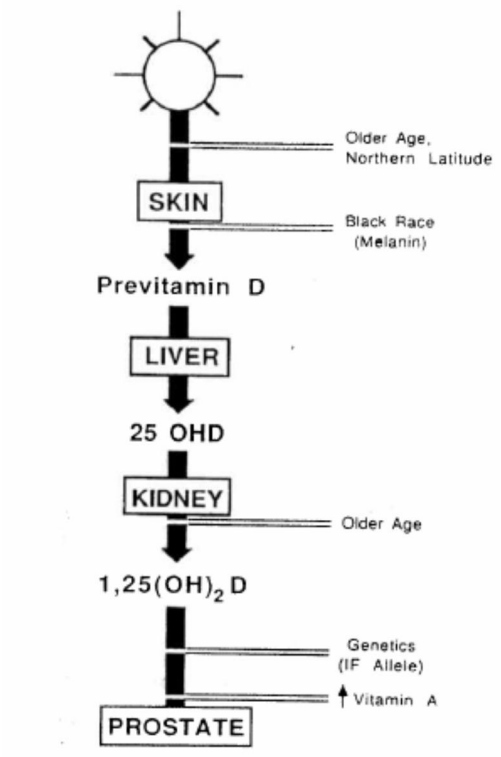


Figure 2: Hypothesised model of prostate cancer in relation to vitamin D deficiency

Other less important factors are anthropometric factors, hormonal profiles, and other co-morbid health factors. They play a minor role in the development and progression of the disease.

ANTHROPOMETRIC FACTORS:

Anthropometric factors like height and obesity and their association with prostate cancer risk has been extensively studied. It was hypothesized that adult height is due to the hormone Insulin like growth factor. This hormone carries significant risk for the development of prostate cancer. Regarding obesity, it was hypothesized that increased obesity reduces sex hormone binding globulins, therefore more availability of free sex hormones in the circulation which can stimulate cancer progression (14). But both the hypothesis has not been proved so far.

Dietary	
Saturated fat	+
Alpha-linolenic acid	+
Red meat	+
Dairy food (and/or calcium)	+
Selenium	-
Lycopene (tomato foods)	-
Vitamin E supplements	-
Legumes (incl. soy)	-
Anthropometric	
Height	+?
Abdominal obesity	+?
Hormonal	
Elevated intraprostatic androgens	+
Elevated IGF-1 (bioactive fraction)	+

Figure 3: List of risk factors in prostate cancer (14)

+ indicates positive association, - indicates inverse association

An increased level of IGF-1 that mediates the action of growth hormone was identified as a independent risk factor for prostate cancer. It was proven that administration of IGF-1 promotes growth of prostate and tumor development in animal models (15)

PATHOGENESIS:

It is the hormone dihydrotestosterone plays a major role in the development of prostatic lesions including benign and malignant lesions.

BPH is characterized by increase in the epithelial and stromal cells commonly in the periurethral zone of prostate.This increased cell number could be due to either increased proliferation of cells or decreased apoptosis (67).

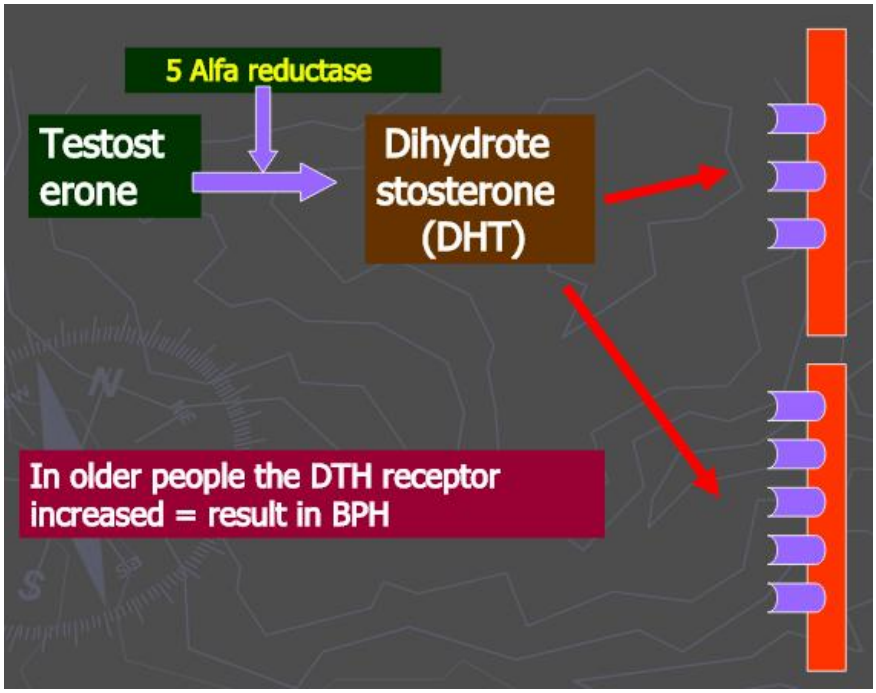


Figure 4 : Pathogenesis of BPH

Dihydrotestosterone (DHT) which is the active metabolite of hormone testosterone is the main androgen responsible for development of BPH.

The stromal cells of prostate gland convert testosterone into DHT through the enzymatic action of 5 Alpha reductase. This DHT is more potent and has more affinity towards Androgen receptor when compared to hormone testosterone. It binds to the androgen receptor (AR) of epithelial and stromal cells thereby stimulating transcription of genes resulting in proliferation of epithelial cells and stromal cells.

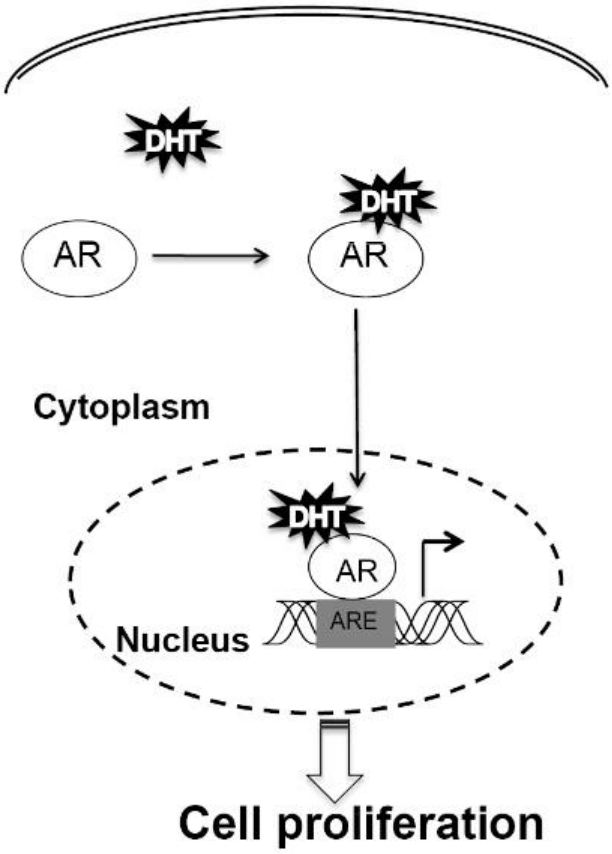


Figure 5: DHT and nuclear transcription.

Though the pathogenesis for the BPH has been well understood, the exact triggering or inciting event is still not clear. One possible hypothesis is that prostatic inflammation could be a triggering factor for the cell proliferation (16). Inflammatory cytokines such as Interleukin 2, Interleukin 6, interleukin 8, interleukin 15 and Interferon alpha causes tissue damage and oxidative stress to the stromal cells. This leads to compensatory cellular proliferation thereby promoting the growth of the gland. (19).

In case of prostatic carcinoma these circulating androgens are essential for the onset of prostate cancer through their interactions with Androgen receptor. Therefore surgical treatment like bilateral removal of testes which is the source for androgens and antiandrogen drugs causes disease regression. But some of the tumors become androgen resistant by following mechanisms:

(I) Androgen receptor gene (AR) amplification results in hypersensitivity to even low levels of androgens.

(II) Mutation in androgen receptor gene causing ligand independent AR activation.

In addition to the androgens, prostatic cancer usually acquires large number of genetic alterations including point mutations, deletions, amplifications and translocations. There are prostate cancer specific chromosomere arrangements. It commonly involves E26 Transformation specific (ETS) gene family. ERG (ETS-related gene product) is the oncogene

which belongs to the ETS family, fuses with Transmembrane protease serine 2 (TMPRSS2) resulting in Androgen independent tumor progression. This results in overexpression of transcription factors that causes upregulation of matrix metalloproteinase. Increased matrix metalloproteinase makes the malignant prostatic epithelial cells more invasive.

Benign prostatic hyperplasia (BPH) and normal epithelium are negative for ERG rearrangements and fusion transcripts. TMPRSS2: ERG fusions are reported in 10 – 21% of high grade prostatic intraepithelial neoplastic lesions (17) and 29 – 59 % in hormone refractory and metastatic prostatic carcinoma. (18)

Other common genetic alterations in prostate cancer includes mutation in BRCA 2 and PTEN tumor suppressor gene, MYC oncogene and at later stages TP53 and RB gene mutations.

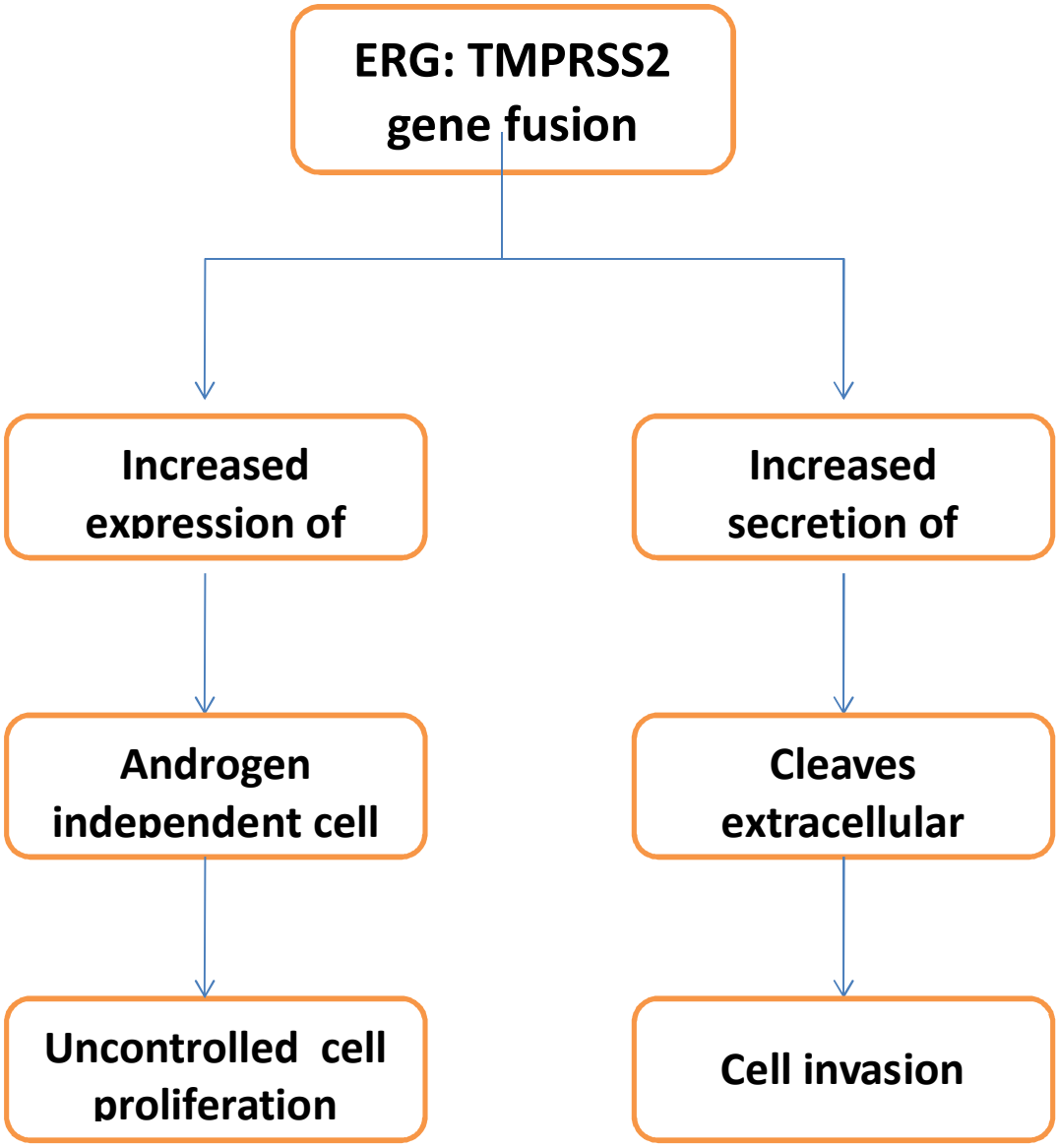


Figure 6: Pathogenesis of prostatic carcinoma

COMMON DISEASES OF PROSTATE:

1. INFLAMMATION:

Inflammation of the prostate is known as prostatitis. Presenceof inflammatory cells in the prostate is commonly seen in the biopsy specimen. Prostatitis is further divided into asymptomatic inflammatory prostatitis, acute prostatitis and granulomatous prostatitis.

Acute prostatits:

It most commonly results from ascending infection from urethra, bladder and epididymis. Patient often presents with acute symptoms like fever, dysuria, urinary urgency intense pelvic pain. Microscopically prostatic biopsy composed of neutrophils in the glandular lumen and macrophages in the stroma. It cannot be diagnosed by histology alone. Often combined clinical picture, urine culture aids in the diagnosis.

Asymptomatic inflammatory prostatitis:

It includes the presence of inflammatory cells like neutrophils, lymphocytesand histiocytes in the prostate but most of the patients are without any clinical symptoms. Since inflammation of the prostate also raises the serum PSA level and since the patient is also asymptomatic it can be clinically misdiagnosed for prostatic carcinoma.Therefore it is important to mention in the biopsy report. Inflammation is commonly associated with benign prostatic hyperplasia.

Granulomatous inflammation:

Granulomatous prostatitis is a rare entity. This inflammation is due to the release of prostatic secretions into the stroma. It elicits the granulomatous reaction in the prostate. This condition is also seen in postbiopsy, after instillation of BCG injection into the bladder for bladder carcinoma patients. Histologically it is composed of granulomas destroying the glands often associated with multinucleated giant cells, histiocytes, lymphocytes and plasma cells. Differential diagnosis could be tuberculosis which often consists of caseating necrosis or could be of fungal etiology, metastatic deposits which are very rare causes.

2. METAPLASIA:

Metaplasia of prostatic epithelium is a secondary phenomenon.It occurs in response to inflammation or injury.Various metaplasias in prostate are squamous, eosinophilic, mucinous, urothelial etc. Reversibility of these metaplasias is very unlikely. These changes are not the preneoplastic conditions.

Squamous metaplasia: It is most commonly an incidental finding. This is mostly a secondary phenomenon due to infarction of the usual nodular hyperplasia, or after post hormonal and radiotherapy for prostatic carcinoma. Histologically it is composed of nests of squamous epithelium with adjacent areas of infarction.The main differential diagnosis could be Squamous cell

carcinoma and Urothelial carcinoma which shows more cytological atypia and stromal invasion.

Mucinous metaplasia: It is very rare entity composed of acini lined by tall columnar cells filled with mucin secretion with basally located nucleus. Clinical significance is that it can mimic adenocarcinoma prostate which should be differentiated by the lack of infiltrative nature, cytological atypia, and presence of basal cells

Urothelial metaplasia: It is defined as urothelial lining the prostatic acini and larger ducts. It is a great mimicker of Urothelial carcinoma spreading via prostatic ducts. Cytologically these two conditions can be differentiated by the presence of nuclear atypia in malignancy and also with the help of immunohistochemistry. Other differential diagnosis is high grade prostatic intraepithelial neoplasia that shows prominent nucleoli.

3. HYPERPLASIA:

Hyperplasia of the prostate includes both epithelial and stromal cell proliferation. It is a benign condition. It includes

a. Usual nodular hyperplasia:

It is the most common microscopic finding in the patient clinically diagnosed of BPH. It commonly arises from the transition zone and periurethralzone. This causes urethral obstruction leading to urinary

symptoms. It is composed of glandular and stromalelements with varied percentage. The glands are more complex with prominent luminal infoldings. Cystic dilatation of glands can also occur.

b. Basal cell hyperplasia:

This subtype includes the proliferation of basal cells. It defines the presence of two or more layers of basal cells with scant cytoplasm around the prostatic ducts and acini.

PROSTATIC INTRAEPITHELIAL NEOPLASIA:

It is the proliferation of epithelial cells with features of atypia in the ducts and acini.

Originally PIN was classified as grade I, II, III, according to increasing degrees of abnormality. Nowadays it is been termed as low grade PIN (grade I) and high grade PIN (grade II, III)

Low grade PIN was formerly known as mild dysplasia. LGPIN mostly do not progress and carries no significance for the subsequent development of prostatic carcinoma. Microscopically the glands show complex architecture with intact basal cell layer and cellular stratification. Cytologically the cells have eosinophilic cytoplasm with enlarged nuclei with increased variability in nuclear size and nuclear hyperchromasia and indistinct nucleoli.

High grade PIN has epithelial cell proliferation in four different architectural patterns: Tufting, micropapillary, cribriform and flat. Regarding the basal cells, high grade PIN shows reduced number of basal cells.

When nuclear features are taken into consideration High grade PIN has increased nuclear atypia and prominent nucleoli.

The significance of reporting high grade PIN is that the patients should be considered for rebiopsy (time interval for rebiopsy not standardised) and they are at increased risk of development of carcinoma.

PROSTATIC CARCINOMA:

Prostatic carcinoma causes significant morbidity among elderly individuals. It has been classified by WHO 2016 as follows.

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF PROSTATE (2016)

EPITHELIAL TUMOURS

Adenocarcinoma(acinar)

- Atrophic
- Pseudohyperplastic
- foamy
- colloid
- Signet ring

- Microcystic variant
- Pleomorphic giant cell adenocarcinoma
- Sarcomatoid carcinoma

Prostatic intraepithelial neoplasia (PIN), High grade

Intraductal carcinoma NOS

Ductal adenocarcinoma

Cribriform

Papillary

Solid

Urothelial tumors

Urothelial carcinoma

Squamous tumors

Adenosquamous carcinoma

Squamous cell carcinoma

BASAL CELL TUMOURS

Basal cell adenoma

Basal cell carcinoma

NEUROENDOCRINE TUMOURS:

Adenocarcinoma with Neuroendocrine differentiation

Well differentiated neuroendocrine tumor

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

MESENCHYMAL TUMOURS

Stromal tumor of uncertain malignant potential

Stromal sarcoma

Leiomyosarcoma

Rhabdomyosarcoma

Chondrosarcoma

Angiosarcoma

Synovial sarcoma

Inflammatory myofibroblastic tumour

Osteosarcoma

Undifferentiated pleomorphic sarcoma

Hemangioma

Chondroma

Leiomyoma

Granular cell tumor

Solitary fibrous tumor

Solitary fibrous tumor , Malignant

HEMATOLYMPHOID TUMORS

Diffuse Large B cell lymphoma

Chronic lymphocytic leukemia/ Small lymphocytic lymphoma

Follicular lymphoma

Mantle cell lymphoma

Acute myeloid leukemia

B lymphoblastic leukemia/Lymphoma

MISCELLANEOUS TUMOUR

Cystadenoma

Nephroblastoma

Rhabdoidtumor

Germ cell tumors

Clear cell adenocarcinoma

Melanoma

Paraganglioma

Neuroblastoma

SECONDARY/METASTATIC TUMOUR

NEWER ENTITY ADDED IN WHO 2016:

Intraductal carcinoma

New variants of acinar adenocarcinoma

Microcystic variant

Pleomorphic large cell variant

Large Cell carcinoma of prostate

Though we have numerous histological classification of prostatic tumor, the term prostatic carcinoma commonly refers to prostatic adenocarcinoma.

Prostatic adenocarcinoma usually arises in the peripheral zone of prostate gland. Grossly the tumor is grey white, irregular or nodular in appearance.

Histologically prostatic adenocarcinoma is characterised by abnormal glandular architectural pattern with single layer of epithelial cells and with absence of basal cells.

Numerous variants of acinar adenocarcinoma have been described. Microcystic variant and Pleomorphic giant cell variant are the newly added entity in WHO 2016 blue book.

VARIANTS OF PROSTATE ACINAR ADENOCARCINOMA:

ATROPHIC VARIANT:

Atrophic variant is a rare entity. The criteria to report this entity is presence of malignant atrophic glands occupying at least 50 % of the tumor. The glands should be of infiltrative nature. The glands are lined by flattened cells with scant cytoplasm and hyperchromatic nuclei with prominent nucleoli. This variant has been graded as Gleason pattern 3 or 4 and grouped under moderately differentiated carcinoma.

PSEUDOHYPERPLASTIC VARIANT:

The glands are benign looking ,dilated with papillary infoldings but show malignant cytological features like enlarged hyperchromatic nuclei with prominent nucleoli. Mostly graded as Gleason's grade 3 and generally has good prognosis. (53)

FOAMY GLAND VARIANT:

The glands will have foamy appearance with round pinpoint nuclei rather than prominent hyperchromatic features. Infiltrative nature of glands proves a diagnostic feature. It has got a good prognosis and mostly comes under Gleason's grade 3 (54)

MUCINOUS VARIANT:

Atleast 25% of the tumor should have aggregates of tumor cells floating in mucin lakes. No significant intracellular mucin is seen.This variant has to be differentiated from metastasis before diagnosing the same. These tumors are given Gleason grade 4 and considered as aggressive variant. Since only few cases have been reported the exact behavior is not known (51)

SIGNET RING VARIANT:

25% of the tumor should have single cells with intracellular mucin vacuole that pushes the nuclei to the periphery.This need to be differentiated from metastasis. It is assigned Gleason grade 5 and has aggressive behavior. It is regarded as high grade adenocarcinoma with poor patient survival. (52)

MICROCYTSIC VARIANT:

It is composed of cystically dilated glands with gland diameter 10 times more than that of malignant acinar glands. Nuclear atypia is difficult to assess

in atrophied cells due to cystic dilatation of the glands. It has been graded as Gleason 2 or 3, with favorable prognosis (55)

PLEOMORPHIC GIANT CELL VARIANT:

It is the highly aggressive variant of adenocarcinoma composed of giant cells with marked pleomorphism, lack of cohesiveness and areas of extensive necrosis (56). It has a poor outcome and generally assigned high Gleason score 9 or 10.

The different architectural patterns forms the basis for Gleason's grading system which forms the important prognostic factor in the management of prostatic carcinoma. It is the most commonly used grading system which consists of five basic grades. The original Gleason's grading system was developed by Dr. Donald Gleason, who is a pathologist worked at Minneapolis Veterans Affairs Hospital. He developed it in the year 1965 (45)

Then it underwent several modifications and popularly called as modified Gleason's grading system. It was first modified by International Society of Urological Pathology (ISUP) consensus meeting in the year 2005 (46). Again in the year 2014 ISUP Prostate Cancer Grading Panel consensus meeting made further alterations.

Grade 1 and 2 encloses well differentiated tumor. It is composed of well circumscribed nodules of closely packed small glands of uniform sizes. The stroma in between the glands is more in case of grade 2.

Grade 3: It is the most common grade and considered as well differentiated grade like grades 1 and 2. It consists of small and irregularly dilated infiltrating individual glands with wide stromal separation. The cells are darker when compared to the normal epithelial cells. WHO 2016 includes microcystic and pesudohyperplastic glands in this grade and removed cribriform glands from this grade.

Grade 4 consists of attempted glandular formation glands fusion, glands with cribriform pattern and glands with glomeruloid pattern.

Grade 5 composed of sheets, cords and in single cells. WHO 2016 also includes linear arrays, solid nests which was not recognized by WHO 2004.

Post hormonal and post radiotherapy tumors are excluded from Gleason's grading.

EVOLUTION OF GLEASON GRADING:

Donald Gleason included cribriform pattern of glands in both grade 3 and grade 4. He doesn't forms a strict criteria to differentiate cribriform pattern of Gleason grade3 from Gleason grade 4.

In 2005 ISUP modified and developed strict criteria to overcome the above difficulties.

Cribriform Grade 3 : It includes small round glands with round contours with evenly spaced round lumens.

Cribriform Grade 4: It includes irregular gland with irregular contours with irreguilar lumens or slit like lumensunder pattern 4.

According to 2014 ISUP modification all the cribriform pattern are considered as grade 4.

Newer modifications in Gleasons grading system in WHO 2016 are as follows:

1. All cribriform patterns are grouped under pattern 4.
2. Glomeruloid pattern gouped under pattern 4.
3. Mucinous adenocarcinoma may be pattern 3 or pattern 4.
4. Intraductal carcinoma not to be graded.

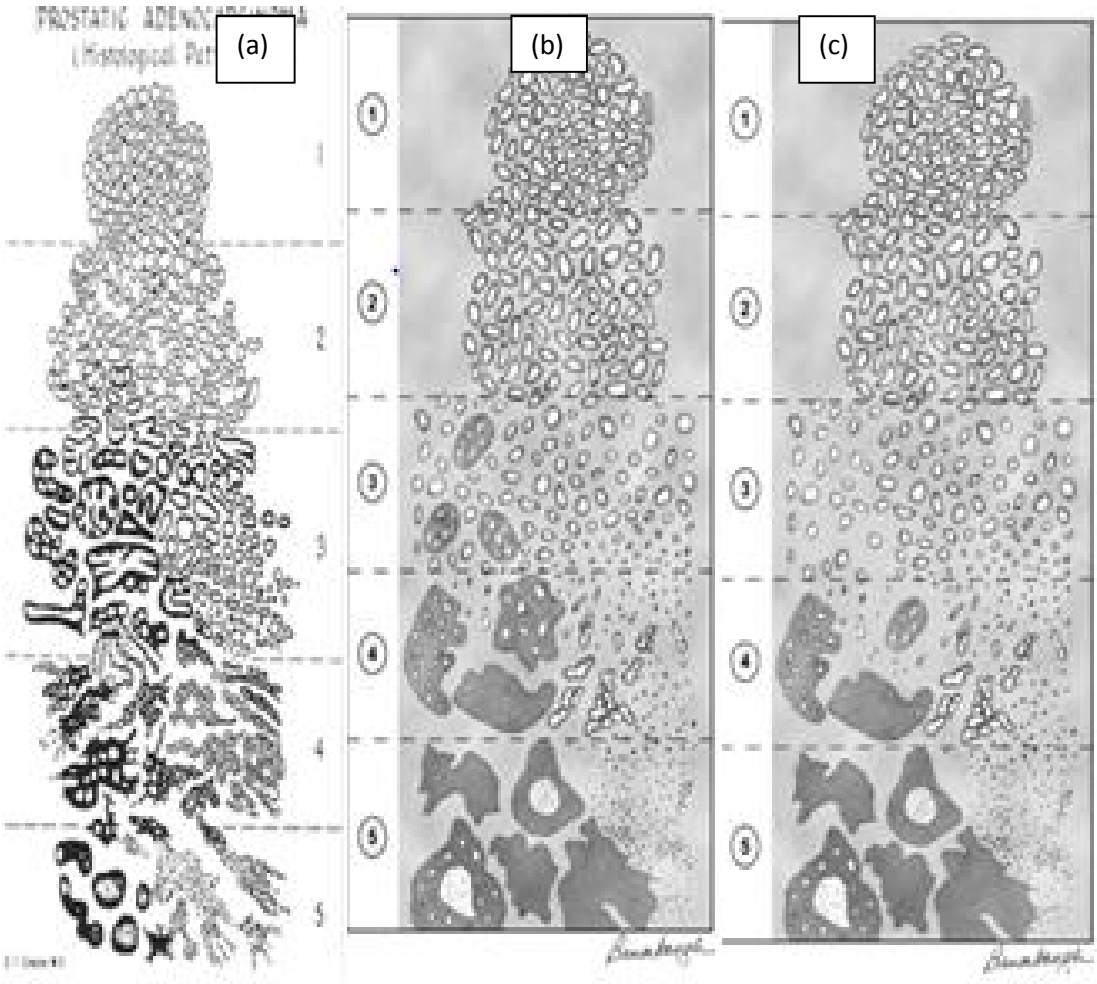


Figure 7: Evolution of Gleason grading system

(a) Original Gleasom grading system

(b) 2005 ISUP Modification of Gleason grading system

(c) 2014 Isup Modificationof Gleason grading system

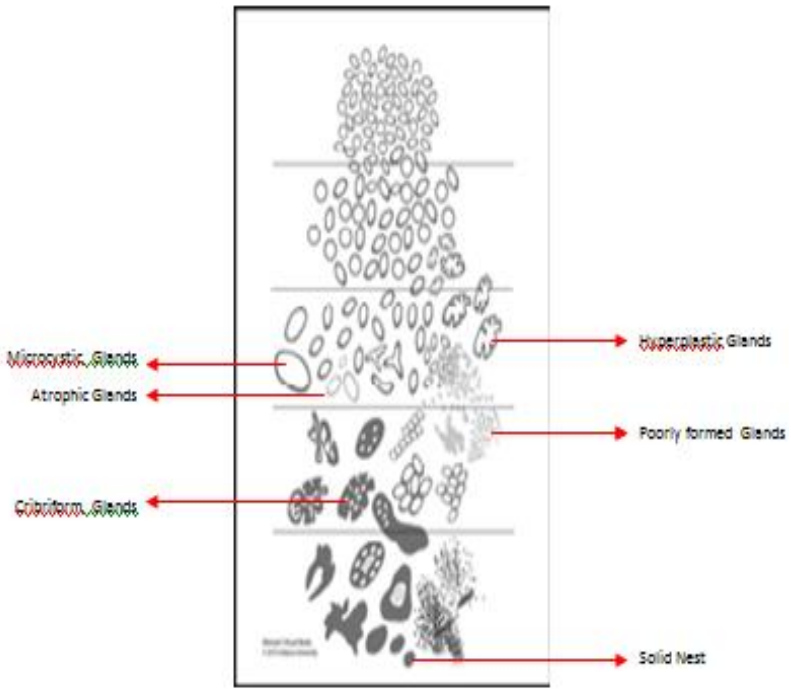


Figure 8 : 2016 WHO modified Gleason grading system.

Reporting of Gleasons grading

Based on the above patterns in H and E sections the tumors are scored.

The most predominant pattern is called as primary pattern and the second most common pattern is known as secondary pattern and the least pattern is known as tertiary pattern.

Generally the primary and secondary pattern is added together for Gleason's scoring in cases of radical prostatectomy and TURP specimens. In case of needle biopsy the most common grade and worst grades are added since underdiagnosis is common in biopsy specimen.

The lowest possible Gleason score is **2** (1 + 1), where both the primary and secondary patterns have a Gleason grade of 1.

The highest possible Gleason score is **10** (5 + 5), when the primary and secondary patterns both have the most disordered Gleason grades of 5.

Based on Gleason's score prostatic adenocarcinoma is divided into well differentiated, moderately differentiated and poorly differentiated tumors.

Gleason's score 2- 6 : Well differentiated tumors, with excellent prognosis.

Gleason's score 7 (3+4): Moderately differentiated tumors.

Gleason's score 7(4+3): Moderately to poorly differentiated tumors.

Gleason's score 8 – 10: Poorly to undifferentiated tumors, aggressive in nature.

Recently based on Gleason'sscore prostatic carcinoma is divided into 5 prognostic groups by ISUP (International Society of Urologic Pathology). The prognostic groups are as follows:

Grade group 1 (Gleason score 3 + 3 = 6): Only individual discrete well-formed glands.

Grade group 2 (Gleason score 3 + 4 = 7): Predominantly well-formed glands with lesser component of poorly formed/fused/cribriform glands.

Grade group 3 (Gleason score 4 + 3 = 7): Predominantly poorly formed/ fused/cribriform glands with lesser component of well-formed glands.

Grade group 4 (Gleason score 8) - Only poorly formed/fused/cribriform glands or predominantly lacking glands and lesser component of well-formed glands

Grade group 5 (Gleason scores 9–10): Lack of gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands.

They analysed that the 5 year risk free survival for grade groups 1 to 5 are 96%, 88%, 63%, 48% & 26% respectively (fig 9)

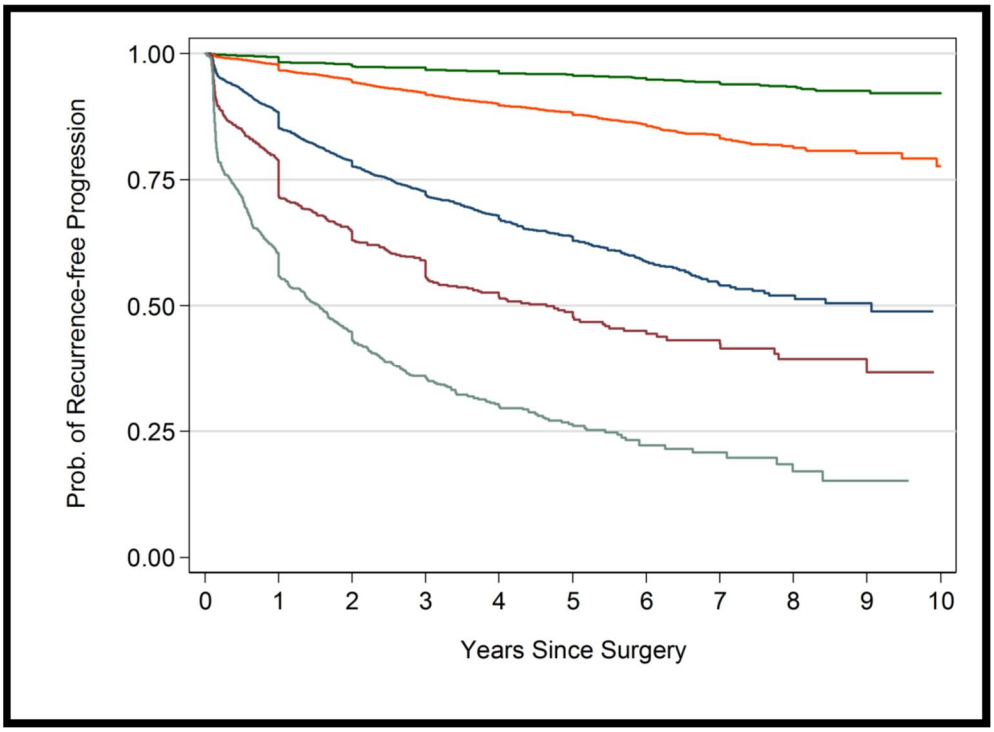


Figure 9: Recurrence-free progression following radical prostatectomy stratified by grade (green line -Gleason score 6 [Grade Group 1], orange –Gleason score 3+4 [Grade Group 2], dark blue -Gleason score 4+3 [Grade Group 3], brown –Gleason score 8 [Grade Group 4], gray –Gleason score ≥9 [Grade Group 5]).

RISK STRATIFICATION:

Based on diagnostic serum PSA level , Gleason score and clinical stage of localized prostate cancer, D'Amico et al in the year 1998 (50) proposed risk stratification that was included in the WHO 2016 Blue book. They provide better options for treatment recommendations than just using stage of cancer alone. It forms the basis for initial treatment for men with prostate cancer and it avoids overtreatment for early stage of cancer (49).

Low risk -Diagnostic PSA <10.0 ng/ml and highest biopsy Gleason score <6 and clinical stage T1c or T2a.

Intermediate risk - Diagnostic PSA >10.0 ng/ml but <20ng/ml or highest biopsy Gleason score =7 or clinical stage T2b.

High risk – Diagnostic biopsy >20 ng/ml or highest biopsy Gleason sc ore >8 or clinical stage T2c /T3.

Thus according to ISUP recommendations It was decided that any biopsy report for prostate carcinoma it is mandatory that it should contain modified Gleason scoring system and Prognostic groups. Treatment options are categorized according to risk stratification.

PROSTATIC INTRADUCATL CARCINOMA (IDC – P)

It is added as a new entity in the recent WHO 2016 classification of prostatic cancers.It is the involvementof preexisting ducats by adjacent high

grade (Gleason 4 and 5) adenocarcinoma. The basal cells are focally preserved.

Rather than infiltrating borders the edges are smooth. It may have any one of the following three architectural and cytological features (57)

1. Solid cribriform architecture – less than 50 % cribriform spaces
2. Loose cribriform architecture> 50 % of lumen formation.
3. Marked nuclear pleomorphism with 6 times larger than the normal and associated with comedo necrosis.

The main differential diagnosis is high grade PIN, which can be distinguished from Intraductal carcinoma Prostate by the absence of nuclear pleomorphism, comedo necrosis.

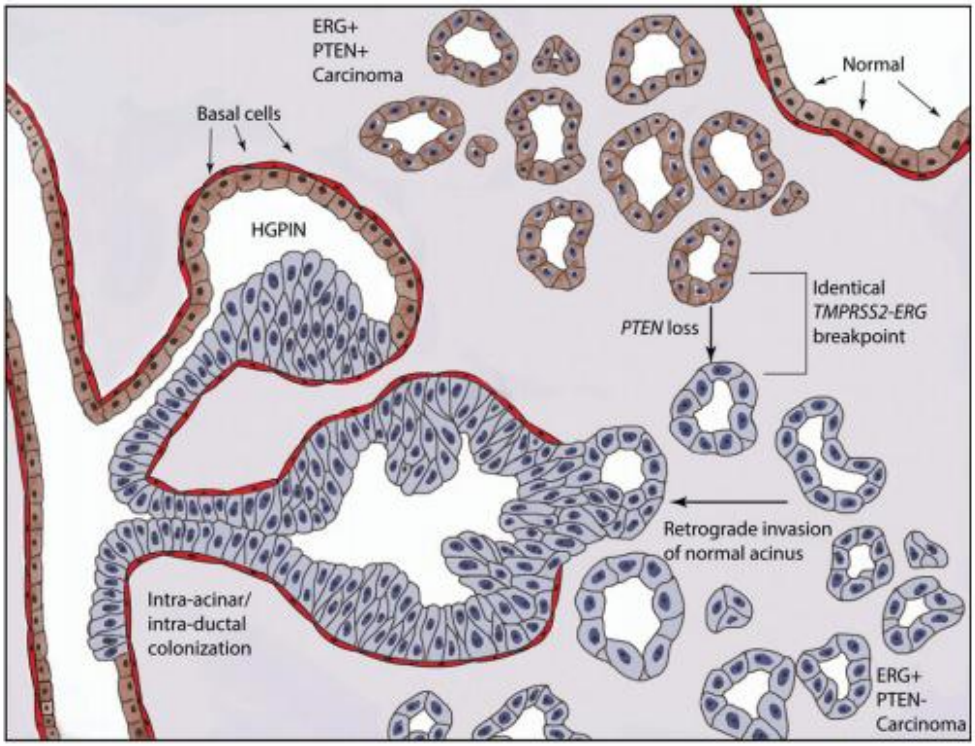


Figure 10: proposed model of intraductal carcinoma

IMMUNOHISTOCHEMISTRY IN PROSTATIC CARCINOMA:

Though predominantly architectural pattern of glands and their cytological features helps in differentiating benign and malignant, immunohistochemistry proves to be a valuable tool in a subset of cases.

There are separate immunostains for basal cells and malignant epithelial cells.

The loss of basal cells is an early and the most important diagnostic feature in prostate carcinomas.. The lack of basal cell layer staining must be validated against the simultaneous demonstration of a positive basal cell layer in adjacentfociof benign glands. Basal cell markers are cytokeratins (CK HMW, CK 5/6, CK 14) and p63.

AMACR (Alpha-methylacyl-CoA racemace) stains the malignant epithelial cells.AMACR is a mitochondrial and peroxisomal enzyme that is involved in beta-oxidation of branched-chain fatty acids. It is expressed in the cytoplasm of malignant epithelial cells.

It is highly sensitive marker showing positivity in both PIN and prostatic carcinoma.

When it is combinedly used with basal cell markers we can significantly increase the diagnostic accuracy and thereby avoiding unnecessary re-biopsies.

Prostate-specific antigen (PSA) is widely used marker to confirm the prostatic origin of metastatic carcinoma.

In case of distinguishing from poorly differentiated prostatic carcinoma and poorly differentiated urothelial carcinoma prostatic carcinoma shows positivity for PSA, PSAP (prostate specific acid phosphatase) and negative for thrombomodulin and Uroplakin. Others newer marker used in diagnosing prostatic origin are Prostein and NKX3.1

CD 10 MARKER:

CD 10 commonly called as CALLA antigen is a neutral endopeptidase present on many cell surface. Their major function is to inactivate biopeptides (20). It is widely used as cell surface marker for categorizing acute leukemia and malignant lymphomas. It is expressed by germinal center B cells, and lymphoid precursor cells. The role and expression of CD10 in non hematolymphoid tissue in both normal and pathological state has been well documented in many literatures.

CD 10 IN NORMAL TISSUE:

Normal Tissue in Which CD10 Antigen Was Detected are myoepithelial cells of breast and in apocrine metaplasia of breast tissue**,** apical surface of normal epithelial cells of small and large intestines (25)**,** glomerular cells and proximal convoluted tubules of kidney**,** Apical surface of large prostate ducts (23)**,** Apical surface of epididymalducts**,** Endometrial stromal cells (26)**,** Bone marrow stromal cells**,** Liver canaliculi (24) and alveolar epithelial cells in lung (21)

CD10 IN HEMATOPOIETIC TUMORS:

CD10 is used as a diagnostic marker in B-lymphoblastic leukemia/ lymphoma and in mature B-cell lymphomas like plasma cell myeloma, follicular lymphoma, diffuse large B-cell lymphoma and Burkitt lymphoma and very rarely in T-cell lymphoma.

CD 10 positivity in B cell lymphoma indicates a good prognosis. Such patients receive less intensified chemotherapy, proving it to be prognostic marker as well.(27)

CD10 IN SOLID TUMORS

From the extensivestudies on CD10, it was revealed that CD10 is notspecific to hematopoietic malignancies but is also expressed by several non hematopoietic tumors such as solid tumors of childhood like nephroblastoma and neuroblastoma (28), and in several carcinomas originating from kidney (30), lung (31), pancreas (33), prostate (34), liver (35), breast (36), stomach (37), cervix (36), and bladder (38), Malignant melanoma (29) and various other skin tumors (32) also show CD10 positivity.

ROLE OF CD 10 IN EPITHELIAL CELLS:

CD 10 is a transmembrane enzyme present over the surface of epithelial cells has got dual functions.

The extracellular portion of CD10 has peptidase enzymatic activity which cleaves the several peptides like endothelin, bombesin, enkephalin etc. These cleaved peptides can either activate or inactivates the stem cells

resulting in either proliferation and differentiation towards the epithelial cell lineage or it can be inhibited from proliferation.

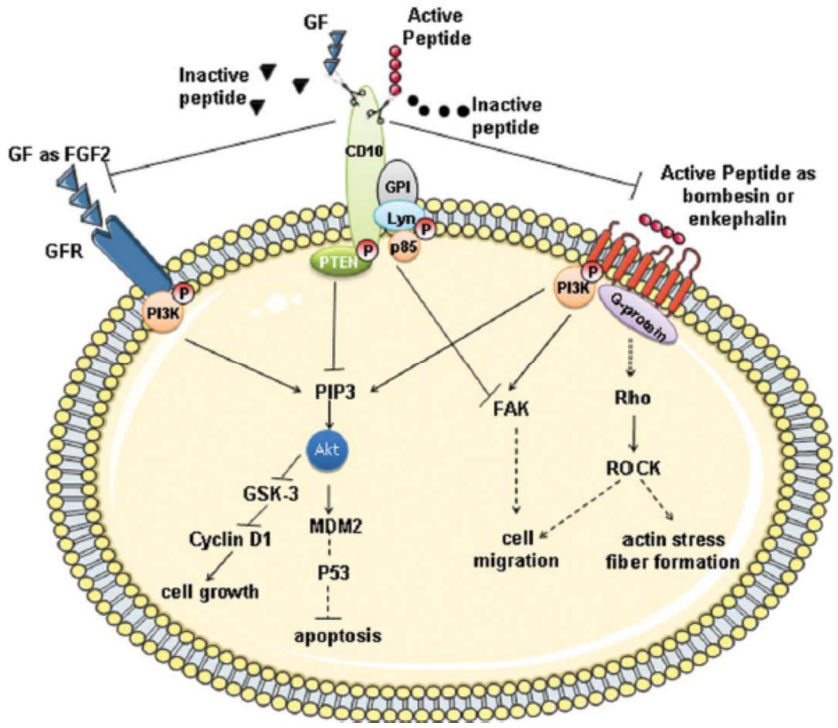


Figure 11: CD10 structure and function

The intracellular portionof CD10 governs major signaling pathway that are required for cell proliferation. Thus it can integrate signals from extracellular and intracellular compartment and can bring important changes in the cells according to the microenvironment present around the cells.

ROLE OF CD 10 IN NORMAL PROSTATE:

In normal prostate gland CD 10 is expressed by luminal epithelial cells. Some studies state that it also expressed by basal cell layer of prostate (39) .This strongly signifies the role of CD 10 that it cleaves excessive peptides thus preventing and controlling the unwanted proliferation of epithelial cells.

ROLE OF CD10 IN PROSTATE CANCER:

CD10 is strongly expressed by normal prostatic luminal epithelial cells. Primary tumors of the Prostate especially adenocarcinoma shows different pattern of expression of CD10 .Low Gleason grade tumors shows loss of expression whereas high Gleason grade tumors shows altered and strong expression. The expression of CD10 is cytoplasmic when compared to apical membranous of normal epithelial cells. Cytoplasmic accumulation of CD10 may activate signaling pathway constantly leading to uncontrolled proliferation and invasion. But the hypothesis has not been proved yet (60)

MOLECULAR ANALYSIS OF CD 10 EXPRESSION IN PROSTATE:

The neutral endopeptidase or CD10 gene was analysed by several molecular studies including tissue microarray, human cell lines study in vitro and in vivo etc. It was identified that transcription of this gene is androgen hormone mediated in prostate cancer cells. They have also identified in the NEP gene an androgen responsive element (NEP-ARE) and an androgen responsive region (NEP-ARR) suggesting the role of androgen ion their expression. Thus both the regions are involved in the transcriptional activation of CD10. **(**41).

After transcription, translation of CD10 is enhanced by methylation of gene promoter (42). There is evidence of hypermethylation of gene promoter in case of high grade carcinoma suggesting a role of promoter region

mutation. So far no studies have demonstrated the mutations in CD10 gene per se.

It has been well analysed that CD 10 is expressed in the membranes of prostatic cells. High grade tumors of prostate showed cytoplasmic positivity and low grade tumors shows absence of expression. This variation in cytoplasmic localization of CD10 possibly due to following mechanisms:

1. Internalization of membrane bound CD10 (43)
2. Strong association of CD10 with intracytoplasmic heat shock proteins.(44)

The reason for absence of its expression in low grade tumors is not well understood.

Most lymph node metastases of prostatic carcinomastrongly express CD10 positivity in the malignant epithelial cells thus contributing to the fact that it could be involved in the pathogenesis of lymph node metastasis.(39).

High CD10 expression directly correlates with advanced Gleason score, and thus tumor expressing CD10 may be considered aggressive tumors with poor pathological outcome.

Because of the strong association of CD10 with high grade tumors and in lymph node metastasis one can consider the possibility that CD10 can be used to categorise the lesion as aggressive and such patients can be closely followed up for lymph node metastasis.

Anti CD10 drug therapy can also be used to treat the patients, if its exact role in the pathogenesis is identified.

An immunohistochemistry based test can be used in the clinical setting to identify CD10-positive tumors on prostate needle biopsies, which may warrant more aggressive initial therapy or closer surveillance post-operatively.

A number of drugs against CD10 are available and potential targeted therapies could be formulated based on these drugs, including monoclonal antibody mediated-delivery of chemotherapy.

MATERIALS AND METHODS

Study Place: Department of Pathology, Chengalpattu Medical College and Hospital, Chengalpattu.

Study Design: The present study is an observational study conducted in the Department of Pathology during the period of June 2012 to May2016 .

Ethical clearance for the study was obtained from the Institutional Ethics Committee of Chengalpattu Medical College, Chengalpattu.

A total sample of 40 cases of prostatic lesions was analyzed during the period of June 2012 to May 2016.

Study Population

INCLUSION CRITERIA

Tissue blocks of patients who are diagnosed as having benign and malignant prostatic lesions.

EXCLUSION CRITERIA:

Tissue blocks of patients who are diagnosed as prostatic carcinoma and underwent preoperative Radiotherapy or Chemotherapy.

During the period of June 2012 to May 2016, as per the inclusion and exclusion criteria, biopsies received in the Department of Pathology were included.

History written in the histopathology request form was recorded on predesigned and pretested proforma (Annexure I).

MATERIALS USED

Tissue sections prepared from paraffin embedded formalin fixed tissues

Haematoxylin and eosin staining kit

CD 10 monoclonal antibody kit

Secondary antibody kit

Positive control

Negative control

METHOD:

Formalin fixed paraffin embedded blocksand haematoxylin eosin stained sections of 40 prostatic biopsies are taken up for the study. On histopathological examination, they were categorized as follows:

1. Benign prostatic hyperplasia,
2. Benign prostatic hyperplasia with prostatitis
3. Prostatic intraepithelial neoplasia high grade and low grade,
4. Prostatic adenocarcinoma.

Prostatic adenocarcinoma was assigned Gleason grade ranging from grade 1 to grade 5 according to modified Gleason grading system.

Immunohistochemistry was performed on the tissue sections taken from the blocks along with positive and negative control.

Immunohistochemistry

Procedure

1. 4µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. Rehydrated through descending grades of alcohol as follows :

   (i)Absolute alcohol x 2 changes 5 minutes each

   (ii) 90% alcohol x 5 minutes

   (iii) Washed in distilled water 2 changes, 2 minutes each
5. Heat induced antigen retrieval was done with microwave oven at 150 degree Celsius with citrate buffer (pH 6.0) for 15 to 20 minutes.
6. Then cooled for 10 minutes.
7. Washed in distilled water 2 changes, 2 minutes each.
8. Washed in Tris Buffer Saline (TBS) for 2 minutes.
9. Endoperoxidase blocking was done by adding hydrogen peroxide on the section and kept for 5 minutes.
10. Washed in the wash buffer for 2 minutes twice.
11. Primary antibody CD 10(Mouse monoclonal;prediluted) was added and kept for 30 minutes in a moist chamber.
12. Then washed in wash buffer 2 minutes 2 times each.
13. Poly excel target binder reagent was added and kept for 15 minutes.

14. Washed in two changes of buffer 2 minutes each.
15. Poly excel HRP (Horse Radish Peroxidase) was added and incubated for 15 minutes.
16. Washed with buffer – 2 minutes, 2 changes.
17. Working DAB Dchromogen (1ml DAB buffer + 1 drop chromogen, mix well) was added and kept for 2-5 minutes.
18. Then washed in distilled water.
19. Counter stained with hematoxylin for 30 seconds.
20. The slides were washed in running tap water for 3 minutes.
21. The slides were air dried, cleared with xylene and mounted with DPX.

Positive control included blocks containing normal prostatic gland-internal control Negative control included Primary antibody replaced with PBS.

Immunostained sections were reviewed for CD 10 expression

CD10Expression:

1. CD 10 immunoreactivity was observed and assigned as positive or negative.
2. CD 10 immunreactivity was analysed for pattern of expression.

Pattern of expression as follows:

1. Apical membranous positivity
2. Diffuse membranous positivity
3. Membranous and cytoplasmic positivity
4. Cytoplasmic positivity
5. Negative

STATISTICAL ANALYSIS

Datas obtained were coded and entered into the Microsoft excel spread sheet (Annexure II). Datas were compared between groups using Pearson Chi-square or Fisher‘s exact tests ($p<0.05$).

All statistical analysis was performed using SPSS statistical software version 11. Charts were prepared using Microsoft excel 2007.

OBSERVATION AND RESULTS

We took a sample size of 40. Among the 40 cases .We have the following distribution

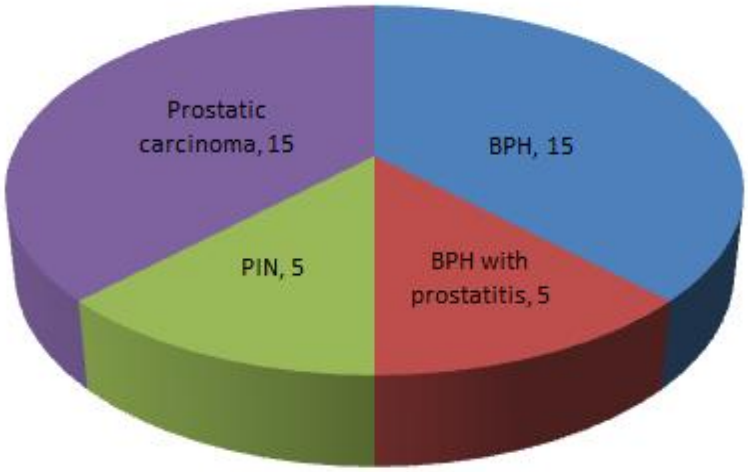
15 cases - Benign prostatic hyperplasia

15 cases -Prostatic adenocarcinoma

5 cases - Benign prostatic hyperplasia with prostatitis,and

5 cases - Prostatic intraepithelial neoplasia.

The case distribution is represented as follows



AGE WISE DISTRIBUTION OF PROSTATIC LESION:

The age wise distribution of prostatic lesions in the present study showed the following observations.

For benign prostatic hyperplasia majority of the patients are in the age group of 60 – 80 years.

Benign prostatic hyperplasia with prostatitis, 70 – 80 years

Prostatic intraepithelial neoplasia - 60 – 80 years

Prostatic adenocarcinoma - 60 – 70 years.

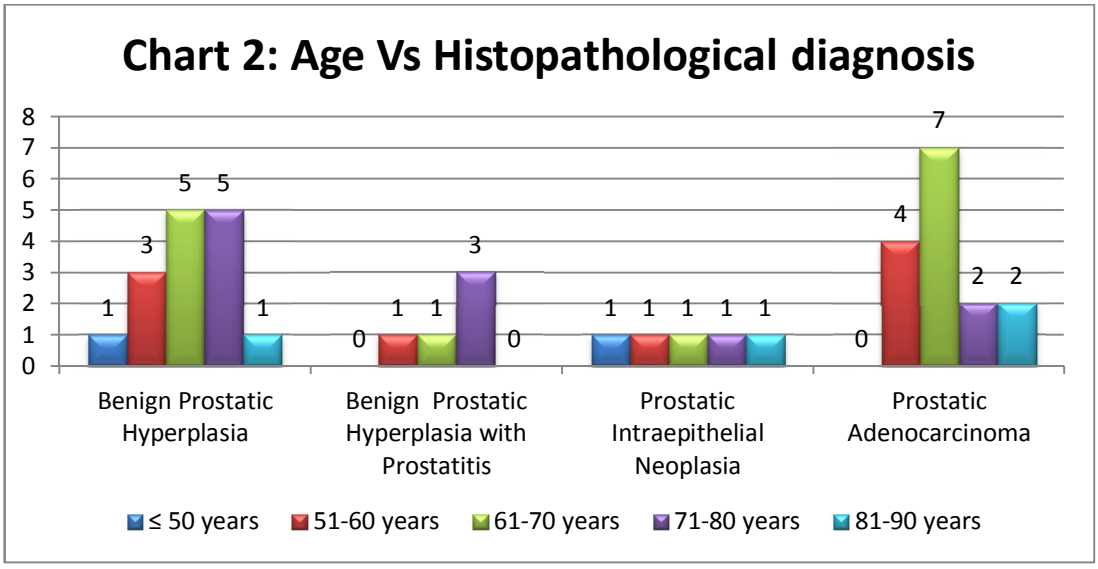
The mean age group for various lesions is as follows

Benign prostatic hyperplasia–67years

Benign prostatic hyperplasia with prostatitis-70years

Protatic intraepithelial neoplasia –66yearsand

Prostatic carcinoma -68years.



Age Vs HPE	Benign Prostatic Hyperplasia	%	BPH with Prostatitis	%	Prostatic Intraepithelial Neoplasia	%	Prostatic Adenocarcinoma	%
≤ 50 years	1	6.67	0	0.00	1	20.00	0	0.00
51-60 years	3	20.00	1	20.00	1	20.00	4	26.67
61-70 years	5	33.33	1	20.00	1	20.00	7	46.67
71-80 years	5	33.33	3	60.00	1	20.00	2	13.33
81-90 years	1	6.67	0	0.00	1	20.00	2	13.33
Total	15	100	5	100	5	100	15	100

Table 1: Age and histopathological diagnosis

HPE Distribution	Benign Prostatic Hyperplasia	BPH with Prostatitis	Prostatic Intraepithelial Neoplasia	Prostatic Adenocarcinoma
Mean	67.93	70.80	66.80	68.47
SD	10.26	7.85	12.83	9.53
P value Single Factor ANOVA Test				0.9307

GLEASON SCORE AND PROSTATIC ADENOCARCINOMA :

The prostatic carcinoma cases were scored according to Gleason score as<3+3, 3+4, 4+3, >4+4. Their distribution as follows:

Table 4: Gleasons score and prostatic carcinoma.

Gleason Score	Prostatic Adenocarcinoma	%
<3+3	6	40.00
3+4	2	13.33
4+3	3	20.00
>4+4	4	26.67
Total	15	100

6 cases were having the score of <3+3

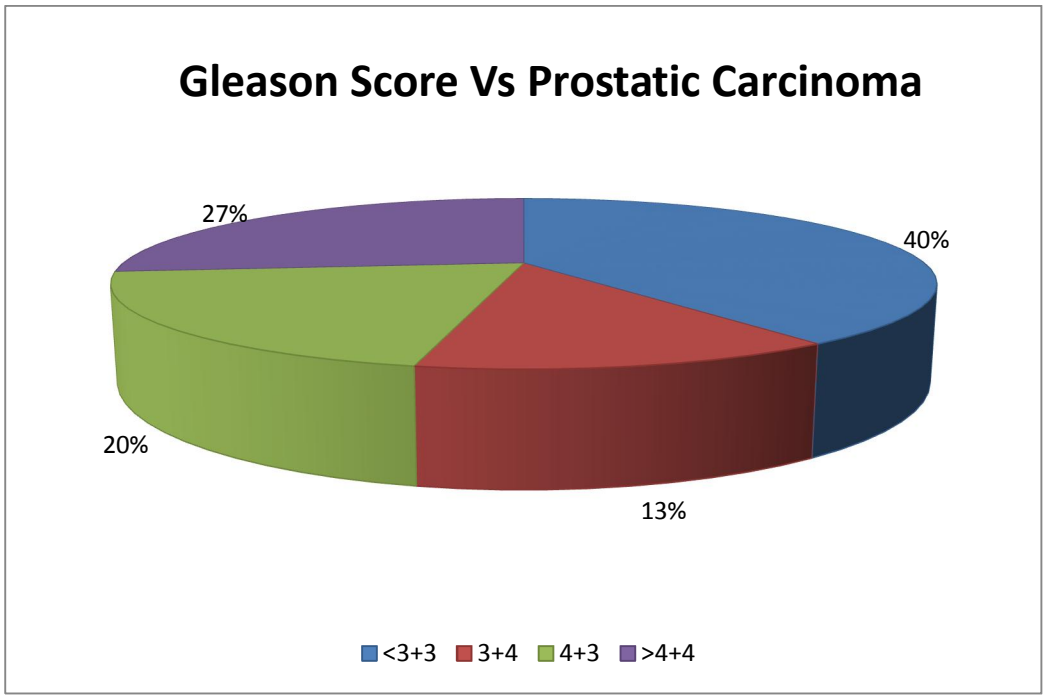
2 cases were having the score of 3+4

3 cases were having the score of 4+3

4 cases were having the score of >4+4 .

Their percentage is represented as follows:

Among the study cases 40% showed Gleason score <3+3, 26% showed Gleason score >4=4, 20 % showed with Gleason score 4+3, and 13.33% showed with Gleason score 3+4.

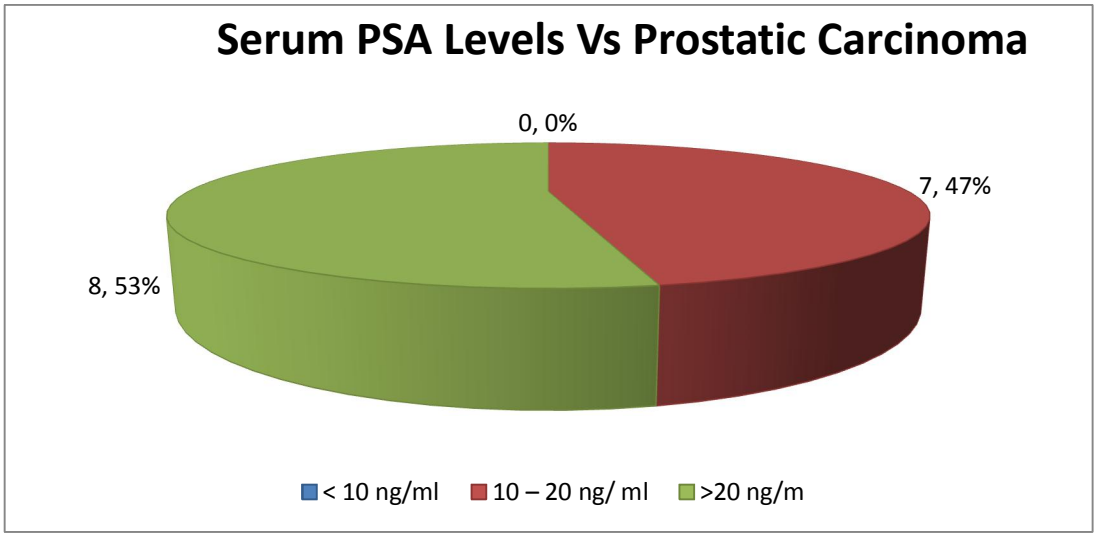


SERUM PSA LEVEL AND PROSTATIC CARCINOMA:

We collected Serum PSA level from the patients with prostatic carcinoma. The cases were categorised as follows

Table 5: Serum PSA level vs prostatic carcinoma

Serum PSA Levels	Prostatic Adenocarcinoma	%
< 10 ng/ml	0	0.00
10 – 20 ng/ ml	7	46.67
>20 ng/m	8	53.33
Total	15	100



In our present study,

There were no cases under <10 ng/ml,

There were 7 cases under the range of 10 – 20 and

There were 8 cases coming under range of >20 ng /ml.

The percentage of cases are 46.67% in the range of 10 – 20ng/ml, and 53.33% for >20 ng/ml.

SERUM PSA LEVEL AND GLEASON SCORE:

Analysing serum PSA levels with Gleason score we had the following results

Serum PSA level 10 – 20ng/ml,

Gleason score <3+3 - 83.33%

Gleason score 3+4- Nil cases

Gleason score 4+3 - 33.33% and

Gleason score>4+4. - 25%

For serum PSA level more than 20 ng/ml, we have the following percentage

Gleason score<3+3 - 16.67%

Gleason score 3+4- 100%

Gleason score 4+3 - 66.67% and

Gleason score>4+4. - 75% .

Using fischers exact test increases serum PSA level shows strong association with high Gleason score with the P value being <0.001

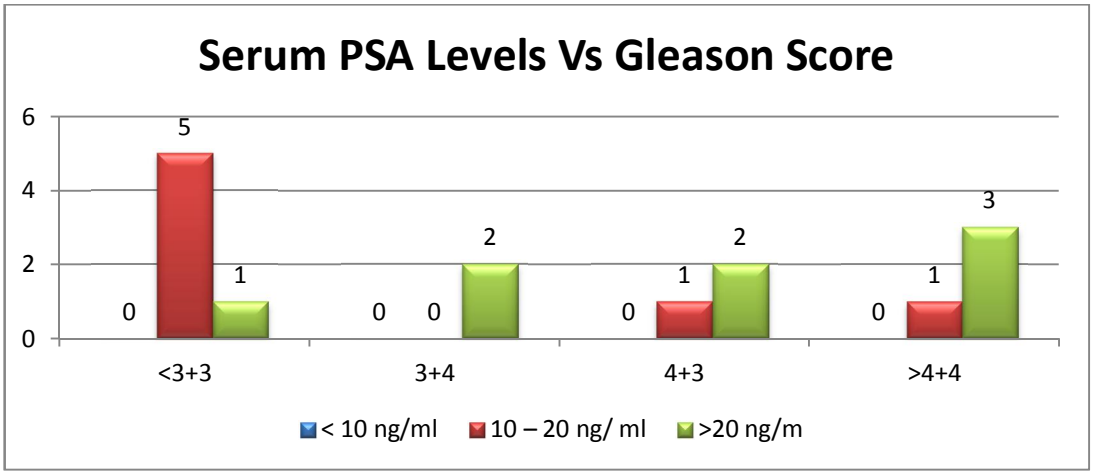


Table 6: Serum PSA level vs Gleason score

Serum PSA Levels Vs Gleason Score	<3+3	%	3+4	%	4+3	%	>4+4	%
< 10 ng/ml	0	0.00	0	0.00	0	0.00	0	0.00
10 – 20 ng/ ml	5	83.33	0	0.00	1	33.33	1	25.00
>20 ng/m	1	16.67	2	100.00	2	66.67	3	75.00
Total	6	100	2	100	3	100	4	100
P value Fishers Exact Test							<0.0001	

AGE AND GLEASON SCORE:

In our present study we analysed the age wise distribution of Gleason score. The distribution and mean age group for the different gleason score are as follows.

For Gleason score<3+3 the mean age group is 68 years

For Gleason score 3+4 the mean age group is 62 years

For Gleason score 4+3 the mean age group is 66 years

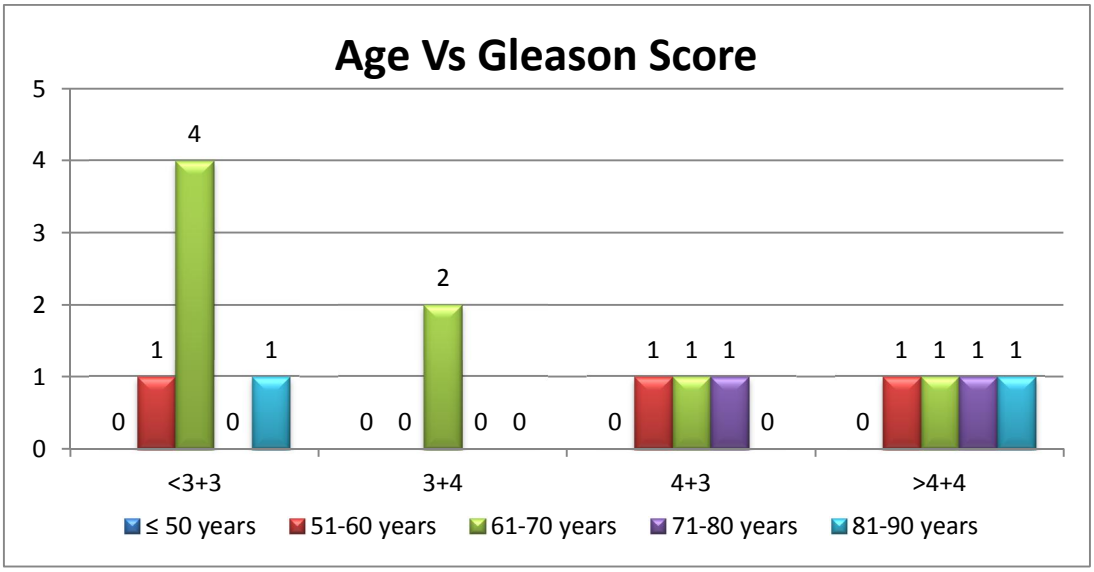
For Gleason score >4+4 the mean age group is 73 years.

Age Vs Gleason Score	<3+3	%	3+4	%	4+3	%	>4+4	%
≤ 50 years	0	0.00	0	0.00	0	0.00	0	0.00
51-60 years	1	16.67	0	0.00	1	33.33	1	25.00
61-70 years	4	66.67	2	100.00	1	33.33	1	25.00
71-80 years	0	0.00	0	0.00	1	33.33	1	25.00
81-90 years	1	16.67	0	0.00	0	0.00	1	25.00
Total	6	100	2	100	3	100	4	100

Table 7: Age vs Gleasons score

Age Vs Gleason Score Distribution	<3+3	3+4	4+3	>4+4
Mean	68.33	62.50	66.67	73.00
SD	10.29	3.54	7.64	12.25
P value Single Factor ANOVA Test			0.8036	

Gleasons score <3+3 and 3+4 Gleason score was observed predominantly in the age group of 61 – 70 years constituting 66.67% and 100% respectively.Gleason score 4+3 and 4+4 shows equal distribution of age groups each constituting about 33.33% and 25 % respectively..



DIFFERENT PROPORTION OF GLEASON GRADE AMONG PROSTATIC CARCINOMA:

We also analysed the percentage of Gleason grade 2, 3, 4 and 5 among the total sample of 15 malignant lesions. Their percentage are as follows

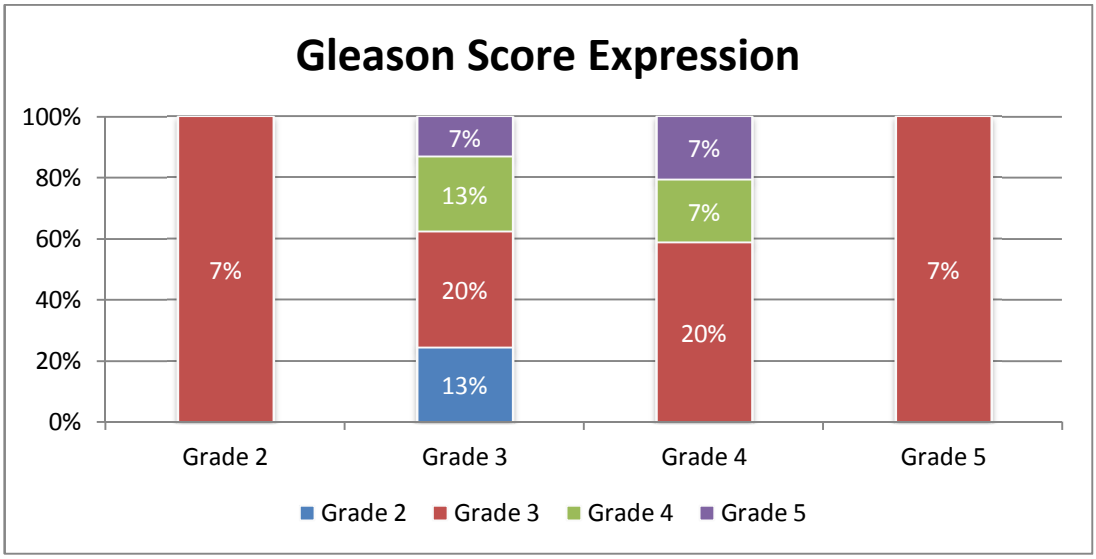
Grade 2 – 7%

Grade 3 – 53%

Grade 4 – 34%

Grade 5 – 7%

In our study Gleason pattern 3 form major proportion of cases.



We also analysed the different combinations among various Gleason pattern. The results are as follows:

Table 8: Different combination of Gleason pattern 2,3,4,5

Gleason Score Expression	Grade 2	%	Grade 3	%	Grade 4	%	Grade 5	%
Grade 2	0	0%	1	7%	0	0%	0	0%
Grade 3	2	13 %	3	20 %	2	13 %	1	7%
Grade 4	0	0%	3	20 %	1	7%	1	7%
Grade 5	0	0%	1	7%	0	0%	0	0%

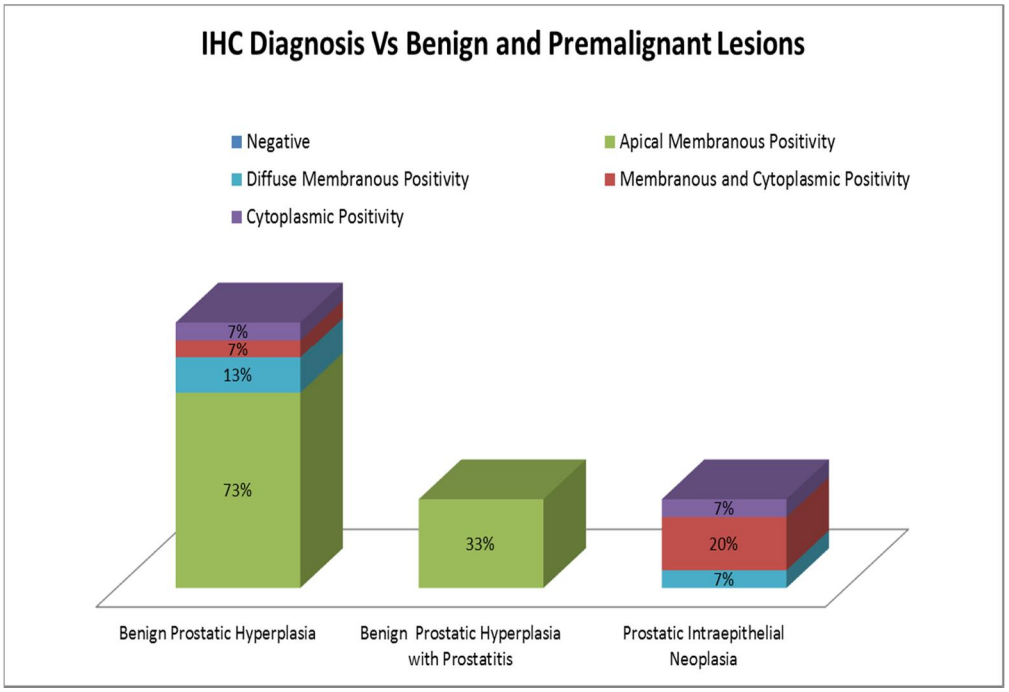
IMMUNOOHISTOCHEMICAL EXPRESSION IN BENIGN AND PREMALIGNANT LESIONS:

Regarding the immunohistochemical expression of CD10 in benign and premalignant lesions the results are as follows. In case of benign prostatic hyperplasia 73% showed apical membranous staining whereas only 13% showed diffuse membranous staining and 7% cytoplasmic staining. The

staining pattern is only apical membranous in all the 5 cases of BPH with prostatitis. In case of PIN 20% showed diffuse membranouspositivity, predominantly around 60 % showed both membranous and cytoplasmic positivity and 20 % showed only cytoplasmic positivity.. This showed a significant correlation with p value <0.0021

Table 3: Immunohistochemical diagnosis vs benign and premalignant lesions

IHC Diagnosis Vs Benign and Premalignant Lesions	Benign Prostatic Hyperplasia	%	Benign Prostatic Hyperplasia with Prostatitis	%	Prostatic Intraepithelial Neoplasia	%
Negative	0	0.00	0	0.00	0	0.00
Apical Membranous Positivity	11	73%	5	100.00	0	0.00
Diffuse Membranous Positivity	2	13%	0	0.00	1	20.00
Membranous and Cytoplasmic Positivity	1	7%	0	0.00	3	60.00
Cytoplasmic Positivity	1	7%	0	0.00	1	20.00
Total	15	100	5	100	5	100
P value Fishers Exact Test				0.0021		



GLEASON GRADE AND CD10 EXPRESSION:

Having been categorized the malignant lesions of prostate according to the age, Gleason score, and serum PSA levels, we also analysed the immunohistochemical pattern of expression of CD 10 and its significance with different Gleason score and serum PSA levels.

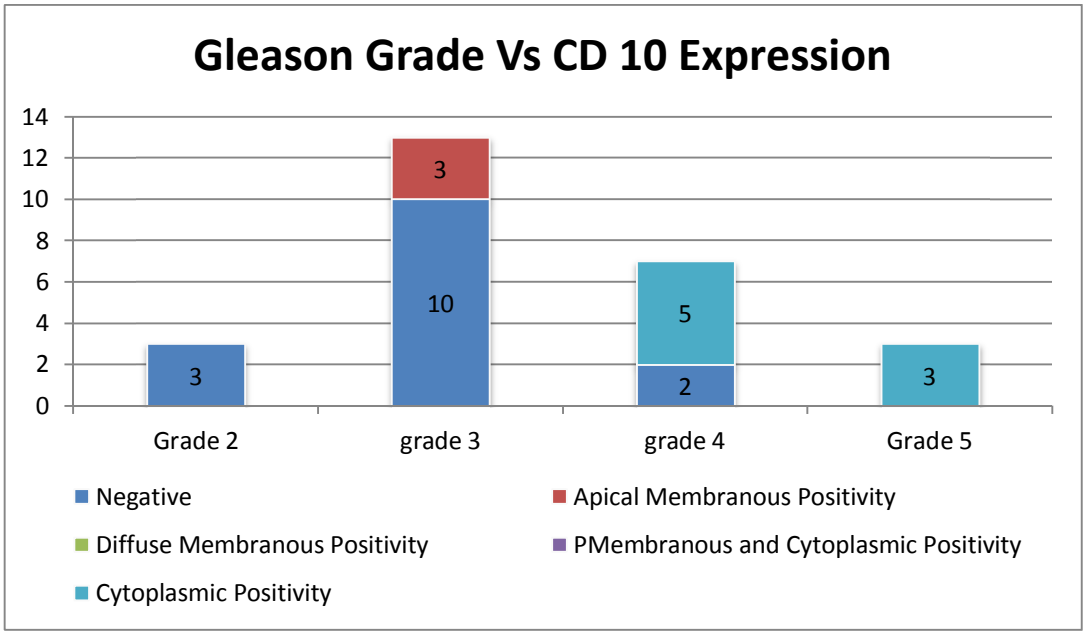
All the prostatic carcinoma cases were graded according to the Gleason grade and the percentage of each grade was estimated. We had the following results:

Out of 15 cases grade 3 component was seen in 13 cases, grade 4 component in 7 cases, grade 2 and grade 5 component in 3 cases each. The percentage of staining in each pattern was estimated. we observed the following results.

Table 9: Gleason grade vs. CD10 expression

Gleason Grade Vs CD 10 Expression	Negative	Apical Membranous Positivity	Diffuse Membranous Positivity	Membranous and Cytoplasmic Positivity	Cytoplasmic Positivity	Total
Grade 2	3	0	0	0	0	3
grade 3	10	3	0	0	0	13
grade 4	2	0	0	0	5	7
Grade 5	0	0	0	0	3	3
P value, Fishers Exact Test				0.0021		

Analysing the expression of CD10 all the grade 2 components showed absence of expression (100%), 76.92% of grade 3 componentsshowed absence of expression and 23.07% showed apical membranous positivity.None of the grade 3 lesions showed combined and cytoplasmic positivity. Among grade 4 lesions 71.43% showed intense cytoplasmic positivity and 28.57% showed absence of expression. All cases of grade 5 lesions (100%) showed diffuse cytoplasmic positivity with intense staining pattern.



SERUM PSA LEVEL AND CD10 EXPRESSION:

In the present study we classified the serum PSA level of the prostatic carcinoma cases as <10ng/ml, 10-20 ng/ml, >20ng/ml.

CD 10 expression also varied depending upon the PSA level.

In the PSA range of 10 – 20 ng/ml

4 cases showed absence of expression

2 cases showed apical membranous positivity and

1 case with cytoplasmic positivity.

In cases with serum PSA level >20ng/ml, we had

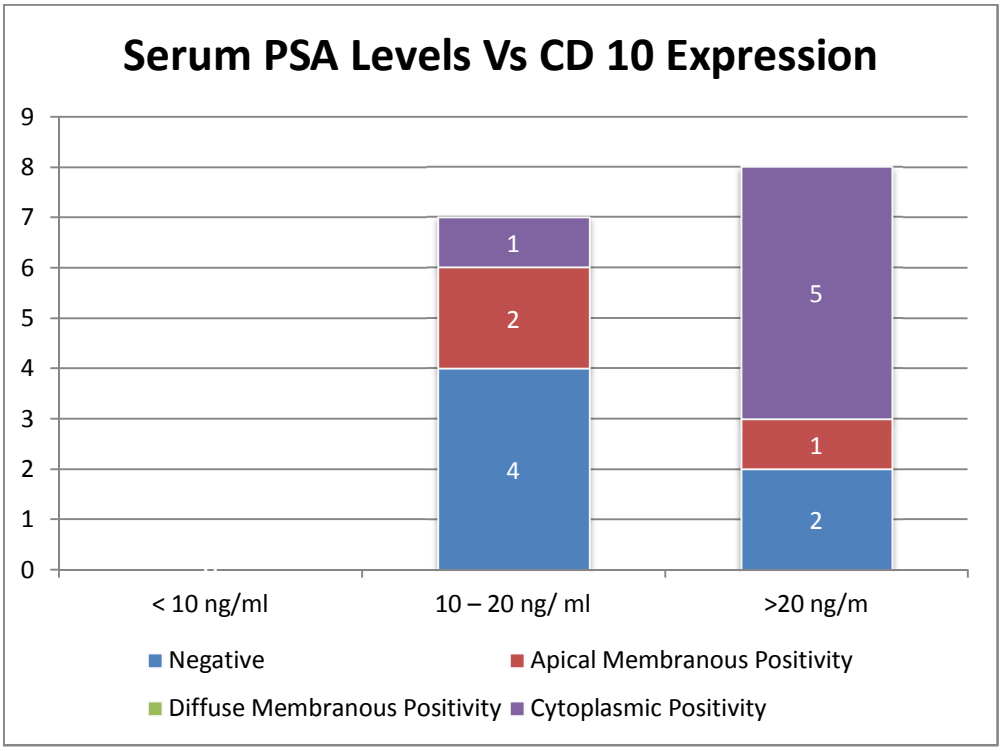
2 cases withneagtive expression

1 case with apical membranous positivity

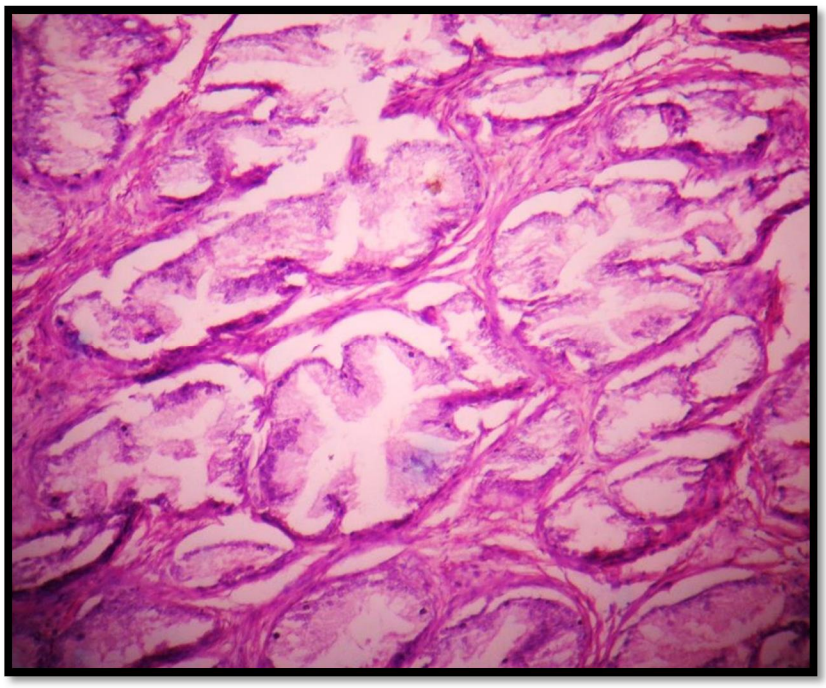
5 cases with cytoplasmic positivity.

Table 11: Serum PSA level vs CD 10 expression

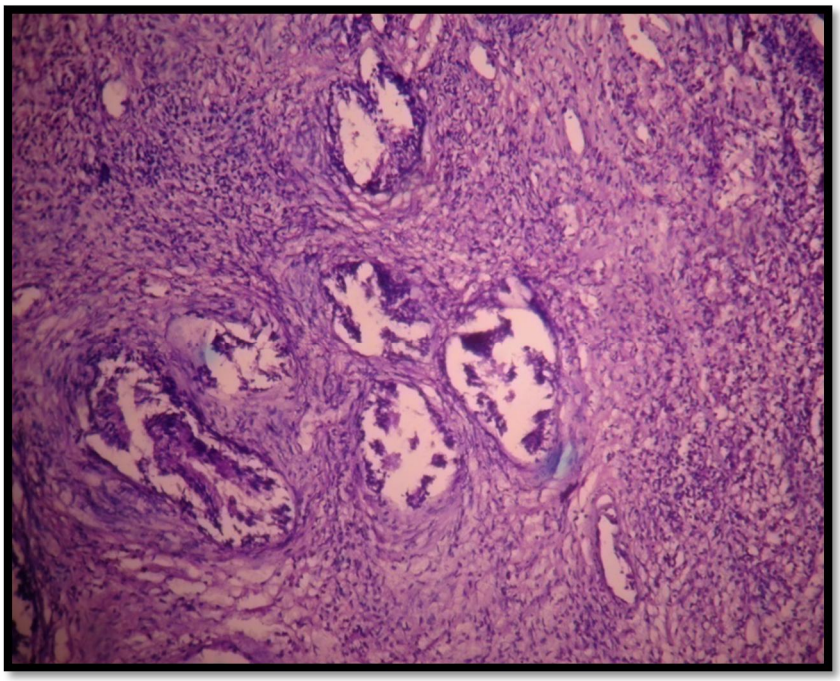
Serum PSA Levels Vs CD 10 Expression	Negative	Apical Membranous Positivity	Diffuse Membranous Positivity	Cytoplasmic Positivity	Total
< 10 ng/ml	0	0	0	0	0
10 – 20 ng/ ml	4	2	0	1	7
>20 ng/m	2	1	0	5	8
P value , Fishers Exact Test				0.0492	



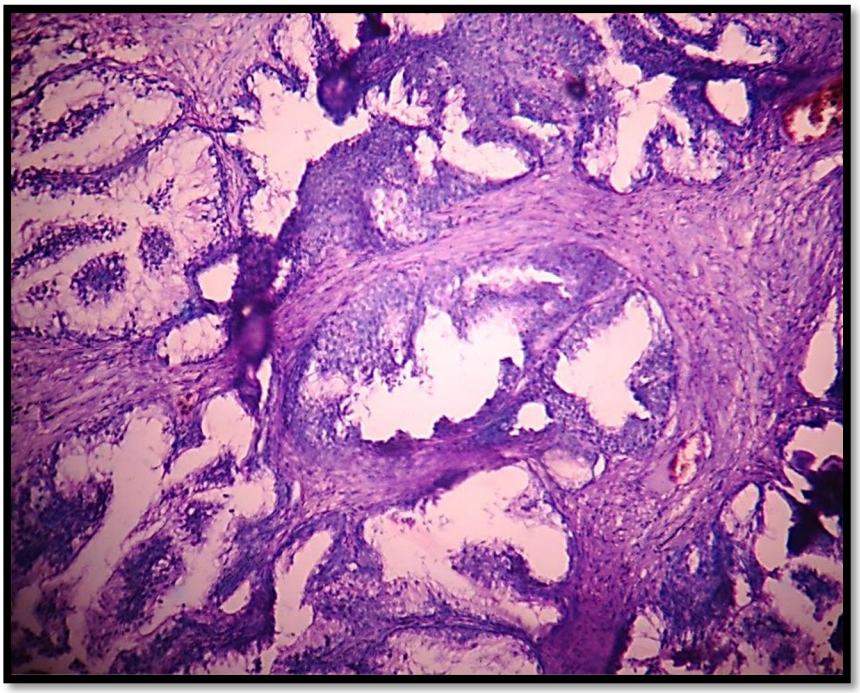
As the serum PSA level increases there is a shift from negative expression to cytoplasmic expression. P value showed significant association between increased serum PSA and increased cytoplasmic expression.



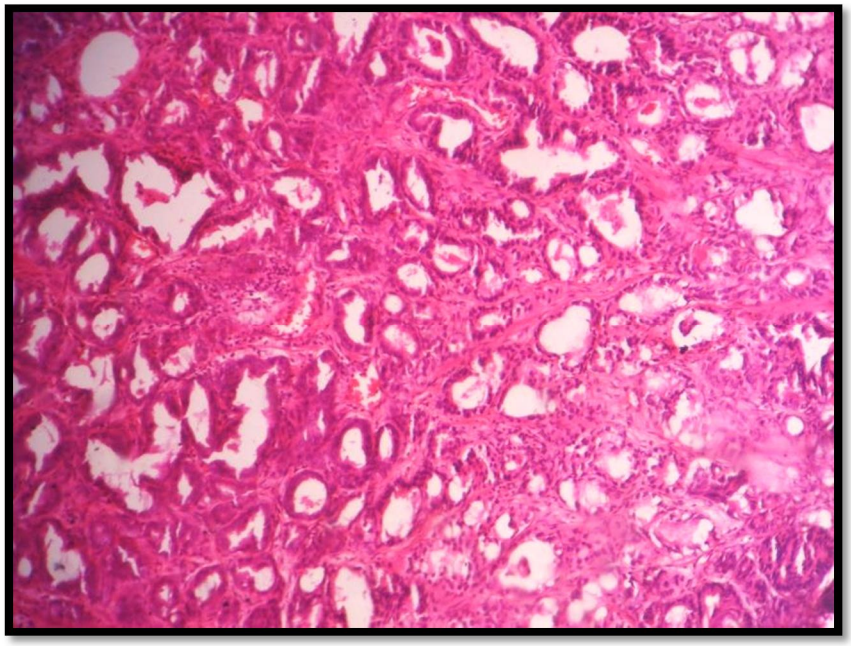
Colour plate 1: H&E, Benign prostatic hyperplasia, 10x



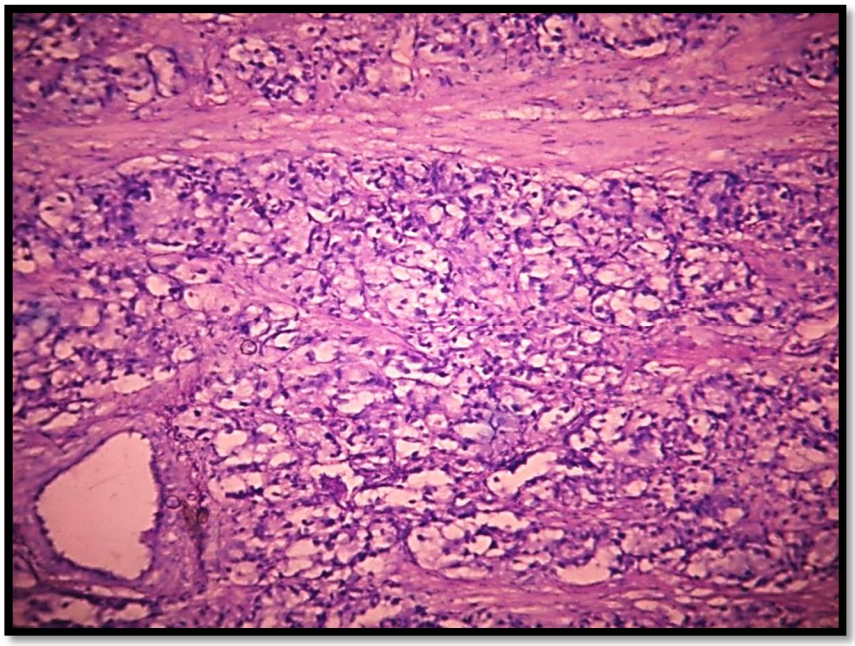
Colour plate 2: H&E, Benign prostatic hyperplasia with prostatitis, 10x



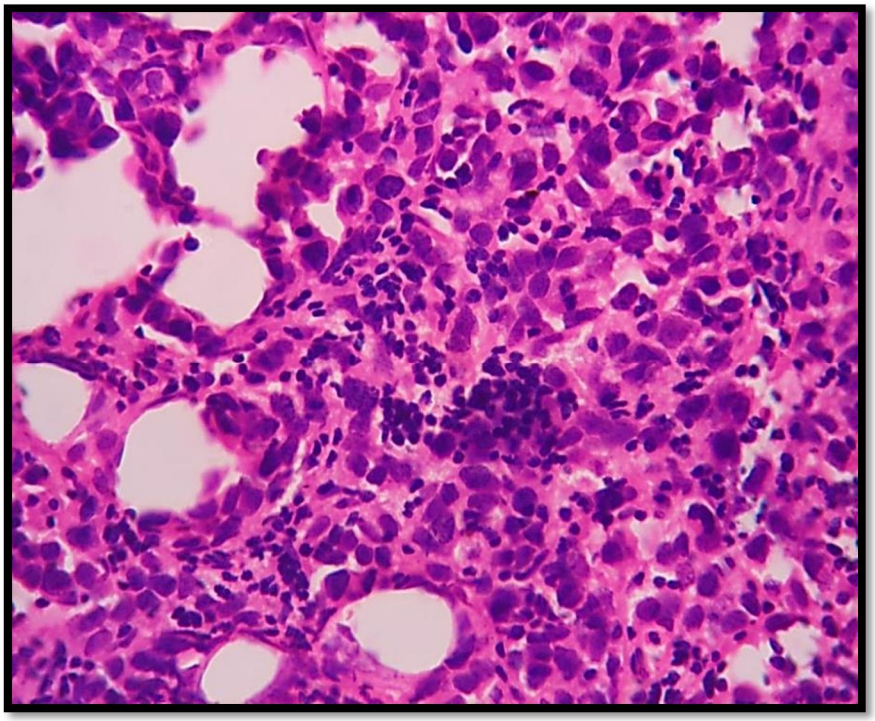
Colour plate 3: H&E, Prostatic intraepithelial neoplasia, 10x.



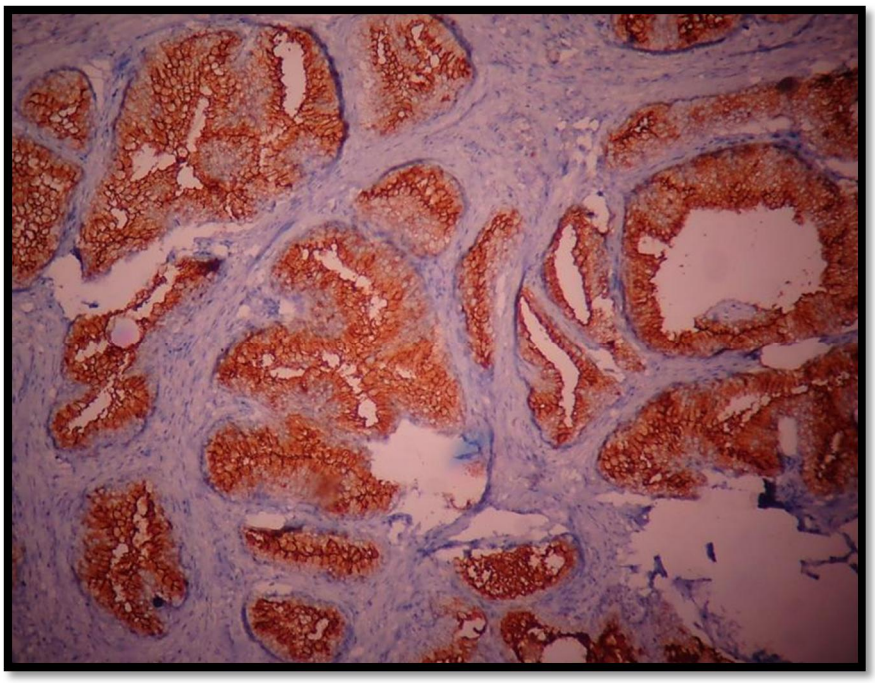
Colour plate 4: H&E, Gleason grade 3, 10x.



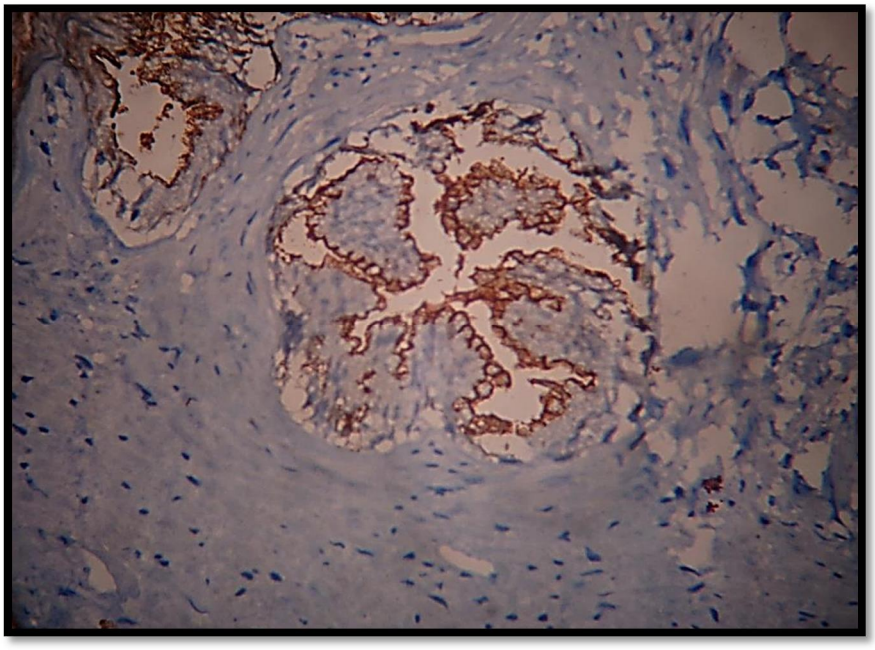
Colour plate 5: H&E, Gleason grade 4 (hypernephroid pattern), 10x



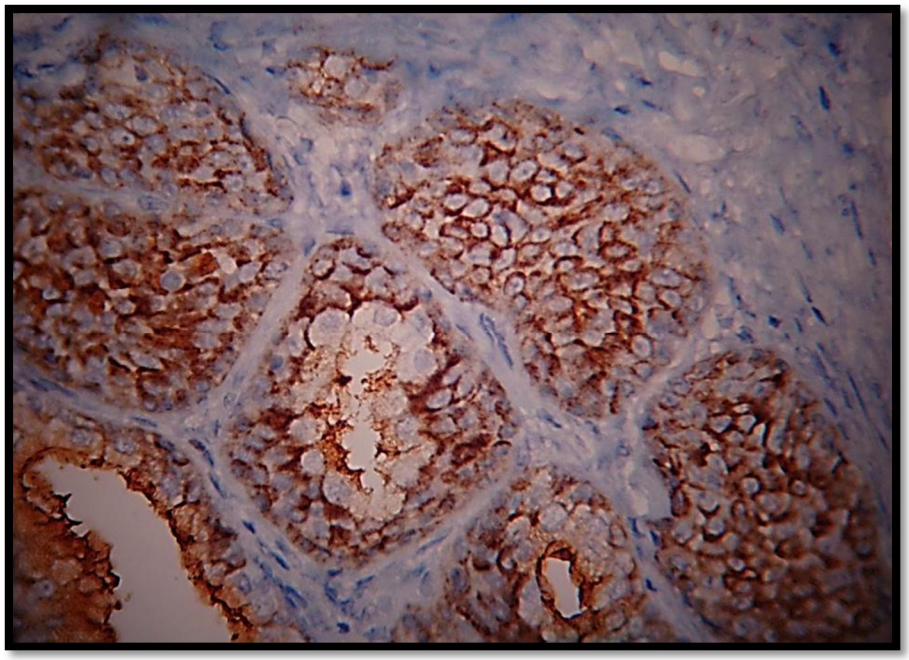
Colour plate 6: H&E, Gleason grade 5, 40x



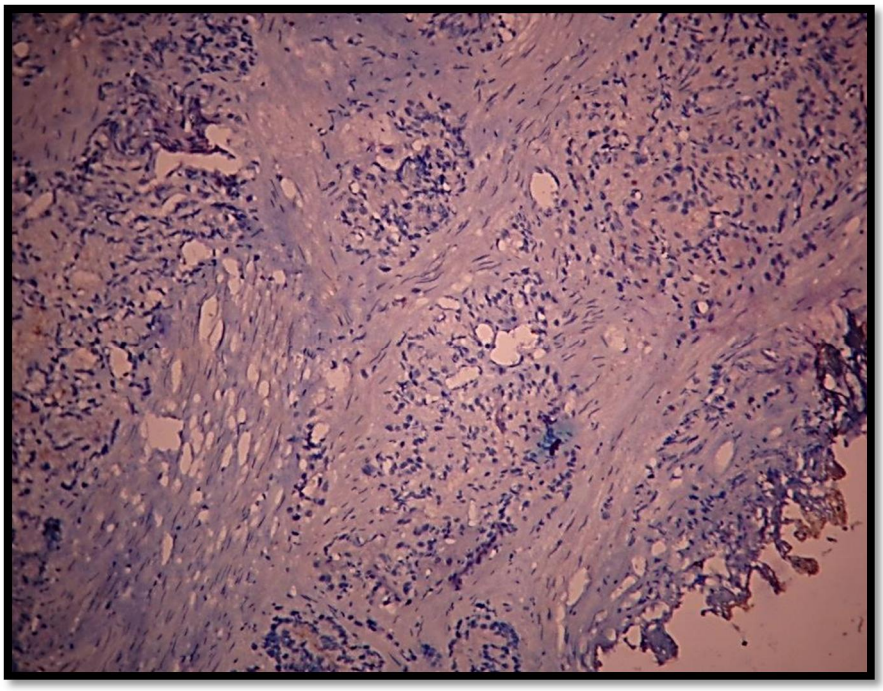
Colour plate 7: CD10 positivity, Benign prostatic hyperplasia, 10x



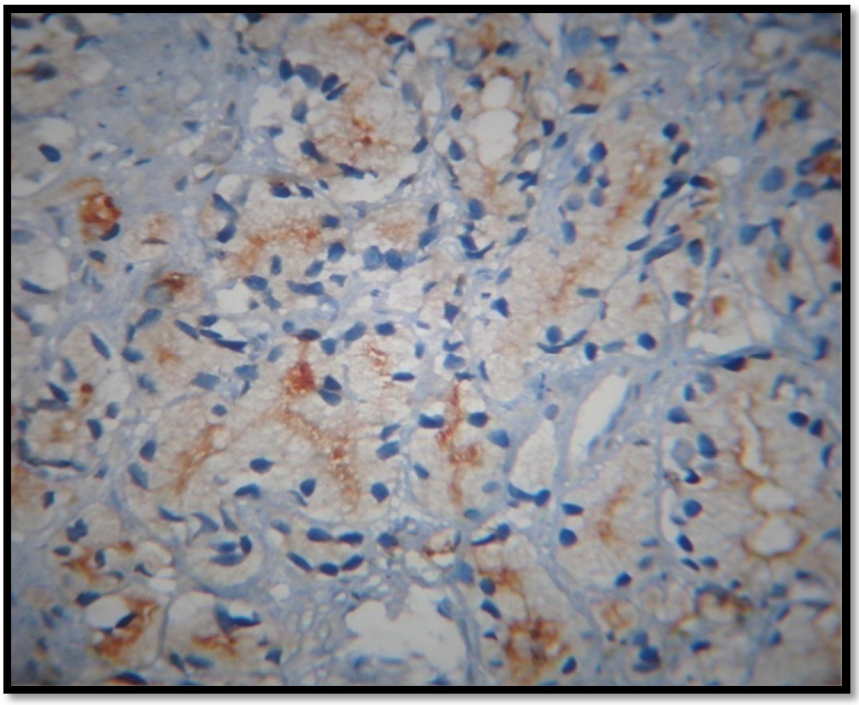
Colour plate 8: CD10 positivity, Apical membranous staining in benign glands, 40



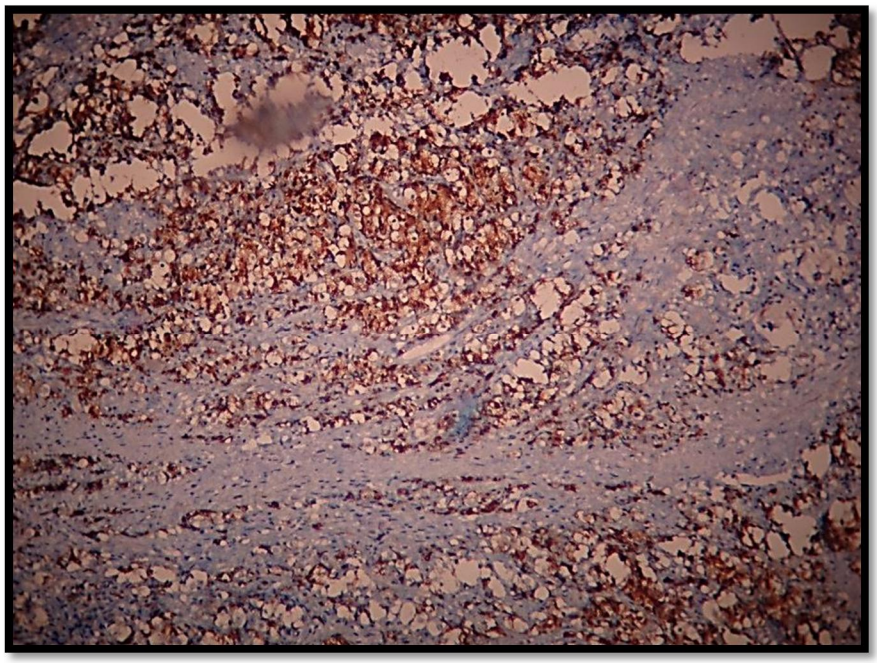
Colour plate 9: CD 10 Positive, Diffuse membranous positivity, 40x



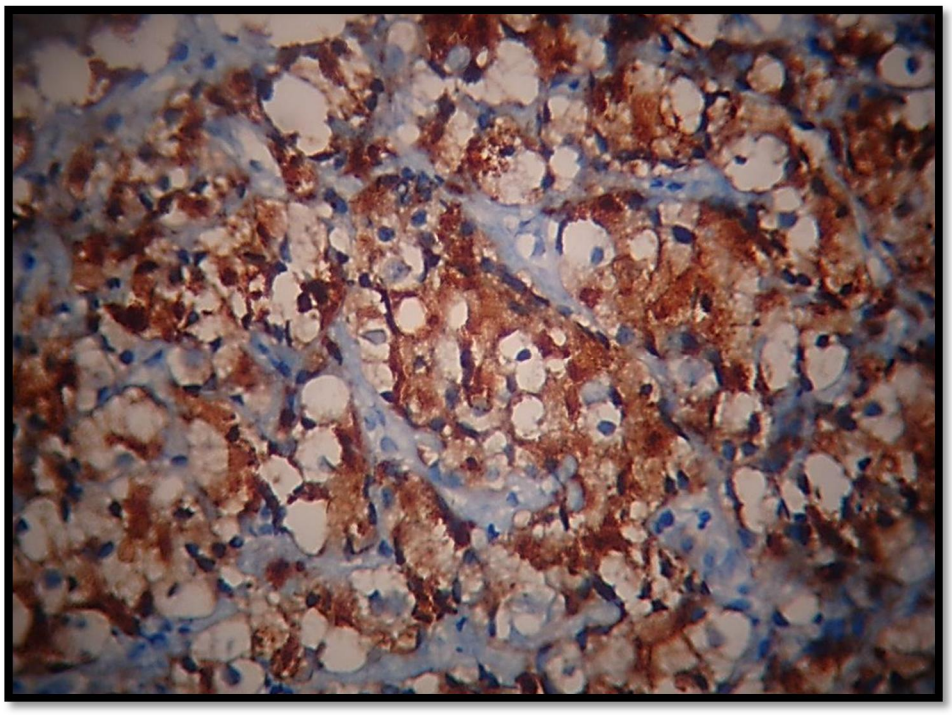
Colour plate 10: CD10 negative, neoplastic acinar glands, 10x



Colour plate 11: CD10 negative, Gleason grade 3, 40x.



Colour plate 12 : CD10 positive, Gleason grade 4, 10x



Colour plate 13: CD10 positivity, Gleason grade 4, cytoplasmic positivity, 40x

DISCUSSION

Prostatic lesions whether it is a nonneoplastic or neoplastic are responsible for significant morbidity and mortality among the males worldwide (1). Of all the lesions, prostatic carcinoma causes significant mortality and has to be viewed seriously. It has got long indolent course with varied presentations ranging from asymptomatic, urinary outflow obstruction to widespread lymph node and skeletal metastasis.

Our study is an attempt to use a hematological marker CD10 and to evaluate its expression and significance in various lesions of prostate. CD 10 is a transmembrane ectopeptidase which generally cleaves the peptides is thought to have a role in prostatic cancer.

Based on the fact that variety of CD antigens was used to identify and classify several leukemias, we are keen in knowing whether the same could be applicable for prostatic lesions also.

On comparing age wise distribution of prostatic carcinoma with other studies we had only one case with similar comparison.

Only study by Achim Fleischman et al., analysed age wise distribution of prostatic carcinoma cases. He had predominant cases of 1807 in the age range of 60 – 70 years, contributing 59%. In our study we also had maximum number of cases in the age group of 61 – 70 years contributing 46.67% of total prostatic carcinoma cases.

Table 11: Age wise distribution of prostatic carcinoma cases – Comparison

	Age range	Number of cases	Percentage of cases
Our study	51-60 years	4	26.67
	61-70 years	7	46.67
	71-80 years	2	13.33
	81-90 years	2	13.33
Achim Fleischman. et al	<50	83	2.7%
	50 – 60	998	32.6%
	60 – 70	1807	59%
	>70	175	5.7%

Similarly by analysing Gleason score wise distribution of prostatic carcinoma cases with study by Achim Fleischman et al., we had the following comparison:

	Gleason score	Number of cases	Percentage
Our study	<3+3	6	40%
	3+4	2	13%
	4+3	3	20%
	>4+4	4	26.6%
Achim Fleischman. et al	<3+3	1426	45.9%
	3+4	1311	42.2%
	4+3	313	10.1%
	>4+4	55	1.8%

In our present study majority of the cases 40% are under Gleason score <3+3 which is similar to Achim Fleischman et al., study who had 45.0% under Gleason score <3+3.

It clearly indicates that benign, preneoplastic and neoplastic lesions show different expression patterns from the statistical analysis.

In our study it was observed that in normal glands and in benign prostatic hyperplasia CD 10 showed intense apical membranous staining. Very few glands showed circumferential membranous staining also and even less than 1% showed granular cytoplasmic positivity. The complete membranous staining by epithelial cells produced a honey comb appearance. Some of the glands also showed staining of corpora amylacea of varying intensity. Since we are considering about the luminal epithelial cells all other findings was not seriously analysed.

Study conducted by Peiguochu et al (29) concludes that CD10 expression wasseen in luminal epithelial cells and ductal epithelial cells of normal prostatic gland.It was the first study to analyse the expression of CD10 in nonhematopoietic tissues including normal and pathological forms. But he didn't mention about the pattern of expression in such cells.

Complete membranous staining was observed in benign prostatichyperplastic and normal glands in the study of Iman Osman et al (40). He didn't stress about the apical membranous positivity.

Sherif Tawfic et al., (38) study showed similar results to our study. In our study normal prostatic glands and benign hyperplastic glands shows similar pattern of expression when compared with his study. But he also stated that CD 10 expression in addition to luminal epithelial cells is also seen in basal cells. The cytoplasm and membrane of basal cells in benign glands

shows CD10 positivity. He uses this feature of basal cell positivity in differentiating benign and malignant lesions of prostate..

We also observed that in prostatitis cases there is no alteration in the CD10 pattern of expression of hyperplastic glands. All glands showed intense apical membrane staining pattern. So we can conclude that inflammation adjacent to the glands doesn't change the expression pattern in the hyperplastic glands. No other studies have considered CD 10 expression in prostatitis. So our results could not be compared.

In case of Prostatic intraepithelial neoplasia there is a variable expression pattern of CD10. We included 2 cases of low grade PIN and 3 cases of high grade PIN. Low grade PIN showed diffuse membranous positivity whereas high grade PIN showed negative in 2 cases and diffuse cytoplasmic positivity in 1 case.It is well known that low grade PIN does not carry any risk for subsequent development of carcinoma. The expression pattern also displayed same feature as that of benign glands. No other study has taken low grade PIN into consideration.

There are 3 case studies which showed absence of CD10 expression in high grade PIN. Studies by Freedland et al (58) and Zellweger et al (59**)**.on tissue microarray of radical prostatectomy samples showed absence of membranous positivity in High grade PIN. Study by Sherif tawfic et al (38) analysed twelve cases of PIN also showed absence of diffuse membranous and

cytoplasmic expression of CD 10 in all the cases. Our study also showed absence of membranous positivity in 2 cases.

Thus when we analyse the CD 10 expression in benign and premalignant lesions of prostate it was clearly observed that there is a progressive loss of membranous CD10 expression thus signifying its role in the pathogenesis from benign to premalignant conditions. In normal glands and in benign conditions the extracellular peptidase activity of CD10 cleaves the unwanted peptides that could acts as a growth factor for the cells, thereby controlling the cell proliferation (23). In case of intraepithelial lesion their absence of expression leads to loss of cleavage activity thereby resulting in uncontrolled proliferation of cells

Analysing the expression of CD10 in various grades of prostatic carcinoma, we have heterogenous expression in different grades. In our study almost all the neoplastic acinar glands showed complete membranous and cytoplasmic loss of CD 10 expression in Gleason grade 2 and Gleason grade 3. It is important to mention that in all the grade 2 and grade 3 lesions the adjacent benign glands shows typical membranous positivity. Thus we can easily distinguish benign from the malignant counterparts even at a lower magnification itself.

Sherif tawfic et al (38**)** and Mellisa et al (61) in their study, also observed similar pattern of expression in low grade tumors (grade 2 and 3).

Study by Achim Fleischmann et al (60) showed variable expression patterns in grade 3. In his study he observed that 40 % of grade 3 lesions showed total absence of expression, whereas 30 % showed membranous expression and few cases showed cytoplasmic expression.

In our study we observed that malignant cells of Gleason pattern 4 and 5 showed increased cytoplasmic expression (71% and 100%) respectively. In all the cases the adjacent normal glands showed membranous positivity and therefore the pattern of expression is easily compared.It is evident that there is a sharp alteration in the subcellular localisation of CD10, shifting from membranous in benign to cytoplasmic in malignant. Among the malignant lesions there is again a shift from absence of expression in lower grade to increased expression in higher grade.

Table 11: Gleason grade and CD 10 expression- comparison

Our study	**Gleason Grade**	**Negative**	**Apical Membranous Positivity**	**Membranous and Cytoplasmic Positivity**	**Cytoplasmic Positivity**	**P value**
	Grade2	100%	0	0	0	0.0021
	Grade 3	76.92%	23.07%	0	0	
	Grade 4	28.57%	0	0	71.43%	
	Grade5	0	0	0	100	
Achim Fleischmann	**Gleason score**	**Negative**	**Apical Membranous Positivity**	**Membranous and Cytoplasmic Positivity**	**Cytoplasmic Positivity**	**P value**
	<3+3	53.5	34.1	18	4.3	<0.0001
	3+4	46	27.1	22.1	4.8	
	4+3	33.5	26.5	25.9	14.1	
	>4+4	36.4	10.9	23.6	29.1	
Iman Osman,	**Gleason score**	**Negative**	**Heterogenous**	**Positive**	**P value**	
	<7	45.9	18	36.1	0.700	
	>7	48.5	13.9	37.6		

Sherif tawfic et al **(**38**)** in his study observed that in higher Gleason pattern 4 and 5 neoplastic cells arranged in glandular pattern showed absence of expression whereas infiltrating cells, single cells and cribriform pattern showed cytoplasmic expression. He insisted that the histopathological pattern of malignancy determines the CD10 expression rather than higher grade of the tumor (Gleason pattern 4 and 5).

Osman et al. (40), in his study, divided primary prostatic carcinoma into two groups based on Gleason score as < 7 and >7. He observed that both the groups <7 and >7 Gleason score showed predominantly absence of expression in 45.9% and 48.5% respectively. He mainly analysed the presence or absence of CD10 expression and their association with PSA recurrence during follow up.

There are handful of reasons for the altered expression of CD10 in various lesions of prostate. Most of them are only hypothesis arrived by prostate cancer cell specific microarray studies.

One such study by Usmani et al.,(63) stated that the loss of CD 10 expression could be due to hypermethylation of promoter region. After transcription, translation is necessary for the synthesis of protein. When there is hypermethylation in the promoter region during the process of translation CD 10 synthesis cannot take place thereby resulting in reduced expression in case of PIN (preneoplastic lesion) to absence of expression in the next stage of

disease progression (Gleason pattern 2 and 3). Thus thishypothesis explained the possibility of reduced expression in high grade PIN to absent of expression in early stage of tumor.

The cytoplasmic localization of Cd10 in high grade could be due to increased bound forms of CD10 with cytoplasmic heat shock proteins.This intracytoplasmic accumulation drives the cell to constant signaling pathway that is independent of the growth factor signaling (64)

Marc A Dall'Era, Achim Fleischmann et al (60), Melissa E Ho et al (61) studied the association of CD10 expression and lymph node metastasis as well as with recurrence free survival. All the studies concludes that increased expression of CD10 is associated with high grade tumors, lymph node metastasis and also with decreased recurrence free survival.

This is contradictory to the study by Sumitomo M eta l (62) who proves that CD 10 inhibits cell migration in prostate cancer. This is strongly against the possibility of Cd10 expression and lymph node metastasis.

Dall'Era et al (65) .and Achim Fleischmann et al (60) suggested that CD10 expression in malignant lesions of prostate is an unfavorable risk factor for carcinoma prostate. Achim Fleischmann et al also added that PSA recurrence–free survival significantly declines from membranous over membranous-cytoplasmic to exclusively cytoplasmic CD10 expression.

In contradicting the above studies, Osman et al (40) observed that loss of CD10 expression is associated with an unfavorable patient outcome. He stated that complete loss of CD10 expression was associated with PSA recurrence after radical prostatectomy. He also stated that person with prostate cancer showing complete loss of expression are 2 times at a higher risk for relapse. Thus he concludes that absence of CD10 expression is an independent risk factor for relapse.

Tissue micro array study by Melissa E Ho et al (61) who analysed the expression of two markers AGR2 and CD10 simultaneously in prostate cancer specimen observed that in case of PIN, the CD10 expression decreases. The author also analysed the association of CD10 expression with clinical outcome of the patient. He observed that cases with low CD10 expression have longer recurrence free survival. He hypothesised that downregulation of CD10 expression in PIN andabsence of the same in low grade tumors strongly suggests its role in the tumor initiation and progression.

Regarding serum PSA level and CD10 expression our study showed that as the PSA level increases there is a shift from absence to cytoplasmic expression of CD10 thereby concluding that increased serum PSA level is associated with increased cytoplasmic expression of CD10 in malignant glands. Thus Serum PSA level directly correlated with increased cytoplasmic positivity.

Table 12: Serum PSA level and CD10 expression – Comparison

Our study	**Serum PSA Levels**	**Negative**	**Apical Membranous Positivity**	**Diffuse Membranous Positivity**	**Cytoplasmic Positivity**	**P value**
	< 10	0	0	0	0	0.04
	10 – 20	4	2	0	1	
	>20	2	1	0	5	
Achim Fleischmann et al.	**Serum PSA level**	**Negative**	**Membranous Positivity**	**Combined Positivity**	**Cytoplasmic Positivity**	**P value**
	<4	57%	20.7%	8.1%	4.1%	0.001
	4 – 10	47.5%	27.4%	19.7%	5.4%	
	10 – 20	44.9%	23.9%	23.9%	7.3%	
	>20	41.3%	25.3%	24.4%	8.9%	
Osman et al	**Serum PSA**	**negative**	**heterogenous expression**	**positive**	**P value**	
	<5	14	13	11	0.088	
	5 – 10	44	9	28		
	>10	37	20	32		

We have a significant p value of 0.04 which is been correlated with other studies. Achinm et al (60) states that as the serum PSA level increases the absence of CD10 expression decreases and cytoplasmic positivity increases, with a positive p value of 0.001.

Osman et al finding is contradictory from our findings. He states that there is no significant association Of CD10 with serum PSA value. His p value was 0.088.

Finally we cannot expect the same role of CD10 in various tumors. In some tumors it inhibit their growth, for eg lung cancer and pancreas and in some it promotes their growth for eg liver and gastric cancer. Thus CD10 has tissue specific role and in the same tissue it can perform different functions at different phases of pathology.

Since many studies have strongly shown an association between CD10 and outcome of prostate cancer, including disease free survival, lymph node metastasis etc. This can be used as a potential target for the treatment by anti CD10 drugs. CD 10 can also process peptide prodrugs due to their cleavage activity thereby increasing the drug concentration around the malignant cells and by promoting the drug cytotoxicity (66)

By analyzing the CD10 expression on prostatic cancer biopsy specimen we can categorise the prostatic adenocarcinoma as high grade and low grade tumors. It will help to follow up high grade tumors for lymph node metastasis and guide to treat them more aggressively

SUMMARY

Prostatic lesions causes significant morbidity and mortality among the elderly males worldwide. The symptoms due to prostate lesions whether benign or malignant are related to urinary symptoms, thus cannot be differentiating between these two entity.

Our objective was to (i) identify and to analyse the expression of CD10 in various lesions of prostate, (ii) to evaluate the expression of CD10 in the malignant lesions of prostate, (iii) to correlate the CD10 expression with age, histopathological grading, serum PSA level, of prostatic tumor cases.

A total sample of 40 cases were analysed during the period of June 2012 to May 2016.We categorized the total cases as benign, premalignant and malignant lesions, their age wise distribution was analysed. The cases of prostatic cancer was anlysed according to various Gleason grade, Gleason score, serum PSA levels. We performed IHC detection in sections of formalin fixed paraffin embedded tissue of prostatic biopsy cases and correlated the various patterns of CD10 expression among the different lesions of prostate with respect to histopathological diagnosis.

In our study we found that benign prostatic hyperplasia showed predominantly apical membranous staining, BPH with prostatitis shows same apical membranous positivity, PIN showed differential expression with membranous positivity in low grade and absence to combined positivity in high grade PIN. In case of malignant lesions absence of expression in majority

of Gleason grade 2 (100%) and grade 3 (76%), cytoplasmic positivity predominance in high grade Gleason grade 4 (71%) and grade 5 (100%). Increased CD10 cytoplasmic expression is seen with increased serum PSA levels (>20 ng/ml), with predominantly negative staining in serum PSA levels of 10 – 20 ng/ml.

CONCLUSION

Our study is an observational study on "**Analysis Of Immuno histochemical Expression Of CD10 In The Lesions Of Prostate** was conducted in the Department of pathology from June 2012 to August 2016.

Our study showed different pattern of expression of CD10 in various lesions of prostate. Apical membranous positivity in benign prostatic hyperplasia, same pattern of expression in BPH with prostatits, decreased membranous expression in high grade PIN, and altered expression in prostatic carcinoma. In low grade tumors we noted absence of expression, and in the high grade tumors we noted cytoplasmic expression.

Still the exact mechanism and the role of CD10 in the pathogenesis of prostatic carcinoma is under study, one of the hypothesis states that cytoplasmic positivity is due to localization of CD10 molecule in the cytoplasm. This intracytoplasmic accumulation of CD10 drives the cell to constant signaling pathway leading to uncontrolled cell proliferation. Our study also favors this hypothesis as there is cytoplasmic expression in high grade tumors.

In future this marker could be used as a diagnostic marker in differentiating benign and malignant lesions, to categorise the low grade and high grade tumors, and to determine the aggressive nature of the neoplasm.

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ANNEXURE - I

PROFORMA

Date:

1. Name : OP/IP No :
2. Age :
3. Sex : Male Female
4. History :
5. Serum PSA level :

Clinical Diagnosis :

Histopathological confirmation and grading of H&E stained section:

1. Benign prostatic hyperplasia,
2. Benign prostatic hyperplasia with prostatitis
3. Prostatic intraepithelial neoplasia high grade and low grade
4. Prostatic adenocarcinoma

    Gleason grade 2

    Gleason grade 3

    Gleason grade 4

    Gleason grade 5

CD10 expression

Negative

Positive

Apical membranous

Diffuse membranous

Cytoplasmic

ANNEXURE II

MASTER CHART

1. AGE

1. <60
2. 60 – 70
3. 71 – 80
4. >80

2. SEX

1. Male

3. HPE DIAGNOSIS:

1. Benign prostatic hyperplasia
2. Benign prostatic hyperplasia with prostatitis
3. Prostatic intraepithelial neoplasia.
4. Prostatic adenocarcinoma.

4. GLEASONS SCORE:

1. <3+3
2. 3+4
3. 4+3
4. >4+4

5. IHC DIAG- CD 10 EXPRESSION:

0. Negative
1. Apical membranous positivity
2. Diffuse membranous positivity
3. Membranous and cytoplasmic positivity
4. Cytoplasmic positivity

6. PRE OPERATIVE SERUM PSA LEVEL:

1. < 10ng/ml
2. 10 – 20 ng/ ml
3. >20ng/ml

S.NO	HPE NO	Age	Sex	Age group	HPE diag	Gleason score	Serum. PSA level	IHC diag
1.	G750/16	46	1	1	1	nil	nil	1
2	G751B/16	86	1	4	1	nil	nil	1
3	G841/16	67	1	2	1	nil	nil	2
4	G890/16	80	1	3	1	nil	nil	3
5	G920/16	60	1	2	1	nil	nil	1 and 4
6	G923A/16	72	1	3	1	nil	nil	2
7	G919/16	60	1	2	1	nil	nil	1 and 4
8	G924/16	75	1	3	1	nil	nil	2
9	G1012/16	60	1	2	1	nil	nil	1
10	G925/16	62	1	2	1	nil	nil	1
11	G961/16	78	1	3	1	nil	nil	1
12	G962/16	65	1	2	1	nil	nil	1 and 5
13	G963/16	65	1	2	1	nil	nil	1
14	G964/16	78	1	3	1	nil	nil	1
15	G974/16	65	1	2	1	nil	nil	1
16	G948/15	66	1	2	2	nil	nil	1
17	G1017/15	75	1	3	2	nil	nil	1
18	G1381/15	80	1	3	2	nil	nil	1
19	G273/16	73	1	3	2	nil	nil	1
20	G975/16	60	1	2	2	nil	nil	1
21	G1367/13	50	1	1	3	nil	nil	3
22	1769/14	60	1	2	3	nil	nil	3
23	G1259/15	65	1	2	3	nil	nil	4
24	G211/16	81	1	4	3	nil	nil	3
25	G356/16	78	1	3	3	nil	nil	2
26	G775/12	65	1	3	4	1(3+2)	2	0 and 0
27	G2/13	60	1	2	4	1(3+2)	2	0 and0
28	G1317/13	75	1	3	4	3(4+3)	3	0 and1
29	G316/14	65	1	2	4	3(4+3)	3	4 and 0
30	G346/14	88	1	4	4	1(3+3)	2	0 and 0
31	G564/14	60	1	2	4	3(4+3)	2	4 and 0
32	G1182/14	66	1	2	4	1(2+3)	2	0 and 0
33	G977/15	80	1	3	4	4(3+5)	3	0 and 4
34	G1087/15	65	1	2	4	2(3+4)	3	0 and 4
35	G1875/15	60	1	2	4	2(3+4)	3	0 and 4
36	G67/15	61	1	2	4	1(3+3)	2	0 and 0
37	G197/16	67	1	2	4	4(4+5)	2	4 and 4
38	G421/16	70	1	2	4	1(3+3)	3	0 and 0
39	G842/16	59	1	1	4	4(4+4)	3	4 and 4
40	G960/16	86	1	4	4	4(5+3)	3	4 and 0

ANNEXURE – III

GLOSSARY

CD 10	:	Cluster of differentiation
DAB	:	Di amino benzidine
H&E	:	Haematoxylin & Eosin
IHC	:	Immunohistochemistry
PBS	:	Phosphate buffer solution
TBS	:	Tris buffer solution
BPH	:	Benign prostatic hyperplasia
PIN	:	Prostatic Intraepithelial neoplasia
DHT	:	Dihydrotestosterone
PSA	:	Prsotate Specific Antigen
IDC – P	:	Intraductal carcinoma prostate
AMACR	:	Alpha-methylacyl-CoA racemace
WHO	:	World Health Organisation
ISUP	:	International society of Urologic Pathology
NEP	:	Neutral Endopeptidase
NEP-ARE	:	NEP gene an androgen responsive element
NEP-ARR	:	NEP androgen responsive region
CALLA	:	Common Acute Lymphoblastic Leukemia Antigen
PSAP	:	Prostate Specific Acid Phosphatase
TURP	:	Transurethral Resection of Prostate
TMPRSS2	:	Trans Membrane Protease Serine 2
ERG	:	ETS-related gene product
ETS	:	E26Transformation specific gene family