# To Establish Role of P63 in Evaluating Prostatic Proliferative Lesions.

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**Abstract: Background -** Prostatic cancer is second most frequent cancer in world and is the sixth leading cause of mortality in men due to malignancies. Histopathological diagnosis of various prostatic proliferative lesions may pose problems due to presence of mimickers. Hence, p63 is used as an immunochemical marker. P63 is a nuclear protein. It is confined to basal cells/myoepithelial cells of prostate gland.

**OBJECTIVES** :

To confirm and diagnose prostatic proliferative lesions by histopathological examination.
  i.   To evaluate prostatic proliferative lesions by using IHC marker P63.
 ii.   To compare and correlate findings of histopathology and P63 staining.

**Methods –** The present study is an observational, analytical, cross-sectional and prospective study, to be conducted for a duration of two years in the Department of Pathology, JNMC, Sawangi(Meghe), in coordination with the Department of General Surgery, Acharya-Vinoba-Bhave-Rural-Hospital, Sawangi(Meghe), Wardha. In this study, 55-60 biopsies will be taken and studied with help of immunostaining by P63 and results will be studied through well-tabulated master chart.

**Results -**The observations will be depicted in a well-tabulated master chart.

**Conclusion -** Conclusion will be drawn from the results obtained from the study.

**Keywords:** Prostate, P63, Proliferative lesions,

## INTRODUCTION:

Prostatic diseases cause high morbidity and mortality in elderly men all over the world. Prostatic cancer is second most frequent cancer in world and is the sixth leading cause of mortality in men due to malignancies. [1] It accounts for 1,276000 new cases and 35900 deaths in 2018.[2] In 2040 the world prostate cancer burden will grow upto 2.3 million new cases and 740,000 deaths. In India average annual incidence rate for prostate cancer ranges to 5.0 – 9.1 per 100,000/year. The main cause of this increase in burden will be due to growth and aging of the population.[3]

The prostate is both an accessory gland of male reproductive system and muscle driven mechanical switch between urination and ejaculation. The **prostate** is small, rubbery, walnut-sized gland located in front of rectum between bladder and penis. It weighs around 7 to 16

grams on average 11 grams.  Urethra runs from center of prostate from bladder to penis letting urine out of the body. The gland produces and secretes part of semen. The prostatic secretions help nourish and protect sperms.The prostatic fluid is slightly alkaline and milky white is appearance.  Seminal vesicles are structures located on prostate and secrete a large portion of ejaculate. The neurovascular bundles run across the prostate and controls the erectile function. Prostate gland grows in adolescent age under the control of male hormones called as androgens known as  testosterone and its by productsdihydrotestesterone (DHT). Testesterone is largely made in testis but in small amounts also made in adrenal gland. Main sign of any prostatic proliferative lesion is prostatic enlargement. Symptoms such as lower urinary tract infections, erectile dysfuction are seen in prostatic proliferative lesions. It is surrounded by fibromuscular capsule and contains glandular tissue and connective tissue. Blood supply of the gland is by internal pudendal artery, inferior vesical artery and middle rectal artery. Venous supply is given by prostatic venous plexus, pudendal plexus, vesicalplexsus and internal iliac vein. Nervous supply by inferior hypogastric plexus and lymphatic drainage is done by internal iliac lymph nodes.

It has been observed that by age of sixty years majority of men develop benign prostatic hyperplasia. Recent reports state that lifetime risk of man to get Benign prostatic hyperplasia is fifty percent in age group 51-60 years which increases to seventy percent in age group of 61-70 years. In India the incidence rate of benign prostatic hyperplasia is 6.7-14 percent.[3] Black race, family history also has increase chances of developing the disease. There are some other risk factors reported recently like body fatness, dairy products, high calcium diet, low plasma selenium and alpha tocopherol levels to cause the disease.

The prostate gland is made up of glandular and connective tissue. Tall columnar cells form the lining epithelium of the gland. The epithelium is highly variable at some they are pseudostratified epithelium. Cuboidal and transitional epithelium is also present in some outer regions of longer ducts. Gland consists of many follicles which drains into 12-20  main ducts which drains into urethra. Connective tissue of the gland is made up of fibrous tissue and smooth muscles.
Morphologically there are various types in prostatic lesions like  acinar, cribriform, papillary, trabecular and solid.[3]  Prostatic adenocarcinoma  can be usually studied on morphological methods. But, the problems lie during differentiating the malignant adenocarcinoma cells form the benign looking lesions.[4]

Disorders of prostate include enlargement, inflammation, infection and malignancy. There are various prostatic lesions like prostatitis, benign prostatic hyperplasia, prostatic intraepithelial neoplasia and adenocarcinoma prostate. Gross anatomy consists lobes and microanatomy consists of zones. There are five lobes i.e anterior lobe or isthmus, posterior lobe, right and left lateral lobes and median or middle lobe. There are five zones in prostate gland i.e central zone, fibromuscular zone, transitional zone, peripheral zone, peripheral urethral gland region. Most prostatic malignancies start at peripheral zone near the rectum. Benign prostatic hyperplasia arises from transitional zone whereas prostatic adenocarcinoma arises from epithelial cells located in peripheral zone and small percentage arises from transitional zone of prostate gland.

There are evidences in which long standing benign prostatic hyperplasia converts into a malignant form of adenocarcinoma. Patients developing prostate cancer have a very slow progression resulting into them being asymptomatic for a long time during initial stages. In current practice , screening modalities like digital rectal examination (DRE) and elevated levels of prostatic specific antigen (PSA) are used with non reliable findings as results.[5]

This is then followed by some advanced diagnostic techniques such as transrectal ultrasonography (TRUS) and guided biopsies.[5] There is increased used of prostate needle biopsies due to increase awareness. Diagnosis of malignancy on biopsies is difficult due to small volume biopsies that's why biopsies done to detect prostatic lesions is challenging and needs expertise. This is where the role of immunohitochemical markers (IHC) has gained popularity for reaching a reliable diagnosis. There are many immunohistochemical markers such as P63, cytokeratin, AMACR, calponin etc.[6] P63 is most potent in immunohistochemical staining with basal cells. Immunohistochemistry was carried out using streptavidin biotin immunoperoxidase technique. There is evidence of basal, luminal, and neuroendocrine epithelial cells of prostate originate from the differentiation of stem cells seen in basal component.

P63 is homologous to P53. P63 is located on 3q27-28 chromosome. P63 is a nuclear protein and shows nuclear staining in basal cells.[7] It is also involved in epithelial differentiation and proliferation. This transcription encodes two classes of protein which are opposing oncogenic and tumour suppressor including transactivation, cell proliferation and apoptosis. In prostatic adenocarcinoma P63 is under expressed. Negative staining for P63 is useful for diagnosis of benign mimickers. Over expression of P63 is useful for identifying cancer progression, poor progression of several malignant sites like ovaries and oral squamous cell carcinoma. This gene is important for development of epithelial tissue in many organs and is essential for epithelial stem cell regulation and maintenance. For establishing an accurate diagnosis between the confusion of benign lesions and malignant lesions, IHC stands superior to all the other modalities. Reason behind the accuracy of IHC being the affinity to basal cells.[8] P63 is confined only to the myoepithelial layers within prostate. P63 IHC marker stains prostatic basal layer whose presence or absence is a diagnostic criteria for clearly defining two totally opposite entities such as benign prostatic hyperplasia from prostatic carcinoma.

Prostatic diseases have been on the rise since recent past, compelling us to review/ study more confirmatory diagnostic tool. There by, the main concern regarding diagnostic studies in prostatic diseases sum up to difficulty regarding differentiation between benign prostatic disease and malignant prostatic tumours . So the role of Immunohistochemistry markers plays a significant role for the study.

**METHODOLOGY:**

**STUDY DESIGN -** Observational, analytical, cross-sectional and prospective.
**PLACE OF STUDY –** Department of Pathology, JNMC, Sawangi(Meghe), Wardha, Maharashtra.
**DURATION –** 2020 to 2022 (2 years)
**METHODS –**
Study dosen't involve major or minor issues offending to human subjects.
Biopsy from clinically suspected cases will be submitted for histopathological examination to the Department of Pathology, Jawaharlal Nehru Medical College (J.N.M.C).
All cases which are confirmed on ultrasonography and biopsy tissue as prostatic lesions.
The cases confirmed as "prostatic lesion" on histopathological examination will be respectively operated on and the resected specimens will be received in the Department of Pathology, J.N.M.C. in 10% Formalin.

The resected specimens will be kept in 10% Formalin for 12-24 hours for fixation.
Specimens will be subjected to routine tissue processing after which routine Haematoxylin and Eosin (H & E) staining will be carried out.

Immunostaining for P63 will be carried out to differentiate between benign prostatic hyperplasia and prostatic adenocarcinoma.

After which, comparison of histopathology and P63 will be done.

- **Sample size:**
  Sample size formula with desired error of margin:

$$n = (Z\frac{\alpha}{2})^2 X\ p\ X\ (1-p) / d^2$$

where,

$Z\frac{\alpha}{2}$ is the level of significance at 5% i.e. 95% confidence interval = 1.96

p = prevalence of prostatic lesions = 0.035%

d = desired error of margin = 5% = 0.05

$n = (1.96)^2\ X\ 0.035\ X\ (1 - 0.035) / (0.05)^2$

= 51.90

= **55-60 patients needed in each study group**

**Inclusion Criteria:**

All cases presented with prostatic enlargement and confirmed on ultrasonography and biopsy tissue.

**Exclusion Criteria:**

- All cases which are previously treated for prostatic adenocarcinoma, benign prostatic hyperplasia and other prostatic lesions.
- All cases which have recurrence.
- Cases which have metastasis from other cancers.

**EXPECTED RESULTS:**

The study will be conducted for a period of 2 years and all the observations will be depicted in a well-tabulated master chart.

**DISCUSSION:**

Prostatic proliferative lesions include benign prostatic hyperplasia, prostatic intraepithelial lesions and prostatic adenocarcinoma. Prostatic adenocarcinoma is sixth leading cause of mortality worldwide. For screening of these prostatic lesions Prostate specific antigen and Digital rectal examination is done. IHC plays a crucial role to differentiate between benign and malignant lesions.

P63 is a homologous to P53 which is a nuclear protein and stains nucleus in basal cells. P63 marker stains prostatic basal layer whose presence or absence is a diagnostic criteria for differentiating from various prostatic lesions.

In a study conducted in 2018 by Ahemad Ibn EdrissMohmaed, et.al entitled "Optimizing detection of prostate cancer by AMACR and P63 on prostatic needle biopsy Sundanese experience" with sample size of 250 specimens of prostatic biopsies which concluded that P63 had 95% sensitivity and 95% specificity, it was significantly expressed in benign prostatic hyperplasia.[1]

In a study carried out by S Premalatha, et.al in year 2019 entitled " Role of expression of P63 and Calponin in prostatic biopsies" with total sample size of 30 biopsies out of which 93%

cases of benign prostatic hyperplasia showed positive staining for P63 and 100% cases of prostatic adenocarcinoma showed negative staining for the same.[3]

In a study conducted by Dr. Muthukumar M, et.al in year 2019 entitled "Diagnostic utility of immunohistochemistry using basal cell markers in distinguishing benign from malignant prostatic lesions" carried out a study on 40 prostatic biopsies. The study showed P63 staining in benign and malignant glands with sensitivity and specificity to be 100%. In 40 specimens, 30 were stained positive and was diagnosed for benign prostatic hyperplasia and 10 were stained negative and was diagnosed as prostatic adenocarcinoma.[9]

In a study conducted byHayam E Rashed, et.al in 2015 entitled " Minimal adenocarcinoma in prostate needle biopsy tissue immunohistochemical study" on 60 prostatic needle biopsy specimen which concluded that there was a significant difference in cytoplasmic P63 expression between benign prostatic hyperplasia and prostatic adenocarcinoma.[10]

A number of immunohistochemical studies were reported [11-14]. Few of the studies in this region were reviewed [15-18].

In a study conducted by Muhammad Kashifbaig, et.al in 2012 entitled " Role of P63 in differentiating morphologically ambiguous lesions of prostate" in which they took 30 prostate biopsies which inferred that prostatic adenocarcinoma was negative for P63 and positive for benign prostatic hyperplasia.[4]

In a study conducted by Michael H Weinstein, ShabinaSignoretti, Massimo Loda et.al in 2002 entitled "Diagnostic utility of immunohistochemical staining for P63, a sensitive marker of Prostatic basal cells." With sample size of 70 specimens that concluded that p63 staining is sensitive in identifying basal cells in benign lesions and will not lead to false positive diagnoses of malignancy in needle biopsies of prostate.[7]

## CONCLUSION:

Conclusion will be drawn from the results obtained from IHC study using P63 for different prostatic proliferative lesions.

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