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Expression of P63 in Prostatic Biopsies

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ABSTRACT

Introduction- *This study helps us to identify the expression of p63, a new basal cell marker of the present era in prostatic biopsies.* ***Aim-*** *A descriptive study to evaluate the expression of p63 in benign and malignant lesions of prostate.*

Materials and methods- *35 Prostatic biopsies were collected of which 20 were benign and 15 were malignant. Histopathological evaluation (HPE) was done. These formalin fixed, paraffin – embedded blocks were sliced 3-4 um thickness for Immunohistochemistry (IHC) with p63.The avidin biotin complex detection system was used on specimens of formalin-fixed, paraffin embebbed tissue section. Nuclear staining of basal cells with p63 was taken as positive.* ***Results-*** *Out of 20 benign cases,17 cases showed positivity towards p63 immunostaining and 3showed negative immunostaining.All the15 malignant cases showed negative immunostaining.****Conclusion-*** *P63 is found to be expressed in a large majority of benign prostatic lesions and negative in all the cases of prostatic adenocarcinoma.*

KEYWORDS : prostatic biopsies, P63

INTRODUCTION

Prostatic diseases causes significant morbidity and mortality in men of elderly age group throughout the world. Benign prostatic hyperplasia and prostatic carcinomas are the two prostatic diseases which are of increasing incidence with aging. The prevalance of these diseases are currently showing a tendency to increase. In particular the prevalence rates of prostatic diseases and the mortality rates due to prostatic carcinomas are noteworthy.

The diagnosis of prostatic malignancy can be usually distinguished by morphological features. However, the diagnosis of prostatic cancer on routine biopsies like Trucut and Transurethral resection of prostate(TURP)specimens can be challenging and difficult when certain problems arises when limited or insufficient tissue samples are received, small foci of carcinoma or benign lesions that mimic prostatic cancer like for example atrophy, basal cell hyperplasia, atypical adenomatous hyperplasia are noted.

Hence, in such challenging instances the application of immunohistochemistry to distinguish between prostate cancer from its benign mimickers and to confirm the diagnosis becomes more necessary and specific, especially in equivocal cases.

This study mainly aims at evaluating p63 in distinguishing benign prostatic lesions from prostatic malignancies.

P63 antibody targets the nuclear protein p63 which is homologous to the TP53 tumor suppressor gene and has been proven to selectively stain the basal cell nuclei(2). p63 immunostaining provides greater specificity because of its nuclear localization which may have greater potential for nonspecific reaction.

MATERIALS AND METHODS

iia)EXPERIMENTAL DESIGN

This study was conducted at the Meenakshi Medical College and Research Institute Hospital, Enathur, Kancheepuram.Thirty five cases were studied from August 2014- till July 2016 and it was diagnosed by a single pathologist to avoid inter- observer variations.Among the 35 cases of prostatic lesions, 25 cases were obtained from TURP and 10 cases were obtained from TRUCUT biopsies. These specimens were obtained from both out patients and in patients in Meenakshi medical college.

Informed consent was obtained from each patient before surgery for the examination of prostatic tissue Histological features of all cases were studied with hematoxylin and eosin(H&E). A total of 35 specimens were collected of which 20 cases were diagnosed as benign prostatic lesions and 15 cases were diagnosed as prostatic adeno carcinoma

INCLUSION CRITERIA

Cases from August 2014 to July 2016 were selected. All cases irrespective of age and other physical conditions were selected for study over a period of 2 years from August 2014 to July 2016.The paraffin blocks of patients with prostatic lesion were subjected to H&E and p63 immunohistostaining study

EXCLUSION CRITERIA

Samples were excluded which fell out of the time frame.

IMMUNOHISTOCHEMICAL STUDY

Hematoxylin-eosin slides of all the tissues were evaluated, and for each case, the best paraffin blocks, highest tumor content were chosen in order to prevent artefact staining. These formalin fixed, paraf-

fin – embedded blocks were sliced 3-4 um thickness for IHC .p63 immunostaining was done in those sections. The avidin biotin complex detection system was used on specimens of formalin-fixed, paraffin embebbed tissue section.

iii)STATISTICAL ANALYSIS

As the study was designed as a descriptive study, no statistical analysis was done.

iv)ETHICAL CONCERN

Ethical clearance was obtained from the Ethical committee meeting conducted at Meenakshi Medical College, Kanchipuram, Tamil nadu, India.

V) RESULTS AND OBSERVATION

Total number of 35 cases were selected. Among these cases 20 cases (57%) were benign and 15 (43%) cases were malignant.

Majority of the patients in our study presented with history of increased frequency, hesitancy and urgency in micturition, nocturia and incomplete bladder emptying.

TABLE 1: FREQUENCY OF BENIGN AND MALIGNANT LESIONS IN TURP AND TRUCUT SPECIMENS .
Total number of TURP specimen received = 25, TRUCUT=10

GROSS	TURP	TRUCUT
BENIGN	12(48%)	8(80%)
MALIGNANT	13(52%)	2(20%)

Among the 20 benign lesions, 12 cases were reported in TURP specimen and 8 cases in Trucut biopsy. Among the 15 malignant lesions, 13 were diagnosed in TURP samples and 2 in Trucut biopsy samples.

TABLE- 2: INCIDENCE OF VARIOUS LESIONS IN PROSTATE

Sl.No	HP diagnosis	Percentage
1	Nodular hyperplasia	10 (28.5%)
2	Basal cell hyperplasia	2 (5.7%)
3	Squamous metaplasia	1 (2.8%)
4	Prostatitis	4 (11.4%)
5	Atrophy	3 (8.5%)
6	Carcinoma	15(42.8%)
7	Total	35 (100%)

TABLE – 3AGE WISE DISTRIBUTION OF BENIGN LESIONS

AGEWISE DISTRIBUTION	NUMBER OF BENIGN LESIONS	PERCENTAGE
40-49	2	10%
50-59	3	15%
60-69	10	50%
>70	5	25%

Among 20 benign lesions, majority of the benign cases belonged to the age group of 60 - 69 years.

TABLE – 4AGE WISE DISTRIBUTION OF MALIGNANT LESIONS

AGEWISE DISTRIBUTION	NUMBER OF MALIGNANT LESIONS	PERCENTAGE
40-49	0	0
50-59	2	13%
60-69	5	33%
>70	8	54%

Among 15 malignant lesions, majority of the cases were seen in age group of more than 70 years.

TABLE-5: FREQUENCY DISTRIBUTION OF GLEASON'S SCORE IN ADENOCARCINOMA

	Gleasons score	Total number of cases	Percentage
In our study	2-4	2	13.3
	5-7	9	60.1
	8-10	4	26.6
	TOTAL	15	100

Majority of the malignant cases showed Gleason's scoring ranging between 5 – 7.

EVALUATION OF IHC

20 benign and 15 malignant cases were subjected to p63 immunostaining of basal cells. Intense positive of immune staining for basal cells was demonstrated for the antibody in majority of the benign prostatic glands - specific immune staining with p63 which is localized to the nucleus. For p63, positive immune staining was taken as an evidence of benignity whereas negative immune staining was taken as evidence of carcinoma . Basal cell staining was considered positive only if > 10 % of the glands were stained and negative if only ≤ 10 % of the gland were stained.

TABLE 6:RESULTS OF P63 STAINING IN BENIGN AND MALIGNANT PROSTATIC GLANDS

RESULTS OF P63 STAINING	BENIGN	MALIGNANT	TOTAL
POSITIVE	17	0	17
NEGATIVE	3	15	18
TOTAL	20	15	35

Out of the 20 benign lesions, 17 showed positivity(fig 1, 2, 3) for p63 immunostaining and 3 showed negativity.

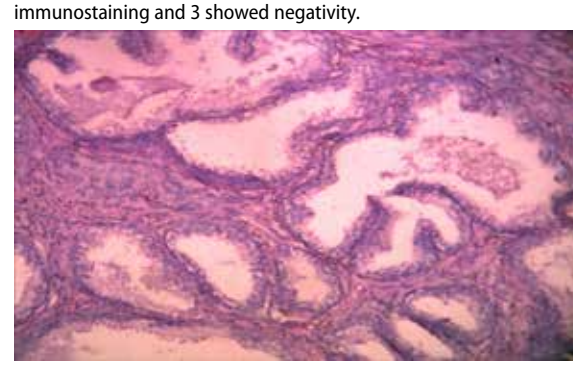


FIG-1 Benign prostatic hyperplasia – Showing hyperplasia of both glandular and stromal components(H&E)100X

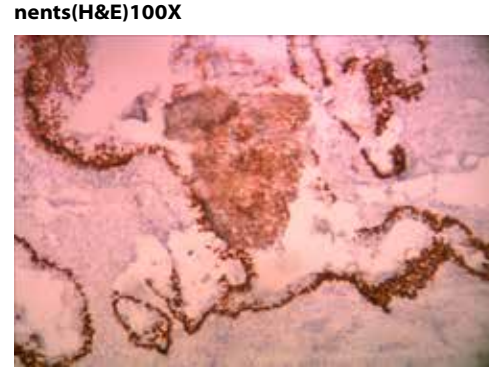


FIG-2 : BPH- Diffuse nuclear positivity for p63(IHC) - 100X

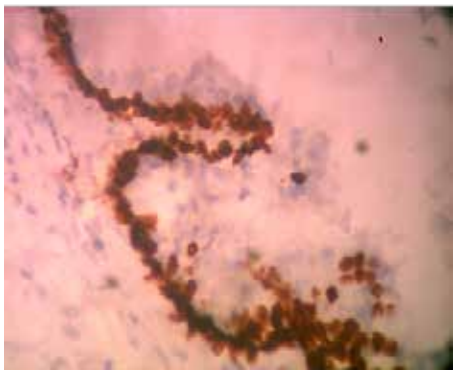


FIG 3: BPH- Diffuse nuclear positivity for p63(IHC) - 400X

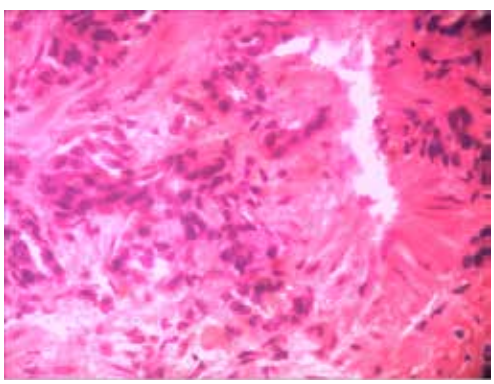


FIG- 4Prostatic adenocarcinoma – Gleason's pattern 3 – composed of small to medium sized glands (H&E) 400X

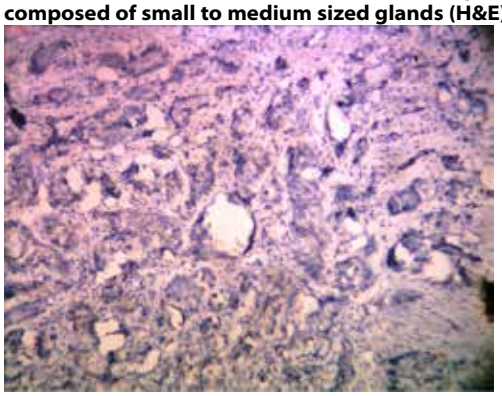


FIG-5 Adenocarcinoma-Negative for p63(IHC)–100X

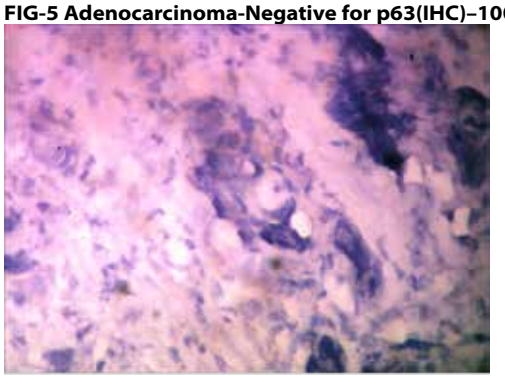


FIG 6: Adenocarcinoma – Negative for p63(IHC) – 400X

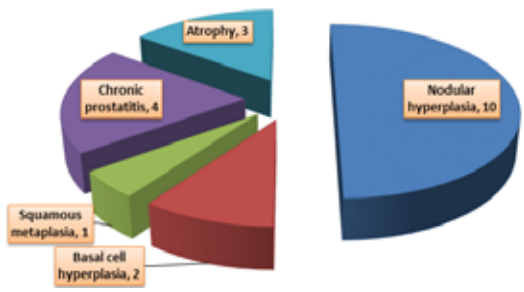
DISCUSSION

prostatic enlargement is common in the geriatric age group. Benign prostatic hyperplasia and carcinoma of the prostate have an increasing frequency with advancing age.

The distinction between Prostatic adenocarcinoma and benign conditions is traditionally made purely on morphologic grounds, but is often problematical. As a result, immunohistochemical methods have been introduced in the differential diagnosis of these conditions(3).

In the present study, the incidence of benign lesions was 57 %. Nodular hyperplasia alone was noted in 28.5 % as seen in graph no:1

GRAPH:1 Microscopic findings in benign lesions



Age incidence of nodular hyperplasia range from 60 – 69 years in our study. This correlates well with studies done by Anjorin et al(4) and Elizabeth George and Sosama(5). According to these studies the peak age for nodular hyperplasia incidence was 60-69 years.

In the present study 2 cases showed basal cell hyperplasia along with nodular hyperplasia in the age group of 55 - 75 years. It is characterized by small uniform darkly staining basal cells forming solid nests, tubules and cords with peripheral palisading appearance.In a study by Mittal BV et al(6) the percentage of basal cell hyperplasia was accounting for 5.33 % among the prostatic lesions which is well correlating with the present study which shows 5.7%. In a study by Cleary et al,(7) all the patients were above the age of 60 years and all had nodular hyperplasia, in addition to basal cell hyperplasia which correlated well with or study.

The present study showed 1 case of squamous metaplasia, in addition to nodular hyperplasia, thus accounting for 2.8% of total cases studied. However study by Mittal et al(6) showed Metaplastic epithelium in 10.27% of cases.

In the present study, 3(8.5%) cases had atrophy along with Nodular hyperplasia out of the 35cases . Mittal BV et al(6) showed 1.32% cases of atrophy, present along with Nodular hyperplasia in their study. Wenle Wang M D et al(8) showed 4.35% of cases with partial atrophy.

Prostatic cancer contributes to the overall cancer burden, being the most frequent malignancy in men world wide. The total number of cases had continuously increased over the past decades, due to higher life expectancy, western life style characterized by a high fat intake, obesity, high calorie diet and lack of physical exercise.The Incidence of prostate cancer vary greater than 25 fold worldwide, highest rates are seen in Australia, Newzealand, Western and Northern Europe, North America, largely because the practice of PSA testing and subsequent biopsy has become widespread in those region(10,11).

In our study peak age incidence of prostatic adenocarcinoma was seen above 70 years. This correlates well with studies done by Lee and Shanmugaratnam et al (12), L Stanford et al(13), Hamwakyoma et al(14), Roberto Deanglessetal(15), F Aragona et al(16), Anjorini et al(4). According to these studies, the peak age group of prostatic adenocarcinoma ranged between 60 – 80 years.

According to a study done by Dr. Hamwakyoma and Dr. J L. Magandi(14) , 61% of cases showed a gleasons scoring of 5-7 and 33% showed a gleasons scoring of 8-10. This study is well correlated with the present study.

p63 has recently generated much interest due to its expression in the basal cells of the prostate, and it is essential for prostate development. Signorett et al(17)also highlighted the role of p63 in the development of prostate gland and showed that p63 is expressed in virtually all the basal cells of prostatic glands.

The results of our study demonstrates that p63, is specific for basal cells in the prostate gland. None of the 15 cases with histologically unequivocal prostatic carcinoma demonstrated immunoreactivity for p63.

With regard to p63 staining in our study, out of 35 cases, 17 benign cases showed positivity and 3 cases showed negativity. This correlated with the study of Shah et al(18) who reported the absence of basal cell staining with p63.

This absence of basal cell staining may be attributed to the diminished or absence gene expression of basal cell markers, technical variables, including those resulting from surgical procedures and antigen retrieval methods could be another important source of negative basal cell IHC reactions.(19,20)

Conclusion

P63 is found to be expressed in a large majority of benign prostatic lesions and negative in all the cases of prostatic adenocarcinoma and there by helps in distinguishing benign and malignant histopatholgically challenging cases.

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