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DIAGNOSTIC UTILITY OF IMMUNOHISTOCHEMICAL MARKER, P63 IN PROSTATE BASAL CELLS TO DIFFERENTIATE PROSTATE CARCINOMA FROM BENIGN LESIONS OF PROSTATE

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ABSTRACT

Introduction: Prostate carcinoma (PC) is the fifth most common cancer in the world and the is emerging as one of the leading causes of cancer in both developed and developing countries. Needle core biopsies make it very challenging in view of small volume of biopsies and presence of benign mimickers of PC. p63, a basal cell marker for prostatic myoepithelial cells are absent in prostate adenocarcinoma and their identification in prostatic glands can be used to rule out the diagnosis PC. **Method:** Routine HPE was done along with IHC with p63 on the tissue sections along with positive and negative controls. **Results:** Out of 57 samples, 19 cases were neoplastic of which 1 case was reported as PIN and remaining 18 cases were malignant. 39 cases were reported as benign. The most common mimicker found was BPH with and without prostatitis accounting to 73.6% cases followed by Basal Cell Hyperplasia and Prostatic Atrophy. 5.8% cases were missed on H&E but showed negative p63 staining indicating PC and 2.5% of benign mimickers were diagnosed as PC on H&E which showed strong nuclear positivity of p63 in suspected malignant glands. **Conclusion:** In our study, benign lesions showed strong basal nuclear positivity of p63. Malignant cases showed negative nuclear staining in glands but was seen positive in benign glands entrapped in between. The premalignant lesion showed focal and patchy staining by p63. AMACR showed positive in malignant cells but comparatively weak staining was noted in adenocarcinomas having cribriform pattern (Gleason pattern 4). Routine H&E still remains to be the gold standard (94.4%) in diagnosing all PC cases and IHC in an ancillary study done in selected cases only and should not be used as a screening test.

KEYWORDS

Prostate carcinoma, mimickers, benign, p63

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INTRODUCTION

Prostate carcinoma (PC) is the fifth most common cancer in the world and the second most common cancer and is emerging as one of the leading causes of cancer in both developed and developing countries.1(Baig MK, Hassan U, Mansoor S),2(Grisanzio C) Needle core biopsies and TURP chips require accurate interpretation for early diagnosis which is very challenging in view of small volume of biopsies and presence of benign mimickers such as atrophy, atypical adenomatous hyperplasia, basal cell hyperplasia, atypical small acinar proliferation and high grade PIN. Further the threshold for detecting small foci of cancer in needle biopsies is very low.3(Humphrey PA) In recent years various treatment modalities have been developed for the management of PC. And hence it is very important to correctly diagnose and prevent over or under- diagnosis. Prostatic needle biopsy is the preferred and widely practiced method for diagnosing early PC. Even though the diagnosis of prostatic lesions is analysed mainly through histopathological examination, sometimes diagnosis can be difficult when pathologists are faced with problems such as small foci of carcinoma commonly seen in needle biopsy specimens or benign mimickers of PC.14(Cuzick J) With the help of p63, a basal cell nuclear marker for prostatic myoepithelial cells, it is easier when dealing with puzzling cases of prostate or when tangential sections from PIN/Prostatic carcinoma are studied. Benign mimickers will show strong positivity for p63 and premalignant lesions show patchy staining for the marker.6(Srigley JR Benign mimickers of prostatic adenocarcinoma) Basal cells are absent in prostate adenocarcinoma. The identification of these basal cells in prostatic glands can be used to rule out the diagnosis PC. This very information helped give rise to the use of immunohistochemical markers to identify basal cells, especially in reporting cases like benign mimickers or other non- pathologic mimickers of PC.3(Humphrey PA)

MATERIALS AND METHODS

The present study was a prospective study over a period of eighteen months from 1st March 2021 to 1st December 2022 conducted in Histopathology section, Central Laboratory, Department of Pathology of M.G.M. Medical College, Kamothe, Navi Mumbai. Data was collected by using proforma from patients fulfilling the inclusion criteria and exclusion criteria. Written and informed consent was taken from them. All the biopsies and surgical specimens of prostate, TURP chips and needle biopsies of prostate were included in the study except needle or core biopsies inadequate or suboptimal in nature and autolyzed samples. 57 specimens were received and foxed in 10 % neutral buffered formalin and routine paraffin processing was done, followed by hematoxylin and eosin (H&E) staining of sections. All the slides were examined under a microscope and each case was studied according to age, clinical presentation, Serum PSA levels, DRE findings and microscopic picture. Based on these parameters, a diagnosis was made The cases were divided into benign, malignant and pre-malignant lesions. Gleason grading was used for grading of adenocarcinomas. Immunohistochemistry was performed on the tissue sections taken from the blocks along with positive and negative control. All the data collected was entered in MS Excel. p value was calculated using SPSS test of significance. The results were documented in tables and graphs.

RESULTS

In our study we observed that out of 57 samples, 19 cases were reported as neoplastic and rest 39 cases were reported as benign. 1 case was reported PIN(2%) amidst remaining 18 malignant cases(31%) in the neoplastic group. 16 needle core biopsies were reported as acinar adenocarcinoma and 2 prostatectomy specimens both cases were reported as benign prostatic lesions.

Table 3: Distribution according to Nature of Specimen into benign, premalignant and malignant lesions

Nature of Specimen	Benign	PIN	Malignancy	No.of Cases	%
Prostate biopsy	0	0	16	16	30.1
TURP chips	32	1	2	35	66
Prostatectomy specimen	2	0	0	2	3.7
Total	38	1	18	57	100

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Maximum number of cases were in 71-80 years of age group, of which 13 cases were reported malignant, 7 cases were benign and 1 case was reported as PIN. 26 cases had S. PSA ≤ 4ng/ml of which 24 were reported as Benign. Out of 16 cases with >10ng/ml, 15 were malignant and 1 case of BPH. Our study also showed that BPH with prostatitis show raised S. PSA levels. In contrast, Urothelial carcinoma and Neuroendocrine tumour both cases showed S. PSA < 2ng/ml. Maximum Prostate adenocarcinoma cases belonged to Gleason Grade group 4(4+4=8). S. PSA > 10ng/ml in 15 out 16 Prostate adenocarcinoma cases.

p63 expression studied showed strong nuclear positivity in basal cells of glands of benign lesions, with few cases showing patchy positivity. Whereas all adenocarcinoma showed negative p63 staining in malignant glands. It correlated with positive AMACR staining in tumour cells. Sensitivity of p63 was 88.8% and specificity was 92.1% (p=0.001).

Out of 18 prostatic carcinoma cases one was Urothelial Carcinoma of Prostate, one was Neuroendocrine and rest 14 cases were Adenocarcinoma and all showed negative basal staining with p63.

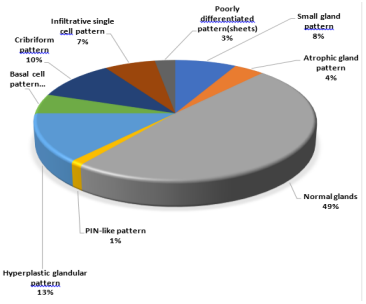

Chart 13: Distribution of different growth patterns

Table 17: Numbers of Specimens for which p63 Staining Was Informative in Final Diagnosis

P63 staining informative	PROSTATIC LESIONS					Total
	BPH	Adenocarcinoma	Partial Atrophy	Basal Cell Hyperplasia	Other Malignancies	
Yes	21	16	2	1	0	40
No	4	1	1	4	1	11
Total	25	17	3	5	1	51

H&E and p63 stained slides were examined together for each case. Staining for p63 was considered informative only when the absence of basal cell nuclear staining with p63 was demonstrated in the lesions that had been diagnosed as malignant on H&E. Good internal positive control was required for comparison and accurate reporting. Staining was additionally considered informative when basal cell nuclei stained was positive with p63 in lesions that were diagnosed as benign on H&E.

On p63 staining, it was found that 1 out 17 cases of Adenocarcinoma and 1 out of 40 benign cases were missed on H&E diagnosis.

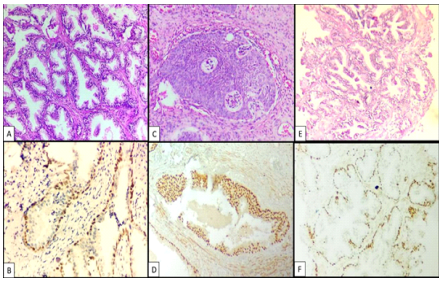

Image 1: A) Benign Prostatic Hyperplasia H&E (40x); B) p63 basal positivity in BPH (40x); C) BCH mimicking PC (cribriform pattern); D) BCH, strong nuclear positivity shown by basal cells; E) Hyperplastic gland pattern H&E(10x) F) p63 basal positivity in Hyperplastic glands H&E (40x)

DISCUSSION

BPH and Prostate Carcinoma are increasingly frequent with advancement of age and are not common before the age of 40 years. Asians, particularly vegetarian, men consume low-fat, high-fiber diets rich in weak dietary phytoestrogens, which have been proposed as chemo-preventive agents. In India, the incidence of PC is estimated at 8/100,000 persons.79(Bid HK) Thus, it is important to differentiate prostatic carcinoma from its mimickers. Many basal cells markers have been studied for the purpose of increasing accuracy in diagnosis and reducing number of missed cases of prostatic carcinomas.

Serum PSA is a sensitive tumour marker and thus is widely used as a screening tool for the detection of prostate carcinoma. Many conditions such as BPH with or without prostatitis, urinary retention, instrumentation, and infarction also can lead to elevation of PSA levels.80(Sindhwani P) In present study we have used p63 stain, which is lost in prostatic adenocarcinoma.

PROSTATIC LESIONS

50 cases were included is a study done by Mahapatra Q et al81 in which 22 cases(44%) were diagnosed as benign and 28 cases(56%) as malignant. The higher incidence of malignancies in this study was due to the exclusion of BPH which usually forms the majority of cases in various studies. In our study, 38 cases were diagnosed as benign, majority being BPH, 1 case of PIN and 18 cases were reported to be malignant. Age wise distribution of cases in our study were in concurrence with studies done by Hirachand S et al82 and Mahapatra Q et al81.

SERUM PSA LEVELS

Table 21: Comparison of PSA levels in different studies

Serum PSA level		≤4	4 – 10	>10
Sharma M et al83	Benign	29	15	5
	Malignant	2	6	3
	Total	31	21	8
Josephine A et al84	Benign	7	8	14
	Malignant	0	3	17
	Total	7	11	31
Mahapatra Q et al81	Benign	19	3	0
	Malignant	0	3	25
	Total	19	6	25
Present study	Benign	24	12	1
	Malignant	2	1	15
	Total	26	13	16

Similar to our study, studies done by Gui-zhong L et al87 and Irani et al88 showed that there was a proportional rise seen in total PSA levels and the grade and extent of inflammation of prostate due to disruption of glands. Urothelial carcinoma and Neuroendocrine tumour were both cases which didn't show increased S. PSA levels.

BENIGN MIMICKS OF PROSTATE CARCINOMA

Our study showed BPH with Prostatitis as the most common benign mimicker of prostate carcinoma similar to study conducted by Murgod PS et al.91 In contrast, study by Mahapatra Q et al81 showed Basal Cell Hyperplasia as the most common mimicker.

Table 23: Mimickers of Prostate Carcinoma in various studies

	BPH with Prostatitis	PIN	Basal Cell Hyperplasia	Partial Atrophy	Adenomatous Hyperplasia	Clear cell cribriform hyperplasia
Mahapatra Q et al81	-	4	13	2	-	2
Murgod PS et al91	97	18	9	0	3	1
Netto GJ.et al58	-	-	-	2	4	7
Present study	7	1	5	3	2	-

In our study, out of the total 5 cases of BCH reported, 1 case was reported as adenocarcinoma on H and E staining but was finally reported as BCH after p63 staining showed basal cell proliferation pattern mimicking adenocarcinoma prostate.

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PARTIAL ATROPHY (PA)

PA is especially confused with the partial atrophy variant of adenocarcinoma prostate.81(Mahapatra Q) The cells in PA have a pale cytoplasm giving rise to pale staining glands that more closely mimic cancer. Herawi M et al92 found partial atrophy as most common mimicker, especially on needle core biopsies. Atrophy is a common ageing process and so is categorized among benign lesions of prostate but is often misdiagnosed as carcinoma on H&E. In our study, we reported 3 cases of partial atrophy, one of which was confirmed with the help of p63 staining.

ADENOMATOUS HYPERPLASIA (AH)

Other common mimicker is adenomatous hyperplasia, also known as adenosis. AH, is defined as a well-circumscribed, nodular proliferation of densely packed small to round glands without cytologic atypia. It resembles well-differentiated (Gleason's grades 1, 2 and 3) PC, but lacks prominent nucleoli or other atypical nuclear features of the latter.56(Grignon DJ).

In our study, 2 cases were diagnosed as AH on H and E which accounted for 3.5% of all cases and this correlated with the majority of the studies.

BASAL CELL HYPERPLASIA (BCH)

It is characterized by a complex cribriform proliferation of glands lined by pale and clear cells confusing them with Gleason pattern 4 lesions. These cells are cuboidal to low columnar showing bland cytologic features and are surrounded by basal cells which help in differentiating them from Gleason grade 4 cribriform variant of adenocarcinoma.

BPH WITH PROSTATITIS

Histomorphological features show broken or disrupted glands leading to raised S. PSA levels making it a clinical as well as histological mimicker of PC.

In our study, BPH with prostatitis was the most common mimicker found, of which 1 case even showed S. PSA > 10 ng/ml.

HISTOLOGIC PATTERNS IN ADENOCARCINOMA PROSTATE

The diagnosis of prostatic cancer requires combination of architectural, nuclear, cytoplasmic and intraluminal features as presence of individual features may also be seen in benign conditions. Architectural features include presence of uniform, small crowded glands infiltrating into stroma in well differentiated carcinoma. Less differentiated tumours show poorly formed, fused or large cribriform glands or infiltrative pattern of growth as single cells and solid sheets. Presence of corpora amylacea mostly indicates a benign lesion.45(Epstein JI)

In our study, we found that predominant pattern seen in prostatic adenocarcinoma cases were hyperplastic glandular pattern contributing 10 cases followed by cribriform pattern contributing 8 cases of all cases.

p63 IMMUNOHISTOCHEMICAL STUDY

Table 24: Sensitivity of immunohistochemical staining for p63 in cases of prostatic carcinoma, summary of published literature

Study	Year	N	Sensitivity
Singh V. et al94	2014	470	90%
C. Boran et al95	2009	98	93.4%
Shiran M. S. et al96	2007	72	88.37%
Wu HH et al97	2004	100	99.44%
Present study	2022	57	88.8%

Protein p63, along with p73, is a structural and functional homolog of the tumor suppressor gene. It is a regulator of growth and development of the cutaneous epithelium, uterine cervix, breast, urogenital tract, and prostate.95(Boran C).

Our study is in concordance with all the above studies except 3 cases of BPH that showed focally negative staining accounting for 5.2%. This finding appears similar to that as with Fahd et al100. This negativity could be explained by prolonged fixation in formalin as the antigenicity will be masked by prolonged fixation or could be due to cautery artefact also. 1 case of Prostatic Intraepithelial Neoplasia reported showed patchy nuclear staining. Among the 16 Prostatic adencarcinoma studied none showed positive nuclear staining for p63. In most of the cases we could see the malignant glands infiltrating benign glands, which on immunohistochemical study showed presence of islands of trapped benign glands showing strong basal positivity with p63 in between by negatively staining malignant glands.

In case of distinguishing from poorly differentiated prostatic carcinoma and poorly differentiated urothelial carcinoma prostatic carcinoma shows positivity for PSA, PSAP and negative for thrombomodulin and Uroplakin. Others newer marker used in diagnosing prostatic origin are Prostein and NKX3.1.102 In our study, NKX3.1, GATA3, Synaptophysin and p63 were used for diagnosis of Urothelail Carcinoma of Prostate and Poorly differentiated Neuroendocrine carcinoma of Prostate.

CONCLUSION

Prostatic carcinoma, one of the leading cause of cancer worldwide, needs to be distinguished from its benign mimickers to provide optimal treatment. Our study concluded that serum PSA level was >10ng/ml in case of all malignant cases except for Urothelial Carcinoma and Neuroendocrine carcinoma. So low PSA levels doesn't always rule out prostate malignancy.

Most common benign mimicker found in our study was BPH with and without prostatitis accounting to 73.6% of cases followed by basal cell hyperplasia(13.1%) and prostatic atrophy(7.8%).

Benign lesions showed strong basal nuclear positivity of p63. All 16 cases acinar adenocarcinoma showed negative nuclear staining in malignant glands but was seen positive in benign glands entrapped in between them. On the other hand, PIN showed focal and patchy staining by p63.

Sensitivity of p63 in our study was calculated to be 88.8% and specificity 92.1%. 5.8% cases were missed on H&E being diagnosed as BPH but showed negative p63 staining indicating PC and 2.5% of benign mimickers were diagnosed as PC on H&E which showed strong nuclear positivity of p63 in suspected malignant glands and report was revised as BCH.

Cases studied with additional IHC markers like AMACR showed strong positivity in malignant cells but comparatively weak staining in glands arranged in cribriform pattern (Gleason pattern 4).

Routine H&E still remains to be the gold standard (94.4%) in diagnosing all PC cases and IHC in an ancillary study done in selected cases only and should not be used as a screening test.