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UTILITY OF P-63 AND 34βE12 AS BASAL CELL MARKER FOR DIAGNOSIS OF PROSTATIC ADENOCARCINOMA AND TO DIFFERENTIATE IT FROM CANCER MIMIKERS

Pathology

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ABSTRACT

world wide incidence of prostate cancer increased last few decades as a result of early detection by core needle biopsy. Differentiation of prostate adenocarcinoma from benign prostate lesions and hyperplasia sometimes cannot be done on the sole basis of morphologic findings. Diagnosis of prostatic carcinoma on routine biopsies can be challenging when pathologists are faced with certain problems such as limited tissue sample, small foci of carcinoma, or benign mimics of prostate cancer like atrophy and atypical adenomatous hyperplasia, basal cell hyperplasia. In these cases, the diagnosis can be made according to the presence or absence of the basal cell layer, considering the fact that in the prostatic adenocarcinoma, there is no basal cell layer but benign lesions have encirclement by this layer. Hence, using basal cell immunohistochemistry markers including p63 and 34βE12 seems useful in distinguishing these two important categories of prostate lesions. Immunohistochemistry (IHC) is a valuable adjunctive in the diagnosis of minute foci of prostatic carcinoma and to differentiate it from benign mimickers and precursor lesions. Objective of this study is to find the usefulness of basal cell markers namely p63 and 34βE12 for diagnosis of adenocarcinoma and to differentiate them from cancer mimickers. Another objective of this study is to compare sensitivity and specificity of p63 and 34βE12 as immunomarker in distinguishing prostatic carcinoma from benign prostatic lesions.

KEYWORDS

Prostate adenocarcinoma, Basal cell marker, 34βE12, p63

INTRODUCTION

Cancer of the prostate is typically a disease of men over age 50[1]. Herbert *et al*. compared data available from various cancer registries and observed that the average annual cancer incidence rate for prostate cancer in India ranged 5.0-9.1 per 100,000/year, whereas the comparative rate in the United States were 110.4 for whites and 180.9 for blacks.[2] Risk of prostatic cancer abruptly increase after 5th decade and mean age of incidence is 65 years [3]

world wide incidence of prostate cancer increased last few decades as a result of early detection by core needle biopsy. Prostate cancer is now the 5th most common cancer in the world and 2nd most common cancer in men [4]. The accurate diagnosis is of great importance for early detection of malignancy.

This directs the line of management of patients towards a lesser invasive procedure instead of more radical one associated with significant morbidity and mortality [5].

Differentiation of prostate adenocarcinoma from benign prostate lesions and hyperplasia sometimes cannot be done on the sole basis of morphologic findings. In these cases the diagnosis can be made according to the presence or absence of the basal cell layer, considering the fact that in the prostate adenocarcinoma there is no basal cell layer but benign lesions have encirclement by this layer. Many benign lesions of prostate are looks malignant on its apparent look. But detailed study of architectural and cellular morphology along with immunohistochemistry differentiates them from malignant counterpart. Approximately 50%of cases with an atypical diagnosis have cancer on repeat biopsy. Repeat negative biopsy is not enough to exclude the presence of carcinoma because of prostatic needle biopsy are associated with fairly high false negative rates of up to 25%[6]. It is incumbent on the pathologists in these cases to have the initial biopsy sent off for consultation or to try to resolve the initial biopsy with ancillary techniques such as immunohistochemistry with antibodies like high molecular weight cytokeratin or p63[7].

Hence, using basal cell immunohistochemistry markers including p63 and 34βE12 seems useful in distinguishing these two important categories of prostate lesions. Also a typical small acinar proliferation that are suspicious for carcinoma may be found in up to 9.0% of all prostate biopsies, in which up to 59% are found to be malignant after using immunohistochemical markers[8,9]

Immunohistochemistry (IHC) is a valuable adjunctive in the diagnosis of minute foci of prostatic carcinoma and to differentiate it from benign mimickers and precursor lesions[10]

The objective of this study is to find the usefulness of basal cell markers namely p63 and 34βE12 for diagnosis of adenocarcinoma and to differentiate them from cancer mimickers. Another objective of this study is to compare sensitivity and specificity of p63 and 34βE12 as immune marker in distinguishing prostatic carcinoma from benign prostatic lesions.

MATERIAL, METHOD AND RESULT

This study includes20 core needle biopsy and 40 TURP specimens which were received from urology department, over the period of one year. Biopsy specimens were fixed in 10% formalin and processed. We studied the H&E stained microscopic slides for histomorphological study. Morphologically confirmed adenocarcinoma cases were reported with Gleason score. Out of total 60 cases 14 cases was diagnosed as adenocarcinoma and 36 cases was diagnosed as Benign hyperplasia of prostate(BHP). Five specimens showed features of prostatic atrophic glands and one specimen showed features of adenosis. Features of High grade intraepithelial carcinoma (HGPIN) were seen in 3 specimens. Features of atypical crowed acinar proliferation with suspicion of malignancy were seen in one specimen. (Table-1)Out of 14 adenocarcinoma cases most predominant Gleason score was 7(3+4) which is observed 10 cases. We also found carcinoma mimicker like, cautery artifact and basal cell hyperplasia in some of the specimens.

Immunohistochemical staining was performed for basal cell markers, including high molecular weight cytokeratin HMWCK (34βE12) and p63.We evaluated result of immunohistochemistry done with both p63 and 34βE12 in all 60 specimens. We found 36 BHP case positive for both the markers. All adenocarcinoma cases were negative for both the markers. (Table-1).36 BHP specimens showed 85% to 100% basal cell positivity with p63 (mean 96.4%) marker and 34 β E12 staining showed 80-100% positivity (mean 92.05%). Three HGPIN cases showed positivity of 48.3% for p63 and 44.66% for 34β E12 marker.

We found a single case of adenosis which showed basal cell positivity of 85% with p63 and 80% with 34βE12 markers.

However p63 had less positivity than 34βE12 in atrophic glands and p63 also failed to stain basal cells in one such case. In 4 specimens with atrophic glands reveled 10-40% p63 positivity of basal cell with mean of 41%.

All five specimens with atrophic glands revealed wide variable range of 30-80% positivity (mean of50%) of basal cells with34βE12 marker.

In one core needle biopsy, we found focal crowded glands where comments on basal cell lining were not possible. We found 0% expression of both basal cell markers so it diagnosed as micro invasive adenocarcinoma.

We found multiple foci of basal cell hyperplasia. All shows strong positivity of both markers.

Both the markers had 100% specificity .But 34βE12 shows 100% sensitivity and P63 shows 97.77% (Table-2&3).Both markers had p - value less than 0.001 and thus according to our study any of the marker can be used as basal cell marker to rule out malignancy.

Overall 15 malignant cases (including one case of micro invasive carcinoma) showed no staining with both the basal cell markers. Out of 45 benign cases including BHP, HGPIN, adenosis and atrophic glands ,12(26.66%) benign cases had same percentage of basal cell positivity for the both the basal cell markers. But 24(53%) cases showed more positivity of p63 than 34βE12 marker, but it had less positivity in 8(20%) cases than 34βE12 stain. One case with atrophic glands failed to stain with p63 marker for basal cells.

Unusual low positivity of p63 than 34βE12 in spite of having more positivity in cases of benign lesions was due to its low positivity in 4 cases of atrophic glands and failure to stain basal cells in atrophic glands in one specimen.

Table-1

Hispathological Diagnosis	P63		34βE12	
	Positive	Negative	Positive	Negative
BHP	36(96.4%)	00	36(92.04%)	00
ADENO CA	00	14	00	14
HGPIN	03(48.3%)	00	03(44.66%)	00
Atypical Gland	00	01	00	01
Adenosis	1(85%)	00	1(80%)	00
Atrophy	4(41%)	1	5(50%)	0

Table-2

P63	Benign Lesion And Hgpin	Adenocarcinoma	Total
Positive	44	00	44
Negative	01	15	16
Total	45	15	60

Sensitivity of P63 marker: 44/ (44+1) =97.77% Specificity: 15(15+00) =100%

Positive predictive Value: 100% Negative predictive value: 93.75%
P -value was obtained by Fisher's exact test and was highly significant

Table-3

34betaE12	Benign Lesion And Hgpin	Invasive Lesion	TOTAL
Positive	45	00	45
Negative	00	15	15
Total	45	15	60

Sensitivity and Specificity of 34βE12 marker: 100%

Positive and Negative predictive Value: 100%

P -value was obtained by Fisher's exact test and was highly significant (p less0.001)

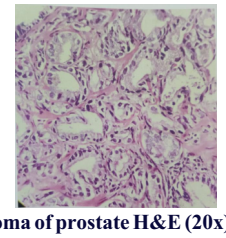

Fig-1. Adenocarcinoma of prostate H&E (20x)

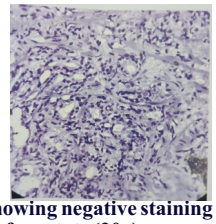

Fig-2. P63 marker showing negative staining of basal cells in a case of Adenocarcinoma of prostate (20x)

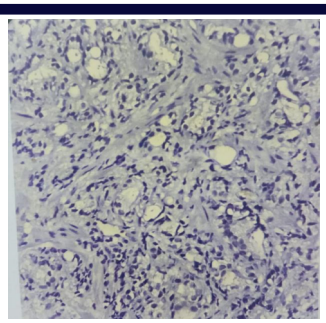

Fig-3. 34βE12 marker showing negative staining of basal cells in a case of Adenocarcinoma prostate (20x)

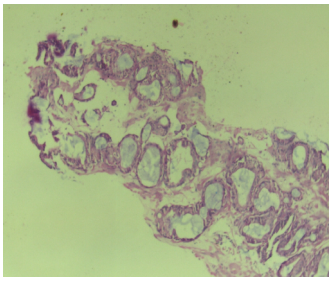

Fig -4. Atypical prostatic glands, suspicious of malignancy (H&E) 20x

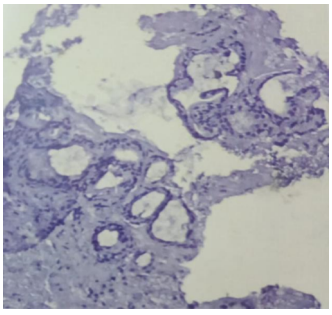

Fig-5. P63 marker showing negative staining of basal cells (20X) of atypical glands

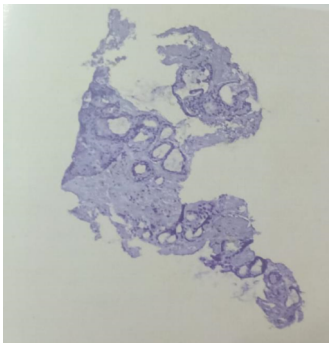

Fig-6. 34βE12 marker showing negative staining of basal cells (20X) of atypical glands

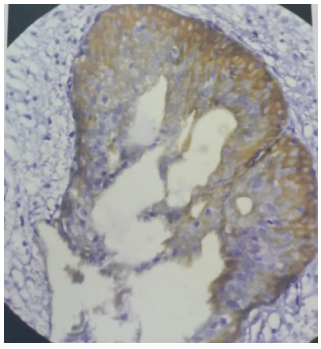

Fig-7. Basal cell hyperplasia showing positive staining with 34βE12 marker (20X)

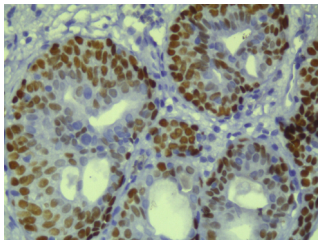

Fig-8. Basal cell hyperplasia showing positive staining with P63 marker (20X)

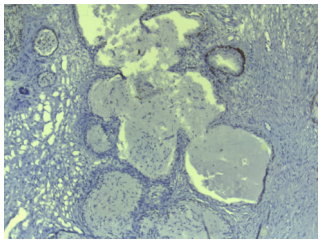

Fig-9. P63 maker showing negative staining of Atrophic glands (20X)

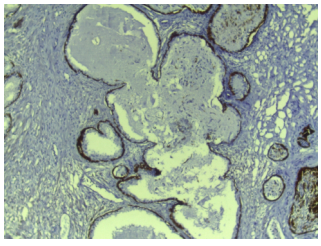

Fig-10. 34βE12 maker showing positive staining of Atrophic glands (20X)

DISCUSSION

The diagnosis of Prostatic carcinoma on routine biopsies can be challenging when pathologists are faced with certain problems such as limited tissue sample, small foci of carcinoma, or benign mimics of prostate cancer like atrophy and atypical adenomatous hyperplasia. It has been well documented that benign prostatic glands retain their basal cells while infiltrating adenocarcinoma do not. Therefore histologically, absence of a basal cell layer provides supportive evidence for prostatic carcinoma.

In 1953 it was observed by Totten etal that basal cells are invariably lacking in prostatic adenocarcinoma[11].In 1984, Gown and Vogel reported for the first time that, high molecular weight (HMW) anti keratin antibody stained prostatic basal cells specifically[12] .Results of other later studies showed that basal cells exist only in normal prostatic glands but not in prostatic adenocarcinoma[13, 14]. Wojno and Epstein showed that in highly suspicious for prostate cancer cases, the proof of basal cell absence by 34βE12 marker is very helpful to confirm prostate adenocarcinoma diagnosis[15]. Signoretti *et al* reported that all basal cells express p63, therefore, this marker also can be useful in distinguishing benign lesions from prostate malignancy[16] .The most commonly used basal cell specific markers are high molecular weight cytokeratin [HMWCK] and newly described basal cell marker p63[17]. HMWCK (34βE12) shows cytoplasmic positivity where as p63 shows nuclear positivity[18].Signorette *et al.* also highlighted the role of p63 in the development of prostate glands and showed that p63 is expressed in virtually all the basal cells of prostatic glands[16]

Also a typical small acinar proliferation that are suspicious for carcinoma may be found in up to 9.0% of all prostate biopsies, in which up to 59% are found to be malignant after using immunohistochemical markers[8,9] . The diagnosis of prostatic adenocarcinoma, especially in needle biopsy samples may be difficult either due to presence of small foci (limited ≤1mm carcinoma in needle tissue), or the difficulty in distinguishing prostatic carcinoma from benign mimickers [19]. Considering the fact that the loss of basal cell layer is a hallmark of prostate adenocarcinoma, the basal cell markers can help to differentiate prostate adenocarcinoma from cancer mimickers [7]. 34βE12 staining is a useful tool in confirming, establishing, or changing the diagnosis in questionable foci seen in the everyday practice of surgical pathology[21]. The intensity of the 34βE12 and P63 was classified as negative, weak, moderate, and strong [20].

1985 Brawer et al showed that, the manner in which staining for high molecular weight cytokeratin could be used to distinguish a variety of benign and potentially pre neoplastic lesions from invasive carcinoma.[22]

A cross sectional study was conducted by kalntari MR et al which showed that the specificity and sensitivity of p63 and 34βE12 in true adenocarcinoma and benign lesions are high, but in cancer mimickers especially when morphologic differentiation is impossible between benign and malignant lesion, p63 sensitivity is significantly higher [7]. M.H. Weinstein *et al.* proved that p63 is more superior in demonstrating prostatic basal cells when compared to HMWCK [23]

In the study of Wang et al 30% of partial atrophy cases were p63 and 34βE12 marker negative [25].

The results of our study demonstrate that p63 and HMWCK are specific for basal cells in the prostate glands. This correlated with the study of Shah *et al.* Who reported the absence of basal cell staining both with HMWCK and p63 in prostatic adenocarcinoma [20] Person *et al* showed that p63 is expressed in normal basal cells and benign prostate hyperplasia (BPH). It is focally expressed in prostate atrophy and HGPIN, but there is no p63 expression in the majority of prostate adenocarcinoma[24]. However, some studies showed p63 and 34βE12 negativity in some benign lesions such as adenosis, atrophic glands, and HGPIN[14], [26,27,28,29]

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Boran etal. who reported that the best combination of basal cell markers used together may be the 34βE12 and p63 because 34βE12 is the best cytoplasmic marker and p63 is the only nuclear marker among basal cell markers [29] Shah et al., stated that p63 is more sensitive than 34βE12 in staining benign basal cells, offering slight advantage over 34βE12 in diagnostically challenging cases, so p63 may be used as an alternative to 34βE12 stain for minimal carcinoma. [21]

Other than selection bias in sampling, the specificity and sensitivity of p63 and 34βE12 in true adenocarcinoma and benign lesions (BPH) are high, but in cancer mimickers, especially, when morphologic differentiation is impossible between benign and malignant lesions, p63 sensitivity is significantly higher than34βE12.[7]Moreover; 34βE12 nonspecific staining in benign lesions is a problem in interpretation of the results[7].

In clinical practice, combination of basal cell markers are helpful to improve differentiation of prostate cancers from mimickers.

Howard Her-juing Wu studied 100 cases of prostatic carcinoma which showed specificity of absence of basal cell staining in the malignant glands for 34βE12 and p63 was 98.63% and 98.60%, respectively which matches with our study[30].Study done by RB Shah showed, out of 108 positive stained specimens, 57(53%) cases with same percentage of basal cell positivity for both markers.45(41%) cases showed more basal cell positivity with p63 and 6(6%) cases reveled more positivity with 34βE12 marker. However one case failed to stain with p63 in their study. [20]

In our study, both p63 and 34βE12 markers had 100% specificity but 97.77% and 100% sensitivity respectively(Table-2&3) .We found basal cell positivity in all cases of BPH and basal cell negative in all cases of adenocarcinoma. Therefore both the markers are highly significant. In our study we conclude that any of the markers can be used as basal cell marker to differentiate adenocarcinoma from benign lesions of prostate and cancer mimickers. However further study of both the markers on partial atrophy of prostatic glands are suggested.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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