Role of p63 in Differentiating Morphologically Ambiguous Lesions of Prostate
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ABSTRACT
Objective: To observe the role of p63 staining in evaluation of morphologically ambiguous lesions of prostate.
Study Design: Descriptive (case series) study.
Place and Duration of Study: Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, from March to September 2010.
Methodology: p63 immunohistochemistry staining was performed on 30 cases diagnosed as morphologically ambiguous lesions of prostate by histopathologist. Patient’s name, age, histology numbers, morphological features, expression of p63 and histopathological diagnosis were recorded and described as frequency percentages.
Results: The prostatic adenocarcinomas were negative for p63 and benign lesions of prostate were positive for p63. Atypical lesions show positive staining in 77.7% and negative staining in 33.3% of cases.
Conclusion: p63 immunohistochemistry staining can be used as a reliable marker to distinguish between benign from malignant morphologically ambiguous lesions of prostate.

applications to those of high molecular weight cytokeratins in the diagnosis of prostatic adeno-carcinoma, but with certain advantages. It stains a subset of 34-betaE12 negative basal cells, is less susceptible to the staining variability of 34-betaE12 (particularly in transurethral resection of prostate (TURP) specimens with cautery artifact), and it is easier to interpret because of its strong nuclear staining intensity and low background. Prostatic adeno-carcinomas have occasional p63 immunoreactive cells, most representing entrapped benign glands or intraductal spread of carcinoma with residual basal cells.

Treatment options and prognosis of prostatic adenocarcinomas and benign lesions differ significantly, so, they must be diagnosed with accuracy. This requires application of immunohistochemical stains for basal cells especially in morphological ambiguous cases (p63 shows nuclear staining in basal cells of benign prostate lesions and no staining in prostatic adenocarcinoma).

The objective of this study was to observe the role of p63 staining in morphologically ambiguous lesions of prostate.

**METHODOLOGY**

This study was carried out in Pathology Department of Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH and RC), Lahore, from March to September 2010. It was a case series study. Approval of study was taken from Institutional Review Board (IRB) of SKMCH and RC. A total of 30 male patients who were diagnosed on pathology as ambiguous lesions of prostate were studied. Sampling technique was non-probabilistic purposive. Needle biopsies, transurethral resections, prostatectomy specimens and morphologically ambiguous lesions of prostate were included. Poorly preserved or poorly fixed specimens were excluded.

The specimens were collected from pathology department. Each was given a case number and medical record number and demographic details of patients were recorded. The specimen were fixed in 10% buffered neutral formalin. After appropriate gross examination, sections were processed and stained with haematoxylin and eosin (H&E). Microscopic features were noted. Only those cases were selected in which prostatic adenocarcinoma could not be distinguished from benign lesions on the basis of light microscopy alone and immunohistochemistry for basal cells was considered essential. p63 immunohistochemical stain was used to elucidate prostatic basal cell layer, whose presence or absence is a reliable criterion for differentiating prostatic adenocarcinoma from benign prostate lesions. Immunohistochemical stain p63 was performed according to the specifications given by the manufacturer. The whole section was scanned at low power in order to assess the general level of intensity throughout. Brown nuclear staining was considered positive (weak, moderate, bright). The pathologist recorded the findings.

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 14.0. Mean and standard deviation were calculated for quantitative variable like patient's age. Frequencies and percentages were calculated for qualitative variables like result of p63 immunohistochemistry, and morphological features (small atypical glands, glands of variable size/shape, crystalloids, corpora amylacea, basophilic mucinous fibroplasias, glomerulation, perineural invasion, nuclear size variation, hyperchromasia, prominent nucleoli, mitotic figures).

**RESULTS**

During the study period, 30 cases which were diagnosed by pathologist as ambiguous lesion of prostate were studied and expression of p63 was noted. The ages ranged from 56 to 90 years with mean age of 70.93 ± 6.23 years. There were 10 TURP specimens, 13 specimens of needle biopsy and 7 specimens of prostatectomy. A total of 29 samples showed small atypical glands. Out of those, 16 (55.2%) were negative for p63, 6 (20.6%) were positive with moderate expression and 7 (24.2%) were positive with bright expression for p63. One sample did not show small atypical glands. Variable sized and shaped glands were seen in 28 cases, out of which 6 (21.4%) cases were positive with moderate expression, 7 (25%) with bright expression and 15 (53.6%) samples were negative for p63. Two samples did not show glands of variable sizes and shapes.

Fourteen samples revealed crystalloids in glands; 3 (21.4%) were positive for p63 and 11 (78.6%) were negative for p63. About 6 samples showed corpora amylacea within glandular lumina of 5 (83.3%) were positive for p63 and 1 (16.7%) was negative for p63. None of the samples revealed basophilic mucin/mucinous fibroplasias, glomerulation and perineural invasion.

Variation in nuclear size of cells lining the glands was seen in 18 samples, out of which 1 (5.5%) was positive with moderate expression and 2 (11.1%) were positive with bright expression and 15 (83.4%) samples were negative for p63. Nuclear hyperchromasia was seen in 15 samples, out of which 1 (6.6%) was positive with moderate expression, 2 (13.4%) with bright expression and 12 (80%) samples were negative for p63. About 15 samples revealed glandular cells with prominent nucleoli; 2 (13.4%) were positive with moderate expression, 2 (13.4%) were positive with bright expression and 11 (73.2%) samples were negative for p63. Only two samples showed mitotic figures in glands which were negative for p63.
Prostatic adenocarcinoma was the primary prostate tumour in 14 (46.7%) of the patients. On the other hand, 6 (23.3%) cases were diagnosed as benign prostatic hyperplasia, 6 (23.3%) as atypical small acinar proliferation and 3 (10%) cases as atrophic glands. p63 IHC stain was applied to all these samples. The prostatic adenocarcinoma revealed no staining while benign prostatic hyperplasia and atrophic glands were positive for p63. There were 6 cases of ASAP, out of which 4 (66.6%) showed p63 staining and 2 (33.4%) were negative for it.

**DISCUSSION**

There are a wide variety of morphological patterns encountered in prostate pathology that may be confused with one or more of the diverse patterns of prostatic adenocarcinoma. Most of these lesions are readily recognized and easily separated from malignancy but problems can be encountered by a histopathologist, especially when dealing with limited sampling in TURP/needle biopsies. False-positive cancer diagnosis may be rendered in some cases leading to serious clinical, psychological and medicolegal consequences. Prostatic biopsy pathology has been identified as a problem area which may lead to litigation.

The most likely conditions giving rise to false-positive malignant diagnosis are atrophy, post-atrophic hyperplasia, atypical adenomatous hyperplasia (adenosis) and seminal vesicle, coxaver gland hyperplasia, cribiform hyperplasia etc. Knowledge of the differential diagnosis of prostatic adenocarcinoma is very important to diagnose limited carcinoma in small biopsy samples. Pathologist should always be aware of the potential of false-positive cancer diagnosis whenever looking at prostate biopsies and to utilize appropriate consultation and ancillary studies to arrive at a correct diagnosis. The management and prognosis of these carcinomas and ambiguous benign conditions are quite different, so making a correct diagnosis is essential for the patient care.

The diagnosis of prostatic cancer is based on a combination of histological features, none of which is absolutely sensitive and specific. The presence of an enlarged nucleolus in the epithelium is a marker for malignancy. However, the limits of normal size are not well-defined. The measurement of nucleolus is considered to be precise but not practical for routine use. One of the defined histological criteria for the diagnosis of prostatic adenocarcinoma is the absence of basal epithelial cells (basal cells) in malignant glands. However, stromal fibroblasts may mimic basal cells and may sometimes pose problems on H&E. Moreover, basal cells may be inconspicuous in some benign glands. Hence, the more specific and sensitive immunohistochemical recognition of these cells with basal cell specific markers has become a practice to diagnose morphologically difficult cases. There are many immunohistochemical markers which have been used for the identification of these tumours but most of them are less sensitive. Immunohistochemical stains help is used in different clinical settings. The most frequent application, often in needle biopsies, is to distinguish low-grade prostatic cancer from a plethora of benign mimics. In addition, it is applied generally in transurethral resection specimens or metastatic tumour samples, to establish the prostatic origin of a poorly differentiated carcinoma. Immunohistochemical studies which focus to identifying prostatic basal cells (34βE12, CK5/6, and p63), prostatic secretory cells (PSA, PAP, and CD57), neuroendocrine cells (chromogranin, synaptophysin) and inflammatory cells (LCA, CD68) may be required to resolve this diagnostic issues.

The patterns which were identified during our study were benign prostatic hyperplasia, atypical small acinar proliferation (ASAP), atrophic glands and prostatic adenocarcinoma. In every case, p63 staining was informative which is very evident from the fact that all the cases of prostatic adenocarcinoma (100%) were negative for p63 confirming the absence of basal cells (Figure 1 and 2). All the benign lesions (100%) including benign prostatic hyperplasia and atrophic glands (Figure 3 and 4) were positive for p63 thus highlighting the presence of basal cells. About 66.6% of ASAP were positive for p63. ASAP cases which are positive for p63 could be diagnosed as benign. On the other hand, negative staining was found in 33.4% of ASAP cases. Although this later finding was suggestive of malignancy but it should not be interpreted as diagnostic of carcinoma, as this may represent a false negative result either due to the small amount of the sample, or to the conditions of the immunohistochemical procedures.
especially fixation. In this situation, a close follow-up and repeat biopsy along with serial PSA levels are suggested.

There were 10 cases of prostatic adenocarcinoma which showed prominent nucleoli whereas 4 did not. Three cases of ASAP showed prominent nucleoli and 3 cases did not show this feature. There was one case each of BPH and atrophic glands which revealed prominent nucleoli and others did not show this feature. Nuclear size variation was observed in 13 cases of prostatic adenocarcinoma and 5 cases of ASAP. All benign lesions showed normal sized nuclei. In addition, one case each of prostatic adenocarcinoma and ASAP showed corpora amylacea which is more commonly associated with benign lesions of prostate. About 10 cases of prostatic adenocarcinoma, 3 cases of ASAP and one case of BPH showed crystalloids. This feature is not indicative of malignancy. Similar findings were noted by multivariate approach of Cavalcanti et al. towards proliferative lesions of prostate.

In a retrospective study by Weinstein et al., they compared p63 staining with 34βE12 in 70 specimens in which the differential diagnosis included prostatic adenocarcinoma. High molecular weight cytokeratin (34βE12) staining was considered gold standard. There was less false negative staining for p63 as compared to 34βE12. No false positive staining was seen with p63. This study was prospective and validated the results of p63 staining of this study.

The results of p63 staining on prostatic adenocarcinoma also support the results of Grisanzio et al., who performed p63 on 130 cases of invasive prostate cancer and found p63 negativity in 126 (97%) cases. Four cases showed p63 positivity in < 1% of tumour cells. This study also supports Shah et al., who compared the utility of p63 and 34βE12 immunostaining in the work-up of diagnostically challenging cases. They concluded that p63 offered advantage over 34βE12, particularly in TURP specimens.

Subsequently, Parsons et al., studied p63 expression in a large series and described strong diffuse p63 protein expression in basal cells of normal and hyperplastic prostate glands, and patchy strong expression in proliferative atrophy and high-grade PIN. Our study also illustrated the same results.

Another study described the use of cocktail immunohistochemical staining (p63/alpha methyl coenzyme A racemase p504s) to diagnose prostate cancer in ambiguous lesions of prostate. The cost of the test was more than the p63 alone as this antibody is made of mixtures of two antibody and it also had few false negative results. In this study p63 stain all benign prostate glands. There were no false negative results during this project.
There was no published local study done in Pakistan to compare with. This is one of the reasons to conduct this study in our settings to facilitate and encourage the use of p63 IHC stain for the definitive diagnosis. Unfortunately due to the cost, unawareness and lack of technical staff to perform this test, it is not widely used in most of the places in Pakistan.

CONCLUSION

Prostatic adenocarcinomas were p63 negative and most of the benign ambiguous lesions of prostate were p63 positive. Hence, it is concluded that p63 is a reliable basal marker and can be used in morphologically difficult cases when the differential diagnosis is adenocarcinoma of prostate.

REFERENCES


