Diagnostic utility of immunohistochemical marker prostein for evaluation of primary and metastatic prostatic carcinomas

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ABSTRACT

Context: The diagnosis of prostatic adenocarcinoma on histopathology depends on architectural and cytomorphological features supported by immunohistochemistry (IHC). Though all the prostate markers show excellent specificity, the sensitivity and percentage positivity vary. Aims: In this study, we aim to study the expression of prostein in normal, benign, and malignant (primary and metastatic) lesions with particular emphasis on its utility in the differential diagnosis of poorly differentiated and metastatic prostatic adenocarcinoma along with a standard panel of IHC markers. Settings and Design: This was both a prospective and retrospective as well as descriptive and observational study. Subjects and Methods: All samples from patients with clinically suspected carcinoma prostate from both primary and metastatic sites from June 2015 to May 2016 were included in the study. Samples with difficulty in diagnosis on hematoxylin and eosin staining were subjected to a panel of IHC markers along with prostein. Statistical Analysis Used: Receiver operating curve analysis and Chi-square test. Results: Prostein showed a 100% sensitivity and specificity to identify normal prostatic epithelium, benign and premalignant lesions, and prostatic adenocarcinoma. Prostein showed a specificity of 100% in differentiating prostatic carcinoma from poorly differentiated urothelial carcinoma and in differentiating metastatic prostatic carcinoma from adenocarcinoma of nonprostatic origin. Conclusions: Prostein is a new and promising prostate-specific marker that showed slightly more sensitivity and specificity than prostate-specific antigen. Thus, adding prostein to the IHC panel will greatly improve the detection of poorly differentiated primary and metastatic lesions of the prostate.

KEY WORDS: Immunohistochemistry, prostate adenocarcinoma, prostein

INTRODUCTION

Prostate cancer is the second most frequently diagnosed cancer as well as the sixth leading cause of death in males with increasing incidence worldwide. Several Indian registries have revealed an increasing trend in the incidence of prostate cancer and the mean annual percentage change has ranged from 0.14 to 8.6%. The diagnosis of prostatic adenocarcinoma on histopathology depends on architectural and cytomorphological features supported by immunohistochemistry (IHC). The utility of IHC in prostate cancer is primarily for confirming the diagnosis of carcinoma in biopsy material containing atypical glands. In addition, IHC helps confirm the prostatic origin of the tumor in the primary or metastatic setting of high-grade prostatic adenocarcinoma and differentiate that from nonprostatic carcinomas. Basal cell markers (high molecular weight cytokeratins, p63, CK5/6) and α-methylacyl-CoA racemase (AMACR) are used to confirm malignancy. Prostate-specific antigen (PSA), prostate-specific acid phosphatase (PSAP), and prostate-specific membrane antigen (PSMA) are used to confirm the prostatic origin of the
tumor.\textsuperscript{[4,5]} Though all the prostate markers show excellent specificity, the sensitivity and percentage positivity vary.\textsuperscript{[6]} P501S (prostein) is a prostate-specific marker that is expressed in the cytoplasm of benign and malignant prostatic glandular cells. It has not been detected in any other normal or malignant tissues.\textsuperscript{[7]} The new IHC markers include prostein (P501S) and NKX3.1. Prostein (P501S) is a prostate-specific 553 amino acid protein identified by complementary DNA (cDNA) subtraction. It is an organ-specific marker for benign and malignant prostatic epithelial cells. Its expression is restricted to prostatic tissues and unrelated to Gleason grade.\textsuperscript{[8]} It shows characteristic diffuse granular cytoplasmic (Golgi) staining and provides an additional valuable IHC marker for detection of metastatic prostatic carcinoma.\textsuperscript{[9]}

In this study, we aim to study the expression of prostein in normal, benign, and malignant (primary and metastatic) lesions with particular emphasis on its utility in the differential diagnosis of poorly differentiated and metastatic prostatic adenocarcinoma along with a standard panel of IHC markers. To our knowledge, there are no studies from India using prostein.

**SUBJECTS AND METHODS**

This was both a prospective and retrospective as well as descriptive and observational study. All samples from patients with clinically suspected carcinoma prostate from both primary (core biopsy, transurethral resection of the prostate [TURP] and radical cysto-prostatectomy) and metastatic sites from June 2015 to May 2016 were included in the study. Demographic, clinical, and imageology data were retrieved from the laboratory information system for the retrospective cases, and data was collected for the prospective cases. Ultrasound (US) or magnetic resonance imaging (MRI) data and serum PSA values were obtained, wherever available. Samples with inadequate tissue/nonavailability of paraffin blocks as well as normal prostatic tissue with no suspicion of malignancy were excluded. The study was approved by the Institutional Ethics Committee. The date of approval is 04.02.2016.

Surgical samples as per inclusion criteria were fixed in buffered formalin, followed by paraffin embedding and stained with hematoxylin and eosin (H and E). Samples diagnosed as adenocarcinoma were scored according to the World Health Classification (2016) criteria, graded using Gleason’s grading system, and assigned with new prognostic grade grouping system.\textsuperscript{[9]}

For samples where there was a difficulty in diagnosis on H and E, appropriate sections were prepared on poly-L-Lysine coated slides and were subjected to IHC using selective antibodies CK 5/6, p63, AMACR, Ki67. In poorly differentiated carcinoma in the prostate, a panel of antibodies (CK7, AMACR, and GATA-3) was used whereas, in metastatic lesions where prostate carcinoma was suspected on morphology, a panel of antibodies (Pancytokeratin, TTF-1, glypican-3, synaptophysin, chromogranin, CD56, and PSA) depending on the site and morphology was used. The clone, dilution, and antigen retrieval method as well as manufacturer of the various antibodies used in the study are given in [Table 1].

**Prostein (P501S) immunohistochemistry**

Immunohistochemistry (IHC) was performed on Ventana automated stainer. The antibody used was FLEX monoclonal mouse antihuman prostein antibody (Dako, USA; test code: IR088; test clone: 10E3; prediluted, ready to use [RTU]). The positive control was normal prostatic tissue showing the granular cytoplasmic staining of luminal epithelial cells, and negative control was section treated with a tris-buffer solution instead of primary antibody.

The intensity of positivity was scored from 0 to 3 as follows: score 0 = nonstained; score 1 = weak; score 2 = moderate; and score 3 = strong. The percentage of positively stained cells for each staining intensity was estimated in the respective lesions.\textsuperscript{[7]}

Expression of prostein was studied in 60 samples including noneoplastic prostatic tissue (adjacent to malignancy in radical prostatectomy) in 8, benign prostatic hyperplasia (BPH) in 10, high-grade prostatic intraepithelial neoplasia (HGPIN) in 3, prostatic adenocarcinoma in 17 (including poorly differentiated carcinomas), urothelial carcinoma (UC) in 2, metastatic prostatic adenocarcinoma in 10, metastatic carcinoma of nonprostatic origin in 5. Prostein was also studied in normal bladder epithelium, kidney, and testis.

The intensity of prostein expression was compared (i) between benign and malignant lesions, (ii) between different grades of Gleason in primary prostatic adenocarcinoma and (iii) between primary and metastatic prostatic adenocarcinoma.

**Table 1: List of antibodies with a clone, dilution, antigen retrieval method, and details of the manufacturer**

| Antibody | Clone | Dilution | Antigen Retrieval | Manufacturer         |
|----------|-------|----------|-------------------|----------------------|
| p63      | AM41815M | RTU | Tris/EDTA, 90°C | BioGenex             |
| CK7      | Ovi12/30 | RTU | Tris/EDTA, 90°C | Dako                 |
| Ki67     | mib1   | RTU | Tris/EDTA, 90°C | Dako                 |
| CK5/6    | EP1601Y | 1:50 | Tris/EDTA, 90°C | Cell Marque          |
| TTF-1, Glypican-3, GATA-3 | | 1:100 | Tris/EDTA, 90°C | Cell Marque          |
| Synaptophysin, CD56, Chromogranin | MRQ-40, MRQ-42, LK2H10 | 1:100, 1:100, RTU | Tris/EDTA, 90°C | Cell Marque          |
| AMACR    | 13H4   | 1:100 | Tris/EDTA, 90°C | Dako                 |
| PSA      | 15S14  | 1:100 | Tris/EDTA, 90°C | Dako                 |
| Prostein | 10E3   | 1:400 | Tris/EDTA, 90°C | Dako                 |

RTU: Ready to use, AMACR: α-methylacyl-CoA racemase, PSA: Prostate-specific antigen
Diagnosis After Immunohistochemistry

**RESULTS**

The total number of patients included in the study was 106 and six patients were excluded because of nonavailability of blocks. Hence, the sample size was 100. The age of the patients ranged from 44 to 86 (median 65) years with 40% being in the 61 to 70 years age-group.

Patients presented predominantly with complaints of acute urinary retention and lower urinary tract symptoms. Patients of metastatic prostatic lesions presented with lymphadenopathy, predominantly iliac and para-aortic lymph nodes and metastatic deposits in bones and/or paravertebral regions, or as abdominal mass. The PSA levels of 70 patients were available. Patients of benign prostatic lesions presented with PSA levels ranging from 0.1 to 350 ng/ml. Primary and metastatic prostatic carcinomas had PSA levels ranging from 0.1 to >5000 ng/ml. All the patients on US and/or MRI pelvis revealed grade II to grade III prostatomegaly. Patients with suspicion of carcinoma showed an ill-defined lesion in the prostatic parenchyma with or without the involvement of lymph nodes and adjacent structures.

The specimens included 85 from the prostate (core needle biopsies in 61, TURP in 13, both core biopsy and TURP in 3, radical prostatectomy in 8, core or incisional biopsies from suspected metastatic sites in 15). The metastatic sites included lymph nodes (5), bones (6), liver (2), and one each as abdominal mass and peritoneal deposit.

**Diagnoses based on morphology and IHC**

Based on morphology alone on H and E, the diagnosis was made in 44, and metastatic adenocarcinoma suspicious of prostatic origin in 5. The metastatic sites included lymph nodes (3), bones (6), liver (2), and one each as abdominal mass and peritoneal deposit.

Using IHC with protein in addition to CK5/6, p63, AMACR, and Ki67 index, the diagnosis of atypical glands suspicious of malignancy was resolved as BPH (CK5/6, p63, protein positive, AMACR negative, and Ki67 index of 2% to 5%) in 3; HGPIN (CK5/6, p63, protein, AMACR positive, and Ki67 index of 25%) in one; and prostatic adenocarcinoma (CK5/6, p63 protein, AMACR positive in 7 and equivocal in one, protein positive in all and Ki67 index of 26% to 98%) in 8. Prostate was positive in all the cases. The case with equivocal staining with AMACR had high-serum PSA levels and was positive for protein; hence the diagnosis of prostatic adenocarcinoma was given.

Using IHC with protein in addition to AMACR, CK7, and GATA-3, the four cases of poorly differentiated carcinoma where there was difficulty in differentiating prostatic from UC were resolved as prostatic adenocarcinoma (protein positive in 3, AMACR positive in 2, and CK7 and GATA-3 negative in all) in 3 and UC (protein and AMACR negative and CK7 and GATA-3 positive) in one.

Using IHC with protein in addition to AMACR and PSA, the metastatic adenocarcinomas suspicious of prostatic origin were resolved as prostatic adenocarcinoma (protein positive in all, PSA positive in 9, and AMACR positive in 7) in 10 cases. The remaining five cases were resolved as neuroendocrine carcinoma in two (CD56, synaptophysin, and chromogranin positive); lung in one (TTF-1 positive); hepatocellular carcinoma in one (glypican-3 positive); and one with unknown primary (PCK, AMACR, PSA, protein, TTF-1, CK5/6, synaptophysin, and CD56 negative) [Table 2].

After the application of IHC, the diagnosis was resolved as BPH in 19, HGPIN in one, prostate adenocarcinoma in 64 (associated HGPIN in 2), UC in one, metastatic prostatic carcinoma in 10, metastatic adenocarcinoma, and nonprostatic in 5.

**Prostatic adenocarcinoma (n = 64)**

Majority (61) of primary prostatic carcinomas were diagnosed as adenocarcinoma, acinar (not otherwise specified) and three cases as adenocarcinoma with neuroendocrine differentiation. The various patterns observed were well-defined glandular pattern, ill-defined glands, fused glands, cords, cribriform pattern, solid sheets, hypernephrom, glomeruloid, and comedo necrosis with a...

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**Table 2: Diagnostic problem on morphology and resolution after application of immunohistochemistry in benign and malignant prostatic lesions (n = 31)**

| Diagnostic Problem | Diagnosis After Immunohistochemistry |
|--------------------|--------------------------------------|
| Biopsies with atypical glands, suspicious for malignancy (n = 12) | BPH (n = 3); CK5/6: +; p63: +; Prostate: +; AMACR: −; Ki67: 2-5% |
| Poorly differentiated carcinoma (n = 4) | Prostatic adenocarcinoma (n = 3); AMACR: + in 2; Prostate: + in 3; CK7: −; GATA-3: − |
| Metastatic carcinoma suspicious of prostatic primary (n = 15) | AMACR: + in 7; PSA: + in 9; Prostate: + in all |
| HGPIN (n = 1) | Prostatic adenocarcinoma (n = 8); CK5/6: −; p63: +; Prostate: +; AMACR: +; Ki67: 26-98% |
| UC (n = 1) | AMACR: −; Prostate: −; CK7: +; GATA-3: + |
| Neuroendocrine carcinoma: 2 (synaptophysin: +; chromogranin: +; CD56: +) | Lung adenocarcinoma: 1 (TTF-1: +) |
| Hepatocellular carcinoma: 1 (glypican-3: +) | Unknown primary: 1 (pan CK+, synaptophysin, CD56, CK5/6, TTF-1, PSA, AMACR, protein: negative) |

BPH: Benign prostatic hyperplasia, HGPIN: High-grade prostatic intraepithelial neoplasia, AMACR: α-methylacyl-CoA racemase, UC: Urothelial carcinoma, PSA: Prostate specific antigen
glandular pattern being the most common. Morphology diagnosis of prostatic adenocarcinoma on H and E sections was done in 53/64 (83%) and in the others following IHC. The Gleason score was 4 + 4 (most common) followed by 4 + 3, 4 + 5, 3 + 3, 5 + 4, 3 + 4, and 5 + 5. The prognostic grade group were assigned as per the Gleason score [Figure 1a-f].

**Expression of prostein in normal, benign, and malignant (primary and metastatic) prostatic and nonprostatic tissues (n = 60)**

Prostein was expressed in 8/8 nonneoplastic prostatic tissue (adjacent to malignancy in radical prostatectomy), 10/10 BPH, 3/3 HGPIN (one which was resolved after IHC and two samples adjacent to carcinoma in radical prostatectomy), 17/17 prostatic adenocarcinoma, and 10 metastatic prostatic adenocarcinomas. It was negative in UC; metastatic carcinoma of nonprostatic origin; and normal bladder, kidney, and testis epithelium.

Granular staining at the apical aspect of cytoplasm, predominantly, adjacent to the nuclei, was observed in the normal and benign prostatic lesions. In weakly stained cases, the granules were relatively faint and punctuate, but they were still visible in the apical region of the cells using higher magnifications. The staining intensity score of nonneoplastic tissue adjacent to malignant glands was 1.8 to 2 with one case showing focal loss of expression and it was 2 to 2.8 in BPH. The score for HGPIN was 2 to 2.2 and the score for prostatic adenocarcinoma was 1 to 1.5. There was focal loss of expression in two adenocarcinomas; however, the score was not significantly different from that of the above nonneoplastic group, and it was not statistically significant. There was no correlation of the expression of prostein to the Gleason grade (in the samples studied).

Metastatic prostatic carcinomas also demonstrated a granular apical staining pattern with P501S antibody and the scores ranged from 0.8 to 1.5, which were lower than those of the above benign prostatic lesions and carcinoma of the prostate. P501S was negative in all the five metastatic carcinomas of nonprostatic origin as well as normal epithelium from the bladder, kidney, and testis [Figure 2 and Table 3].

**Statistical analysis**

In our present study, the overall sensitivity, specificity, positive predictive value, and the negative predictive value of prostein in primary and metastatic prostatic lesions was 93.75%, 100%, 100%, and 80%, respectively. It was useful in differentiating poorly differentiated prostatic carcinoma from UC. In addition, it was superior to the expression of AMACR and PSA in diagnosing metastatic prostatic lesions. Statistical analysis of prostein by a ROC curve analysis showed a value of 0.943, indicating that it is accurate in diagnosing prostatic lesions [Figure 3].

**DISCUSSION**

Prostatic adenocarcinoma is primarily a disease of elderly men and is uncommon before the age of 40 years. In the present study, the predominant age group was between 61 and 70 (mean 69.9) years and in agreement with earlier studies. The diagnosis

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**Table 3: Expression of prostein in normal, benign, and malignant lesions of prostate and other organs (n=60)**

| Type of Tissue                      | Prostein Expression | Intensity Score | Comment                                                                 |
|------------------------------------|---------------------|-----------------|------------------------------------------------------------------------|
| Normal prostatic epithelium (n=8)  | Positive (100%)     | 1.8-2          | Focal loss of expression in one normal epithelium and two prostatic adenocarcinomas; no statistically significant difference in intensity scores between benign and malignant lesions; and prostein expression had no correlation with Gleason grade. |
| BPH (n=10)                         | Positive (100%)     | 2.2-2.8        |                                                                       |
| HGPIN (n=3)                        | Positive (100%)     | 2.2            |                                                                       |
| Primary prostatic adenocarcinoma (n=17) | Positive (100%) | 1.5            |                                                                       |
| Metastatic prostatic adenocarcinoma (n=10) | Positive (100%) | 0.8-1.5        |                                                                       |
| UC (n=2)                           | Negative (100%)     | Nil            |                                                                       |
| Metastatic adenocarcinoma, nonprostatic origin (n=5) | Negative (100%) | Nil            |                                                                       |
| Normal urothelium, kidney, and testis (n=5) | Negative (100%) | Nil            |                                                                       |

BPH: Benign prostatic hyperplasia, HGPIN: High-grade prostatic intraepithelial neoplasia, UC: Urothelial carcinoma
is usually made using a needle biopsy and TURP specimens to provide specific information on the grade and extent of the tumor. However, IHC is required in cases where suspicious foci of carcinoma are seen, differentiation is lost, or metastasis from the prostate is suspected. The percentage of suspicious for malignancy category varied from 4.83 to 26.3 in various series, and it was 16% in the present study. The diagnosis of poorly differentiated carcinoma of the prostate may pose a problem, especially to differentiate from poorly differentiated UC. The IHC markers CK7 and AMACR are usually used to resolve the diagnosis. While CK7 is positive in UC and negative in prostate carcinoma, AMACR is expressed in some nonprostate cancers, including UC; hence, it is not useful as a single marker in the differential diagnosis of poorly differentiated carcinoma. Jiang et al. recommended a panel of AMACR, HMWCK, and p63. Among the other markers, PSA and PSAP were most commonly used to establish the prostatic origin of tumors, but their expression was significantly decreased in poorly differentiated prostatic cancers. Prostein was used in the present study to decide the prostatic origin of the tumor, both in primary and metastatic sites.

Protein is a prostate-specific 553 amino acid protein identified by cDNA subtraction. Its expression is restricted to prostatic tissue and not detected in normal heart, kidney, liver, lung, or colon. Moreover, protein expression is not related to Gleason grading; hence, it is a useful IHC marker to discriminate a prostatic origin of cancer from tumors of the bladder and the colon. Prostate carcinoma metastatic to lymph node, bone, and liver also express high levels of protein; therefore, they were regarded among the best validated immunohistochemical markers of prostatic origin.

Expression of protein (P501S) in primary prostatic lesions
In the present study, protein was studied in 60 samples that included nonneoplastic prostatic tissue surrounding the malignancy, BPH, HGPIN, and prostatic adenocarcinoma both in primary and metastatic sites. Protein was positive in all the primary prostatic lesions and negative in the normal (bladder, kidney, and testis) and malignant nonprostatic tissues (UC), confirming its specificity. The intensity of staining had no statistical significance. These results were comparable with those of Parwani et al. who studied nonneoplastic prostatic tissue (36), BPH (35), HGPIN (35), prostate adenocarcinoma (135), metastatic adenocarcinoma (60), and nonprostatic tissues (20). In a study of 38 poorly differentiated prostatic carcinomas and 35 poorly differentiated UC, Chuang et al. observed that the sensitivity for labeling prostatic cancers for PSA, p501S, PSMA, and NKX3.1 was 97.4%, 100%, 92.1%, and 94.7%, respectively.

Expression of protein to differentiate carcinomas of prostatic and urothelial origin
In the present study, there were four poorly differentiated carcinomas where IHC was performed with protein along with other markers. Protein was positive in 3/4 carcinomas. These three cases were negative for CK7 and GATA-3, but AMACR was positive in only two cases. The biopsy that was negative for protein was positive for GATA-3; hence, it was diagnosed as UC. Hence, the addition of protein and GATA-3 to the panel including CK7 and AMACR helped in differentiating poorly differentiated carcinoma of prostatic origin from the urothelial origin. These observations were in agreement with earlier studies. In a study of 38 poorly differentiated prostatic carcinomas and 35 poorly differentiated UC, Chuang et al. observed that the sensitivity for labeling prostatic cancers for PSA, p501S, PSMA, and NKX3.1 was 97.4%, 100%, 92.1%, and 94.7%, respectively.
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Parwani studied 132 patients with high-grade UC and 23 patients of prostatic carcinoma with p63 and P501S. They observed that p63+/P501S− immunoprofile had 90% sensitivity and 100% specificity for UC and p63−/P501S+ immunoprofile had 96% sensitivity and 100% specificity for prostatic carcinoma.[22]

Expression of prostein in metastatic adenocarcinoma
In the present study, 10 cases were diagnosed with having a prostatic origin using the IHC panel of AMACR, PSA, and prostein. The positive expression of AMACR, PSA, and prostein was 70%, 90%, and 100% respectively. Among the other five metastatic carcinoma cases, two were diagnosed as neuroendocrine carcinomas and one case each of pulmonary adenocarcinoma, hepatocellular carcinoma, and unknown primary. In all these cases, prostein was negative. These observations were comparable to other studies.[7,23]

Parwani et al. studied the expression of PSA and prostein in 54 metastatic prostatic carcinomas (30 lymph nodes and 24 distant metastases), where PSA was expressed in 87% and prostein in 86.7% of samples. The mechanisms responsible for the diminished expression of P501S in metastatic prostatic carcinomas are unknown but could be similar to those for PSA.[19] Queisser showed sensitivity of PSA, PSMA, and androgen receptor to be 97%, 94%, and 91%, respectively and concluded that sensitivity can be increased up to 98% to 100% with the combined use of PSMA and P501S [Table 4].[23]

Studies found a loss of PSA expression in distant metastases in 10% to 20% of the investigated cases.[11,13] The specificity of PSA IHC is limited, as immunoreactivity for PSA has additionally been detected in some nonprostatic tissues.[24] Netto and his coworkers suggested that the combination of PSA and P501S is better than either alone in confirming prostatic origin in workup of metastatic or locally advanced lesions.[23] In our study, prostein was positive in all the 10 metastatic carcinomas, whereas PSA was positive in only nine cases. Though the number of cases studied was limited, prostein showed better sensitivity compared to PSA.

CONCLUSIONS
Prostein is a new and promising prostate-specific marker, which showed 100% sensitivity and specificity to identify normal prostatic epithelium, benign and premalignant lesions, and prostatic adenocarcinoma. Prostein showed a specificity of 100% in differentiating prostatic carcinoma from poorly differentiated UC and metastatic prostatic carcinoma from adenocarcinoma of nonprostatic origin. Prostein showed slightly more sensitivity and specificity than PSA. Thus, adding prostein to the panel of IHC, including PSA and AMACR, will greatly improve the detection of poorly differentiated primary and metastatic lesions. However, as the number of cases studied in our work were limited, prostein needs to be studied in more cases to apply it for routine diagnostic use.

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Conflicts of interest
There are no conflicts of interest.

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