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Study of Utility of p63 Immunohistochemical Stain to Differentiate Urothelial Carcinomas of Urinary Bladder from Adenocarcinomas of Prostate

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Abstract

Introduction: Cancer of the bladder and of prostate are commonmalignancies all over the world. In some cases it is difficult to point to the exact primary due to the anatomical location and morphology of the tumors from these two sites. Various immunohistochemical markers can be used to identify the primary site and p63 is one such marker. *Aim of the study:* To study the expression of p63 in urothelial carcinoma and prostatic adenocarcinoma so as to differentiate between the two carcinomas. *Materials and Methods:* In this study, formalin fixed paraffin blocks from 60 cases of urothelial carcinomas of urinary bladder and 20 cases of prostatic adenocarcinomas were examined on light microscopy and tissue sections from all 80 cases were studied for immunohistochemical staining with p63 marker. *Observations and Results*: The mean age of patients of urothelial carcinoma and of prostate carcinoma was 54.1 and 64.5 years respectively. There were 28 cases (46.6%) of low grade and 32 cases (53.3%) of high grade urothelial carcinomas. The Gleason score for prostatic carcinoma was 5-6 for 8 patients, 7-8 for 9 patients and 9-10 for 3 patients.In 60 urothelial carcinomas 90% of cases showed p63 positivity. All the 20 cases of prostatic adenocarcinomas were negative for p63. *Conclusion*: p63 is a reliable marker of urothelial differentiation and it can be used in morphologically difficult cases when the differential diagnosis is between poorly differentiated adenocarcinoma of prostate and high grade urothelial carcinoma of urinary bladder.

Keywords: Urothelial Carcinoma of Bladder; Prostatic Carcinoma; p63 Staining.

Introduction

Cancer of the urinary bladder is quite common worldwide and accounts for 6.5% of all cancers. It is the fourth most common cancer in men and the eighth in women. It has a high incidence in industrialized countries. In India also this malignancy is common and incidence is slightly higher in males than females [1]. Bladder carcinoma on histopathology shows urothelial cell morphology in 90% cases, squamous cell morphology in 3% to 8% cases and adenocarcinoma in 1% to 2% cases [2]. Prostate cancer has a high incidence in western countries and is a leading cause of cancer related death in men, second only to lung cancer [3]. It has a high geographic variability and is influenced by race, genetic factors, life style and diet of the population.It has more or less similar prevalence in different parts of India [4]. In India, prostate cancer has a lower incidence compared to the United States [5].

p63 is a nuclear protein encoded by a gene on chromosome 3q27-29 with homology to p53 (a tumor suppressor gene). It has shown to regulate growth and development of various epithelia of viscera and skin. Specific isotopes are expressed in basal cells of prostate, myoepithelial cells of breast, urothelium, and squamous epithelium [6,7]. p63 has similar applications to those of high-molecular-weight cytokeratins in the diagnosis of prostatic adenocarcinoma. p63 has added advantages compared to high-molecular-weight cytokeratins and that are : Stains a subset of 34 beta E 12 negative basal

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cells, Less susceptible to the staining variability of 34 beta E12 (particularly in TURP specimens with cautery artifact), It is easier to interpret because of its strong nuclear staining intensity and low background.

Aim of the Study

To study the expression of p63 in urothelial carcinoma and prostatic adenocarcinoma so as to differentiate between the two carcinomas based on p63 expression.

Materials and Methods

The present study was a prospective study conducted from June 2011 to May 2013 in Upgraded department of Pathology, Osmania Medical College (OMC) and General Hospital, Hyderabad. OMC general hospital is a tertiary referral centre and it also caters to the needs of various districts of neighbouring states.

The study group consisted of a total of 80 cases out of which 60 were transurethral resection of bladder tumors (TURBT) and 20 were transurethral resection of prostate (TURP) specimens. Clinical details of all the patients were noted.

Inclusion Criteria

The study included transurethral resection of bladder (TURBT) and transurethral resection of prostate (TURP) specimens which were diagnosed on histopathology as urothelial carcinoma or prostatic adenocarcinoma.

Exclusion Criteria

Non-urothelial malignancies of bladder and non-adenocarcinomas of prostate were excluded.

Already known cases of carcinoma on treatment were excluded.

Cases that had recurred in the study period were excluded.

Complete clincopathological data like age, gender, chief complaints and histopathological diagnosis of the cases were noted.

The tissue biopsies received in the department of Pathology were fixed in 10% formalin, processed in an automatic tissue processor and were embedded in paraffin. Sections were cut at 4 micron thicknessand were stained with hematoxylin and eosin stains.

All the slides were examined for the morphology and grade of tumors. For prostatic carcinomas, Gleason grading system was used in addition. These 80 cases were further subjected to immunohistochemical staining for p63.

Immunohistochemistry

IHC staining was performed on all 80 tissue sections.

Immunohistochemical staining of p63 (clone 7 JUL, DAKO) was done using peroxidase–antiperoxidase method according to the instructions by the manufacturer.

Observations and Results

A total of 80 cases were studied of which 60 were urothelial carcinomasand 20 were prostatic adenocarcinomas.

Age Wise Distribution

The patients of urothelial carcinoma were between 23 and 79 years and the mean age was 54.1years. There were 52 males and 8 females, the male to female ratio being 6.5:1.

The patients of prostatic adenocarcinoma were between 55 and 80years and the mean age was 64.5 years.

Table 1: Age distribution of Urothelial and Prostatic carcinoma

Age (years)	Urothelial carcinoma No. of cases (%)	Prostatic Carcinoma No. of cases (%)
<40 years	6 (10%)	-
41-50years	12 (20%)	-
51-60	18 (30%)	9 (45%)
61-70	13 (21.6%)	10 (50%)
71-80	11 (10.9%)	1 (5%)

Clinical Features

In our study, patients with urothelial carcinoma presented with combinations of symptoms and signs like hematuria, dysuria and abdominal pain. Of the 60 patients, 95% presented with painless hematuria followed by dysuria (3%) and frequency (1%).

Table 2: Clinical features of Urothelial and Prostatic carcinoma

Main Clinical Features	Urothelial Carcinoma No. of Cases (%)	Prostatic Carcinoma No. of Cases (%)
Hematuria	57 (95%)	-
Dysuria	2 (3.33%)	-
Frequency	1 (1.66%	2 (10%)
Difficulty in passing urine	-	17 (85%)
Pain or discomfort in perineal region	-	1 (5%)

Patients with prostatic adenocarcinoma presented with combinations of symptoms with 85% of patients having difficulty in passing urine (85%), frequency (5%), and pain in perineal region (3%).

Distribution of Urothelial Carcinomas

Urothelial carcinomas were graded according to WHO classification (2004) into Low grade and High grade papillary urothelial carcinomas.

There were 28 cases (46.6%) of low grade and 32 cases (53.3%) of high grade urothelial carcinomas.

Gleason Scores of the Patients with Prostatic Adenocarcinoma

There were total 20 cases of carcinoma of prostate on histopathology. The Gleason score was 5-6 for 8 patients, 7-8 for 9 patients and 9-10 for 3 patients.

Evaluation of p63 Expression

Immunohistochemical expression was assessed semi-quantitatively for staining intensity and percentage of positive tumor cells with brown nuclear staining. Only moderate or strong staining in at least 5% of the tumor cells was considered positive. (Figure 1). Basal cells of the benign glands of prostate were taken as internal control.

The whole section was scanned at low power in order to assess the general level of intensity throughout. The average intensity of the staining corresponds to the presence of negative, weak, moderate, and strong staining.

Immunohistochemical staining scores for p63 were individually compared between Urothelial and Prostate cancers, and a p-value less than 0.05 was considered statistically significant.

Urothelial Carcinomas

In our study in 54 out of 60 (90%) cases of urothelial carcinomas moderate to strong nuclear positivity to p63 was seen.Negative p63 immunoreactivity was seen in 6 cases.

Out of 28 Low grade urothelial carcinomas, 25 (89.2%) showed positive staining with p63.

Out of 32 High grade urothelial carcinomas, 29 stained positively with p63 (90.6%).

Prostatic Adenocarcinoma

In our study, we received 20 cases of Prostatic Adenocarcinoma with Gleason scores between 5 to10.

All the 20 cases were negative for p63 expression.

Comparison between Urothelial Carcinomas and Prostatic Adenocarcinomas

In 60 urothelial carcinomas 90% of cases showed p63 positivity. All the 20 cases of prostatic adenocarcinomas were negative for p63.

Table 3: Expression of p63 in urothelial and prostatic carcinomas

p63 staining	Low grade Urothelial carcinomas	High grade Urothelial carcinomas	Prostatic Adenocarcinoma	Total
Positive	25(89.2%)	29(90.6%)	-	54 (90%)
Negative	03 (10.7%)	03(9.3%)	20 (100%)	26
Total	28	32	20	80

In our study p63 expression showed sensitivity of 90% and specificity of 100%.

P value for p63 was statistically significant (P value 0.00001).

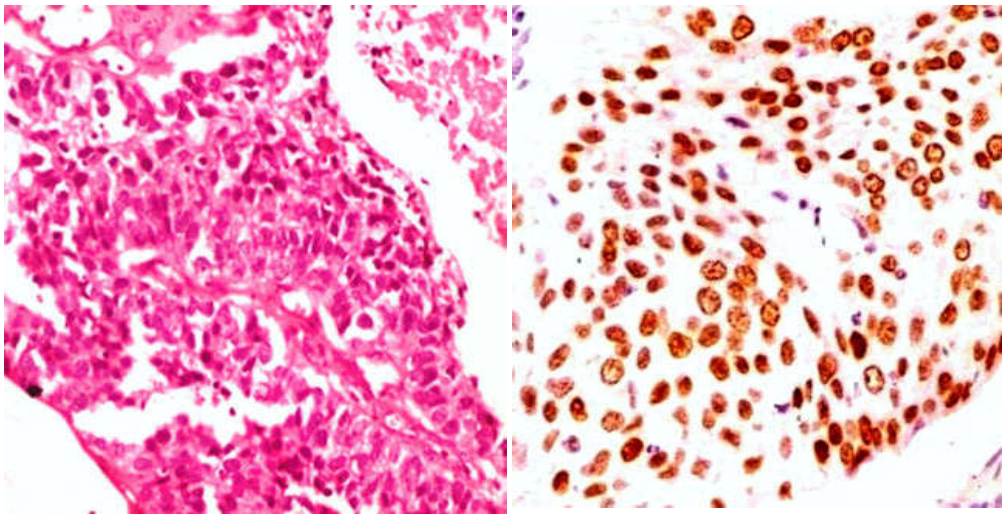

Fig. 1: High grade Urothelial carcinoma (Hematoxylin and eosin and IHC for p63 100 X)

Discussion

Cancer of the urinary bladder is one of the common malignancies worldwide.

Prostate cancer is common in western countries and its incidence increases with advancing age. It is one of the leading causes of cancer related death in men [3].

p63 is a nuclear protein encoded by a gene on chromosome 3q27-29 and has similarity to p53 which is a tumor suppressor gene. It plays a vital role in growth and development of various epithelial cells of skin, cervix, breast, and urogenital tract [6].

Age and Gender of the Patients

In the present study, urothelial carcinoma was more common in the 50-70 year age groups. Biswas et al [8] also reported maximum cases in the higher age groups and 1/4th of their cases were less than 50 years. Male predominance was seen in urothelial carcinomas and the male to female ratio in our study was 6.5:1 which is similar to the observation of Biswas et al as 6:0.7 [8]. In the present study the mean age for carcinoma prostate was 64.5 years which is similar to the observation of Hariharan et al [9] who reported it as 65 years in their study.

Gender Wise Distribution

Biswas et al [9] observed in their study, bladder cancer was six times higher in males as compared to females, whereas, Mungan et al [10] observed it to be 3 to 4 times more common in males. In our study also a definite male predominance was seen in urothelial carcinomas and the male to female ratio in our study was 6.5:1.

Primary Bladder and Prostate Carcinoma

This study attempts to demonstrate the expression of p63 and use it in differentiating high grade urothelial carcinomas from prostatic adenocarcinomas.

Distinction of poorly differentiated prostate cancer from high grade urothelial carcinoma with prostatic extension is a challenging task due to overlapping morphologic characteristics and similar clinical manifestations. Also if the tumor lies near or arises from the bladder neck area, it is extremely difficult to comment if it is primary tumor of bladder or from prostate [10]. Distinction is important because of common occurrence of glandular differentiation and the frequently raised serum prostate-specific antigen in cases of urothelial carcinoma extending into the prostate gland.

This distinction has significant therapeutic and staging implications. The standard surgical procedure for the treatment of bladder cancer in some cases is cystoprostatectomywhich is inappropriate for prostatic cancer when it is confined to the gland proper. Correct diagnosis is required for determination of the tumor stage and also for prognostication as extension of bladder cancer into the prostate as well as prostate cancer into the bladder would signify pT4 disease. Invasive high grade tumors of non-genitourinary origin like carcinomas of anal region and from uterine cervix may also give rise to diagnostic problems especially if the high grade urothelial carcinomas exhibit squamoid morphology [10].

Other markers that can be used for correct identification of urothelial carcinomas are GATA binding protein 3, THROMBO and uroplakin III and HMWCK with variable sensitivity. Sakr et al [11] have used p63, CK7 and PSA to differentiate between urothelial and prostatic carcinoma in a study in Egyptian men and have found p63 as a reliable marker.

Table 4: Comparison of present study with other studies for p63 expression in urothelial and prostatic carcinomas

Study	Urothelial Carcinoma	Prostatic Adenocarcinoma
Kunju et al [12]	92%	0%
Nasir Uddin et al [13]	88%	0%
Srinivasan et al [14]	90%	0%
Present study	90%	0%

Kunju et al [12] performed a panel of immunohistochemical stains including p63 on 36 cases of high grade urothelial carcinomas and 42 cases of poorly differentiated prostatic carcinomas. p63 positivity was seen in 92% of urothelial carcinomas in their study and none of the prostatic carcinomas stained with p63. Our results compare well with this study.They found p63 to be a fairly sensitive and highly specific marker of urothelial carcinoma with consistent diffuse nuclear positivity.

In 2011, Nasir Uddin et al [13]studied the expression of p63 in 50 urothelial carcinomas and 50 prostatic adenocarcinomas. Urothelial carcinomas showed positivity in 44 (88%) cases. None of the prostatic adenocarcinoma showed positivity. The authors concluded that p63 was a reliable marker to distinguish prostatic adenocarcinomas from urothelial carcinomas in difficult cases in conjunction with other markers like PSA. In our study also all the cases of prostatic adenocarcinoma were negative for p63 staining.

In 2011, Srinivasan et al [14] studied the diagnostic utility of a dual immunohistochemical stain p63 and P501S (prostein), in differentiating urothelial carcinoma from prostate carcinoma. p63 /P501S dual-color sequential immunohistochemical staining was performed on 132 patients with high-grade urothelial carcinoma and 23 patients with prostatic adenocarcinoma. p63 was positive in 119/132 of urothelial carcinomas and negative in prostatic adenocarcinomas.

P501S was positive in 22/23 of prostatic adenocarcinoma and negative in urothelial carcinomas. They concluded that p63 and P501S are highly specific and can be useful in distinguishing urothelial carcinomas from prostatic adenocarcinoma. Using these markers is especially useful whenever the tissue material is limited as the test can be performed on a single slide.

Conclusion

We conclude that p63 is a reliable marker of urothelial differentiation and it can be used along with other markers in morphologically difficult cases when the differential diagnosis isbetween poorly differentiated adenocarcinoma of prostate and high grade urothelial carcinoma of urinary bladder.

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