Evaluation of p63 Immunohistochemical Stain as First Line Marker in Differentiating Urothelial Carcinomas from Adenocarcinomas of Prostate

Sonia Agarwal[1], Atul Tiwari[2], Mohan Lal Yadav[3]

[1, 3] Department of Pathology, Mahatma Gandhi Medical College and Hospital, Sitapura Jaipur, Rajasthan, India.
[2]Department of Pathology, S.M.S. Medical College and Hospital, Jaipur, Rajasthan, India.

ABSTRACT

BACKGROUND

Differentiating prostate carcinoma (PCa) arising in the neck of urinary bladder from high grade urothelial cancer (UCa) with prostatic extension can be a difficult task for histopathologist due to similar morphologic characteristics and overlapping clinical manifestations in the two diseases. These two tumours often occur in association with one another but have different potential therapeutic strategies and prognostic implications. We have investigated p63 immunohistochemical (IHC) marker as simple first line marker adjuvant to histopathological examination.

METHODS

In this prospective study, total 50 cases including 25 cases of urothelial carcinoma and 25 cases of prostatic carcinoma were taken. Tumour grade was determined according to standard H&E staining and scoring system. p63 expressions were determined by immunohistochemical staining of all the cases. The obtained results were analysed and evaluated using chi-square statistical test to determine whether p63 IHC can be used as simple first line marker tool with a high sensitivity and specificity.

RESULTS

p63 was not expressed in any of the 25 cases of prostatic carcinoma cases while in urothelial carcinoma it was expressed in 23 of 25 (92 %) cases. p63 IHC staining expression is positive in all histological grades of urothelial carcinomas. 2 out of 25 cases of urothelial carcinomas were negative for p63 IHC expression. None of the prostatic adenocarcinomas expressed p63 staining. Sensitivity of p63 stain in differentiating UCa with PCa was 92 % in our study, specificity of p63 stain in differentiating UCa with PCa was found to be 100 %.

CONCLUSIONS

p63 can be used as a screening first line IHC marker to distinguish urothelial carcinomas from prostatic adenocarcinomas. For challenging and unresolved cases both of these have limited sensitivity; thus, authors recommend two lineage-specific markers one each for UCa (GATA3, S100P) and PCa (NKX3.1, P501S, PSMA) should be used for definitive diagnosis.

KEYWORDS

p63 Immunohistochemistry, Urothelial Carcinoma, Prostatic Adenocarcinoma

Corresponding Author:
Dr. Sonia Agarwal,
Assistant Professor,
Department of Pathology,
Mahatma Gandhi Medical College and Hospital, Sitapura - 302022,
Jaipur, Rajasthan, India.
E-mail: drsoniaagarwal@gmail.com

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BACKGROUND

Differentiating prostate carcinoma arising in the neck of urinary bladder from high grade urothelial cancer with prostatic extension can be a difficult task for histopathologist due to similar morphologic characteristics and overlapping clinical manifestations in the two diseases.[1]

Both carcinoma arise from same anatomical site with many variants, it is difficult to differentiate them accurately through clinical presentation and histopathology. Additionally, serum prostate specific antigen (PSA) levels may be raised in urothelial cancers that infiltrate the prostate gland adding to the diagnostic dilemma.[2]

These two tumours often occurs in association with one another but have different potential therapeutic strategies and prognostic implications. Prognosis of high grade muscle invasive UCa extending up to bladder neck (stage pT2b) is poorer than PCa involving bladder neck.[3] Five and 10 year survival rates in high grade UCa ranges from 70 % - 83 % and 69 % - 78 % respectively, whereas in case of PCa involving bladder neck, survival rates ranges from 95 % to 100 % and 81 % to 93 % respectively.[4]

Therapeutic approach is radical cystectomy and chemotherapy for urothelial cancer[5] and anti-androgen hormonal therapy with radiation for prostate cancer.[6]

Main differentiating feature of urothelial carcinoma from prostatic carcinoma is presence of basal cells in urothelial carcinoma but malignant glands of prostate carcinoma lacks basal cells.[7] This difference of basal cell in these tumours can be highlighted with appropriate marker stains. Our hypothesis is that p63 can be used as first line immunohistochemistry marker in differentiating urothelial carcinomas from adenocarcinomas of prostate.

METHODS

We performed hospital based observational study with analysis of all urothelial carcinoma and prostatic carcinoma biopsy samples after getting approval by the institutional review board between June 2015 and May 2017.

Inclusion criteria was all urothelial and prostatic carcinoma samples received in pathology department from male patients of age more than 50 years during mentioned period. Samples showing inadequate biopsy tissue, improperly fixed specimen, pathology other than UCa and PCa or autolysed tissues along with samples of patients who refuse to give consent were excluded from this study.

Total 33 samples of urothelial carcinoma and 38 samples of prostatic carcinomas were received during study period. All the specimens received were fixed in formalin, processed and paraffin blocks were made. The blocks were cut at 3 – 5 micron thickness and stained with haematoxylin and eosin. Detailed microscopic examination of tumour was done to arrive at a histopathological diagnosis. Out of 33 UCa biopsy samples 5 have scanty tissue, 3 samples were non-malignant on histopathology thus excluded from study. In PCa group out of 38 samples 13 samples were diagnosed with pathology other than PCa (9 benign and 4 metastatic) thus excluded from study. The urothelial lesions were classified as per World Health Organization (WHO) / International Society of Urological Pathology (ISUP) 2017 classification consensus of urinary bladder and prostatic lesions were graded as per Gleason's grading system.

Immunohistochemical staining for p63 using enzyme linked polymer-based detection method was done in each and every case.

Grading of UCa

Grading of urothelial carcinoma usually done according to findings on routine histopathological (HP) examination and classified into two groups –

Low Grade Urothelial Carcinoma

UCa samples which do not show high-grade cytologic features like pleomorphism, mitoses toward surface, nucleoli throughout specimen.

High Grade Urothelial Carcinoma

Urothelial carcinoma with high grade cytological features described as above which show predominant disorderly pattern and moderate to marked architectural and cytologic atypia are classified under this group.

Grading of PCa

Grading of PCa was done according to new Gleason grading system.

- Well differentiated PCa grade group 1 (Gleason score ≤ 6) - Only individual discrete well-formed glands.
- Moderately differentiated PCa grade group 2 (Gleason score 3 + 4 = 7) – Predominantly well-formed glands with a lesser component of poorly-formed / fused / cribriform glands.
  Moderately differentiated PCa grade group 3 (Gleason score 4 + 3 = 7) – Predominantly poorly-formed / fused / cribriform glands with a lesser component of well-formed glands.
- Poorly differentiated PCa grade group 4 (Gleason score 8) - Only poorly formed / fused / cribriform glands or predominantly well-formed glands with a lesser component lacking gland†† or predominantly lacking glands with a lesser component of well-formed glands.
- Poorly differentiated PCa grade group 5 (Gleason scores 9 - 10) – Lacks gland formation (or with necrosis) with or w / o poorly-formed / fused / cribriform glands.

p63 Immunostaining Scoring

The tissue sections were inspected through bright field microscope to evaluate the percentage of IHC positive cells in at least 3 different areas. Positive cells for p63 were recognised by the existence of brown nuclear staining. Nuclear p63 immunoreactivity was assessed with a 12 point calculated scoring system. First, the percentage of positive cells in each area was scored using a 5 point scale 0 for < 5 %, 1 for 5 - 25 %, 2 for 25 - 50 %, 3 for 50 - 75 % and 4 for over 75 %. Second, the intensity of positive cells was

scored using a 3-point scale: 0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining, then, the total score for each area was calculated by multiplying the percentage of positive cells by the intensity of staining score. Finally, the results were grouped as negative (0 - 1), weak (2 - 3), moderate (4 - 6) and strong (7 - 12). Statistical analysis of p 63 expression was done by using chi square test with P value analysis where P-value less than 0.05 is statistically significant.

RESULTS

Out of 25 cases diagnosed as urothelial carcinoma, 23 (92 %) cases were positive on p 63 staining and all 25 cases (100 %) of PCa were negative. Out of 12 low grade urothelial carcinomas, 08 were strong positive stained with p63 (66.67 %) [Figure 1]. Out of 13 high grade urothelial carcinomas, 11 were positively stained with p63 (84.6 %).

P63 status was positive in 23 out of 25 cases (92 %). 40 % cases each were stained strong and moderate with p63 IHC stain. 12 % cases were weakly stained by p63 IHC in our study. [Figure 2]. Statistically significant P value (P value: 0.0003), so p63 expression had an inverse correlation with histological grade of urothelial carcinoma. Significant difference was observed in p63 status in relation with histological grading. Strong positive staining with p63 was observed in 66.7 % of low grade UCa in comparison to 15.4 % of high grade UCa group. Weak positive p63 stain was observed in 23 % of high grade UCa in comparison to 0 % in low grade group. Both data were significantly different according to statistical analysis.

p63 Status	UCa		PCa	
	N	%	N	%
Positive	23	92 %	0	0 %
Negative	2	8 %	25	100 %
	25	100 %	25	100 %

Table 1. Comparison of p63 Expression in UCa and PCa Group

UCa Grade	High Grade	Low Grade	
Strong +	2	8	10
Moderate +	6	4	10
Weak +	3	0	3
Negative	2	0	2

Table 2. Distribution of p63 IHC Staining Expression in UCa Group According to Histological Grade.

Histological Grade		Strong		Moderate		Weak		Negative	
	P63 (+)	n	%	n	%	n	%	n	%
High grade N = 13	11	2	15.5 %	6	46 %	3	23 %	2	15.5 %
		11 / 13 (84.6 % %)						2 /13 (15.5 %)	
Low grade N = 12	12	8	67 %	4	33 %	0	0 %	0	0 %
P value 0.003*		12 / 12 (100 %)						0 / 12 (0 %)	

Table 3. p63 IHC Staining Expression in Urothelial Carcinoma According to Histological Grade

p63 IHC Stain in UCa Group

Out of the 25 urothelial carcinomas 23 stained with p63 (92 %). Overall, 40 % of cases showed strong nuclear staining and 40 % of cases showed moderate nuclear staining tumour cells after multiplying 5-point score to 3-point score. Only two cases (8 %) of UCa group out of 25 were negative for p63 staining. Out of 12 low grade urothelial carcinomas, 08 were strong positive stained with p63 (66.67 %).

p63 IHC Stain in PCa Group

None of the 25 prostatic adenocarcinomas expressed p63 [Figure 3]. Staining status was compared between urothelial carcinomas and prostatic adenocarcinomas in all 50 cases. Basal cells of benign glands of prostate were taken as positive internal controls, [Figure 4]. Positive staining was defined as dark brown homogeneous or punctuate staining limited exclusively to the nucleus. p63 positivity was observed in 92 % of urothelial carcinomas and none of prostatic adenocarcinomas with a P value of 0.001 [Table 1].

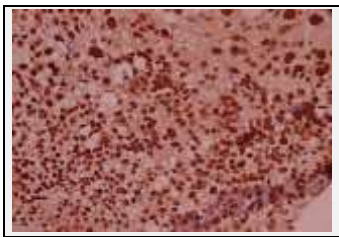

Figure 1. Strong p63 Staining in Urothelial Carcinoma (IHC, 40X)

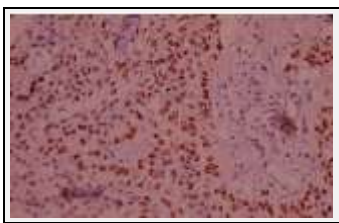

Figure 2. Weak p63 Staining in Urothelial Carcinoma (IHC, 40X)

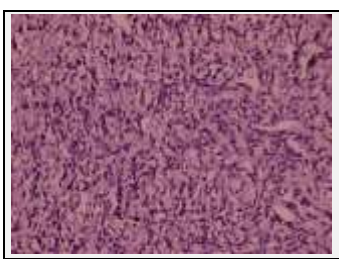

Figure 3. Negative p63 Staining in Prostatic Adenocarcinoma (IHC, 40X)

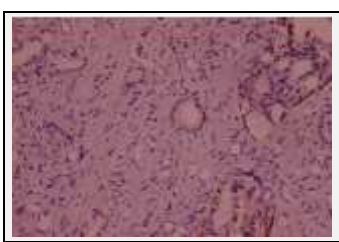

Figure 4. P63 Positivity of Basal Cells of Entrapped Benign Glands (IHC, 40X)

p63 Staining	UCa in HPE	PCa in HPE	Total
Positive	23	0	23
Negative	2	25	27
Total	**25**	**25**	**50**

Table 4 Sensitivity and Specificity

Sensitivity of p63 stain in differentiating UCa with PCa: A / (A + C) X 100: 23 / (23 + 2) 100 = 92 %

Specificity of p63 stain in differentiating UCa with PCa: D / (D + B) X 100: 25 / (25 + 0) 100 = 100 %

DISCUSSION

We had done this study as pilot project thus eligible cases received during study period were taken. Out of 25 eligible cases of UCa, 13 cases (52 %) were of high grade and 12

cases (48 %) were of low grade. This is in consistent with study of Zeenat Ara et al,[8] where 51 % cases were of low grade and 49 % cases were of high grade. In study conducted by AbelWahab et al,[9] 40 % cases were of low grade and 60 % were of high grade.

In the present study expression of p63 with WHO grade was observed as out of 25 cases of UCa, p63 was expressed in 23 cases (92 %). Out of 12 low grade UCa, all 12 cases (100 %) were positively stained with p63, whereas out of 13 high grade UCa, 11 cases (84.6 %) were positively stained with p63. 0verall 40 % cases showed strong nuclear staining. This is consistent with study done by Ud Din et al,[1] in which out of 50 UCa, 44 cases (88 %) stained with p63. p63 positivity was found in 94.7 % of high grade UCa. Present findings is consistent with findings of Langner et al,[10] in which p63 was expressed in 51 cases (96.2 %) of UCa and 23 (92 %) of high grade UCa. In study of Abdelwahab MM[11] p63 was expressed in all low grade UCa similar to present study and 66.7 % of high grade UCa cases. In study done by Elnashar et al,[12] p63 was expressed in 94 % of low grade and 72.7 % of high-grade cases of UCa which is consistent with present study.

Present study showed variation in expression of p63 in high grade and low grade UCa cases. Out of 13 high grade cases 2 cases (15.4 %) were found to be strongly stained, 6 cases (46.2 %) were moderate, 3 cases (23 %) were designated weak and 2 cases (15.4 %) were negative on p63 scoring, whereas among 12 cases of low grade carcinoma 8 cases (66.7 %) were strongly stained, 4 cases (33.3 %) were moderately stained and none of the case (0 %) were found to be weak and negative on p63 scoring.

Afat T Elnasher et al,[12] also observed variation in p63 expression. He studied 50 cases of urothelial carcinoma, among which 33 cases were of high grade and 17 cases were of low grade. He observed that out of 33 cases of high grade 24.2 % were strong, 21.2 % were moderate, 27.2 % cases were weak and 27.3 % cases were negative on p63 score. But out of 17 cases of low grade 64.7 % cases were strong, 17.6 % were moderate, 11.8 % were weak and 5.9 % were negative on p63 score.

Our study results are consistent with the maximum number of cases of low grade UCa with strong p63 score. Abdel Wahab MM[11] observed maximum number of cases with strong staining in low grade UCa. He reported 58.3 %, 25 %, 16.7 % and 0 % cases in strong, moderate, weak and negative p63 scoring respectively. Whereas in high grade UCa cases strong, moderate, weak and negative p 63 score was seen in 16.6 %, 27.8 %, 22.25 % and 33.3 % respectively.

In present study p63 expression was positive in 23 (92 %) cases out of 25 cases in UCa group, while none (0 %) of 25 cases in PCa group was stained with p63 IHC marker. Results of present study are consistent with the results of study of Nasir Ud Din et al,[1] who observed that p63 expression was present in 88 % of UCa and none of PCa. Kaufmann et al,[13] who performed p63 on UCa and PCa found p63 positivity in 87 % of UCa and 2 % of PCa cases.

Kunju et al.[14] found p63 positivity in 94.7 % of high-grade urothelial carcinomas. They performed p63 along with a panel of immunohistochemical stains 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. None of the prostatic carcinoma stained with p63. They found p63 to be a fairly sensitive and highly specific marker of urothelial carcinoma with consistent diffuse nuclear positivity in 92 % of all documented cases of urothelial carcinomas. Our results are also confirming study results of Kunju et al.[14] Like their results, we also found p63 positivity in 92 % of high-grade urothelial carcinomas.

Study by S. Premalathal et al,[15] also found no expression of p63 IHC stain in PCa cases. It can be concluded that p63 can be useful marker in the differential diagnosis of urothelial carcinoma from poorly differentiated prostatic carcinoma.

Sensitivity of p63 stain in differentiating UCa with PCa was 92 % in our study, specificity of p63 stain in differentiating UCa with PCa was found to be 100 %. This data is in accordance with data published by Kunju et al,[14] who showed 91.7 % sensitivity and 100 % specificity. Oh WJ et al,[16] published 73.9 % sensitivity and 100 % specificity of p 63 in UCa group. Therefore p63 has proven its utility in differentiating UCa from PCa effectively.

CONCLUSIONS

Pathological differentiation of UCa with PCa becomes a challenging task on routine histopathological examination. Our aim was to study cost effective, easy and simple IHC marker which can effectively distinguish UCa from PCa. We have used p63 IHC stain with the hypothesis that it selectively stains basal cell nuclei but no other cells.

Results observed in the current study proved the hypothesis that all cases of the prostatic adenocarcinomas were p63 negative and most of the urothelial carcinomas were p63 positive. P63 appears to be a useful marker in distinguishing between UCa and PCa due to its high specificity for UCa. Hence, we conclude that p63 is a reliable marker of urothelial differentiation and can be used as first line marker in differentiating urothelial carcinomas from adenocarcinomas of prostate.

We recommend that for distinguishing UCa from PCa, first line IHC staining panel should be done with p63 and PSA. As both of these have limited sensitivity, for challenging and unresolved cases, two lineage-specific markers one each for UCa (GATA3, S100P) and PCa (NKX3.1, P501S, PSMA) should be used for definitive diagnosis.

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