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Research Article

UTILITY OF IMMUNOHISTOCHEMICAL EXPRESSION OF SMOOTHELIN IN STAGING OF UROTHELIAL CARCINOMA IN TRANSURETHRAL RESECTION SPECIMENS OF BLADDER TUMORS

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A R T I C L E I N F O

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A B S T R A C T

Background: Accurate recognition of muscularis propria **(**MP) and muscularis mucosae (MM) in bladder cancer is crucial as carcinoma confined to lamina propria is treated conservatively, whereas one extending into MP needs radical surgery. Distinguishing MP from MM can be problematic on H&E staining, especially in transurethral resection specimens of bladder tumors (TURBT). Desmin stains both MM and MP equally. Smoothelin is a novel smooth muscle specific marker expressed only in terminally differentiated smooth muscle cells and relatively specific for MP.
Aim: This study was done to evaluate the diagnostic value of Smoothelin and Desmin expression in discrimination between MM and MP.
Methods: This study was conducted in Department of Pathology, Pt. B. D. Sharma, PGIMS, Rohtak, on 55 cases of TURBT specimens. Expressions of Immunohistochemical (IHC) markers - SMA, Desmin and Smoothelin were compared in MM and MP.
Results: SMA and Desminstained MP and MM with equal intensity. Smoothelinshowed absent /weak staining in 96.3% cases and moderate positivity in 3.7% in MM and strong /moderate staining in MP (100%).
Conclusion: The distinct IHC pattern of smoothelin staining in MM and MP proves very useful for accurate staging of urothelial carcinoma.

INTRODUCTION

Accurate pathological staging is crucial in urothelial carcinomas, as pT1 [confined to lamina propria, muscularis mucosae (MM)] carcinomas are treated conservatively while pT2 [invading muscularis propria (MP)] needs radical surgery. Distinguishing MP from MM is problematic on H&E staining, especially in transurethral resection specimens of bladder tumors (TURBT), due to hyperplastic MM, desmoplasia and splayed MP. [1]

Smoothelin is a novel smooth muscle specific marker, expressed in terminally differentiated cells and relatively specific for MP.[2]Desmin shows positivity in both MM & MP.[3] This study was done to evaluate the differential expression of Smoothelin and Desmin in MM and MP.

MATERIAL AND METHODS

Case Selection

This prospective study was conducted on 55 cases of TURBT specimens in the department of Pathology in collaboration with department of Urology, at Pt. B. D. Sharma PGIMS, Rohtak. It was a cross sectional descriptive study.

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Inclusion Criteria

Primary Urothelial carcinoma.

Exclusion Criteria

Other malignancies and Inadequate biopsies

All specimens were formalin fixed, routinely processed, and embedded in paraffin. Four sections were prepared from each tissue block, one of them stained by H&E for re-evaluation; the other three were subjected to the immunohistochemical markers SMA, Desmin, Smoothelin. IHC[4] and H&E staining[5] were carried out according to the standard procedure. Positive and negative controls were run with each batch of IHC stain.

Interpretations of Results

Tumor staging was done as per TNM staging system.[6] Grading of urothelial tumors was done according to the WHO Classification of tumors of Urothelial Tract 2016.[7] Interpretation of immunohistochemical stains in each case were performed semiquantitatively by analyzing the intensity of staining separately in each compartment (myofibroblasts, smooth muscle of the MM, and smooth muscle of the MP). Vascular smooth muscles were used as an internal control.

The intensity and pattern of staining were evaluated and categorized as follows

Intensity of staining

Grading Criteria- 0-Negative, 1+ Any weak or focal staining, 2+ moderate patchy/diffuse or strong and patchy, 3+ strong and diffuse.
Distribution- Negative <5%, focal 5-10%, patchy 11-50%, diffuse _>50%

Pattern of staining

SMA: Positive staining- Brown colour, Cytoplasmic
Desmin : Positive staining –Dark Brown colour, Cytoplasmic
Smoothelin : Positive staining- Brown Cytoplasmic[8]

Statistical Analysis

The whole data was subjected to statistical analysis Using SPSS 24 software. Cases were compared to the control. Chi-square test was used to compare qualitative variables. P value of <0.05 was considered statistically significant. Sensitivity, specificity and accuracy were used to assess diagnostic values of markers.

Biomedical Waste Disposal

All the biomedical waste generated during the study was discarded as per the Biomedical Waste Management and Handling, Rules 2016.[9]

RESULTS

We included 55 cases of urothelial carcinoma in our study. The patient's age ranged from 37-80 years with mean age of 62.5 ± 10.9 years. Male: female ratio was 8.2:1[as shown in Table- 1]. In all 55 cases, expressions of SMA, Desmin and Smoothelin were assessed in MP and MM based on intensity and distribution of staining.

Table no- I Showing age distribution, sex distribution and WHO/ISUP grades of urothelial tumors

Age Distribution	Total		WHO/ISUP Grading of Urothelial Tumors	
		Gender Distribution	High grade	Low grade
<40	4	M – 4 F - 0	4	-
41-50	5	M – 5 F – 0	2	3
51-60	8	M – 8 F - 0	5	3
61-70	25	M – 22 F – 3	20	5
71-80	13	M – 10 F – 3	9	4
TOTAL	55	M – 49 F – 6	40	15

M – Male, F - Female

SMA Immunoreactivity

SMA expression was seen in all the cases in blood vessels, MP (100%) and 94.5% cases in MM.

Desmin Immunoreactivity

Desmin was expressed with almost equal intensity in both MM and MP (91% and 100% respectively) but it was not expressed in blood vessels.

Smoothelin Immunoreactivity

Smoothelin showed positivity in 50.9% cases in MM, 100% cases in MP and 91% cases in BV when any intensity (weak, moderate, strong) was taken as positive. Staining of blood vessels in lamina propria was weakly positive in majority of the cases and was taken as internal control. With this intensity, sensitivity, specificity and accuracy were 100%, 49.1% and 74.5% respectively. But when only moderate and strong intensity of smoothelin were considered as positive, it showed positivity in 100% MP and only 3.7% MM and its sensitivity, specificity and accuracy were 100%, 96.3% and 98.1% respectively. [as shown in Table -2]

Table II Showing IHC expression of Desmin and Smoothelin markers with varying intensities of smoothelin.

	IHC Markers Expression (Positive)		Intensities of Smoothelin				Muscle Invasion	
	Desmin	Smoothelin	0	1+	2+	3+	H & E	Smoothelin
MM	51	2	27	26	2	0	18	32
MP	55	55	0	0	4	51	34	20
P VALUE	0.010						0.001	

On comparing the expression of desmin and smoothelin in MM, it was seen that Desmin was positive in 92.7% cases while smoothelin in only 3.6% cases and this difference was statistically significant with P value of 0.010. When Desmin and Smoothelin expressions were compared in MP, both were found to be highly sensitive with Sensitivity of 100%.

In our study, based on H&E staining, MP invasion was present in 61.9% cases while smoothelin showed invasion in only 36.4% cases. 14 out of total 55 cases depicted variations in results between H&E and smoothelin staining. This difference in result was statistically significant and TNM stage changed from T2 to T1 in these 14 cases. [as shown in Table -3]

Table III Showing difference in TNM stages depending on H&E and Smoothelin staining.

TNM Stage	Based on H&E Staining	Based on Smoothelin Staining
Tis	3	3
T1	18	32
T2	33	19
T3	1	1
T4	0	0
TOTAL	55	55

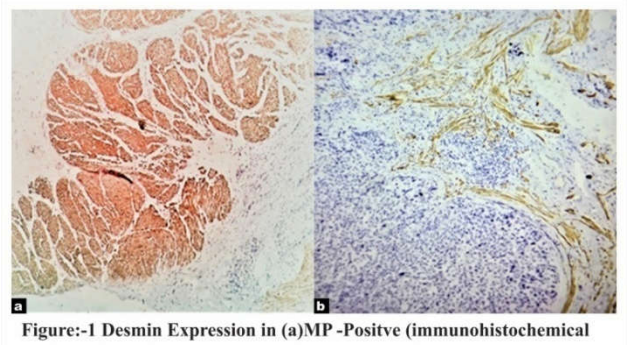

Figure:-1 Desmin Expression in (a)MP -Positve (immunohistochemical [IHC], x 100) & (b)MM -Positve (IHC x 100)

Figure I Desmin expression (IHC) in MP (a) and MM(b) – Positive

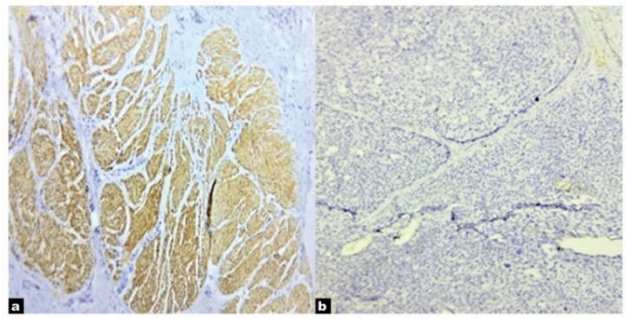

Figure:-2 Smoothelin Expression in (a)MP -Positve (IHC x 100) & (b)MM -Negative (IHC x 100)

Figure II Smoothelin expression (IHC) in MP (a) – Positive and MM (b)- Negative

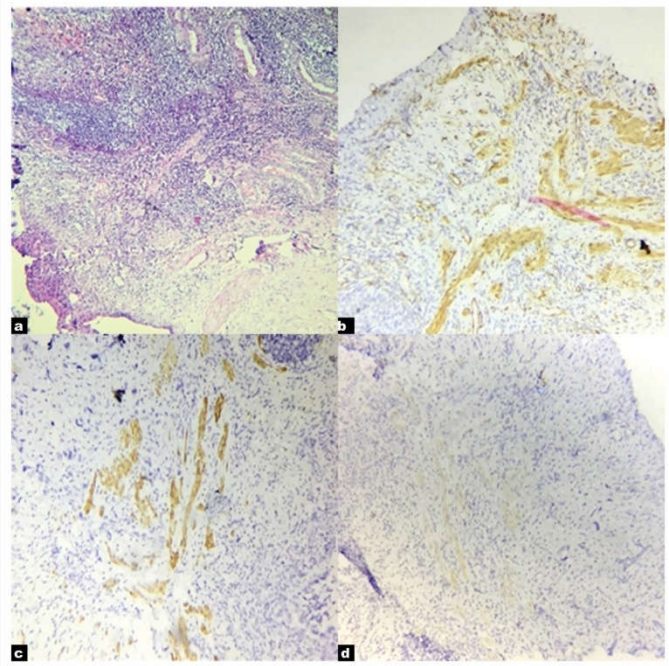

Figure:-3 (a) H&E Stained Section Showing MM (b) SMA -Positive (IHC x 100) (c)Desmin -Positive (IHC x 100) (d) Smoothelin - Negative (IHC x 100)

Figure III (a)H&E stained section showing MM, (b)SMA expression (IHC) in MM – Positive, (c) Desmin expression (IHC) in MM – Positive, (d) Smoothelin expression (IHC) in MM - Negative

DISCUSSION

The single most important prognostic factor in urothelial carcinoma is the pathological stage which includes the anatomic depth of invasion. The discrimination between MM and MP is crucial as carcinoma confined to the lamina propria is usually treated conservatively, whereas one extending into the MP almost always dictates a more radical surgical management. Distinguishing bladder MP from MM can be problematic especially in small biopsies including TURBT specimens where fragmentation, tangential sectioning and thermal artifacts can lead to poor orientation.[1] Therefore, there is an increasing need for additional diagnostic techniques. IHC has emerged as a powerful, adjunctive tool for accurate staging of urothelial cancer.[10] Especially useful in the difficult cases where desmoplastic reaction, hypertrophic MM resembles MP and splaying of MP can mask the differentiation of MM and MP.[1]

We found a difference in Male: Female ratio between India and western countries which was probably due to different lifestyle and increased smoking habits among females in the western countries.[11]

We selected our cases based on the presence of MP on histopathological examination. We also included diagnostically difficult cases with equivocal areas of MP and MM on routine histopathology. IHC analysis of all the cases were done using SMA, Desmin and Smoothelin. SMA was taken as internal control as it stains all the smooth muscles of blood vessels, MM and MP with equal intensity.

Expressions of Desmin and smoothelin were compared in differentiating MM and MP. Desmin showed positivity in MM in 92.7% of cases while smoothelin stained MM as negative in 96.7% of cases and this difference in staining pattern was statistically significant (p value = 0.010). This differential staining of Desmin (positive) and Smoothelin (negative) in MM was used for confirmation of MM and differentiation from MP in our study.

When all smoothelin expressions were taken as positive irrespective of intensity, it's sensitivity, specificity and accuracy were 100%, 49% and 74.5% respectively. But when only strong and moderate expression was considered as positive and mild and weak as negative, smoothelinsensitivity was 100%, specificity 96.3% and accuracy 98.1%.

On H&E staining, 60% of the cases were found in T2 stage and 32.7% cases were in stage T1. In fourteen cases, there was absence of smoothelin staining in areas of muscle invasion found on H&E. In all these cases, smoothelin was strongly positive in muscles present elsewhere in the section or in the control section run in the same batch. On the other hand, Desmin was strongly positive in all these areas. Keeping in mind strong positivity of desmin in both MP and MM and negative staining of smoothelin in MM, these muscles were interpreted as hyperplastic MM. So stage changed from T2 to T1 in 14 cases and this difference was statistically significant with P value of 0.001.

However, interpretation of smoothelin in TURBT specimens may warrant caution. Some studies have reported 2+ staining in MM and 1+ staining in classic MP in some of the cases and have attributed these flaws either to fragmented biopsies, suboptimal staining quality, false positive reaction and topographical variations as seen in trigonal area of bladder.[13] Kamel *et al*[12] also made certain recommendations for optimal smoothelin staining interpretation which includes - 1) Use of concomitant bladder control sections with the test section on the same slide to show differential staining pattern and validating the smoothelin staining to be optimal. 2) Use of optimal dilution to limit false positive results although it may compromise sensitivity due to weaken MP staining.

Paner *et al*[13] in their study included the cases with features of stromal desmoplasia, cautery effect and topographical variations of MP especially in trigonal area where muscles are thin and superficial and virtually resemble MM and confirmed their presence by 3+ smoothelin staining in 2 TURBT specimens. All areas of stromal desmoplasia showed completely negative smoothelin staining. Smoothelin staining was not impacted in any section with thermal effects.

Miyamoto *et al*[14] also confirmed a relatively distinct staining pattern of smoothelin between MM and MP in their study. They have recommended to maintain the caution while using

smoothelin IHC as a diagnostic tool for MP invasion due to overlap of intensity of staining between MM and MP.

Differentiation between MM and MP in bladder tumors is critical for staging of tumors, however may not be straightforward in some overlapping cases, where IHC has a potential role. Smoothelin is a novel smooth muscle specific contractile protein expressed only by fully differentiated smooth muscle cells (MP) and not by proliferative non-contractile smooth muscles (MM) and myofibroblasts. Our data also confirmed the relatively distinct staining pattern of smoothelin between MM and MP. Although we have used this distinct staining pattern in diagnosis and staging of tumors, none of the other studies in the literature have used smoothelin as a diagnostic tool for staging, they used it only as a supplement to H&E staining. Further evaluation of specificity of the smoothelin in a larger group is recommended to determine its ultimate use in diagnosis and clinical practice.

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Conflict of interest

We declare that there are no conflicts of interest amongst the authors. The manuscript has been read and approved by all the authors, the requirements for authorship have been met and each author believes that the manuscript represents honest work, if that information is not provided in another form.

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