Original Article

Does desmin immunohistochemistry have a role in assessing stage of urothelial carcinoma in transurethral resection of bladder tumor specimens?

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ABSTRACT

Context: Pathological stage is the most important determinant of clinical outcome of bladder carcinoma patients. pT_1 carcinoma is defined by invasion into lamina propria, including muscularis mucosae (MM), but not into muscularis propria (MP) (pT_2). However, pathological staging of the tumor is a complicated task for pathologists. "Splitting of MP" or "hypertrophy of MM" caused by tumor invasion are important causes of interpretation subjectivity leading to intra-pathologist variation and disagreement. The aim of the study was to prospectively evaluate the utility of desmin immunohistochemical expression for evaluation of muscle invasion in transurethral resection of bladder tumor (TURBT) specimens. **Materials and Methods:** A total of 40 TURBT cases was taken. Specimens were processed, stained with H and E, graded and evaluated to determine whether MP invasion was present. Desmin immunohistochemistry (IHC) was used to assess muscle invasion and compare the result with H and E stained sections. Tumors with radiological evidence of gross invasion and those of stage T_{is} were excluded. **Results:** Among 40 TURBT cases, there were 37 cases of transitional cell carcinoma. Among them, 17 were high grade and 20 were low grade. On H and E, 17/37 cases showed MP invasion, 8/37 cases showed no MP invasion and the rest 12/37 had questionable MP invasion. Desmin staining intensity was graded from 0 to 3+. MM showed negative (0) and moderate (2+) staining in one case each, mainly (35/37) showed mild (1+) staining intensity. MP showed moderate (2+) (3/37) to strong (3+) (34/37) staining intensity. Among 12/37 questionable cases (on H and E) desmin staining showed definite MP invasion in eight cases. **Conclusions:** Although morphology remains the gold standard, desmin IHC has diagnostic utility in the evaluation of questionable MP invasion and hence in staging of urothelial carcinoma.

Key words: Desmin, staging, urothelial carcinoma

INTRODUCTION

Transitional cell carcinoma (TCC) is one of the important causes of cancer-related mortality and morbidity. Proper staging of carcinoma is of utmost importance to predict the prognosis as well as determining the mode of therapy in individual patients. Lamina propria (LP) invasion by the tumor is staged pathological stage T1 (pT_1). It may be accompanied by hypertrophy of smooth muscle cells of LP, muscularis mucosae (MM). The thin wisps of muscle fibers in these cases become thickened muscle bundle that can simulate muscularis propria (MP).[1] This can cause difficulty in interpretation of transurethral resection of bladder tumor (TURBT) specimens where orientation is a big challenge. Invasive carcinoma alters the appearance of LP making it more desmoplastic, stroma becomes more myofibroblastic in morphology.[2] Nonetheless cauterization artefacts, necrosis are the inherent drawbacks of TURBT biopsy. All these are very confounding factors for even an experienced pathologist. Tumor staging may be falsely upgraded in these conditions. Furthermore, when carcinoma invades the MP, pathological stage T2 (pT_2), it may cause splitting of muscle fibers, which on routine

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H and E stained section mimics MM due to inadequacy of the biopsy material.[3] However, this discrimination of MM and MP is the first and foremost task for a pathologist as it signifies the stage, 5 year's survival (better for pT_1 than pT_2) and directs clinicians to institute appropriate therapy to the patients.

To sort out this problem, various immunohistochemical (IHC) methods were applied, but with conflicting results. In our study, urothelial carcinoma were staged first using light microscopy and H and E staining and then using IHC for desmin.

Desmin is a muscle type intermediate filament, found mostly in smooth and striated muscle and in a lesser amount in myofibroblast.[4] It is one of the earliest protein markers for muscle tissue in embryogenesis as it is detected in the somites.[5] Although it is present early in the development of muscle cells, it is only expressed at low levels, and increases as the cell nears terminal differentiation. Desmin knockout mice develop normally and only experience defects later in life.[6]

Desmin is also important in muscle cell architecture and structure since it connects many components of the cytoplasm.[7] There is some evidence that desmin may also connect the sarcomere to the extracellular matrix (ECM) through desmosomes, which could be important in signaling between the ECM and the sarcomere, which could regulate muscle contraction and movement.[8]

Desmin is primarily used for the identification of smooth muscle and skeletal muscle tumors.[9,10] Positivity for desmin associated with negativity for actin is a feature of a subset of cells of myofibroblastic appearance,[11] and of those of hormone-dependent stroma (vagina, breast).[12] Desmin expression has also been described in Ewing's sarcoma/primitive neuroectodermal tumors, desmoplastic small round cell tumors, neuroblastoma, mesothelial cells and tumors, the blastemal component of Wilms' tumor, giant cell tumors of the tendon sheath, ossifying fibromyxoid tumors of soft parts and angiomatoid fibrous histiocytomas.[13,14] Though desmin positivity has been used in an important IHC marker in various neoplasms, we here in our study are using desmin not as a diagnostic marker for tumor cells rather using its property to assess the depth of invasion of tumor cells thus aiding in staging of urothelial neoplasm.

The study was undertaken to look for differential staining property of MM and MP for desmin IHC and thus aid in assessing muscle invasion. It would be of great clinical value to be able to stage cases of urothelial carcinoma in TURBT specimens appropriately using desmin IHC along with H and E.

MATERIALS AND METHODS

This is a prospective and observational study done over a period of approximately 2 years, from January 2011 to December 2012, after approval from Institutional Ethical Committee and informed consents from the patients who came to our Urology Department during the study period. A total of 40 suspected cases of urothelial carcinoma in patients attending the Urology Department of our institute was taken up.

Inclusion criteria

1. Transurethral resection of bladder tumor specimens of the patients with radiological or cystoscopic features suggestive of bladder wall invasion
2. Patients who gave the consent and fully co-operated during the study period
3. Patients with full preoperative clinical history.

Exclusion criteria

1. Cases of *in-situ* urothelial carcinoma and cases with histological diagnosis other than TCC were excluded from our study, after examination of H and E stained slides
2. Patients with radiological evidence of tumor extending beyond serosa.

Our study was designed after Strobe observational studies in epidemiology.

Transurethral resection of bladder tumor specimens received from the Department of Urology of our institute were fixed in 10% neutral buffer formalin (NBF) for 12 h. On each occasion we received two containers; one with curettage of the tumor proper and another from the tumor bed including the muscle. After proper tissue processing all the chips were embedded in paraffin and H and E stain was done. Histological categorization was done according to the WHO guidelines in H and E stained sections.[15] Histological grading and staging of the urothelial carcinoma were then evaluated by two pathologists independently. MP invasion was reported as "yes", "no" and "questionable" based on H and E stained sections without prior knowledge of desmin immunostaining status.

Immunohistochemical analysis

All the specimens were fixed in NBF and routine paraffin sections were made. Sections were cut at 3 μm thickness and mounted on poly-L-lysine coated slides. The sections were deparaffinized in xylene and rehydrated in alcohol. Antigen retrieval was done using a pressure cooker method.[13] Endogenous peroxidase was blocked with 3% H_2O_2. Later, the sections were incubated in humidifying chamber with primary antibodies (desmin) for 60 min.

For desmin, Novocastra Lyophilized mouse monoclonal antibody (D33 monoclonal antibody from Cell Marque; US) was reconstituted with 0.1 ml of sterile distilled water and working dilution of 1:50 was prepared. The slides were then washed with Tris buffer, and secondary antibody was applied for 30 min. Then polymer HRP was added and incubated for 30 min. Thereafter the chromogen, di-amino-benzidine was added for 10 min as a substrate chromogen solution to produce a brown color. The slides were counterstained with Harris hematoxylin.

The specificity of desmin immunostaining was controlled by using vascular plexus in the LP, which is known to stain positively.

Evaluation of immunohistochemical stain

Intensity of desmin expression was graded semi-quantitatively as follows:

Negative was 0, weak was 1+, moderate was 2+, and strong was 3+.

The medium-sized blood vessels in LP were taken as internal control in each case, and the staining intensity of these blood vessel wall was marked as 1+ according to the scoring stated above [Figure 1]. This differentiated MP invasion from MM given the difference in their staining intensity [Figure 2a and b]. Based on desmin immunostaining MP invasion was reported in two categories as "yes" or "no" without prior knowledge of H and E staining status.

Finally, results of both H and E and IHC slides in discrepant cases were discussed between two pathologists to reach a final decision.

The results were tabulated, compared, and statistical analysis done.

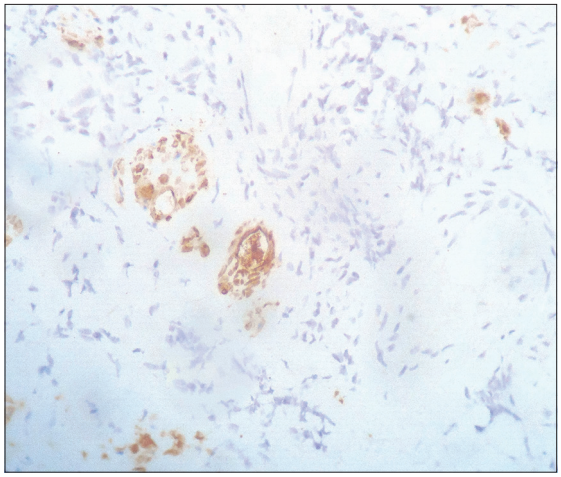

Figure 1: Vascular plexus in lamina propria showing desmin immunostaining grade I (IHC, ×400)

RESULTS

We found a marked male predominance in our study (male: female = 4.3:1) and the history of smoking was present in 75.68% (n = 37) cases. The patients presented mainly with dysuria.

There were 40 cases altogether. One case each of adenocarcinoma, squamous cell carcinoma, and papillary urothelial neoplasm of low malignant potential (PUNLMP) were excluded.

Table 1 shows the distribution of the cases according to routine (H and E) histopathological diagnosis, which reveals 37 out of 40 TURBT cases were TCC. Among 37 cases taken up 17 were high grade and 20 were low grade TCC. The clinicopathological details of 37 cases taken up for the study were summarized in Table 1.

On H and E stain definite MP invasion was found in 15 cases (13 were high grade and 2 were low grade), 10 cases were negative for invasion (2 were high grade and 8 were low grade), whereas 12 cases had questionable muscle invasion (2 were high grade and 10 were low grade) [Figure 3, Table 2].

Desmin staining, on the other hand, showed a higher percentage of MP invasion. MP invasion was present in 20 cases after desmin immunostaining. Among them, 15 were

Table 1: Clinicopathological details of the cases

Clinicopathological parameters	Cases (n=37)	Percentage
Sex distribution		
Male	30	81.1
Female	7	18.9
Presenting features		
Pain	21	56.8
Pain and hematuria	16	43.2
Smoking history		
Yes	28	75.7
No	9	24.3
Preoperative history		
Yes	7	18.9
No	30	81.1
Histopathological grade		
High grade	17	45.9
Low grade	20	54.1

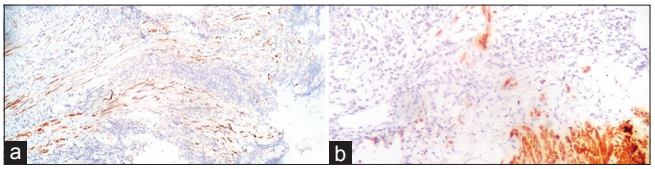

Figure 2: (a) Thin whips of muscularis mucosae showing desmin immunostaining grade I or weak desmin staining intensity (IHC, ×100). (b) Muscularis propria showing desmin immunostaining grade III or strong desmin staining intensity (IHC, ×100)

high grade, and five were low grade TCC [Figure 4, Table 2]. Thus, desmin IHC picked up muscle invasion in two cases of high grade and three cases of low-grade TCC, which were missed in H and E staining alone. Hence, desmin staining changed the diagnosis of muscle invasion status in both grades of TCC, more so in low grade (from 10% to 25%, $n = 37$) than in high grade (from 76.47% to 88.24%, $n = 37$) [Table 2].

Desmin staining intensity was used to discriminate between MM and MP muscle fibers. In most of the cases, MP showed strong (3+) desmin immunostaining. On the other hand, blood vessels and MM showed much weaker degree of staining intensity, majority showed weak (1+) desmin immunostaining [Table 3]. There was a definite difference in intensity score of MM and MP in all the cases and in the majority of them (94.6%, $n = 37$) the difference was of 2 levels in a scale of 0 to 3+.

DISCUSSION

Pathological stage is the most important determinant of treatment and prognosis for bladder cancer.[16-20]

Pathologic staging of bladder tumors is based on review of TURBT, partial cystectomy or radical cystectomy specimens. pT_1 carcinoma is defined by invasion into LP, and not into MP. MM, in contrast to MP, consists of thin and wavy fascicles of smooth muscle frequently associated with thin-walled blood vessels.[21] Recognition of LP invasion is indeed challenging. Pathologists should be aware of various diagnostic pitfalls which are inherent to TURBT specimens such as tangential section, poor specimen orientation, obscuring inflammation, deceptively bland cytology in some variants of urothelial carcinoma and pseudoinvasive nests of benign proliferative urothelial cells.[22] pT_1 carcinomas often invade the underlying stroma as single cells or irregularly shaped nests of tumor cells causing diagnostic difficulties.[21] Thermal injury or cautery artefact can also render an accurate diagnosis of invasion difficult. Thus, the invasion of LP (pT_1) can be very subjective. Invasion of LP (pT_1) carries a better prognosis compared with the invasion of MP (pT_2). MP invasion can be difficult to determine if there are insufficient muscle bundles to distinguish between hypertrophic MM and true MP.

A couple of previous studies have reported that smoothelin IHC was useful in the discrimination between MM and MP in archival cystectomy specimens with unequivocal morphological findings.[2,23] In this study, we have demonstrated that desmin too can serve as an ancillary tool in the evaluation of MP invasion by bladder carcinoma in diagnostic bladder biopsy specimens.

Desmin immunostaining allows distinction of MP from MM, given the difference in intensity of expression. MM consistently displayed negative or weak reactivity, whereas MP was consistently of stronger intensity. In our study, MM showed negative or weak reactivity in 97.29% cases ($n = 37$)

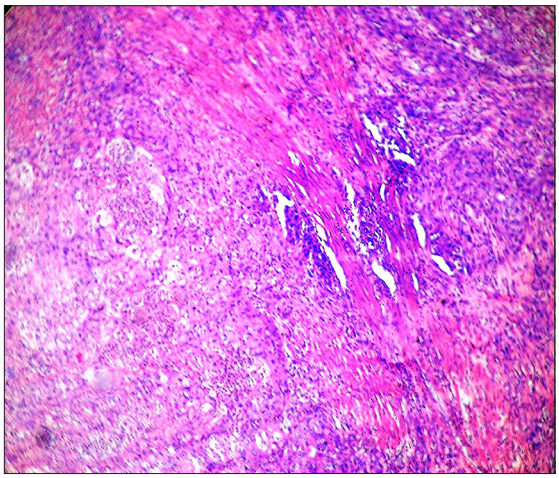

Figure 3: Urothelial carcinoma invading into muscularis propria (H and E, ×100)

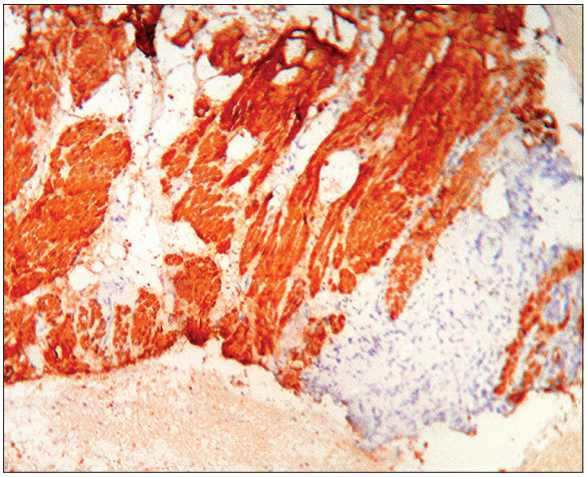

Figure 4: Urothelial carcinoma invading into muscularis propria confirmed by desmin immunostaining (IHC, ×100)

Table 2: Grades, HP features of invasion and desmin staining

Grade of tumors	HP showing invasion into MP	HP showing no invasion into MP	HP showing questionable invasion into MP	Desmin stain showing invasion into MP in questionable cases	*P* value
High grade	13	2	2	2	<0.01
Low grade	2	8	10	3	<0.01
Total	15	10	12	5	<0.01

MP: Muscularis propria, HP: Histopathology

Table 3: Summary of desmin immunoreactivity intensity score

Desmin intensity	Structures other than MP (including MM)	Percentage (*n*=37)	MP	Percentage (*n*=37)
0 (negative)	1	2.7	0	0
1+ (weak)	35	94.6	0	0
2+ (moderate)	1	2.7	2	8.1
3+ (strong)	0	0	34	91.9

MP: Muscularis propria, MM: Muscularis mucosae

and MP had strong or moderate reactivity in 97.3% of cases (n = 37). Every case (100%, n = 37) had at least one level (i.e. weak to moderate) of increased IHC intensity between MM and MP, and 94.6% of cases had 2 levels of intensity difference between MM and MP.

One important observation of our study was that desmin staining intensity was similar in MM and medium-sized blood vessels, like those of LP vascular plexus [Figure 1]. Using smoothelin IHC Bovio *et al*. found that the reactivity of medium-sized blood vessels of LP vascular plexus is related to the expression of different isoforms of smoothelin.[2] Similar to their study we also found that the reactivity of both medium-sized vessel and MM is important because it can serve as an internal intensity quality control for IHC since vessels are virtually present in all nonsuperficial TURBT specimens. Presence of internal quality control is very important because difference in intensity of immunostaining may be caused by variation in tissue fixation, processing time and variation in IHC technique. Significant variation in intensity was not observed in our study, but differences in reactivity may be seen due to inter-lab variation due to different fixation times and processing methods, and IHC protocol.

It is likely that hypertrophic MM misinterpreted as MP is the cause of some tumors being over staged, that is, pT_2 at TURBT or biopsy and pT_1 at cystectomy.[24] Moreover, the recent observation that at trigone and at the ureteral insertion, MP extends into superficial LP has complicated the problem further.[25]

A follow-up study has demonstrated that these more superficial bundles of MP show strong reactivity with smoothelin in keeping with MP, but studies using the frequently used smooth muscle marker desmin is lacking in such areas.[23] Other situation in which this assessment is problematic includes cases of invasion of the tumors into the MP, in which the normal thick bundles of MP are splayed apart and distorted by invasive carcinoma, and in cases of intense desmoplastic response of myofibroblasts in LP mimicking MP.[3] In both of these settings, IHC with desmin may be a useful adjunct as the intensity of reactivity can be used to distinguish MM, even hypertrophic MM, from MP and myofibroblast.

Although desmin is clearly beneficial as an adjunct to thorough light microscopic (H and E) evaluation, it should not be used as the sole determinant of MP invasion. This point was demonstrated by two cases in our series in which the MP was moderately immunoreactive for desmin. However, in both the cases, muscles other than MP showed one scale lower staining intensity.

In 12 cases of our study which showed questionable muscle invasion in routine H and E staining, desmin helped to decide the actual status of muscle invasion and thereby provided a guide to appropriate staging of the neoplasm. Not only that, in a few doubtful cases desmin even helped to assess whether MP was present in the biopsy specimen at all. The two methods (H and E and desmin IHC) showed agreement in 25 out of 37 cases. IHC upstaged the diagnosis of urothelial carcinoma from pT_1 to pT_2 in five cases. It lowered the rate of imprecision and eliminated the disagreement between two pathologists.

CONCLUSION

Although morphology remains the gold standard, we can apply desmin immunostaining to aid pathologists in assessing the muscle invasion in TURBT biopsy specimens, thereby help in staging of urothelial carcinomas.

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