Section: Pathology

Original Research Article

DIAGNOSTIC UTILITY OF SMOOTHELIN IMMUNOMARKER IN DIFFERENTIATING MUSCULARIS MUCOSAE AND MUSCULARIS PROPRIA IN TURBT SPECIMENS

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ABSTRACT

Background: Accurate assessment of muscularis propria invasion is a key determinant in staging and management of urothelial carcinoma. Smoothelin, However, distinguishing Muscularis Mucosae especially hyperplastic Muscularis Mucosae from Muscularis Propria in Transurethral Resection of Bladder Tumor specimens can be challenging on routine Hematoxylin and Eosin staining. Smoothelin, a cytoskeletal protein specific to terminally differentiated smooth muscle, has emerged as a potential immunohistochemical marker for identifying Muscularis Propria.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology, Government Medical College, Thrissur, over 18 months. All Transurethral Resection of Bladder Tumor specimens of urinary bladder malignancies received during the study period were included, except cystectomy specimens and those with inadequate tissue for immunohistochemistry. Formalin-fixed, paraffin-embedded sections were stained with H&E for histopathological typing and assessment of muscle invasion, with Muscularis Mucosae defined as thin, wispy fibres near lamina propria vessels and Muscularis Propria as thick bundles with rounded contours. All Transurethral Resection of Bladder Tumor cases where muscle tissue was present including equivocal ones were further evaluated using Smoothelin immunohistochemistry (anti-Smoothelin antibody R4A), with staining scored as 0–3 (0/1+ for Muscularis Mucosae and 2+/3+ for Muscularis Propria).

Results: Among 74 cases, muscle was present in 30 specimens and absent in 44. Fourteen cases showed muscle invasion—7 involving Muscularis Mucosae (pT1) and 7 involving Muscularis Propria (pT2). Smoothelin demonstrated absent or weak staining in Muscularis Mucosae and moderate to strong staining in MuscularisPropria. For Muscularis Mucosae, the sensitivity and specificity were 95% and 80%, respectively, with a Positive Predictive Value of 90% and Negative Predictive Value of 89%. For Muscularis Propria the sensitivity and specificity were 100% and 95%, respectively, with a Positive Predictive Value of 87% and Negative Predictive Value of 100%. The concordance rate between Hematoxylin & Eosin sections and Smoothelin Immunohistochemistry was 96%.

Conclusion: Smoothelin is a reliable smooth muscle–specific marker that effectively distinguishes MM from MP and enhances diagnostic accuracy in assessing muscle invasion in urothelial carcinoma. Incorporating Smoothelin immunomarker as an adjunct to routine microscopy can aid precise pathological staging and improve therapeutic decision-making.

Keywords: Smoothelin Immunohistochemistry, Muscle Invasion, Urothelial Carcinoma.

INTRODUCTION

Accurate determination of the degree of bladder wall invasion is the most significant predictive factor for tumor staging in bladder carcinoma, and it continues to play a pivotal role in guiding prognosis and subsequent therapeutic management. According to the American Joint Committee on Cancer (AJCC), non-invasive tumors are classified as pTa or pTis, while pT1 tumors are confined to the lamina propria and pT2 stage tumors show invasion into the muscularis propria.[1] Despite its prognostic importance, several histopathological challenges are encountered in accurately determining the depth of invasion on routine Hematoxylin and Eosin (H&E)-stained sections. These include: (a) the uncertainty regarding the presence or absence of muscularis propria in Transurethral Resection of Bladder Tumour (TURBT) specimens; (b) the potential for the Muscularis Mucosae (MM)—which may appear hyperplastic—to mimic the Muscularis Propria (MP); and (c) the distortion of muscularis propria bundles by infiltrating tumor cells, which may disrupt their rounded configuration and cause splaying of muscle fibers, thereby obscuring the true plane of invasion. Under such circumstances, the distinction between MM and MP becomes difficult, often necessitating non-committal or equivocal histopathological reporting.

The introduction of the Smoothelin immunomarker has significantly improved diagnostic precision in these ambiguous cases. Smoothelin, a cytoskeletal protein expressed exclusively in terminally differentiated, contractile smooth muscle cells, is absent or minimally expressed in non-contractile or non-proliferative smooth muscle fibers, unlike classical smooth muscle markers such as Smooth Muscle Actin (SMA).[2,3] In urothelial carcinoma, strong Smoothelin expression is typically observed in the MP while the MM exhibit weak or negative staining. This distinct immunostaining pattern enables pathologists to more accurately distinguish between MM and MP, thus providing better evaluation of the depth of tumor invasion. Morphologically, the MM consists of thin, discontinuous, and often wispy smooth muscle fibers located near the vascular channels of the lamina propria, whereas the MP comprises thick, well-organized bundles with rounded profiles forming a continuous contractile layer.[4]

While cystectomy specimens generally permit straightforward differentiation between MM and MP, this distinction becomes considerably more challenging in small biopsies and TURBT specimens due to tissue fragmentation and sampling limitations. On H&E-stained sections, certain epithelial and stromal characteristics—such as irregular nests, single-cell infiltration, disruption or absence of the basement membrane, finger-like tentacular projections, and paradoxical differentiation characterized by increased eosinophilic cytoplasm—assist in identifying lamina propria invasion.

The present study aims to evaluate the degree of invasion in urothelial carcinoma by delineating the muscularis propria using the Smoothelin immunomarker. The specific objectives are to differentiate between the MM and MP and to assess the concordance between routine H&E microscopy and Smoothelin immunohistochemistry findings. This approach is expected to enhance the accuracy of pathological staging and improve the reliability of prognostic assessment in bladder carcinoma.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, Government Medical College, Thrissur, a tertiary care institution in Kerala, over a period of 18 months from June 2015 to November 2016. The study included all TURBT specimens received for histopathological evaluation during the study period.

Inclusion Criteria:

All TURBT tissue samples diagnosed as malignant lesions of the urinary bladder and submitted to the Department of Pathology, Government Medical College, Thrissur, during the study period were included.

Exclusion Criteria:

Cases with inadequate tissue material for immunohistochemical evaluation and cystectomy specimens were excluded from the study.

Sample Size:

Although the desired sample size was calculated to be 166, all available TURBT cases of urinary bladder carcinoma received during the study period were included due to practical constraints in case availability.

Procedure: Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were prepared from each TURBT specimen. Unstained sections were cut at 4 µm thickness. One section from each case was stained with H&E for routine histopathological examination, including tumor typing, assessment of the type of muscle present, and evaluation of muscle invasion.

The MM was defined morphologically as thin, wispy, and often discontinuous smooth muscle fibers located within the lamina propria, typically in close association with vascular structures. The MP was identified as thick, well-organized muscle bundles with rounded contours situated beyond the submucosa. Cases in which the smooth muscle layer could not be confidently categorized as MM or MP were classified as equivocal.

For all the TURBT cases with muscle tissue available, an additional unstained section was subjected to immunohistochemistry (IHC) using an anti-smoothelin antibody to determine the muscle type and depth of invasion. IHC was performed using the anti-smoothelin (R4A) mouse monoclonal

antibody, an IgG1 isotype, following standard antigen retrieval and detection protocols. Smoothelin staining intensity was evaluated semi-quantitatively and scored according to the method described by Paner et al,[5] as follows:

- 0 = negative staining,
- 1+ = weak staining,
- 2+ = moderate staining, and
- 3+ = strong staining.

For analytical purposes, the presence of muscularis propria was defined by a staining intensity of 2+ or 3+, indicating strong or moderate positivity, whereas muscularis mucosae was defined by 0 or 1+ staining, corresponding to negative or weak expression.

This immunohistochemical approach enabled accurate delineation of muscle layers and allowed for more reliable assessment of the depth of tumor invasion, thereby complementing conventional H&E-based evaluation.

RESULTS

The study population comprised a total of 74 patients diagnosed with carcinoma of the urinary bladder. The cohort showed a marked male predominance, with 63 males and 11 females, reflecting a male-to-female ratio of approximately 5.7:1. The age of the patients ranged from 45 to 82 years, with the majority of cases occurring in the 61–70-year age group, indicating a higher prevalence of bladder carcinoma in the elderly population.

Histopathological examination revealed that papillary urothelial carcinoma (low grade) constituted the predominant subtype, accounting for 45 cases (60.8%), followed by invasive urothelial carcinoma in 14 cases (18.9%), papillary urothelial carcinoma (high grade) in 8 cases (10.8%), adenocarcinoma in 4 cases (5.4%), and squamous cell carcinoma in 3 cases (4.1%) (Figure 1).

Among the 74 TURBT specimens analyzed, muscle tissue was identified in 30 cases (40.5%), while 44 cases (59.5%) lacked demonstrable muscle in the sections examined. Of the 30 cases where muscle was present, 20 cases (66.7%) contained MM,7 cases (23.3%) demonstrated MP, and 3 cases (10%) were classified as equivocal, where differentiation between MM and MP could not be confidently established on routine H&E staining.

Table 1: Smoothelin IIHC Score for Muscle in H&E

SCORE	0	+1	+2	+3
NUMBER	21	2	1	6

All cases of MM in H&E were concordant with IHC. One case of MP was discordant which showed score 0 staining pattern.

Table 2: Comparison between H&E and Smoothelin IHC Findings

EQUIVOCAL IN H&E	IHC	
3	2 MM (score 0) 1MP (score+3)	
	MM	MP
H&E	20	7
IHC CONCORDANT	20	6
IHC DISCORDANT	-	1(score 0)

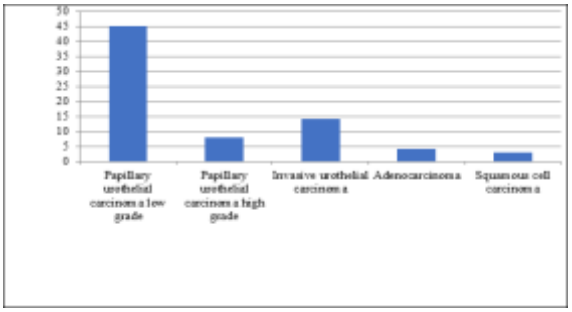

Figure 1: Histopathological Classification of Bladder Carcinoma Cases

Out of the 30 cases in which muscle was present, 14 cases (46.7%) demonstrated muscle invasion, whereas 16 cases (53.3%) showed no evidence of invasion. Among the 14 muscle-invasive cases, 5 (35.7%) exhibited invasion into the MM, 6 cases (42.9%) showed invasion into the MP, and 3 cases (21.4%) were considered equivocal for the depth of invasion. Smooth muscle differentiation was further evaluated using Smoothelin IHC. Based on the IHC scoring of muscles corresponding to areas of invasion observed in H&E-stained sections, 6 cases (42.9%) demonstrated a score of 0, 1 case (7.1%) showed a score of 1+, 1 case (7.1%) exhibited a score of 2+, and 6 cases (42.9%) showed a strong positive score of 3+ (Figure 2).

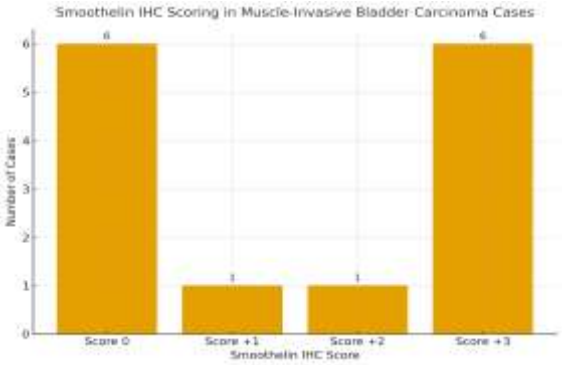

Figure 2: The bar graph illustrating the distribution of Smoothelin IHC scores among muscle-invasive bladder carcinoma cases

Among the 14 muscle-invasive cases, 7 cases (50%) were categorized as stage pT1, indicating invasion into the lamina propria, and 7 cases (50%) were classified as stage pT2, signifying invasion into the muscularis propria. The overall diagnostic accuracy of Smoothelin IHC in detecting smooth muscle in TURBT specimens was subsequently evaluated.

For the identification of MM, Smoothelin IHC demonstrated a sensitivity of 95%, specificity of 80%, Positive Predictive Value (PPV) of 90%, and Negative Predictive Value (NPV) of 89%. In contrast, for the detection of MP, the assay exhibited a sensitivity of 100%, specificity of 95%, PPV of 87%, and NPV of 100%. The overall concordance rate between conventional H&E microscopy and Smoothelin IHC was 96%, reflecting a high level of diagnostic agreement between the two modalities and supporting the utility of Smoothelin as a reliable adjunct in the histopathological staging of bladder carcinoma. The results are summarized in **Table 3**.

Table 3: Diagnostic Accuracy Parameters of Smoothelin IHC

Parameter	Muscularis Mucosae (%)	Muscularis Propria (%)
Sensitivity	95%	100%
Specificity	80%	95%
Positive predictive value	90%	87%
Negative predictive value	89%	100%

Figures 3–6: These figures illustrate the spectrum of Smoothelin IHC staining patterns and their correlation with histomorphological findings in bladder carcinoma.

Figures 3A–D demonstrate the gradation of Smoothelin staining intensity in the MM and MP, ranging from negative (score 0) to strong positivity (score 3+).

Figures 4A-B,5A-B depict cases of infiltrating urothelial carcinoma showing invasion into the MM and MP, respectively, with corresponding Smoothelin IHC highlighting the smooth muscle layers involved.

Figures 6A–B show a case of papillary urothelial carcinoma (low grade) with hyperplastic MM lacking Smoothelin expression, emphasizing the utility of Smoothelin in distinguishing true muscle fibers from hyperplastic smooth muscle bundles.

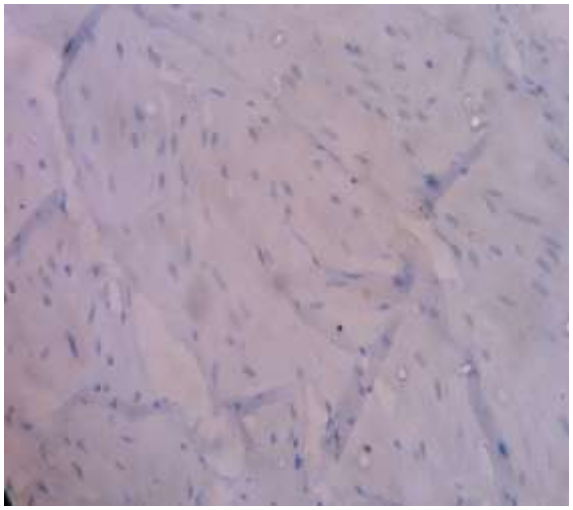

Figure 3A: Muscularis Mucosae showing score 0 (negative) staining with Smoothelin immunohistochemistry (40x)

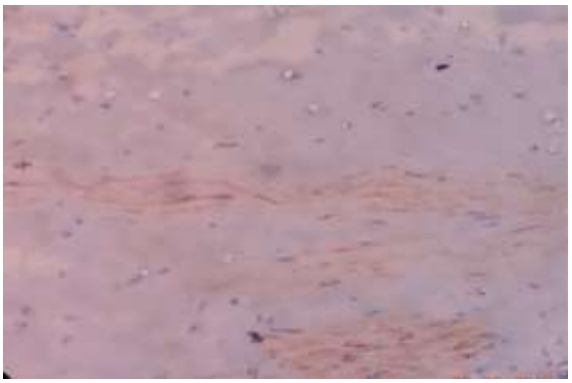

Figure 3B: Muscularis Mucosae showing score 1+ (weak) Smoothelin staining (40x)

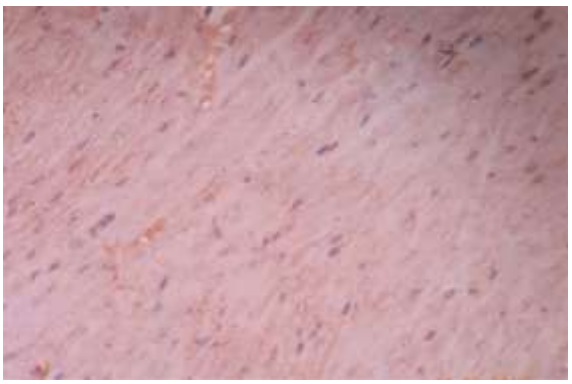

Figure 3C: Muscularis Propria displaying score 2+ (moderate) Smoothelin immunoreactivity(40x)

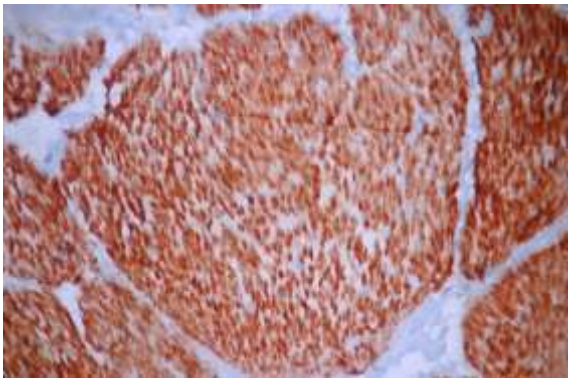

Figure 3D: Muscularis Propria exhibiting score 3+ (strong) Smoothelin positivity(40x)

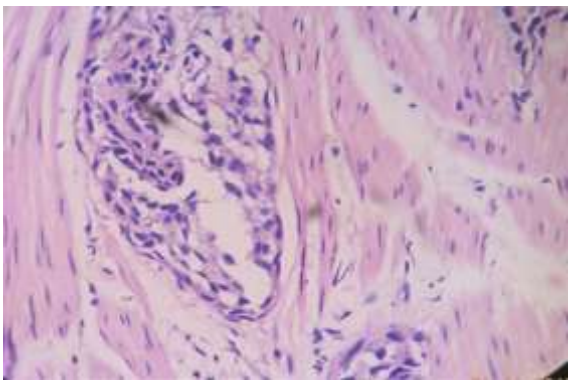

Figure 4A: Infiltrating urothelial carcinoma with Muscularis Mucosae invasion (H&E, 40x).

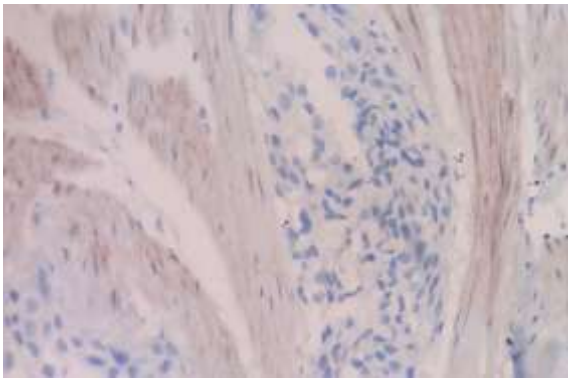

Figure 4B: Corresponding section showing Muscularis Mucosae invasion with 1+ Smoothelin staining(40x)

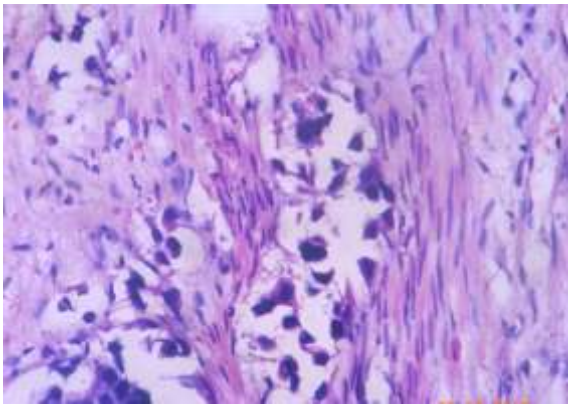

Figure 5A: Infiltrating urothelial carcinoma with Muscularis Propria invasion (H&E,40x).

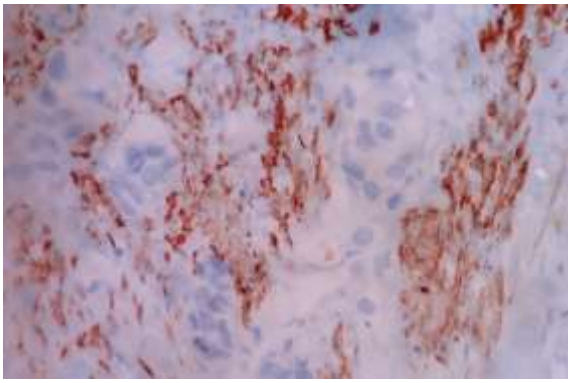

Figure 5B: Muscularis Propria showing strong Smoothelin positivity (3+) in areas of invasion (40x).

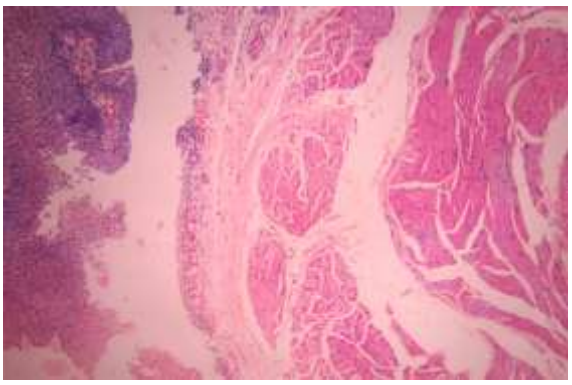

Figure 6A: Papillary urothelial carcinoma (low grade) with hyperplastic Muscularis Mucosae (H&E, 4x).

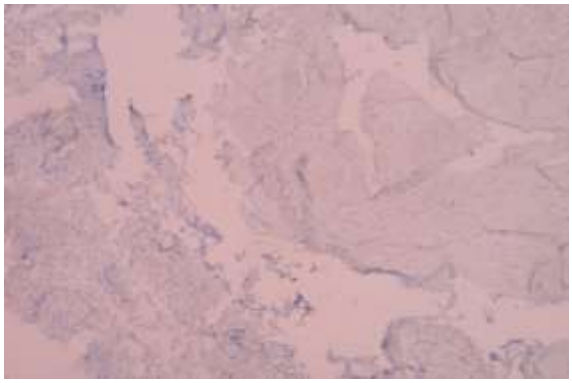

Figure 6B: Corresponding section showing absence of Smoothelin staining in hyperplastic Muscularis Mucosae (4x)

DISCUSSION

Bladder carcinoma is a heterogeneous malignancy exhibiting diverse morphological patterns and biological behavior. Among the various prognostic determinants, tumour stage remains the most critical predictor of patient outcome. Accurate pathological staging depends on precise assessment of tumour invasion depth within the bladder wall. The MP serves as a vital landmark, demarcating lesions suitable for conservative management from those necessitating radical surgical or chemotherapeutic intervention. Hence, correct identification and differentiation of MP from MM is of paramount clinical relevance.[6]

Although H&E staining provides adequate diagnostic clarity in most cystectomy specimens, TURBT samples often present diagnostic difficulties. Fragmentation, cautery artefacts, and the absence of deeper muscle bundles can obscure the interface between MM and MP[7]. Such ambiguity risks understaging or overstaging, which directly affects treatment planning, surveillance intensity, and patient prognosis.

To overcome these limitations, Smoothelin, a smooth muscle–specific cytoskeletal protein, has been recognized as a valuable adjunctive biomarker. Smoothelin is expressed exclusively in fully differentiated smooth muscle cells, demonstrating strong, diffuse cytoplasmic staining in MP and weak

or absent reactivity in MM.[8] This contrast in staining intensity offers a dependable means of distinguishing the two layers, particularly when morphological criteria alone are inconclusive.

In the present analysis of 74 TURBT specimens, Smoothelin expression was evaluated and correlated with H&E findings to determine diagnostic accuracy. The study population displayed a marked male predominance (63 males, 11 females), consistent with the global epidemiological pattern of bladder cancer, which exhibits a male-to-female ratio of approximately 3.5:1 and increasing incidence with advancing age.[9] The majority of cases belonged to the 61–70-year age group.

Histopathological evaluation revealed papillary urothelial carcinoma (low grade) as the most frequent subtype (45 cases), followed by infiltrating urothelial carcinoma (14 cases), papillary urothelial carcinoma (high grade) (8 cases), adenocarcinoma (4 cases), and squamous cell carcinoma (3 cases). Muscle tissue was present in 30 of 74 specimens, in accordance with the recommendation by Furuse et al., who emphasized that all TURBT specimens should ideally include muscle from the tumour base for accurate staging.[10]

Of the 30 muscle-containing cases, 20 demonstrated MM, 7 demonstrated MP, and 3 were equivocal on routine H&E. Following Smoothelin IHC, all MM cases exhibited absent (score 0) or weak (1+) staining, while MP cases showed moderate (2+) to strong (3+) staining, confirming the histologic interpretation. These findings corroborate those of Paner et al., Bovio et al., and Refaiy et al., who reported similar discriminatory staining patterns.[8]

One case initially interpreted as MP on H&E was subsequently reclassified as MM following Smoothelin negativity (score 0), likely representing hyperplastic muscularis mucosae. Prior studies have demonstrated that invasion of the lamina propria can induce hypertrophy of MM fibres, leading to histologic mimicry of MP.[5] Hansel et al. observed that hyperplastic MM within bladder diverticula displayed absent or weak Smoothelin staining in 67.5% and moderate staining in 32.5% of cases, findings that support the present results.[11]

Among the three equivocal cases, Smoothelin IHC allowed reclassification of two as MM (score 0) and one as MP (score 3+). In the 14 muscle-invasive cases, H&E revealed 5 MM, 6 MP, and 3 equivocal invasions. Smoothelin IHC demonstrated complete concordance with these findings, with MP-invasive cases showing moderate to strong positivity (five 3+, one 2+) and MM-invasive cases showing absent or weak staining (four 0, one 1+).

Half of the muscle-invasive cases (7/14) were categorized as pT1, while the remaining 7 cases were pT2. The overall concordance rate between Smoothelin IHC and H&E evaluation in differentiating MM and MP was 96%, comparable to the 97% concordance reported by Paner et al.[8]

The diagnostic performance of Smoothelin was found to be excellent. For MM identification, sensitivity, specificity, PPV, and NPV were 95%, 80%, 90%, and 89%, respectively. For MP detection, the corresponding values were 100%, 95%, 87%, and 100%. These results closely align with Paner et al,[8] who reported a sensitivity of 98%, specificity of 95%, PPV of 98%, and NPV of 71%, and with Refaiy et al.[6], who demonstrated 93.9% sensitivity and 100% specificity in cystectomy specimens.

CONCLUSION

In the present study, a total of 74 cases of urothelial carcinoma were analyzed, comprising 45 cases of papillary urothelial carcinoma (low grade), 14 cases of infiltrating urothelial carcinoma, and the remaining cases including papillary urothelial carcinoma (high grade), adenocarcinoma, and squamous cell carcinoma. The disease predominantly affected males in the 61–70 years age group, consistent with the established epidemiological pattern of bladder carcinoma.

Smoothelin, a robust and specific smooth muscle marker, proved to be a valuable adjunct to routine light microscopy in the evaluation of muscle invasion in bladder cancer. It demonstrated differential staining properties, with MM showing absent or weak staining, and MP exhibiting moderate to strong positivity. This clear staining distinction allowed for accurate identification of the muscle layer involved and, consequently, more precise tumour staging.

A single case of hyperplastic MM was observed, which histologically mimicked MP on H&E but was correctly identified as MM on Smoothelin IHC. Of the three equivocal cases identified on H&E, two were reclassified as MM and one as MP following Smoothelin IHC evaluation.

Among the 14 cases of infiltrating urothelial carcinoma, 7 showed invasion into MM and 7 into MP, corresponding to pT1 and pT2 stages, respectively. A concordance rate of 96% was achieved between H&E microscopy and Smoothelin IHC, highlighting the high diagnostic agreement between the two methods.

Overall, Smoothelin demonstrated high sensitivity and specificity in distinguishing MM from MP, thereby playing a crucial role in accurate pathological staging of bladder carcinoma. Nevertheless, the study's conclusions are limited by the modest sample size, and larger studies are warranted to validate these findings and to further explore potential diagnostic pitfalls associated with Smoothelin IHC.

Finally, it is emphasized that biopsy samples from the tumour bed or muscle layer should be obtained in all TURBT procedures to ensure the inclusion of muscularis propria, which is essential for accurate assessment of invasion depth and staging.

REFERENCES

1. Greene FL, Page DL, Fleming DI, et al., eds. AJCC Cancer Staging Manual. 6th ed. New York: Springer-Verlag; 2002.

2. Krämer J, Aguirre-Arteta AM, Thiel C, et al. A novel isoform of the smooth muscle cell differentiation marker smoothelin. J Mol Med.1999;77:294–298
3. van der Loop FT, Schaart G, Timmer ED, et al. Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. J Cell Biol.1996;134:401–411.
4. Lindh C, Nilsson R, Lindstrom MA, Lundin L, Elmberger G. Intensity of smoothelin immunohistochemical staining in bladder muscularis mucosae is strongly dependent on antigen retrieval—positive staining is not a reliable marker for muscularis propria [abstract]. WashingtonDC: USCAP; 2010.
5. Paner GP, Brown JG, Lapetino S, Nese N, Gupta R, Shen SS, et al. Diagnostic use of antibody to smoothelin in the recognition of muscularis propria in transurethral resection of urinary bladder tumor(TURBT) specimens.Am.J.Surg.Pathol.2010;34:792-799
6. Refaiy A, Muhammad E, ElGanainy E. Semiquantitative smoothelin expression in detection of muscle invasion in transurethral resection and cystectomy specimens in cases of urinary bladder carcinoma.African journal of urology.2011;17(1):6-10.
7. Bovio IM, Al-Quran SZ, Drew PA, et al. Smoothelin expression is a useful adjunct for assessing muscularis propria invasion in bladder cancer. Mod Pathol. 2009;22:160A
8. Paner GP, Shen SS, Lapetino S, Venkataraman G, Barkan GA, Quek ML, et al. Diagnostic utility of antibody to smoothelin in the distinction of muscularis propria from muscularis mucosae of the urinary bladder:A potential ancillary tool in the pathologic staging of invasive urothelial carcinoma. Am.J.Surg.Pathol.2009;33(1):91-8.
9. Eble JN,Sauter G,Epstein JI,Sesterhenn IA.World Health Organisation Classification of Tumours. Pathology and Genetics of Tumours of the urinary system and Male Genital Organs.IARC Press: Lyon 2004.
10. Furuse, H. and Ozono, S. (2010),Transurethral resection of the bladder tumour (TURBT) for non-muscle invasive bladder cancer: Basic skills. International Journal of Urology, 17: 698–699.
11. Hansel D, Paner GP, Amin MB. Bladder diverticula demonstrate limited diffuse smoothelin expression within the muscularis mucosa. Mod Pathol. 2009;22:172A.