ORIGINAL ARTICLE

Proper Staging of Bladder Urothelial Carcinoma Using Smoothelin and Vimentinimmunohistochemical Stains

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ABSTRACT

Background: Urothelial carcinoma of the urinary bladder represents 90% of all primary tumors of this organ . The depth of the invasion particularly for the muscularispropria is the most important prognostic and therapeutic determinator . Therefore, differentiation between muscularispropria and muscularis mucosa is essential for the proper treatment and avoiding over staging or under staging.
Aim: To evaluate the role of smoothelin and vimentin expression in differentiation between muscularis mucosa and muscularisproperia for better assessment of invasion level and give correct staging.
Method: This study was carried out on 60 specimens of primary urothelial carcinoma .
Results: In the sixty examined specimen , muscularispropria positivity for smoothelin was found in all 60 cases, while vimentin positivity for muscularis mucosa was found in 50 cases .Three of the cases which were staged as Ta at the H&E staining were upstaged to T1 after staining with vimentin; while five of the cases which were staged as T1 at the H&E staining were upstaged to T2 after staining with smoothelin . Therefore; smoothelin and vimentin can be used in differentiation between muscularis mucosa and muscularisproperia for better assessment of invasion level and give correct staging.
Conclusions: differentiation of muscularis mucosa from muscularispropria can be made histologically by using smoothelin and vimentinimmunostaining.
Keywords: Muscularis mucosa, Vimentin, Muscularispropria , Smoothelin, Urothelial CA urinary bladder.

INTRODUCTION

Urothelial carcinoma which traditionally known as transitional cell carcinoma represent about 90% of primary urinary bladder tumors[1]. Urothelial carcinoma is one of the ten most common cancer in the Iraq and worldwide. In Iraq and according to Iraqi cancer registry 2016, it represents the sixth most common tumor; in males ,it is the fifth most common cancer whereas in females,it is the sixth most common cancer. The age of incidence usually more than 50 years with male to female ratio about 3:1[2].

According to WHO/ISUP (World Health Organization /International Society of Urological Pathology) consensus classification of urothelial neoplasm of urinary bladder 2016 ,urothelial carcinoma can be divided microscopically into infiltrative and noninvasiveurothelial neoplasms. The noninvasive papillary tumors can be graded into low grade papillary urothelial carcinoma and high grade papillary urothelial carcinoma depending on variations in architectural and cytological parameters, about 75% of newly diagnosed urothelial carcinoma are noninvasive predominately of low grade[3]. The invasion is very important as prognostic and treatment determinator especially muscularispropria invasion which occur predominately in high grade tumors and many staging systems have been used to evaluate the depth of invasion as modification of the classic Jewett scheme which separate the infiltrating carcinoma into superficial (submucosa and inner half of muscle) and deep (outer half of muscle and perivesical tissue and lymphatic) but the current staging system is AJCC[4]. The treatment depend on age, grade , stage and presence of dysplasia or carcinoma in situ elsewhere in the bladder[5].

Study purposes are evaluate the role of smoothelin and vimentin expression in differentiation between muscularis mucosa and muscularisproperia for better assessment of invasion level and give correct staging.

MATERIALS AND METHODS

This study was carried out in the Department of Pathology and Forensic Medicine, Faculty of Medicine, Babylon University, during the period from October 2018 through October 2019. The patients had been selected from laboratory of histopathology in Al-Hilla Teaching Hospital and Ibn Al-Haitham private laboratory, diagnosed as urothelial carcinoma by cystoscopy and TURBT (transurethral resection of bladder tumor). Sampling of cases include the following:

1- Control group: twelve sections ,six sections from breast (low grade ductal carcinoma in situ) as a negative control and six sections from endometrial adenocarcinoma (as a positive control) were used with the six runs of the staining.
2- Study group: The study is cross sectional retrospective study. sixty patients (50 males and 10 females) with urothelial carcinoma of the urinary bladder obtained by TURBT (transurethral resection of bladder tumors) and confirmed by Haematoxylin / Eosin stained histopathological examination were included in this study. The cases were reviewed for diagnosis by two pathologist

Smoothelin is a mouse monoclonal antibody , which is intended for the laboratory use in identification of smoothelin protein byusing immunohistochemistry in formalin-fixed paraffin-embedded human tissue .Clinical interpretation for the presence of a staining or its absence

must be complemented by morphological study byusing proper controls and should be evaluated within the context of the patient's clinical history and diagnostic tests by a qualified pathologist.

Smoothelin expression is exclusively found in the smooth muscle cells .Cells with smooth muscle cells-like characteristics, for example: myofibroblasts, myoepithelial cells, skeletal and cardiac muscle dont contain Smoothelin. Smoothelin expression is only found in fully differentiated smooth muscle cells such as human detrusor muscle . Also found in the human muscularispropria[6,7].

Vimentin is the most widely distributed intermediate filament. Vimentinis expressed in all the mesenchymal tissues[8]. The studies had shown that smooth muscle cells of the muscularispropria rarely and weakly express vimentinwhile smooth muscle cells in the muscularis mucosa show moderate to strong staining pattern[9].

Statistical study was applying program SPSS version 21, and Categorical variables were presented as frequencies and percentages. Pearson's chi square (X2) was applied for significant difference. A P value of <0.05 was reflected as significant.

RESULTS

sixty patients were studied, 50(83.3%) males and 10 females (16.7%). Muscularispropria showed strong to moderate cytoplasmic immunoreactivity for smoothlin, while the muscularis mucosa showed strong cytoplasmic immunoreactivity for vimentin.

Three of the cases which were staged as Ta at the H&E staining were upstaged to T1 after staining with vimentin; while five of the cases which were staged as T1 at the H&E staining were upstaged to T2 after staining with smoothelin as shown in table (IV.VI).

Table I: Distribution of urothelial carcinoma cases according to different clinicopathologic parameters and H&E staining (age, gender, tumor grade and stage) .

Clinicopathologic parameters		**Urothelial carcinoma cases**
Age	Less than 19 yrs.	1(1.7%)
	20 – 39 yrs.	0
	40 – 59 yrs.	4(6.7%)
	60 – 79 yrs.	50(83.3%)
	More than 80 yrs.	5(8.6%)
Gender	Male	50(83.3%)
	Female	10(16.7%)
Tumor grade	Low	38(63.3%)
	High	22(36.7%)
Stage	Ta	7(11.7%)
	T1	37(61.7%)
	T2	16(26.7%)
Microscopic morphology	Papillary	44(73.3%)
	Conventional infiltrating	10(14.3%)
	Infiltrating with divergent differentiation	6(10%)

Table II: Smoothelinimmunoreactivity and sensitivity in muscularispropria cells at variable H – score cutoff values (10).

H-score cutoff	No. of urothelial carcinoma cases positive for smoothelin	smoothelin sensitivity
Any	60/60	100%
> 10	60/60	100%
> 50	60/60	100%
> 100	60/60	100%
> 150	58/60	97.1%
> 200	57/60	95.7%
> 250	55/60	92.9%

Table III: vimentinimmunoreactivity and sensitivity in muscularis mucosa cells at variable H – score cutoff values.

H-score cutoff	No. of urothelial carcinoma cases positive for vimentin	vimentin sensitivity
Any	50/60	83.3%
> 10	50/60	83.3%
> 50	50/60	83.3%
> 100	48/60	80%
> 150	47/60	78.3%
> 200	45/60	75%
> 250	45/60	75%

Table IV: Smoothelinimmunoreactivity in muscularis mucosa and muscularispropria.

Vimentin expression	**MM(n=60)**	**MP (n=60)**
Negative	55(91.7%)	60(0%)
Positive	5(8.3%)	0(100%)
Intensity Negative/mild	10(100%)	0(98.6%)
Moderate/strong	**0**	60(1.4%)

P value (0.02) <0.05 Significant

Table V: Vimentinimmunoreactivity in muscularis mucosa and muscularispropria.

Vimentin expression	**MM(n=60)**	**MP (n=60)**
Negative	10(16.7%)	60(100%)
Positive	50(83.3%)	0
Intensity Negative/mild	10(16.7%)	0
Moderate/strong	50(83.3%)	0

P value is < (0.001) Significant < 0.05

Table VI: staging of urothelial carcinoma after immunohistochemical staining by vimentin and smoothelin according to AJCC\TNM staging system of urothelial carcinoma of urinary bladder.

Stage after immunohistochemical staining	**n**	**%age**
Ta	4	6.7
T1	35	58.3
T2	21	35

Figure (1) :A: Section of the urinary bladder shows low grade papillary urothelial carcinoma by H&E (10X).B: Sections of the urinary bladder shows high grade papillary urothelial carcinoma(black arrow) by H&E (40X).C: Sections of the urinary bladder shows lamina propria(red arrow) invasion by tumor cells(black arrow) by H&E (10x).D: Sections of the urinary bladder shows muscularispropria(blue arrow) invasion by urothelial carcinoma (black arrow)by H&E (10X).

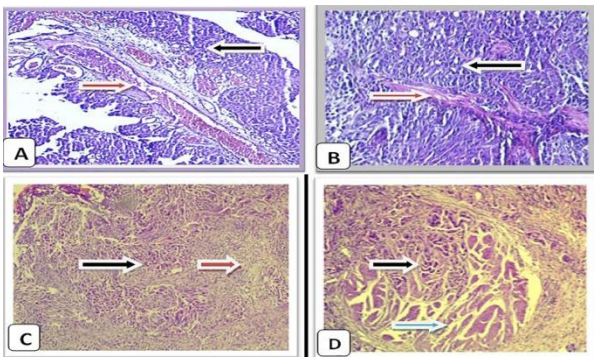


Figure 2: A: Section of the urinary bladder shows lamina propria (black arrow) positive for vimentin without invasion by cancer cells (red arrow)(4X). B: Section of the urinary bladder shows lamina propria (black arrow)positive for vimentin with positive invasion by cancer cells(red arrow) (10X). C: sections of the urinary bladder shows low grade papillary urothelial carcinoma (red arrow) with positive vimentin expression in the lamina proria (black arrow) and cancer cells; which may indicate epithelial mesenchymal transformation (40X). D: Sections of the urinary bladder shows high grade urothelial carcinoma (red arrow) with positive vimentin expression in the tumor cells ; which may indicate epithelial mesenchymal transformation (40X)

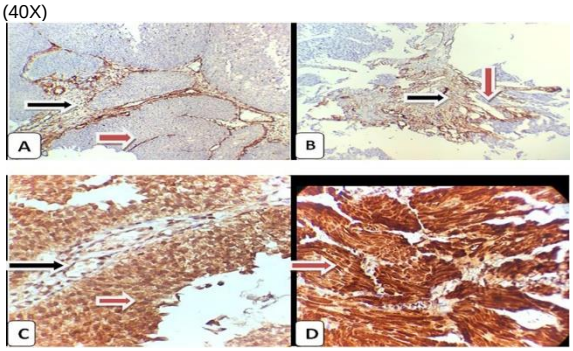
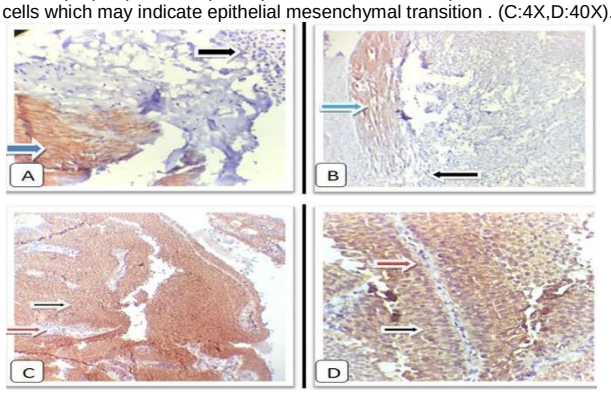


Figure 3: (A)Section of the urinary bladder shows muscularispropria (blue arrow) which is positive for smoothelin without invasion by cancer cells (black arrow)(10X). (B) Section of the urinary bladder which is positive for smoothelin (blue arrow) with invasion by cancer cells (black arrow)(4X). (C)&(D) Sections of the urinary bladder shows low grade papillary urothelial carcinoma(black arrow) that shows negative smoothelin expression in the lamina propria(red arrow) and positive smoothelin expression in the tumor cells which may indicate epithelial mesenchymal transition . (C:4X,D:40X).



DISCUSSION

This study shows that the differential expression of immunohistochemical markers appears to be very useful in distinguishing between the smooth muscle cells (SMC) of the muscularis mucosa and those of the muscularispropria when smoothelin and vimentin especially used in combination.

Independently the sensitivity of each IHC marker was calculated. Sensitivity of smoothelin for muscularispropria was 100% while Pvalue was (0.001). As expected the smoothelin positivity for muscularis mucosa was very low. Only 5/60 (8.3%) cases showed mild positivity, thereby making smoothelin negativity for muscularis mucosa (MM) a very good tool. This confirmed our hypothesis of using smoothelin for exclusively staining the actively contractile MP and not MM.

The expression of the smoothelin marker in smooth muscle cells of the bladder was first detected by Maake et al. He found that the gene and the protein (which is detected by RT–PCR and immunofluorescence) were expressed by the smooth muscle cells of muscularispropria in (8) normal and (13) overactive bladders (7). Then, Kuijpers et al had found that protein expression was confined to the muscularispropria (MP) and has not been detected in muscularis mucosae in eight cystectomy specimens[11]. These findings were recently confirmed in a large study by Paner et al who found that smooth muscle cells of all the (32) evaluated sections of muscularispropria (MP)showed moderate or strong smoothelin expression in contrast to smooth muscle cells (SMC) of the muscularis mucosae , where moderate expression was seen in (2) of (25) specimens of the hyperplastic muscularis mucosae and not in (32) specimens of the conventional muscularis mucosae[12].
Vimentin was used in this study to accurately rule out muscularispropria. The results showed sensitivity of 83.3% positivity for MM layer , while vimentin expression was negative in the muscularispropria in all sixty cases. Pvalue was (0.001). Similar to our study we found a work by Kuijpers et al , in which smooth muscle cells (SMC) of the muscularispropria from all the eight cystectomy specimens which were evaluated by immunofluorescence has been found to be negative for vimentin expression[11]. These findings appear to substantiate our findings in that there is a marked difference in vimentin expression between smooth muscle cells (SMC) of the muscularis mucosae (MM) and smooth muscle cells(SMC) of the muscularispropria (MP).

Another study done by Noha Elkady et al in Egypt in which 59 cases were studied, the study revealed that the Intensity of smoothelin expression showed a significant difference (P value = 0.001) between the muscularismucosa (MM) and the muscularispropria (MP) with a sensitivity of (97.5%). Vimentin was found to be negative in the muscularispropria but showed positive expression in (32) cases (80%) of muscularis mucosa with a statistical significant difference (Pvalue=0.001) providing a sensitivity of (80%). Combined esults of moderate to strong smoothelin and negative vimentin offered a sensitivity of (100%) and a specificity (100%)towards the identification of muscalarispropria (MP)[13].

Only 16 of these 60 cases (26.7%) showed muscle invasion by H&E. While on doing IHC; amongst the 44 noninvasive tumors there were 5 cases which were diagnosed previously as noninvasive , were proven invasive based on IHC by smoothelin. This can be explained by small propria bundles that were missed by us

on the HE stain of the TURBT specimens. These 5 cases were up-staged after IHC. Also, 7 cases out of the 60 cases (11.7%) were staged as Ta by H&E staining. on doing immunohistochemistry with vimentin; three out of the seven cases were found to be invasive for the muscularis mucosa and were up-staged to T1. This supports the importance of carrying out IHC in all TURBT specimens for accurate staging.

Commonest error done by histopathologist is to mistake muscularis mucosa as muscularispropria in disoriented TURBT biopsies. On multiple occasions the MM can have a mild hyperplasia mimicking it as muscularispropria. Tangential sections and effects of cautery further bewilder the pathologists. This falsely changes the stage of the urothelial carcinoma from T1 to T2. This has a direct bearing on the treatment protocol, as BCG instillation is used in T1 tumors, whereas cystectomy is the treatment of choice in T2 carcinomas bladder[14,15,16].

We also found positive staining of the tumor cells by both smoothelin (in 4/60 cases (6.7%) and vimentin (in 5/60 cases 8.3%). this staining included both low grade and high grade urothelial carcinoma which may indicate epithelial mesenchymal transition. This is supported by a research made by Paliwalet al who demonstrated statistically significant association of (cytokeratin, E-cadherin and vimentin) with stage and grade of the bladder cancer. and since these markers form part of the spectrum of changes associated with epithelial mesenchymal transformation[17], the study establishes proof of concept of the existence of this process. This study supports only positivity for vimentin in high grade urothelial carcinoma. While there was no comparative study for positivity of vimentin in low grade urothelial carcinoma or for smoothelin in both high grade and low grade urothelial carcinoma but we mentioned it as a positive findings in our research which may indicate that positive staining does not depend only on the grade of tumor cells but also depend on derangement of the function or kinetics of neoplastic cells.

CONCLUSION

1. Differential expression ofimmunohistochemicalmarkers can be used todistinguish the smooth muscle cells of the muscularis mucosa andthe smooth muscle(MM) cells of the muscularispropria(MP).
2. The proposed immunohistochemical markers can help the pathologist in accurately labeling an invasive bladder carcinoma. The extent of invasion into muscularispropria will decide the treatment protocol.
3. Finally, a caution should be taken in that any number of immunohistochemically stained sections can only supplement andshouldnot replace the morphological findings on haematoxylin and eosin-stained sections.

RECOMMENDATIONS

1. Requesting for IHC as a routine or at any questionable level of tumor invasion.
2. Thinking of IHC before reporting a case of urothelial carcinoma as noninvasive.
3. Future research applying different panels to differentiate fibrous and smooth muscle to pick up the best results for staging urothelial carcinoma.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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REFERENCES

1. 1-Humphrey PA.Urinary bladder pathology: an update.AnnDiagn Pathol.2004;8:380-389.PMID:15614746 DOI: 10.1053/j.anndiagpath.2004.08.012
2. 2-Ammin MH, Alsaed SJ, Alsaraj M. Iraqi cancer registry center. Baghdad-Iraq. 2011;9-11.
3. 3- Peter A. Humphrey , HolgerMoch , Antonio L. Cubilla , Thomas M.Ulbright, Victor E. Reuter. The 2016 WHO Classification of Tumours of the Urinary System and male Genital Organs—Part B: Prostate and Bladder Tumours. European association of Urology. 2016;70(1):106-119.DOI: https://doi.org/10.1016/j.eururo.2016.02.028.
4. 4- L Cheng , A L Weaver, B C Leibovich, D M Ramnani, R M Neumann, B G Scherer,et al. Predicting the survivalof bladder carcinoma patients treated withradical cystectomy. Cancer. 2000; 88(10):2326-32. PMID: 10820355 DOI: 10.1002/(sici)1097-0142(20000515)88:10<2326::aid-cncr17>3.0.co;2-t.
5. 5- Ghoneim MA, Abol-Enein H. Management ofmuscle-invasive bladder cancer: an update. NatClinPract Urol. 2008; 5(9): 501–508.PMID: 18769377 DOI: 10.1038/ncpuro1202.
6. 6- Gladell P Paner, Steven S Shen, Shawn Lapetino, GirishVenkataraman,Güliz A Barkan, Marcus L Quek, et al. Diagnostic utility of antibody to smoothelin in the distinction of muscularispropria from muscularis mucosae of the urinary bladder: a potential ancillary tool in the pathologic staging of invasive urothelial carcinoma. Am J SurgPathol. 2009 Jan;33(1):91-8.PMID: 18936687 DOI: 10.1097/PAS.0b013e3181804727
7. 7- C Maake , M Landman, X Wang, D M Schmid, U Ziegler, H John. Expression of smoothelin in the normal and the overactive human bladder. J Urol. 2006 Mar; 175(3 Pt 1):1152-7. PMID: 16469643 DOI: 10.1016/S0022-5347(05)00315-0
8. 8-Lane EB, Hogan BLM, Kurkinen M, Garrels JI. Co-expression of vimentin and cytokeratins in parietal endoderm cells of early mouse embryo. Nature. 1983;303(5919):701-704. PMID: 6190091 DOI: 10.1038/303701a0
9. 9- Council L, Hameed O. Differential expression of immunohistochemical markers in bladder smooth muscle and myofibroblasts, and the potential utility of desmin, smoothelin, and vimentin in staging of bladder carcinoma. Mod Pathol. 2009;22(5):639-650.PMID: 19252475 DOI: 10.1038/modpathol.2009.9
10. 10-Hironori Ishibashi 1, Takashi Suzuki, Satoshi Suzuki, Takuya Moriya, Chika Kaneko, Touichirou Takizawa, et al. Sex steroid hormone receptors in human thymoma. J ClinEndocrinolMetab. 2003;88(5):2309-17.https://doi.org/10.1210/jc.2002-021353.
11. 11- Kuijpers KA, Heesakkers JP, Jansen CF, Schalken JA. Cadherin-11 is expressed in detrusor smooth muscle cells and myofibroblasts of normal human bladder. Eur Urol. 2007; 52: 1213–22.https://doi.org/10.1016/j.eururo.2007.01.052
12. 12-Gladell P Paner 1, Jeffrey G Brown, Shawn Lapetino, NalanNese, Ruta Gupta, Steven S Shen, et al . Diagnostic use of antibody to smoothelin in the recognition of

muscularispropria in transurethral resection of urinary bladder tumor (TURBT) specimens.Am J SurgPathol . 01 Jun 2010; 34(6):792-799 DOI: 10.1097/pas.0b013e3181da7650 PMID: 20421781

13. 13-Noha Elkady, AsmaaGaberAbdou, Mona Kandil, NohaGhanem. Diagnostic value of smoothelin and vimentin in differentiating muscularispropria from muscularis mucosa of bladder carcinoma. Int J Biol Markers. 2017; 32(3): e305-e312. PMID: 28218359 DOI: 10.5301/jbm.5000252
14. 14- Chang BS, Kim HL, Yang XJ, Steinberg G D. Correlation between biopsy and radical cystectomy in assessing grade and depth of invasion in bladder urothelial carcinoma. Urology. 2001;57:1063–1066; discussion 1066–1067. doi: 10.1016/s0090-4295(01)00998-0
15. 15- Lopez-Beltran A, Cheng L. Stage pT1 bladder carcinoma: diagnostic criteria, pitfalls and prognostic significance. Pathology. 2003;35 (6):484–491. https://doi.org/10.1080/00313020310001619127
16. 16- Abdullah Aydin , RamazanUçak, MetinKarakök, MuhammedEminGüldür, NazimEmrahKoçer. Vascular plexus is a differentation criterion for muscularis mucosa from muscularispropria in small biopsies and transurethral resection materials of urinary bladder? IntUrolNephrol. 2002;34:315–319. PMID: 12899220 DOI: 10.1023/a:1024493518202
17. 17-Paliwal P , Arora D, Mishra AK. Epithelial mesenchymal transition in urothelialcarcinoma:Twist in the tale. India . Indian J PatholMicrobiol.Oct-Dec 2012 ; 55(4):443-9. PMID: 23455777 DOI: 10.4103/0377-4929.107777