

## The Role of Desmin in Assessing and Staging of Urothelial Carcinoma

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### ABSTRACT:

#### BACKGROUND:

Pathological stage of urothelial carcinoma is the most important factor in determining the prognosis and the mode of therapy. Desmin antibody is used to stage urothelial carcinoma by distinguishing the muscular invasion from desmoplasia and differentiating muscularis mucosa from muscularis propria invasion.

#### AIM OF THE STUDY:

To evaluate the usefulness of Desmin expression in differentiation muscularis mucosa from propria invasion and assess muscular invasion in urothelial carcinoma.

#### MATERIAL AND METHODS:

A retrospective study was done on 40 cases with urothelial carcinoma from the histopathology department of Teaching Hospitals and private laboratories in Mosul city, starting in January 2018 till July 2020. Their mean age was 62.1 years. All cases stained by desmin antibody.

#### RESULTS:

It was found that the high-grade Urothelial carcinoma is predominant, which is composed of 80%. Desmin can distinguish the muscularis mucosa from propria by at least one level of difference in the strength of the staining intensity.

#### CONCLUSION:

Desmin marker helps assess the stage of urothelial carcinoma by the differentiation between muscular layer of the bladder wall.

**KEYWORDS:** Urothelial carcinoma, Muscularis mucosa, Muscularis propria, Desmin.

### INTRODUCTION:

Urothelial carcinoma (UC) is one of the important causes of cancer-related morbidity and mortality.<sup>(1)</sup> which is about 6.32% in Iraq.<sup>(2)</sup> It is comprising approximately 90% of all primary cancer of the bladder.<sup>(3)</sup> Cigarette smoking considers the most common risk factor associated with UC.<sup>(4,5)</sup>

The pathological stage is the most important factor that determines the prognosis and the mode of therapy of the UC.<sup>6,7</sup> The carcinoma without basement membrane invasion was staged as pTa (noninvasive papillary urothelial carcinoma) and pTis (carcinoma in situ). While with lamina propria and muscularis mucosal (MM) invasion by the tumor is staged as pT1, and muscularis propria (MP) invasion is staged as pT2. Stage pT3 is given for perivesical soft tissue extension.<sup>(8)</sup>

The orientation of MM and MP is considering a big challenge in the histopathological study, as in desmoplastic reaction,<sup>(9)</sup> hypertrophy of MM<sup>10</sup> and splitting of MP by the tumor cells.<sup>1</sup> So for proper assessing the muscular invasion, immunohistochemical (IHC) marker (desmin antibody) for highlighting the MM and MP was used in this study.

Desmin is a protein, weighted 52 kDa, which is composed of 470 amino acids. It is firstly described in 1977,<sup>(11)</sup> and encoded by the DES gene,<sup>(12)</sup> It is considered a muscle-specific type III intermediate filament essential for the proper muscular structure and mechanical function. It is present in smooth, striated and cardiac muscle and plays a crucial role in providing elasticity and strength for the muscle fiber during its activity.<sup>(11)</sup> Immunohistochemistry for desmin can be used to distinguish muscles from desmoplasia and to highlight muscle contours for distinguishing MM from MP.<sup>(9)</sup>

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### MATERIALS AND METHODS:

This retrospective study of 200 blocks of formalin-fixed, paraffin-embedded tissue was retrieved from the histopathology department of Teaching Hospitals and private laboratories in Mosul city during the period from January 2018 to July 2020. Included 40 cases that undergone TURBT specimen containing muscular layer and diagnosed as UC. And excluded 160 cases that underwent cystectomy or TURBT specimen but did not contain muscular layer, and exclude cases with bladder tumors other than UC. All of the patients are newly diagnosed, and the clinicopathological data, such as age at the time of diagnosis, gender, pathological stage, grades and histological diagnosis, were obtained from the medical reports. Each case was already fixed in 10% formalin, processed, embedded in paraffin wax, cut at four-micron thickness and stained by hematoxylin and eosin (H&E). Stained sections were reviewed for determining the histological type and tumor grade for invasive UC according to the World Health Organization (WHO) classification of bladder tumors 2016 and compared with previous reports. Then 40 cases (paraffin blocks) with identified muscles (MM and/or MP) by H&E stained sections, were selected from 200 cases and included in the study, then stained by Desmin antibody. The expressional intensity of desmin marker was graded as follows<sup>1</sup>:

- 0 = negative stain.
- 1+ = weak stain.
- 2+ = moderate stain.
- 3+ = strong stain.

The statistical studies (frequencies, relative percentages, means, standard deviations and significant correlations with Fisher's Exact test) were performed using the statistical package for social sciences (SPSS) program of version 26.

Values were considered statistically significant when P value  $\leq 0.05$ .

### RESULTS:

In this study, 40 cases of UC were included. Their ages ranged from 36 to 80 years with mean ages $\pm$ SD equal to (62.1 $\pm$ 12.068) years. The peak age distribution has been seen mostly in the sixth decade, which is composed of 18 (45%) cases. The male was the predominant gender (Male: Female=19:1), which is equal to 38 (95%), while the female was just 2 (5%) of cases.

The high-grade UC (HGUC) was 32 (80%) cases, while the low-grade UC (LGUC) was 8 (20%) cases. The grades of UC correlate significantly (P-value <0.001) with the ages of the patient, which means that the grade is higher in the elderly patient, where 26 (65%) cases with HGUC distributed through the interval from 61 to 80 years. As shown in figure (1).

In the evaluation of muscular layers by H&E stain, the MM was found in 34 (85%) cases, and 30 (75%) cases were containing MP. The same number for both MM and MP were detected when IHC (desmin) marker had been used. And when correlate between both stains for muscular layers, the result was not significant (P value=1.0). While the myofibroblast (MF) was detected in 10 (25%) of cases by H&E stain, it shows negative desmin expression, so the correlation between the MF and MP for both stains was significant, p-value < 0.003). as shown in table (1).

To differentiate between the MM and MP, the intensity strength of desmin staining was used as illustrated in table (2) which revealed that the MM was mostly showed weak staining (+1) in 32/34 (80%) cases and just 2/34 (5%) was moderately stained, as shown in Photo (1 B & C) respectively. while out of 30 MP detected cases from 40 studied cases, 20 (66.66%) were strongly stained and 10 (33.33%) were moderately stained, as shown in Photo (1 E & F) respectively. There is at least one level of intensity different between muscular layers, as shown in Photo (2).

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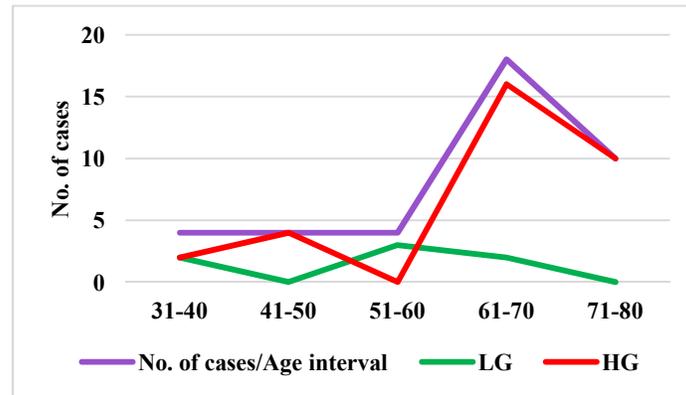


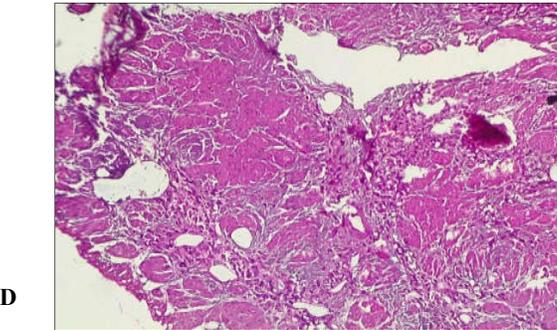
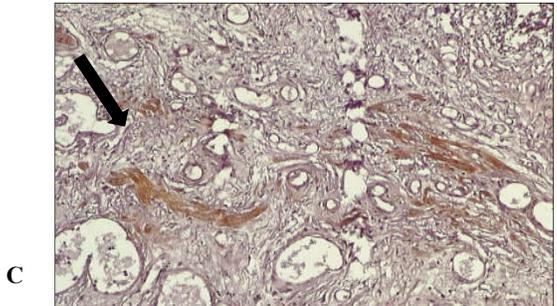
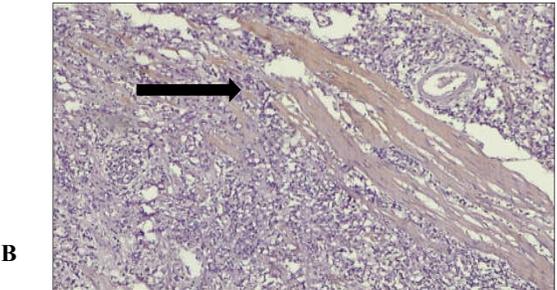
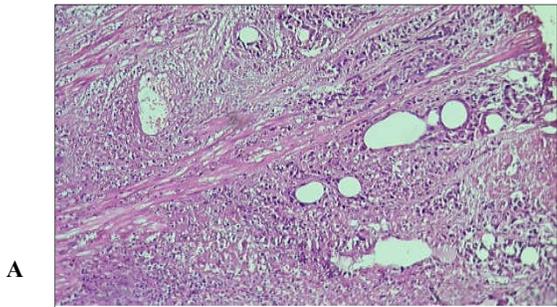
Figure 1: Age distribution in relation to the grades of UC.

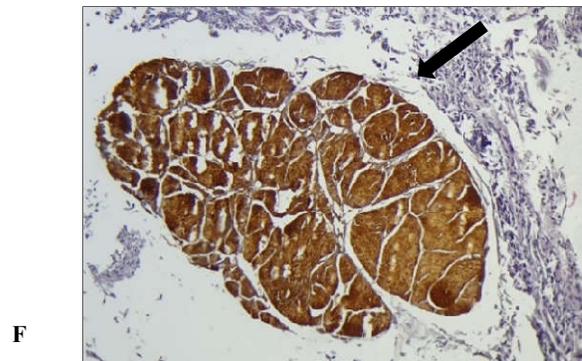
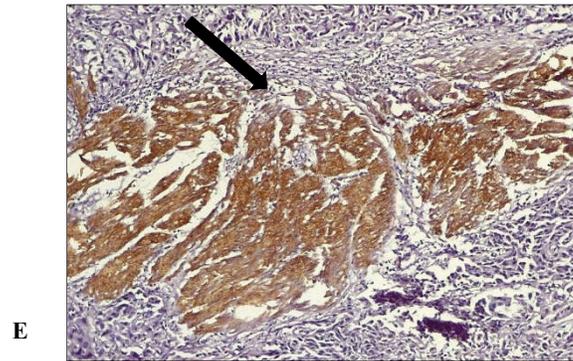
Table 1: Identification of muscular layer (MM & MP) and MF of desmoplasia by routine (H&E) and IHC (desmin) stains and correlation between them.

Stain type	H&E (n=40)		Desmin (n=40)		P value
	Present	Absent	Present	Absent	
Cell type					
MM	34 (85%)	6 (15%)	34 (85%)	6 (15%)	1.0
MP	30 (75%)	10 (25%)	30 (75%)	10 (25%)	
MF	10 (25%)	30 (75%)	0 (0.0%)	40 (100%)	0.003

Table 2: Expressional intensity of desmin IHC marker in the muscular layers (MM & MP).

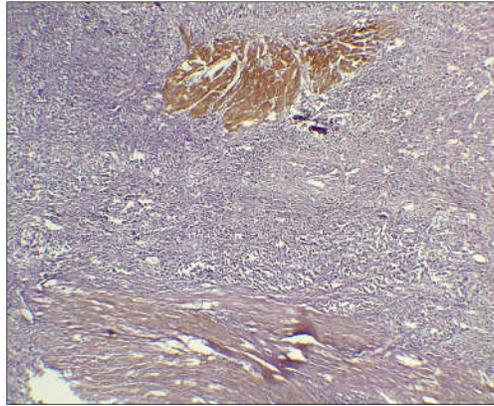
Muscle layer	Desmin staining intensity	Desmin staining intensity			
		0 Negative	+1 Weak	+2 Moderate	+3 Strong
MM	(n=34)	0 (0.0%)	32 (94.11%)	2 (5.88%)	0 (0.0%)
MP	(n=30)	0 (0.0%)	0 (0.0%)	10 (33.33%)	20 (66.66%)
MF	(n=10)	10 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)





**Photo1: Invasive UC of a different cases, shows MM which appears as a thin, interrupted bundles stained by H&E stain in**

- (A) stained weakly by desmin stain in**
- (B) and moderately in**
- (C) Thick bundles of MP invade by neoplastic nests stained by H&E stain in**
- (D) stained moderately by desmin stain in**
- (E) and strongly in**
- (F) (100X).**



**Photo 2: Invasive UC in same case shows MM and MP stained by desmin in different intensity, as the MM stained weakly, while the MP appears moderately (40X).**

### **DISCUSSION:**

Urothelial Carcinoma (UC) in the worldwide comprises the most common type of bladder cancer.<sup>(12)</sup>

In present study, the age distribution of UC is mostly in the interval (61-70) years old, which is constitute 45%. This result is not compatible with Elkady N et al, in which they found that UC is mostly distributed within age interval (45-65) years.<sup>(7)</sup> The difference in the age of detection of UC could be related to the different pathogenesis of UC in Egypt, as parasitic infections play a role in its formation. As well as it could be due to the presence of an early detection program for UC in Egypt, that not present in our locality leading to the delay in the diagnosis of UC.

Regarding the sex distribution in this study, it was found that the male to female ratio is equal to (19:1). When the male is the predominant gender and constitutes 95%. When compared with Elkady N et al, the male is also predominant and equal to 66.1%, but the male to female ratio is also not compatible, which is about 2:1,<sup>(7)</sup> the explanation for this could be that the main cause in Egypt for UC is schistosomiasis; which affects the peasant class in agricultural areas, and the workers there are usually of both genders, so the ratio was almost equal. Saha K et al also found the male is the predominant sex distribution of UC which was equal to 81.1%, and this is a compatible result to this study.<sup>(1)</sup>

About the grades of UC, the results of this study show that 20% of cases were LG, while HG was 80% of cases. A similar result shown in Elkady et

al study as the number of HGUC cases was higher than the LG, when the former was constituted 27.1%, while it was 11.9% in the latter.<sup>7</sup> The study of Li W et al found the HG cases was 45% while the LG cases were 49.2%, which is not compatible with the current results.<sup>(13)</sup> The difference in grade type predominance could be related to the inclusion of the UC of low malignant potential in their grades statistics.

When doing a correlation between the ages of the patients in this study and the grades of the UC, it was found that the correlation is significant (P-value <0.001) and the HG appears in a higher number of cases and older ages, where it was 65% and distributed through the group age from the 61 to 80 years old.

In this study founding the use of desmin stain is important for differentiation between desmoplastic reaction and muscular layers, in which the correlation between them was significant (P-value = 0.003).

The role of desmin stain in the current study is to highlight and reinforce the presence of muscular layers found by H&E, in which the result was appear equal to H&E stained sections, whereas the MM appears in 85% of cases and MP in 75% cases.

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On the other hand, the usage of desmin stain proved its worth in distinguishing the muscular layers (MM from MP) by the difference in the strength of the staining intensity. When 80% of cases containing MM showed (+1) weak staining and 66.66% of cases containing MP were stained (+3) strongly. Founding that there is at least one level of intensity difference between MM and MP layers, and this is the intrinsic benefit of using desmin IHC marker for differentiating between MM and MP and for determining the exact pathological stage of UC.

Parallel result found in Saha K et al study about the discrepancy in the intensity of desmin staining between MM and MP, where they found the MM stained weakly by desmin in 91.9% and MP stained strongly (+3) in 97.3%, and also in all cases of their study the MM and MP staining intensity different at least by one level.<sup>1</sup> Poletajew S et al show nearly similar results in their study, whereas the MM stained weakly in 25% and moderately in 50% of cases, while the MP stained strongly in 60% and moderately in 40% of cases.<sup>(14)</sup>

Another result found in Saha et al and Poletajew S et al studies about a negative expression of desmin when evaluating MM, which was 1% in former and 25% in later,<sup>1,14</sup> and this is compatible with the current study if the reason for that related to the MF of desmoplasia, whereas the whole 100% cases with desmoplasia in this study showed negative staining.

### CONCLUSION:

Desmin marker is helpful in the differentiation between muscular layer of the bladder wall, as it gave a mild to moderate intensity stain for MM, and a moderate to strong intensity stain for MP. In the same case the difference in intensity between MM and MP at least by one level.

### REFERENCES:

1. Saha K, Saha A, Datta C, Chatterjee U, Ray S, Bera M. Does desmin immunohistochemistry have a role in assessing stage of urothelial carcinoma in transurethral resection of bladder tumor specimens?. *Clin Cancer Investig J*. 2014;3:502-7.
2. Iraqi Cancer Board. Results of the Iraqi Cancer Registry 2018. Baghdad, Iraq, Iraqi Cancer Registry Center, Ministry of Health. 2018.
3. Benhayoune K, Tahiri L, Mellas S, Tazi F, Khallouk A, El-Fassi J, et al. Histoprognostic factors in bladder cancer: a case series of 156 patients. *Arch Can Res*. 2018;6:13.
4. Lammers RJ, Witjes WP, Hendricksen K, Caris CT, Janzing-Pastors MH, Witjes JA. Smoking status is a risk factor for recurrence after transurethral resection of non-muscle-invasive bladder cancer. *Eur Urol*. 2011;60:713-20.
5. Polesel J, Bosetti C, di Maso M, Montella M, Libra M, Garbeglio A, et al. Duration and intensity of tobacco smoking and the risk of papillary and non-papillary transitional cell carcinoma of the bladder. *Cancer Causes Control*. 2014;25:1151-58.
6. Wang G, McKenney JK. Urinary bladder pathology: World Health Organization Classification and American Joint Committee on cancer staging update. *Arch Pathol Lab Med*. 2019;143:571-77.
7. Comp erat E, Van der Kwast T. Pathological staging of bladder cancer. *Diagn histopathol*. 2013;19:366-75.
8. Elkady N, Abdou AG, Kandil M, Ghanem N. Diagnostic value of smoothelin and vimentin in differentiating muscularis propria from muscularis mucosa of bladder carcinoma. *Int J Biol Markers*. 2017 ;32:305-12.
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;22:639-50.
10. Vakar-Lopez F, Shen SS, Zhang S, Tamboli P, Ayala AG, Ro JY. Muscularis mucosae of the urinary bladder revisited with emphasis on its hyperplastic patterns: a study of a large series of cystectomy specimens. *Ann Diagn Pathol*. 2007;11:395-401.
11. Costa ML, Escalera R, Cataldo A, Oliveira F, Mermelstein CS. Desmin: molecular interactions and putative functions of the muscle intermediate filament protein. *Braz J Med Biol Res*. 2004;37:1819-30.
12. Magers MJ, Lopez-Beltran A, Montironi R, Williamson SR, Kaimakliotis HZ, Cheng L. Staging of bladder cancer. *Histopathology*. 2019;74:112-34.
13. Li W, Liu M, Feng Y, Huang YF, Xu YF, Che JP, et al. High expression of Notch ligand Jagged2 is associated with the metastasis and recurrence in urothelial carcinoma of bladder. *Int J Clin Exp Pathol*. 2013 Oct 15;6:2430-40.
14. Poletajew S, Wilczek E, Wasutyński A, Górnicka B. Antigenic profile of muscularis mucosae and muscularis propria of the urinary bladder. *Iran J Immunol*. 2015;12:50-63.