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ORIGINAL ARTICLE

Immunohistochemical Expression of NECTIN-4 in Urothelial Carcinoma of the Urinary Bladder

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ABSTRACT

Keyword: Urothelial carcinoma, IHC, Nectin-4.

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Background: Urothelial bladder carcinoma is the most frequent histopathological type of urinary bladder cancer. Immunohistochemistry (IHC), as a diagnostic or prognostic method, could be helpful particularly in the differentiation among muscle invasive and non-muscle invasive urothelial carcinoma (MIUC, NMIUC) as it has completely different implication on clinical management of the patients. Nectin-4 has act as a cancer associated inducer in a number of tumor types including urothelial carcinoma (UC). **Objectives**: This study aimed to assess Nectin-4 expression in invasive and non-invasive urothelial carcinoma and its association with the available clinico-pathological parameters. **Methodology:** This retrospective cohort observational research has been performed on 60 cases of UC to detect the IHC expression of Nectin-4. **Results:** Nectin-4 showed elevated expression in UC of the urinary bladder carcinoma (78.3%). A non-significant upward association was observed between Nectin-4 expression and muscle invasive UC cases. Nectin-4 was higher expressed in larger cancers, high grade and stage UCs, but there was no significant difference. **Conclusions:** These findings suggest an important function of Nectin-4 in predicting the prognosis in UC of the urinary bladder.

INTRODUCTION:

Urothelial bladder carcinoma represents the prevalent histopathological type of bladder tumors, exhibiting peak frequency during the seventh decade of life. The ratio of squamous cell carcinoma to urothelial carcinoma has altered characterized by a decline in squamous cell carcinoma and a rise in urothelial carcinoma (1, 2). smoking is the most frequent risk factor, involved in nearly fifty percent of analyzed urothelial bladder tumors cases. Occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons constitutes additional significant risk factors, whereas the influence of diet and environmental pollution remains less clear (3).

Tumor antigens are found on the surface of neoplastic cells and act as possible drug targets. One of these antigens is tumor-associated Nectin-4, a constituent of the Nectin family within the immunoglobulin superfamily (4).

The Nectin cell adhesion molecule (Nectin) family comprises Ca2+-independent immunoglobulin-like cellular adhesion molecules (nectins 1-4), which included cell adhesion

through homophilic and heterophilic interactions. The Nectin family significantly contributes to the enhancement of cellular viability and movement. Unlike the enrichment of nectins 1-3 in normal tissues, Nectin-4, a protein associated to the poliovirus receptor (pvrl-4), is predominantly elevated in embryonic and placental tissues, exhibiting significantly reduced expression in adult. It is significantly expressed and acts as a tumor-associated inducer across many types of tumors. include lung, urothelial, breast, pancreatic, colorectal, and ovarian carcinomas (5, 6).

Previous studies have revealed that Nectin-4 is widely expressed in urothelial carcinoma (4, 7), however, the relation of this expression to clinicopathological factors is not clear. This investigation aimed to analyze the IHC expression of Nectin-4 in urothelial carcinoma and to detect its association to the available clinicopathological factors related to prognosis of this tumor e.g., tumor size, type, grade, pathological (p) stage.

MATERIAL AND METHOD:

This retrospective cohort observational research has been conducted on 60 formalin-fixed paraffin embedded tissue blocks related to 60 cases of UC obtained from the archived material of Pathology Lab, Aswan University Hospitals in the period from January 2018 to December 2021.

Transurethral resection biopsy (TURB) represent 34/60 cases, while the other 26/60 were radical cystectomy which were selected as half of them (13 cases) were NMIUC and the other half (13 cases) were MIUCs.

The research has been performed following expression from the Ethical Committee of Aswan University Egypt (EC Ref NO.: Asw.Uni / 629 / 5 / 22).

Histological examination:

Tissue blocks were utilized to produce (4µm)-thick tissue slices that were hematoxylin and eosin (H&E) stained. Slides have been examined using a light microscope and the histological diagnosis, tumor grade and pathological stage was conducted in accordance with The WHO 2004/2016 classification system (8).

Immunohistochemical staining:

Four micrometer (4µm)-thick sections have been prepared and mounted on pre-labelled poly-L-lysine coated slides. Immunostaining was done as shown in the product data sheet.

Positive control: Positive control slides from tonsillar tissue were included in each staining session. **Negative controls**: Additional tissue sections were stained simultaneously, but without utilizing the main antibody.

Immunohistochemical detection and scoring of Nectin-4:

Tissue slices were analyzed histologically using a bright-field microscope. The immunoreaction was deemed positive when a brownish cytoplasmic and membranous staining has been detcted. The level of Nectin-4 expression has been assessed utilizing a semi-quantitative technique. A histological scoring technique (H-score), which is a composite measure, takes into account both the proportion of positive cells and the intensity of their staining. The ultimate score varied from 0 and 300. The samples have been categorized depending on a discriminatory threshold. Specimens with scores ranging from 0 to 14, 15 to 99, 100 to 199, in addition 200 to 300 were categorized as having negative, weak, moderate, and high positive, correspondingly (8).

Statistical analysis

The gathered data has been revised, coded, tabulated, and statistically analyzed utilizing the Statistical Package for Social Sciences (SPSS 25), utilizing appropriate analyses based on the nature of the data for each parameter.

Range and Mean ± Standard deviation (± SD) for parametric numerical data, whereas Interquartile range (IQR) and Median for non-parametric numerical data, percentage and frequency of non-numerical data.

RESULTS:

The study was conducted on 60 patients. The mean age of the studied population was 59.78 (± 10.83) years. More than half (63.3%) were males and 36.7% were females. Regarding to 34/60 of cases were smokers, 31 of them were males and only 3 were females. There were 26/60 radical cystectomy cases with tumor size ranging from 3.5cm to 6cm. Most patients had high grade lesion (58.3%). Urothelial carcinoma with squamous differentiation was found in 6/60 (10%) cases **(Table 1).**

The mean Nectin-4 expression for the studied cases was 168.15 (± 104.38) and it was ranged from 2 to 299. More than two thirds of cases 47/60 (78.3%) had positive score "> 15". Most Nectin-4 positive cases (32/47; 68.1%) had strong Nectin-4 score followed by moderate Nectin-4 expression in 10/47 (21.3%) of cases and weak Nectin-4 expression was found in the rest of cases (5/47; 10.6%) as shown in **Table (2).**

Regarding Nectin-4 H score in radical cystectomy cases (MIUC and NMIUC groups), positive score was found in 10/13 of MIUC cases and only 7/13 of NMIUC cases showed positive score.

There was a statistical insignificant variance in expression of Nectin-4 when comparing MIUC and NMIUC groups. Among positive Nectin-4 level there was a statistical insignificant variance in H score among both examined groups **(Table 3).**

There was a statistical insignificant variance in expression of Nectin-4 regarding tumor size, grade and stage **(Table 4).**

There was positive correlation between smoking and cancer grade, and between smoking and Nectin-4 expression. There was significant increase in tumor grade in smokers. There was a statistical insignificant variance in expression of Nectin-4 between smokers and non-smokers' patients (**Table 5).**

Table 1: Clinico-pathological data of the examined cases

Studied parameter	**Results**
Age • Age range	 37-87
Sex • Male • Female	 38/60 (63.3%) 22/60 (36.7%)
Smoking	34/60 (56.7%)
Histological variant • Muscle-invasive • Non-muscle invasive	 13/26 (50%) 13/26 (50%)
Tumor grade • Low grade • High grade	 25/60 (41.7%) 35/60 (58.3%)
Tumor stage • pT1	 34 (56.7%)

• pT2 • pTa	13 (21.7%) 11(18.3%)
Other histological features UCs with squamous differentiation	6/60 (10%)

Table 2: Nectin-4 (H score) in the studied cases

Nectin-4 (H score)		**Results**
H score • Mean ± SD • Median • Range		168.15 ± 104.38 216.5 (67.5 – 255.5) (2 - 299)
H score	Negative	13/60 (21.7%)
	Positive	47/60 (78.3%)
Positive H score (N= 47 "Positive cases")	Weak (15 – 99)	5/47 (10.6%)
	Moderate (100 – 199)	10/47 (21.3%)
	Strong (200 – 300)	32/47 (68.1%)

Table 3: Nectin-4 (H score) in the two studied groups

Nectin-4 (H score)		No. of cases	H&E		Test of significance	
			Muscle Invasive	Non-muscle invasive		
			N (%)	N (%)	p-Value	Sig.
H score Median			177 (17 - 238)	87 (10 - 198)	0.356(M)	NS
H score	Negative	9	3 (23.1%)	6 (46.2%)	0.411(F)	NS
	Positive	17	10 (76.9%)	7 (53.8%)		
Positive H score (N= 17)	Weak (15 – 99)	3	2 (20%)	1 (14.3%)	1.00(F)	NS
	Moderate (100 – 199)	6	3 (30%)	3 (42.9%)		
	Strong (200 – 300)	8	5 (50%)	3 (42.9%)		

(M) Mann-Whitney test of significance, (F) Fisher's Exact test of significance.

Table 4: Relation between marker expression, tumor size, grade and stage

		No. of cases	**H score**		Fisher's Exact test	
			Negative	Positive		
			N (%) Mean ± SD	N (%) Mean ± SD	p-Value	Sig.
Tumor size (Cm)			4.39 ± 0.89	4.76 ± 0.86	0.314(T)	NS
Tumor grade	Low grade	25	6 (46.2%)	19 (40.4%)	0.711(C)	NS
	High grade	35	7 (53.8%)	28 (59.6%)		
Tumor stage	PT1	34	9 (69.2%)	25 (53.2%)	0.157(F)	NS
	PT2	13	3 (23.1%)	10 (21.3%)		
	PTA	11	0 (0%)	11 (23.4%)		
	PTIS	2	1 (7.7%)	1 (2.1%)		

(T) Student-t test of significance, (C) Chi-Square test of significance, (F) Fisher's Exact test of significance.

Table 5: Relation between smoking, Nectin-4 expression & tumor grade

		Smoking		Chi-Square test	
		No	Yes		
		N (Row %)	N (Row %)	p-Value	Sig.
Tumor grade	Low grade	18 (72.0%)	7 (28.0%)	<0.001	S
	High grade	8 (22.9%)	27 (77.1%)		
H score	Negative	5 (38.5%)	8 (61.5%)	0.689	NS
	Positive	21 (44.7%)	26 (55.3%)		
H score		226.5 (70 – 256)	194 (17 – 250)	0.493	NS

NS: non significant, S: significant.

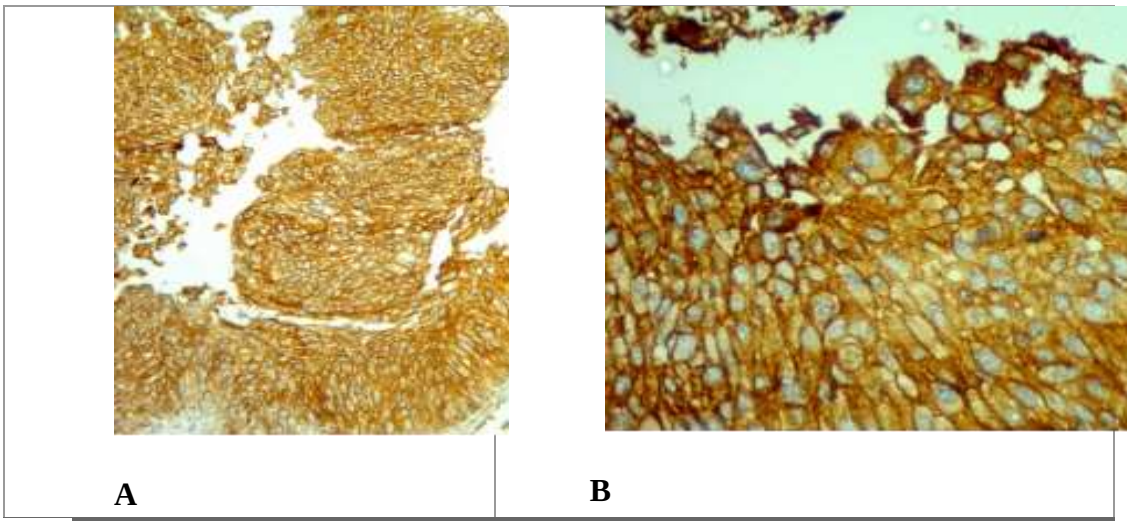

Figure 1: **A** Urothelial carcinoma showing strong Nectin-4 staining (×200)**, B** Urothelial carcinoma showing strong Nectin-4 staining (×400)**.**

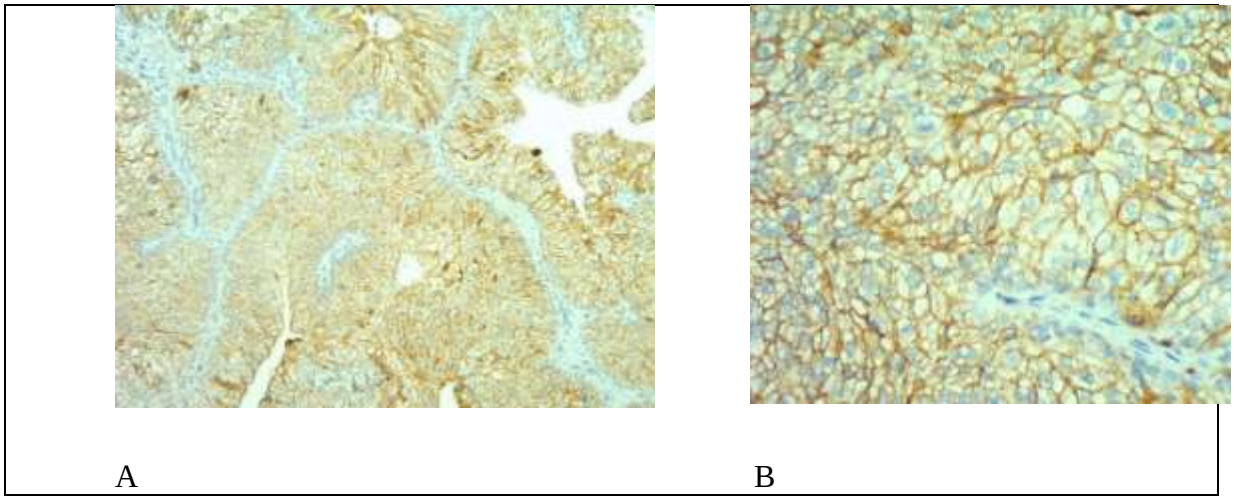

Figure 2: **A** Urothelial carcinoma showing moderate Nectin-4 staining (×200**, B** Urothelial carcinoma showing moderate Nectin-4 staining (×400)**.**

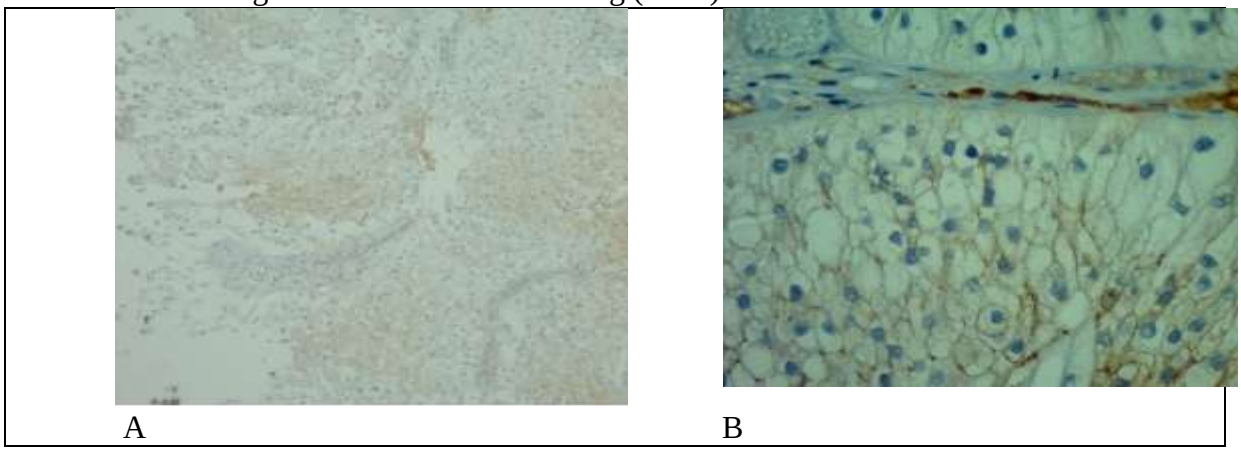

Figure 3: **A** Urothelial carcinoma showing weak Nectin-4 staining (×200)**, B** Urothelial carcinoma showing weak Nectin-4 staining (×400)**.**

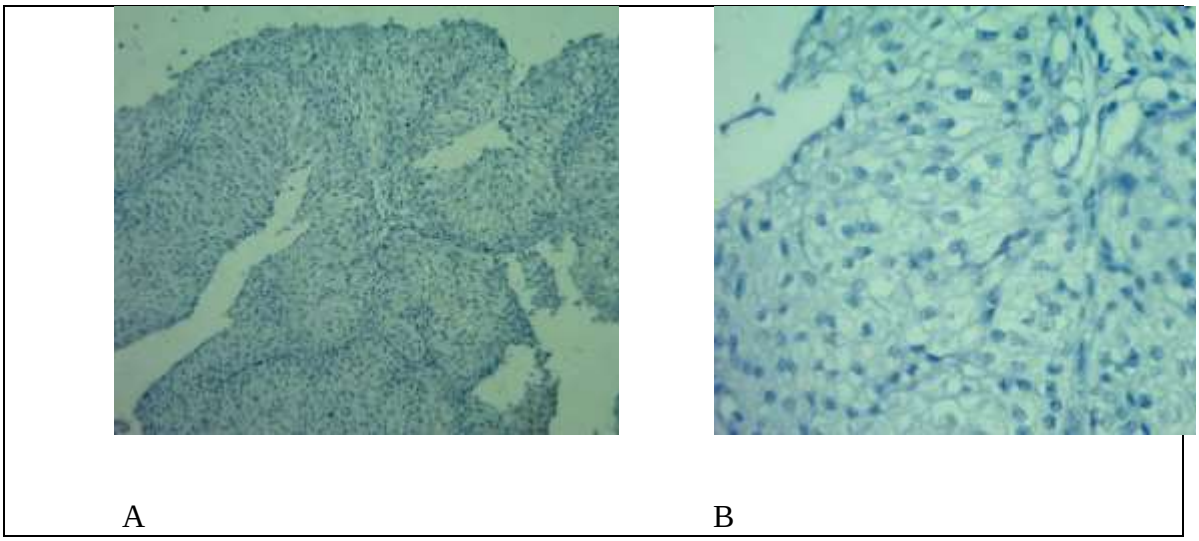

Figure 4: **A** Urothelial carcinoma showing negative Nectin-4 staining (×200)**, B** Urothelial carcinoma showing negative Nectin-4 staining (×400)**.**

Discussion

Urothelial carcinoma is an international public health problem. It ranks as the seventh most prevalent tumor globally among males and the seventeenth among females (3). Differentiation between MIUC and NMIUC by IHC could be helpful as it has completely different implication on clinical management of the patients.

In the present investigation, the age range of the examined cases was 37-87 years. The mean age of UC patients was 59.78 ± 10.83 years. 8/60 (63.3%) of cases were men and 22/60 (36.7%) were women, with male to female ratio of 1.7:1. This showed aligning with Hoffman et al's findings that the average age of UC at diagnosis was 69 years (range 42-86) and male to female ratio of bladder cancer was 3.44:1 (9). Halaseh et al found that three-quarters of bladder tumor cases happen in males (10), while Lobo et al documented that UBC is about fourfold greater in males in comparison with women in females (11).

Nectin-4 was positive in 47/60 (78.3%) and negative in 13/60 (21.7%) of cases of UC, agreeing with Challita-Eid et al's finding that 83% of bladder cancer cases showed positive Nectin-4 expression (12).

Within the 47 Nectin-4 positive cases of UC and according to H score 32/47 (68.1%) cases were strongly positive, 10/47 (21.3%) cases were moderately positive and only 5/47 (10.6%) cases were weakly positive. Rosenberg et al., found that most of his studied UC cases showed strong positive Nectin-4 expression (7).

Of the radical cystectomy cases, 17/26 showed H-score positive Nectin-4 and 9/26 showed H-score negative Nectin-4 expression. Out of the 17 positive cases, 7/17 (41%) were NMIUC.

From 26 radical cystectomy cases 13 were NMIUC cases, 7 of them (7/13, 53.8%) were Nectin-4 H-score positive. Regarding the other 13 MIUC cases, 10 of them (10/13, 77%) were Nectin-4 H-score positive. Hoffman et al., stated that 87% of NMIUC samples showed H-score positive Nectin-4 expression and 68.2% of MIUC cases showed H-score positive Nectin-4 expression (9).

Tomiyama et al., stated that 72% of NMIUC cases showed H-score positive Nectin-4 expression and only 50% of MIUC cases showed H-score positive Nectin-4 expression (13).

Regarding tumor grade, 77% of patients with high grade tumor were smokers and only 28% of patients with low grade tumors were smokers in the recent investigation. This result comes in concordance with Mohseni et al's finding that 90% of cases with high-grade tumors were smokers and only 36.1% of the cases with low-grade tumors were smokers (14).

In the current study, low grade lesions showed H-score positive Nectin-4 expression in 76% of cases, and high grade lesions showed H-score positive Nectin-4 expression in 80% of cases of UC. Tomiyama et al., stated that low grade lesions of upper tract Urothelial carcinoma (UTUC) cases showed H-score positive Nectin-4 expression in 53% of cases, and high grade lesions showed H-score positive Nectin-4 expression in 68% of cases. Both the current study and the previously mentioned study detected slightly higher H-score Nectin-4 expression in higher grades UC cases (13).Non-significant difference was found regarding the relationship between tumor stage and marker expression. H-score positive Nectin-4 expression was 100% in non-invasive papillary urothelial carcinoma (PTA), 73.5% of PT1 showed H-score positive Nectin-4 expression (papillary urothelial carcinomas invading lamina propria) and 76.9% of PT2 showed H-score positive Nectin-4 expression (muscle-invasive urothelial carcinomas). This is in accordance with Chu et al who observed lower Nectin-4 expression in pT1 compared to pT2 tumors (14).

Out of 6 UC cases with squamous differentiation, 5 cases showed H-score positive Nectin-4 expression (83.3%), 4 of them were strong positive (80%) and only one case showed H-score negative Nectin-4 expression. This is comparable with what has been stated by Hoffman et al., who observed that 70% of UC cases with squamous differentiation illustrated H-score positive Nectin-4 expression in the squamous component, 57% of them were strong positive. So the current study and previous findings indicated elevated H-score Nectin-4 expression and strong positivity in UC cases with squamous differentiation (9).

Conclusions:

There is high H-score positive Nectin-4 expression in UC of the urinary bladder. Nectin-4 is relatively increased in larger tumor size, high grade and stage UCs, but with no significant difference. These findings suggest an important function of Nectin-4 in diagnosing UCs of the urinary bladder.

Recommendations

We recommended to study Nectin-4 expression on a larger number of cases with different histological variants of UC of the urinary bladder and to make further comparative studies between Nectin-4 expression in MIUC and NMIUC.

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Conflict of Interest: Nil

Abbreviations: IHC; immunohistochemistry, MIUC; muscle invasive urothelial carcinoma, NMIUC; non-muscle invasive urothelial carcinoma, UC; urothelial carcinoma,

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