Immunohistochemical Expression of Cyclin D1 in Urothelial Carcinoma of Urinary Bladder

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ABSTRACT:

BACKGROUND:

Transitional cell carcinoma is the most common cancer in urinary bladder. Bladder carcinoma is the 5th from the most common ten cancersin Iraq, It is the 2nd in males and the 10th in females according to Iraqi Cancer Registry. It is the 4th most common cancer in inmales, 8thin females in the USA.

OBJECTIVE:

To evaluate the immunohistochemical expression of cyclin D1 in transitional cell carcinoma of urinary bladder and its role in predicting type, grade, stage, progression and prognosis of the tumor.

MATERIALS AND METHODS:

This is retrospective study of 40 bladder biopsy (formalin fixed ,paraffin embedded). Two sections of 5 µmthickness were taken from each block, the first was stained with H&E, the 2nd was stained immunohistochemically for cyclin D1.

RESULTS:

The majority of cases were high grade papillary urothelial carcinoma stage T1 .Nine of cases were score 0 of 4 , six of cases were score 1, three of cases were score 2 ,eleven of cases were score 3,eleven of cases were score 4.

CONCLUSION:

There was no statistical significant correlation between cyclinD1 immunohistochemical expression and tumor stage, grade and type.

KEYWORDS: cyclinD1 expression, Transitional cell carcinoma

INTRODUCTION:

Worldwide, bladder cancer is the 7th most common cancer in males and the 17th most common cancer in females[1] . It is the 2nd most common cancer in men and the 10th in women according to Iraqi Cancer Registry[2]. It is Twice as common in white men vs. black men[3]. it is a devastating and a leading cause of death worldwide[4]. Urothelial carcinoma is the most common type of bladder cancer and accounting approximately 90% of all primary tumors in the urinary bladder. It is range from papillary to flat, noninvasive to invasive and low grade to high grade. Low-grade carcinomas are always papillary and are rarely invasive, but they may recur after removal. High-grade cancers are also papillary but occasionally flat; they may cover larger areas of the mucosal surface, and invade deeper[5].despite important advances in cancer treatment, the disease continues to pose a big challenge to clinicians due to high recurrence rates[6]

Bladder cancer etiology involves several molecular alterations and complex biological pathways that regulate cellular processes, such as proliferation, differentiation, angiogenesis, metastasis and apoptosis[7]. Invasive urothelial carcinoma of the bladder is characterized by alterations in cell-cycle regulatory pathways, Cyclin D1 was identified as a protein that plays a crucial role in mitogen-driven cell-cycle progression[8]. Defects in the expression of cyclin D1, have been implicated in progression of various types of cancer including urothelial carcinoma of bladder. Cyclin D1 protein expression found to be higher in non-invasive tumors than in muscle-invasive urothelial carcinoma [9].

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AIM OF STUDY:

To evaluate the immunohistochemical expression of cyclin D1 in transitional cell carcinoma of bladder and its role in predicting type ,grade ,stage, progression and prognosis of the tumor.

MATERIAL AND METHODS:

This is a retrospective study ofFormalin fixed, paraffin embedded tissue blocks were collected from the Ghazi Al –Hariri specialized surgical hospital in Baghdad (including the period from November 2017to May 2018) ,this study was approved by the patients, hospital management and ministry of health.

The paraffin blocks represent 40 cases of bladder urothelial carcinoma, 36 were transurethral biopsy specimens, 3 were cup biopsy and 1 was radical cystectomy. Two sections of 5 µmthickness were taken from each block, the first was stained with hematoxylin and eosin, the other section was stained immunohistochemically for cyclin D1.

The inclusion criteria of the patients:

The patients were adult collected regardless to sex, age and all of them newly diagnosed and they were not receiving any chemotherapy.

The exclusion criteria: any patient with comorbidity (HT,D.M) was excluded, any fragmented unrepresentive tissue block was excluded.

Procedure of immunohistochemical staining:

1. **Fivemicrons** sections were obtained from formalin fixed-paraffin embedded tissue blocks and mounted on PathnSitu positively charged slides.
2. **Slidebaking**: the slides were placed in a drying oven (hot air oven) at .65C over night
3. **Deparaffinizing** and **rehydration**
4. **Antigenretrieval**: slides are placed in a plastic jar filled with 250 mL of target antigen retrieval solution citrate buffer(pH 6) .then the jar placed in microwave oven together with two other jars containing distilled water and all placed as a triangle to balances the microwaves power
5. **PAPpen**: was used to draw a circle around the tissue section .
6. **Peroxidaseblock**: after draining and carefully blotting around the specimen to remove any remaining liquid. Enough drops of Peroxidase block reagent were applied onto the tissue covering the whole section and incubated at room temperature for 5 minutes in humid chamber, after that the slides were rinsed gently with buffer for a minimum of 15 seconds. Then drained and blotted as before
7. **Primaryantibody (**cyclinD1) (PathnSitu, USA): Primary antibody (Rabbit monoclonalAB) was applied onto each section and incubated at 4 C° overnight in humid chamber, and then slides were rinsed with a stream of buffer from a washing bottle, and then Placed in a fresh buffer bath for 5 minutes. Slides were rinsed again with buffer then drained and blotted gently.
8. **Polyexceltargetbinder** (PathnSitu,USA): cover the tissue section with target binder and incubate for 10 minutes at room temperature . Then the slides were rinsed with a stream of buffer from a washing bottle, and then placed in a fresh buffer.
9. **PolyexcelpolyHRP** (PathnSitu,USA): cover the section with polly Excel poly HRP and incubate for 10 minutes at room temperature in humid chamber. Then the slides were rinsed with a stream of buffer from washing bottle, and then placed in fresh buffer bath for 5 minutes.
10. **Substrate-chromogensolution**.
11. **Hematoxylincounter** stain solution were applied covering the whole section and incubated at room temperature for 5 minutes. After that, the slides were rinsed gently with distilled water then drained and bottled.
12. **Aqueous-base** mounting medium were applied onto the tissue sections and covered with cover slips and left to dry.

The Immunohistochemical expression of cyclin D1 in malignant urothelial cells is diffuse brown intense nuclearstaining and is better evaluated by the percentage of positive tumor cells as to classify the results as[10]:

- Less than or equal to 5% was Negative
- 6-25% = 1+
- 26-50% = 2+
- 51-75% = 3+
- Over 76% = 4+.

Statistical analysis was performed with **SPSS** v18.88(Statistical package for social sciences) and also Excel 2007 programs . Data analysis was done using t-test,chi-square test for tables with frequencies ,percentages , ranges, means standard deviation and standard errors of mean. Values were considered Statistically significant when p-value is equal or less than **0.05** .

RESULTS:

The majority of patients were males 27 of 40 cases,while 13 of cases were females.

The age of patients was range from 25 to 86 years old.

Table (1): Demographic data in this study

Parameter		Value
Age (yr)	Mean±SD	59.88±12.6
	Range	25-88
Sex	Male	27 (67.5%)
	Female	13 (32.5%)

1. **Correlation of cyclin D1 expression with histological type of tumor :**
   There was **NO** stastical correlation between the histological type of urothelial carcinoma and immunohistological expression of cyclin D1 with p value 0.172

Table(2): Relation of score to histological type

Score	Papillary No. (%)	Solid No. (%)	Total	P value
0	5 (14.71%)	4(33.33%)	**9**	**0.172***
1	4 (11.77%)	2 (25.00%)	**6**	
2	3 (8.82%)	0 (0.0%)	**3**	
3	11 (32.35%)	0 (0.0%)	**11**	
4	11 (32.35%)	0 (0.0%)	**11**	
Total	**34**	**6**		

2. **correlation of cyclin D1 expression with tumor grade:**
   There was **NO** significant correlation between tumor grade and immunohistological expression of cyclin D1 with p value 0.091.

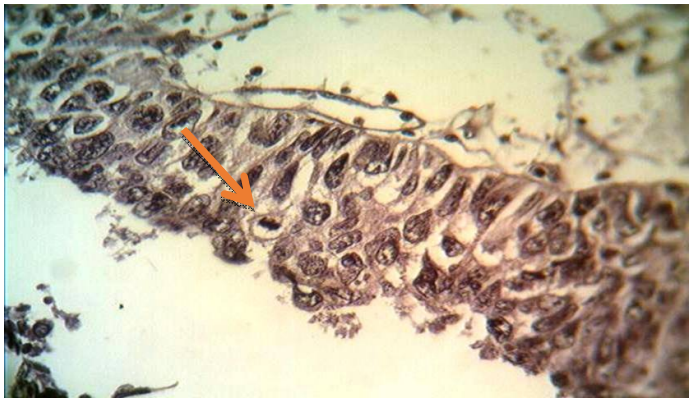
Table(3): Relation of score to histological grade

Score	Low grade No. (%)	High grade No. (%)	Total	P value
0	1 (6.25%)	8 (33.33%)	**9**	**0.091***
1	0 (0.00%)	6 (25.00%)	**6**	
2	1 (6.25%)	2 (8.33%)	**3**	
3	7 (43.75%)	4 (16.67%)	**11**	
4	7 (43.75%)	4 (16.67%)	**11**	
Total	**16**	**24**		

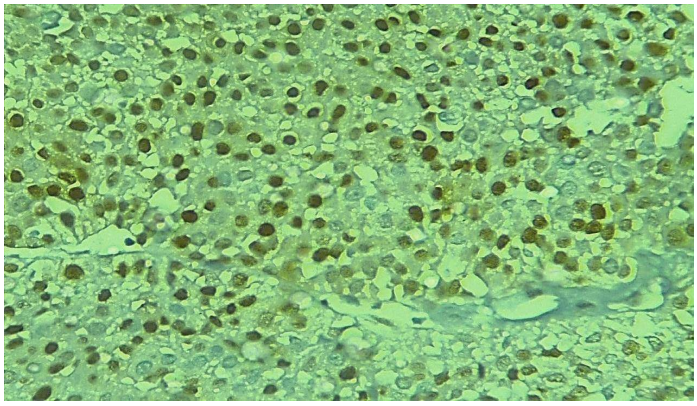
3. **correlation of cyclin D1 expression and tumor stage(pathological stage):**
   There was **NO** significant correlation between immunohistochemical expression ofcyclinD1 and tumor stagewith p value 0.125.

Table (4): Relation of score to pathological staging

Score				Stage				Total	P value
				T1	T2	T4	Ta		
0				7 (22.6%)	0 (0.0%)	2 (100%)	0 (0.0%)	**9**	**0.125***
1				2 (6.4%)	4 (66.7%)	0 (0.0%)	0 (0.0%)	**6**	
2				3 (9.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**3**	
3				11 (35.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**11**	
4	8 (25.8%)	2 (33.3%)	0 (0.0%)	1 (100%)	**11**				
Total	**31**	**6**	**2**	**1**	**40**				



Figure(1): high grade papillary urothelial carcinoma of urinary bladder showing mitotic activity (arrow)H&E(x40)



Figure(2): 1ow grade papillary urothelial carcinoma of urinary bladder showing nuclear immunostaining by cyclinD1 score +3 IHC(X40).

DISCUSSION:

1. Histological type of tumor:

There was **NO** statistical correlation between histological type of tumor and expression of cyclin D1 .

This is in disagreement with the study of Chyi chia et al who evaluated 161 specimen of TCC of urinary bladder; Positive cyclin D1 staining was observed only in papillary TCCs (111 of 161). All the nonpapillary tumors evaluated were negative and the association was statistically **significant** with p value <0.01[11].

Another study did by Liponnen PK et al also detected **significant** correlation between papillary type TCC and cyclin D1 expression in study of 178 cases of TCC with p value 0.006[12].This disagreement with the current study could be contributed to discrepancy in evaluation of immunostaining, tumor heterogeneity, number of retrospectives cases, sample size, type of antibody and antibody dilutional rate.

2. Tumor grade:

In the present study there was **NO** statistical correlation between expression of cyclin D1 and tumor grade . Shin KY et al also detected a **non-significant** correlation between tumor grade and cyclin D1 expression in study of 75 cases of TCC with p value>o.o5% [13].

another study did by Tut V M et al included 150 cases of TCC detected **significant** correlation between tumor grade and immunohistological expression of cyclin D1 with significantly higher expression in G1/G2 47% compared toG3 tumors 14% with p< 0.005[14].this disagreement with the present study may be related to the grading system:3gradesgrade1(welldifferentiated), grade2(moderatelydifferentiated),grade3 (poorly differentiated)system instead of 2 tired system

3. Tumor stage:

In the present study there was **NO** statistical correlation between the expression of cyclin D1 and tumor stage p = 0.125.This is in disagreement with the study of Chyi Chia et al who found **Positive** cyclin D1 staining was limited to Ta and T1 tumors; none of those beyond T1 were positive. Comparison of positive cyclin D1 staining between the two categories, (Ta-T1 vs. T2-T4) revealed a statistically significant difference (p <0.01) [11].This disagreement with the present study may be related to number of samples, size of samples in addition to Interobserver discrepancies in the evaluation of immunostaining of cyclin D.

CONCLUSIONS:

In conclusion There was **NO** statistical significant correlation between cyclin D1 expression and tumor grade, stage and histological type. Hence and according to the results of this study, cyclin D1 may not be a useful ancillary test to assess muscular invasion nor distinguish between different histological tumor types and grade.

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