Immunohistochemical Expression of P53 in Urothelial Bladder Lesions in a Sample of Patients

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ABSTRACT:

BACKGROUND:

TCC is the most common cancer in urinary bladder, despite important advances in cancer treatment, the disease continues to become a big challenges to the clinicians due to high recurrence rate. Invasive urothelial carcinoma is characterized by alterations in cell-cycle regulatory pathways. Altered expression of the TP53 gene has been associated with TCC.

OBJECTIVE:

To evaluate the expression of p53 in urothelial bladder lesions immunohistochemically and to correlate the immunohistochemical expression of p53 with tumor grade and histological diagnosis of lesions.

MATERIALS & METHODS:

This is a retrospective study of 50 bladder biopsy in AL Shaheed Ghazi AL Hariri hospital in Baghdad (covering the period from Jan 2018 to Feb 2019). 49 were transurethral biopsy specimens and 1 was radical cystectomy. Two sections of 5µm thickness were taken from each block, the first was stained (H&E) for histological revision, the other section was stained IHC for TP53.

RESULTS:

The patient's age was in range of 13 to 89 years with mean of 61.24 years. The highest proportion of study patients was aged > 60 years (70%).

The immunostaining of TP53 was nuclear, P53 positive in 4/10 cases (40%) of flat hyperplasia, 12/19 cases (63.2%) of low grade papillary transitional cell carcinoma and 19/21 cases (90.5%) of high grade papillary transitional cell carcinoma.

CONCLUSION:

There was statistical significant correlation between TP53 expression and urothelial bladder lesions, tumor grade and patients gender.

KEYWORDS: TP53, Transitional cell carcinoma, Immunohistochemistry.

INTRODUCTION:

In the world, the bladder cancer is the 7th most common cancer in men and the 17th most common cancer in women [(1)], In the developed world, it is the 4th and 9th most common cancer in men and women, respectively[(2)]. Bladder carcinoma is the 6th from the most common ten cancers in Iraq. It is the 2nd in males and the 10th in females according to Iraq Cancer Registry.[(3)] Urothelial carcinoma is the most frequently type of bladder cancer and accounting about 90% of all primary tumors in the urinary bladder. It is papillary to flat, noninvasive to invasive and low grade to high grade. Low-grade carcinomas are papillary and are rarely invasive, but they may recur after removal[(4)]. High-grade cancers are papillary but may be flat; consist of fused, branching and deliciated papillae with loss of polarity. Nuclear enlargement with marked pleomorphism and hyperchromeasia, multiple prominent nucleoli and atypical mitosis. They may cover large areas of the mucosal surface and invade deep[(4,5)]. Grading of TCC of urinary bladder is of great prognostic importance and significantly affects patient management. A recent classification adopted by the WHO, found arare benign papillary group of papillary urothelial tumors of low malignant potential, and two grades of carcinoma low and high[(4)]. Flat hyperplasia means thickened mucosa up to ten or more cell layers, without cytological atypia or mitosis that maintain morphological evidence of maturation from basal cells to superficial cells. It is a symptomatic, may be adjacent to low grade papillary urothelium neoplasm or as an isolated lesion[(6)].

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TP53 is tumor suppressor gene, the mutation of Tp53 represents the most common genetic alteration in tumor. The product of the gene is nuclear protein that involved in control of cell cycle, apoptosis and maintenance of genomic stability. It has an important role in the pathogenesis of TCC especially in advanced grade.

MATERIALS AND METHODS:

This is a retrospective study including Formalin fixed, paraffin embedded tissue blocks were collected from archived materials from the Ghazi Al–Hariri specialized surgical hospital in Baghdad (covering the period from Jan 2018 to Feb 2019). 40 cases of bladder urothelial carcinoma, in addition to 10 cases of urothelial hyperplasia, 49 were transurethral biopsy specimens and 1 was radical cystectomy. Pathological parameters such as histological grade of the tumors were obtained from the available histopathological reports.

Two sections of 5µm thickness were taken from each block, the first section was stained with (H&E) for histological revision and the other section was stained IHC for P53.

Evaluation of immunostaining:

Immunohistochemical expression of P53 is nuclear and is better evaluated by the percentage of positive tumor cells as:

TP53 Scoring system [7]

In this study, the percentage of Tp53-positive tumor nuclei in all major foci of tumor.

The percentage of Tp53 immunoreactive cells was scored as 0 to 3+ in positive regions. Nuclear Tp53 expression in ≥ 10% of tumor cells was scored as positive, < 10% -ve , 10%-30% +, 31%-50% ++, and > 50% +++.

More than 1000 tumor cells, in high-power fields, had been counted for assessing the percentage, the slides check more than one time to exclude any errors.

Colorectal adenocarcinoma considered as a positive control tissue in this study.

RESULTS:

The total number of study cases was 50. Papillary TCC was diagnosed in 40 cases, while flat transitional cell hyperplasia was diagnosed in 10 cases. The total positive cases were 35 cases (70%) while negative cases were 15 cases (30%).

1. Age and gender

Study patients age was ranging from 13 to 89 years with a mean of 61.24 years and a standard deviation (SD) of ± 15.9 years. The highest proportion of study patients was aged ≥ 60 years (70%).

Regarding gender, proportion of males was higher than females (84% verses (16%) respectively with a male to female ratio of 5:1.

2. Histological diagnosis of cases

There was a significant association (P= 0.041) between histological diagnosis of cases and sex and no statistical significant associations (P = 0.49) detected between histological diagnosis of cases and age.

Table 1: Association between the histological diagnosis and both of age and gender.

Variable	Histological diagnosis		Total (%) n= 50	P - Value
	TCC (%) n= 40	Hyperplasia (%) n= 10		
Age (Year)				
< 40	3 (60.0)	2 (40.0)	5 (10.0)	0.49
40 – 59	8 (80.0)	2 (20.0)	10 (20.0)	
≥ 60	29 (82.9)	6 (17.1)	35 (70.0)	
Gender				
Male	36 (85.7)	6 (14.3)	42 (84.0)	0.041
Female	4 (50.0)	4 (50.0)	8 (16.0)	

3. P53 Expression

The highest proportion of cases was shown positive for P53 (70%).

Table 2: Expression of study cases by P53.

P53 Expression	No. (n= 50)	Percentage (%)
Positive	35	70.0
Negative	15	30.0

4. Histological diagnosis and P53 expression
There was a significant association (P= 0.02) between histological diagnosis of cases and P53 expression.

Table 3: Association between histological diagnosis and P53 expression.

Histological diagnosis	P53 Expression		Total (%) n= 50	P - Value
	Positive (%) n= 35	Negative (%) n= 15		
TCC	31 (77.5)	9 (22.5)	40 (80.0)	0.02
Hyperplasia	4 (40.0)	6 (60.0)	10 (20.0)	

5. Tumor grade with P53 expression
There was a significant association (P= 0.038) between tumor grade with P53 expression.

Table 4: Association between tumor grade and P53 expression.

Tumor Grade	P53 Expression		Total (%) n= 40	P - Value
	Positive (%) n= 31	Negative (%) n= 9		
Low	12 (63.2)	7 (36.8)	19 (47.5)	0.038
High	19 (90.5)	2 (9.5)	21 (52.5)	

6. P53 expression score

6.1. According to histological diagnosis
Mean of P53 expression score was significantly higher in TCC cases than that in hyperplasia cases (1.72 versus 0.5, P= 0.003).

Table 5: Comparison between P53 expression score and histological diagnosis

P53 expression score	Histological diagnosis		P- Value
	TCC Mean ± SD	Hyperplasia Mean ± SD	
	1.72 ± 1.2	0.5 ± 0.5	0.003

6.2. According to tumor grade
Mean of P53 expression score was significantly higher in high grade tumor than that in low grade tumor (2.23 versus 1.15, P= 0.004).

Table 6: Comparison between P53 expression score by tumor grade.

P53 expression score	Tumor grade		P- Value
	High Mean ± SD	Low Mean ± SD	
	2.23 ± 1.1	1.15 ± 1.1	0.004

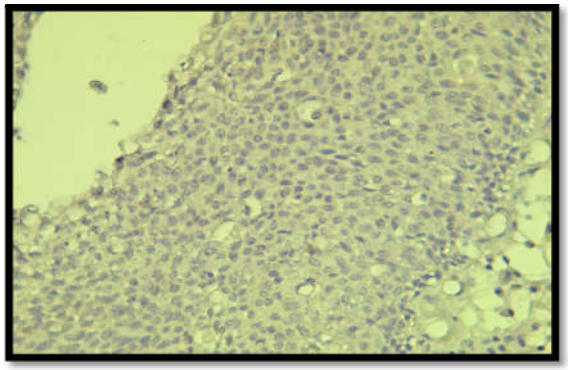

Figure 1: Photomicrograph showing flat urothelial hyperplasia with negative IHC for P53 (x400).

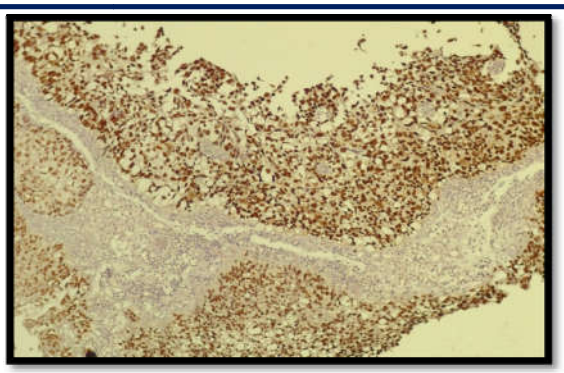

Figure 2: Photomicrograph shows high-grade invasive papillary TCC with positive nuclear expression of P53 by the malignant cells. (x100) .

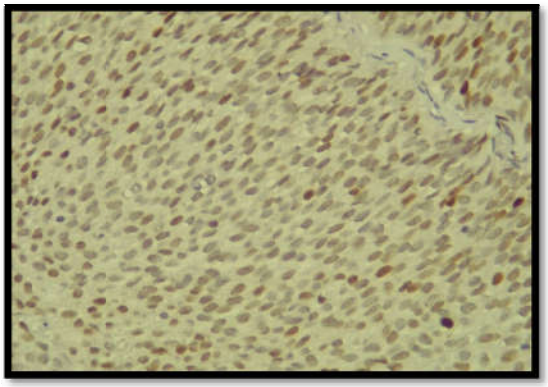

Figure 3: Photomicrograph showing low-grade papillary urothelial carcinoma showing positive weak nuclear expression of P53 by the malignant cells, score 2 (x400)

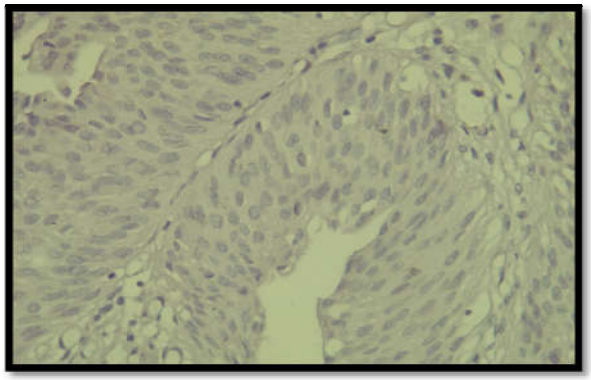

Figure 4: Photomicrograph showing low-grade papillary TCC showing negative nuclear expression of P53 by the malignant cells (x400).

DISCUSSION:

The diagnosis is based on clinical data, imaging and histological features. Transurethral and cystoscopic biopsy stained by H and E stain is the most common procedure used for the diagnosis.

In this study, there was male predominance (85.7%) diagnosed with TCC with significant association (p=0.041) between histological diagnosis and the gender. This is in agreement with studies, **Karam T. Tawfeeq, (2012)**[8] reported the same result forty-five cases (90%) males and five cases (10%) females. Also, **Shazia Mumtaz., (2014)** [9] reported 77 cases were males (81.1%) and 18 (18.9%) cases were females.

The mean age of cases was (61.24 + 15.9) years ranging from thirteen to eighty-nine years, majority of patients were above sixty years (70%).

In this study, there was no significant correlation between age with TCC and hyperplasia, this agrees with study of many investigators, **Baker Sadiq, (2012)**[10] reported the mean age for cases with TCC was (63+10.81) years.

Regarding the assessment of ten cases benign bladder lesion, (40%) of cases were positive for P53 and (60%) of cases were negative (Table 3).

The causes for positivity of P53 in four cases of flat hyperplasia:

- There were atypical cells which could be altered genetically but morphologically normal.
- Flat hyperplasia with atypia of unknown significant which means atypia between reactive and dysplasia, more pleomorphism and hyperchromasia than expected for the amount of inflammation present.
- Could be CIS.

Regarding the assessment of forty cases of papillary transitional cell carcinoma, (77.5%) were positive for P53 immunohistochemical nuclear staining and (22.5%) were negative. There was a significant correlation between histological type and expression of P53 (P value=0.02) (table 3). This finding was in agreement with many other studies worldwide reported by **Jing Du *et al.*, (2003)** [11] found that 82% of cases of bladder cancer were positive for p53 immunostaining. **Roy Chowdhury Anadi, Ranjan Kumar Dey, (2017).** [12] reported P53 positive in 80% of cases. This study disagrees with a study made by **Karam T. Tawfeeq, (2012).**[8] which reported the positivity of P53 in 34% of urothelial carcinoma of the bladder.

The comparison between P53 expression score by histological type is shown in table (5). Mean of P53 expression score was significantly higher in TCC cases than that in hyperplasia cases (1.72 versus 0.5, P= 0.003).

The immunohistochemical analysis of the results showed that P53 expression was positive in (63.16%) and negative in (36.84%) of low grade papillary transitional cell carcinoma. While in high grade transitional cell carcinoma the positivity of P53 in (90.5%) and negativity in (9.5%), it looks that the expression of P53 was increasing as the grade increased, with significant correlation between tumor grade and P53 (P value =0.038). (Table 6).

In agreement with this study, many investigators reported that p53 nuclear expression was associated with the grade of bladder cancer. **Mahmoudreza Kalantari, (2007).** [13] reported the P53 positive in (75%) of low grade papillary TCC and (85%) of high grade TCC with p=0.001.

Stephan J. Cina, Kristen J. Lancaster-Weiss (2001). [14] reported p53 positive in (64.28%) of low grade papillary TCC and (83,87 %) of high grade papillary TCC. This disagrees with the study of **Shazia Mumtaz., (2014)** [9] reported P53 positive in (36.2%) of low grade TCC and (72.9%) of high grade TCC, p=0.001 and **Karam T. Tawfeeq, (2012).**[8] reported positive P53 in (26%) of low grade TCC and (26.5%) of high grade TCC, p=0.152.

Possible causes for this variation may be:

- Number of cases.
- Differences staining procedures.
- Adopted cut off values (scoring system).

The comparison between P53 expression score by tumor grade is shown in table (6). We noticed that 36.8% of low grade cases were scored (0), while 57.1% of high grade cases were scored (3). Mean of P53 expression score was significantly higher in high grade cases than that in low grade cases (2.23 versus 1.15, P= 0.004).

CONCLUSION:

1. P53 expression is well correlated with urothelial bladder lesions, it is positive in TCC.
2. P53 expression is well correlated to the grade of bladder carcinoma.
3. P53 score increases with tumor grade.

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