

## Bcl-2 over-expression in urothelial tumors of the bladder. An immunohistochemical study

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### ABSTRACT

**Objectives:** The aim was, to evaluate the frequency of Bcl-2 over-expression in urothelial tumors of the bladder in Mosul city, to correlate the over-expression with age and sex of the patients, and grade and stage of the tumors, and to compare the results with those of others.

**Methods:** A retrospective case-series study was performed on 50 urinary bladder urothelial tumors. The samples were collected from Al-Jamhuri Teaching Hospital in Mosul city, during a period of 8 months from November 2012 through June 2013.

**Results:** The patients' age was in the range of 23-91 years with a mean of 62.64 year, male:female ratio (9:1). Approximately half of them were in the seventh decade (42%). Bcl-2 immunoreactivity was observed in 42% of the cases, it was positive in 4/11 of papillary urothelial neoplasm of low malignant potential cases, 9/23 of low grade cases, and in 8/15 of high grade cases, and was negative in the one case of papilloma. It was observed in 2/2 of Tis stage, 4/14 of cases with Ta stage, 9/21 of T1 stage, and 6/11 of T2 stage. It was negative in the 2 cases of T4.

Statistically Bcl-2 over-expression was not significantly related to the age and sex of the patients, as well as the grade, and stage of the tumors. However, it was mainly found in the 6th decade of life (42.8%), in males (90.5%), in low grade (42.9%), and in stage T1 (42.9%).

**Conclusion:** Bcl2 over-expression was found in 42% of bladder urothelial tumors. Bcl2 over-expression mainly was observed in the 6<sup>th</sup> decade of life (42.8%), in males (90.5%), in low grade carcinoma (42.9%), and stage T1 (42.9%). Age and sex of the patients, and grade, and stage of the tumors had no significant correlation with Bcl2 over-expression.

**Keywords:** Urothelial tumors, Bcl-2 over-expression, immunohistochemistry.

### فرط تعبير Bcl-2 في الأورام الظهارية للمثانة البولية، دراسة مناعية نسيجية

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#### الخلاصة

**الأهداف:** تم إجراء هذه الدراسة لتقييم حالة الظهور المناعي النسيجي الكيمائي لبروتين Bcl2 في أورام المثانة في مدينة الموصل والربط بينها وبين عمر وجنس المرضى، ودرجة التمايز ومرحلة الورم، ومقارنة هذه النتائج مع نتائج لدراسات أخرى. الحالات والطرق: إن هذه الدراسة دراسة رجعية تم من خلالها جمع 50 حالة من أورام المثانة الظهارية. تم جمع هذه الحالات من مستشفى الجمهوري التعليمي في مدينة الموصل خلال فترة 8 أشهر امتدت من شهر تشرين الثاني 2012 إلى شهر حزيران 2013.

**النتائج:** لقد تراوحت أعمار المرضى بين 23 و 91 سنة بمتوسط قدره 62,64 سنة، نسبة الذكور إلى الإناث (9:1). كان ما يقرب من نصفهم في العقد السابع (42%). ولوحظ فرط ظهور Bcl-2 في 42% من الحالات. فإنه كان إيجابيا في 4/11 من ورم الظهارة البولية الحليمية للإمكانية الخبيثة المنخفضة، 9/23 من حالات سرطان المثانة الحليمي من نوع درجة التمايز المنخفضة، وفي 8/15 من حالات لسرطان المثانة من نوع درجة التمايز العالية، وكان سلبيا في حالة واحدة من الورم الحليمي. لوحظ في

٢/٢ من المرحلة Tis، في ١٤/٤ من المرحلة Ta، ٢١/٩ من المرحلة T1، في ١١/٦ من المرحلة T2، كان سلبيا في الحالتين من T4.

إحصائيا: فرط تعبير Bcl-2 ليس له صلة مع عمر وجنس المرضى، و مرحلة ودرجة تمايز الورم. فرط تعبير Bcl-2 وجد أساسا في العقد ٦ من الحياة (٤٢,٨٪)، في الذكور (٩٠,٥٪)، في درجة التمايز المنخفضة (٤٢,٩٪)، وفي المرحلة T1 (٤٢,٩٪). **الاستنتاج:** لقد لوحظ فرط تعبير Bcl-2 في ٤٢٪ من أورام المثانة الظهارية ولوحظ بالدرجة الاولى في العقد السادس من الحياة (٤٢,٨٪)، في الذكور (٩٠,٥٪)، في درجة التمايز المنخفضة (٤٢,٩٪)، وفي المرحلة T1 (٤٢,٩٪). وقد لوحظ إحصائيا عدم وجود ارتباط بين Bcl2 وعمر وجنس المرضى، و مرحلة ودرجة تمايز الورم.

## INTRODUCTION

Urothelial bladder cancer is one of the most common cancer worldwide, with high incidence in industrialized countries.<sup>1</sup> It ranks fifth among the most common cancers in American men, approximately three quarters of all cases occur in men.<sup>1</sup> In Iraq, bladder carcinoma is recorded as the second most common carcinoma in males,<sup>1</sup> and the sixth most common in females.<sup>1</sup>

The neoplastic changes in the urothelium of bladder is a multistep phenomenon, the genetic events leading to urothelial transformation from normal to neoplastic, involve the activation of oncogenes, inactivation or loss of tumor suppressor genes, and/or alterations in the apoptotic gene products.<sup>2</sup> Loss of apoptotic response in tumor cells is thought to be one of the mechanisms involved in malignant progression and resistance to chemotherapy.<sup>2</sup>

Bcl-2, initially discovered in human B-cell lymphoma, is a proto-oncogene belongs to a family of related genes that regulates the apoptotic pathway, with Bcl-2 promoting a negative influence.<sup>3</sup> However; role of Bcl-2 protein gene expression in TCC is controversial.<sup>3</sup> Retrospective studies have demonstrated a correlation between Bcl-2 gene protein overexpression and poorer overall survival in patients with muscle-invasive disease treated with chemoradiotherapy.<sup>3</sup> Conversely, one of the largest retrospective series involving 119 patients with superficial or locally advanced disease has shown an unexpected association between Bcl-2 protein expression and favorable prognosis in muscle-invasive TCC of the bladder.<sup>3</sup>

The aim of the study was, to find out the frequency of Bcl-2 over-expression in urothelial tumors of the bladder, to correlate Bcl-2 over-expression with age and sex of the patients, and

grade and stage of the tumors, and to compare the results with those of others.

## PATIENTS AND METHODS

A retrospective study based on blocks collected from 50 cases of bladder urothelial tumor. Blocks were collected from Al-Jamhuri Teaching Hospital in Mosul city from November 2012 through June 2013.

Hematoxyline and eosin stained sections from formalin-fixed paraffin-embedded blocks were reevaluated concerning diagnosis, grading and staging of urothelial tumor according to the last WHO classification:<sup>4</sup>

- Urothelial papilloma.
- Papillary urothelial neoplasm of low malignant potential (PUNLMP).
- Papillary urothelial carcinoma, low grade.
- Papillary urothelial carcinoma, high grade.

Expression of Bcl2 protein by immunohistochemical staining was studied and compared in relation to patient's age, and sex, and grade and stage of the tumors. The biopsies were immunostained with Monoclonal Mouse Anti-Human Bcl-2 Oncoprotein Clone: 124, Isotype: IgG1Kappa. Dako Co.

Positive control slides were prepared from adenocarcinoma of colon known to be positive for Bcl2. While negative control slides were prepared from the same tissue block but incubated with tris buffered saline (TBS) instead of the primary antibody.

### Immunohistochemical staining interpretation

Immunohistochemical reaction was scored as follows: negative if  $\leq 10\%$  of cells were stained and positive if  $> 10\%$  of cells were stained. Cytoplasmic staining intensity was scored using a scale of 0 to 3 (0: no staining, 1: weak, 2: moderate, 3:

intense).<sup>5</sup> The marker was placed in one of two categories, altered or not altered (normal). Bcl-2 immunoreactivity was considered altered when samples demonstrated positivity in >10% of tumor cells with an intensity of 2 or 3. <sup>5</sup> Cleaved caspase 3 index was calculated as number of positive cells x100 per total number of cells in 10 random high-power fields (x400) in each tumor. This index was established by counting at least 2000 cells in fields a way from necrotic areas. <sup>5</sup> The sections were checked more than one time to exclude any error.

**Statistical analysis:** The relationship between Bcl2 over-expression and the some clinicopathologic variables was analyzed by the chi-square test. The results were considered statistically significant if the p-value was ≤ 0.05.

## RESULTS

The patients' age was in the range of 23 to 91 years with a mean of 62.64 year. Most of them were in the seventh decade (42%). There were 45 males (90%) and 5 females (10%) with male: female ratio (9:1).

Histologically, there were 1 case of papilloma (2%), 11cases of PUNLMP (22%), 23 cases of low grade carcinoma (46%), and 15cases of high grade carcinoma (30%). Also there were 2 cases in stage Tis (4%), 14 cases in stage Ta (28%), 21 cases in stage T1 (42%), 11 cases in stage T2 (22%), and 2 cases in stage T4 (4%).

### Bcl2 over-expression

Bcl2 immunoreactivity was observed in 21 cases (42%) of the total (Figure1).

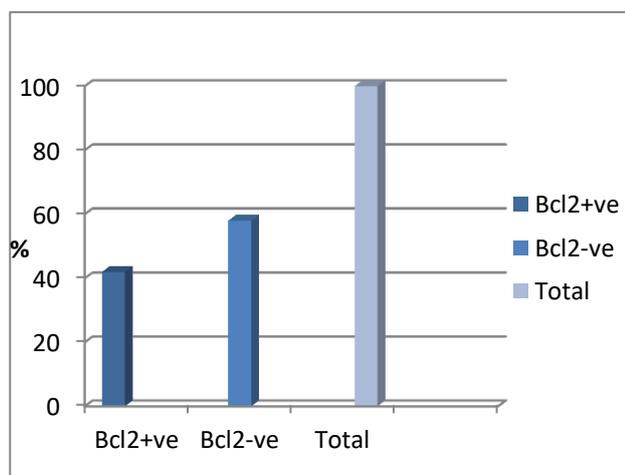


Figure 1. Bcl2 status in urothelial tumors.

In regarding to the Bcl2 over-expression and patient's age, there was no significant correlation with age with p-value of 0.488, (Table 1).

Concerning the patient's sex and Bcl-2 over-expression. There was no significant correlation with sex with p-value of 0.924, (Table 2).

Although no statistical significant correlation was identified between Bcl2 and the grade of the tumors, the higher percentage of positivity was seen in the low grade with p-value of 0.629, (Table 3), (Figures 2 and 3).

Bcl2 over-expression and stage of the tumors: no significant correlation to the stage of the tumors was found with p-value=0.202, (Table 4).

Table 1. Bcl2 over- expression and age of patient.

Age (year)	Total		Bcl2+ve		Bcl2-ve		p-value
	No.	%	No.	%	No.	%	
≤50	8	16.0	2	9.5	6	20.7	0.488
51-60	11	22.0	4	19.1	7	24.1	
61-70	21	42.0	9	42.8	12	41.4	
>70	10	20.0	6	28.6	4	13.8	
<b>Total</b>	<b>50</b>	<b>100.0</b>	<b>21</b>	<b>42.0</b>	<b>29</b>	<b>58.0</b>	

Table 2. Bcl2 over- expression and sex of patient.

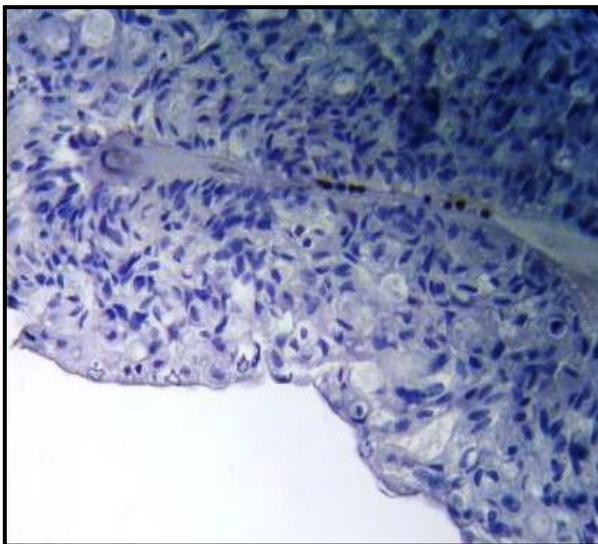
Sex	Total		Bcl2 +ve		Bcl2 -ve		p-value
	No.	%	No.	%	No.	%	
Male	45	90.0	19	90.5	26	89.7	0.924
Female	5	10.0	2	9.5	3	10.3	
<b>Total</b>	<b>50</b>	<b>100.0</b>	<b>21</b>	<b>42.0</b>	<b>29</b>	<b>58.0</b>	

Table 3. Bcl2 over-expression and grade.

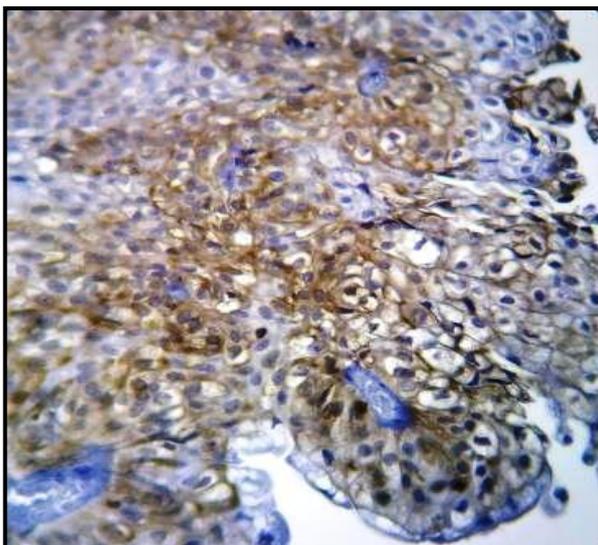
Grade	Total		Bcl2 +ve		Bcl2 -ve		p-value
	No.	%	No.	%	No.	%	
Papilloma	1	2.0	0	0.0	1	3.5	0.629
PUNLMP	11	22.0	4	19.0	7	24.1	
Low grade	23	46.0	9	42.9	14	48.3	
High grade	15	30.0	8	38.1	7	24.1	
<b>Total</b>	<b>50</b>	<b>100.0</b>	<b>21</b>	<b>42</b>	<b>29</b>	<b>58</b>	

**Table 4.** Bcl2 over-expression and stage.

Stage	Total		Bcl2 +ve		Bcl2 -ve		p-value
	No.	%	No.	%	No.	%	
Tis	2	4.0	2	9.5	0	0	0.202
Ta	14	28.0	4	19.0	10	34.5	
T1	21	42.0	9	42.9	12	41.4	
T2	11	22.0	6	28.6	5	17.2	
T3	0	0.0	0	0.0	0	0.0	
T4	2	4.0	0	0.0	2	6.9	
<b>Total</b>	<b>50</b>	<b>100.0</b>	<b>21</b>	<b>42</b>	<b>29</b>	<b>58</b>	

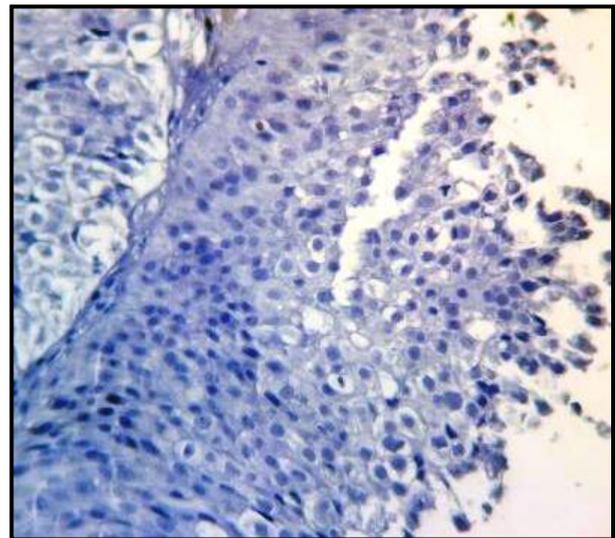


A

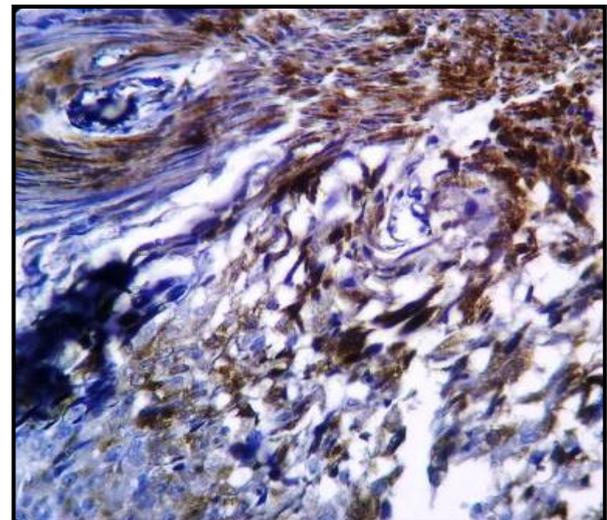


B

**Figure 2.** IHC, Low grade, (A) negative staining, (B) positive staining for Bcl2 protein (x 400).



A



B

**Figure 3.** IHC, High grade, (A) negative staining, (B) positive staining for Bcl2 protein (x 400).

**DISCUSSION**

Urothelial cancer is a common cancers Worldwide (7<sup>th</sup> and 17<sup>th</sup> among males and females respectively).<sup>6</sup> and in western Countries (4<sup>th</sup> and 9<sup>th</sup> in males and females respectively). Bladder cancer is 2<sup>nd</sup> most frequent malignancy of the urinary tract after prostate cancer.<sup>5,7</sup> Transitional cell carcinoma of the bladder accounts for the 90%–95% of urothelial cancers.<sup>6,8</sup>

Oncogenes may contribute to transformation and progression of tumors by being either overexpressed or mutated to produce an

oncoprotein.<sup>9</sup> One of the more important mechanisms by which oncogenes are overexpressed in bladder cancer is through gene amplification.<sup>9</sup> Overexpression of Bcl-2 has been reported in a wide variety of cancers including those of prostate, colorectum, lung, kidney, and bladder.<sup>10</sup> Several studies have provided conclusive evidence that over-expression of Bcl-2 causes resistance to both chemotherapy and radiotherapy and increases the proliferation of malignant cells.<sup>10</sup>

In the current study, 42% of cases with urothelial tumors showed Bcl2 over expression. Other similar studies have shown variable ratios ranging from 33.3% to 69%<sup>(11,12)</sup> as shown in **Table 5**. The reasons for this variation are unknown. However; it might be attributed to; the properties of different antibodies, the scoring methods applied for Bcl2 immunoreactivity, the enzyme and microwave treatments of the tissue, and the tissue fixation procedure, but could be real, due to genetic, demographic or environmental factors.

Patients' age was in the range of 23 to 91 years with a mean of 62.64 year. In western countries, the median age is 65 years.<sup>8</sup> One age-related reason for an increased risk of cancer may be accumulation of somatic mutations in older people.<sup>8</sup> Bcl2 over-expression was mainly found in the 6<sup>th</sup> decade of life (42.8%). Statistically, there was no significant correlation between Bcl2 overexpression and age of the patients. This is consistent with the results of other studies<sup>10,17</sup>. Bcl2 immunoreactivity was 90.5% for males and 9.5% for females.

This difference might be explained by the hormonal differences between men and women and social habits (tobacco smoking is more common in men).<sup>1,18</sup> In addition to the fact that androgen receptors have a major role in development of cancer,<sup>1,18</sup> which is much more active in men than in women.<sup>1</sup>

Statistically, there was no significant correlation between Bcl2 over-expression and sex of the patients. This is comparable to those of other studies.<sup>10,17</sup>

Bcl2 over-expression was mainly found in low grade tumors (42.9%). However, over-expression of Bcl2 in relation to grade was not significant statistically, this result is similar to that as noticed by others.<sup>10,14,15,17,19</sup> However, Abdulmir A *et al*<sup>12</sup> and Baspinar S *et al*,<sup>13</sup> reported significant correlation with high grades, this difference might be because, most of their tumors were in high grade.

These contradictory results might be due to the presence of inter- and intra-observational variations.<sup>8</sup>

The critical importance of tumor histological stage had been recognized in several studies.<sup>8</sup> Tumor growth in the presence of antiapoptotic effect of Bcl-2 is much slower than the one determined by proliferative factors.<sup>13</sup>

In this study Bcl2 over-expression was mainly found in T1 stage (42.9%). Statistically, the over-expression of Bcl2 in relation to stage was not significant confirming the observation by others.<sup>9,14,15,17</sup> On the contrary other studies had reported significant correlation with higher stages.<sup>10,12,13,20</sup>

**Table 5.** Frequency of Bcl2 over-expression in bladder urothelial tumors in different studies of the world.

Study	Year	Site	No. of cases	% of Bcl2+ve cases
Current study	2013	Mosul (Iraq)	50	42%
Baspinar S <i>et al</i> <sup>(13)</sup>	2013	Turkey	84	54%
Enache M <i>et al</i> <sup>(11)</sup>	2012	Romania	45	33.3%
WASAN <i>et al</i> <sup>(2)</sup>	2011	Baghdad (Iraq)	25	34%
Abdulmir A <i>et al</i> <sup>(12)</sup>	2009	Malaysia	82	69%
Maluf1FC <i>et al</i> <sup>(3)</sup>	2006	New York	59	37%
Matsumoto H <i>et al</i> <sup>(14)</sup>	2004	Japan	62	53%
Hani B <i>et al</i> <sup>(15)</sup>	2003	Egypt	49	61.2%
Asci R <i>et al</i> <sup>(16)</sup>	2001	UK	54	45.8%

## CONCLUSION

1. Bcl2 over-expression was found in 42% of bladder urothelial tumors, and this result is within the range observed by others.
2. Bcl2 over-expression in descending order was observed: in low grade carcinoma (42.9%), in high grade carcinoma (38.1%), and in PUNLMP grade (19%).
3. As far as histological stage, Bcl2 over-expression was in descending order; 42.9% in T1 stage, 28.6% in T2 stage, 19% in Ta stage, and 9.5% in Tis stage.
4. Age and sex of the patients, and grade, and stage of the tumors had no significant correlation with Bcl2 over-expression.

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