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ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF BCL-2 AND EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) BIOMARKERS IN UROTHELIAL CARCINOMA OF THE BLADDER LESIONS

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ABSTRACT

Introduction: *Over-expression of Bcl-2 and* ***Epidermal growth factor receptor (*** *EGFR) is seen in many malignancies. However, evidences regarding the expression of these biomarkers in bladder tumors are limited. The aim of this study was to evaluate expression of Bcl-2 and EGFR biomarkers in benign and malignant tumors of urothelial carcinoma of bladder.*

Methods: *This case control study was conducted on 57 bladder tumors (40 malignant tumors and 17 benign tumors) in Shahid Rahnemoun hospital, Yazd, Iran during 2016-2018. Immunohistochemistry method was used for assessing EGFR and bcl-2 expression in tissue tumors.*

Results: *Among all the study participants, the expressions of EGFR and bcl-2 were found to be positive in 10 (17.4%) and 25 (44%) of cases respectively. There was no significant difference between benign and malignant tumors in terms of EGFR (p=0.830) and bcl-2 expression (p=0.094). The mean EGFR in low and high grade was 4.13± 13.22 and 7.69± 26.26, respectively (p= 0.91). The mean bcl-2 in low and high grade was 3.72± 12.81 and 13.31±26.14, respectively (p=0.51). Significant difference was seen between two groups (benign and malignant groups) regarding age and tumor size (p<0.05).*

Conclusion: *According to results of current study, no significant difference was seen between benign and malignant tumors in terms of EGFR and bcl-2 expression. Thus, assessment of these two biomarkers is not proposed in bladder tumors. Moreover, no relation was seen between EGFR and Bcl2 expression with grade. Therefore, expression of these biomarkers didn't affect on grade of the tumor.*

Keywords: *Bcl-2, Biomarkers, Bladder carcinoma, Epidermal growth factor receptor*

INTRODUCTION

Bladder cancer is considered as the fifth most common cancer in the world and the second most common cancer in some areas of Iran (1). The worldwide incidence of bladder cancer is 350-400.000 new cases per year (2). It is assessed that more than 560,000 bladder cancer patients live in United States (3). The incidence of bladder cancer in men is higher than women (4). Common risk factors include cigarette smoking (5-8), certain chemotherapeutic agents, and aromatic hydrocarbons.

Urothelial carcinoma (transitional cell carcinoma) is the most common type of bladder cancer (9). Adenocarcinoma and squamous carcinoma are other types of bladder cancer (4). Sensitive and accurate detection of bladder cancer is critical to diagnose the disease at early stage.

In addition, prediction of prognosis, recurrence, and response to therapy is necessary. Although urine cytology remains as the gold standard technique, several new urinary biomarkers that may help in the diagnosis, prediction of treatment response, and prognostication of the disease have been identified (10).

Abnormal expression of epidermal growth factor receptor (EGFR) is seen in many human cancers. It is considered as a poor prognostic marker (11). This receptor is the product of c-erbB1 proto-oncogene and belongs to type 1 tyrosine kinase receptor family (12). Studies reported assessment of EGFR expression in limited population. Furthermore, bcl-2 protein as a proto-oncogen plays a main role in inhibition of apoptosis.

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Expression of Bcl-2 is seen in many tumors including breast (13), prostate, and head and neck tumors (13). It contributes in oncogenesis by repressing signals that induces apoptotic cell death (14). Its expression is associated with poor prognosis, resistance to current treatment modalities including radiotherapy and hormone therapy in advanced prostate cancer (13).

Enache et al., demonstrated immunoexpression of EGFR in 53.3% of cases of bladder cancer (15), but Hashemi et al., showed lack of EGFR expression in 58.7 % of cases (11). Krish et al. reported that Bcl-2 overexpression is rare in bladder transitional cell carcinoma (16). Korkolopoulou et al. evaluated expression of bcl-2 proteins in bladder carcinomas and showed that 44% of patients were bcl-2 positive (17).
Studies conducted regarding the expression of Bcl-2 and EGFR biomarkers in urothelial carcinoma of the bladder lesions were few and controversial (16-19). So far, no study was conducted regarding expression of these biomarkers in malignant and benign tumor in this area and it was not specified if we can use the expression of these biomarkers for detection of benign and malignant tumors. The aim of this study was to evaluate the immunohistochemical expression of Bcl-2 and EGFR biomarkers in urothelial carcinoma of the bladder lesions and its relation to grade.

MATERIALS AND METHODS

Sample selection and histopathological analysis
This case control study was conducted on bladder tumors in Shahid Rahnemoun Hospital, Yazd, Iran. Paraffin blocks of bladder tumors of benign and malignant groups were taken from Department of pathology. The sample size was assessed according to Cochran formula.
Data of patients (17 benign tumor and 40 malignant bladder tumors) were extracted from medical records. Then paraffin embedded samples were taken from pathology department of Shahid Rahnemoon Hospital, The histopathological diagnosis was performed by criterions according to Lopez et al., 2004 (19). Tumor grade is classified into three groups (grade I, grade II, grade III).

Immunohistochemical analysis of EGFR and Bcl2
Immunohistochemistry method was used for diagnosis of EGFR and Bcl2 biomarkers in tumor samples by poly-L-lysin coated slides. After mounting 3 µm thick histological sections on slide, they were dewaxed at temperature of 60^0C and rehydrated with decreasing concentration of alcohol. Hydrogen peroxide (0.3%) was used for blocking the endogenous peroxidase activity in tissue samples. After washing with tris Then, the sections were exposed with primary antibody (Bcl2 and EGFR antibody) which is prepared from Abcam Company (according to the protocol). In the next step, slides were incubated with secondary antibody (rabbit-mouse antibody) according to DAKO protocol (Denmark). These sections were then incubated with 3, 3-diamino-benzidine tetrahydrochloride and substrate for 10 min and placed in hematoxylin solution for 1 minute and rinsed in tap water. Finally, these slides were immersed in graded alcohol, xylene, and mount.

Statistical analysis
Data were entered into SPSS version 22. Mann Whitney U Test and Chi Square test were used for analysis of data. $P<0.05$ was assumed significant.

Ethical consideration
After obtaining consent from patients, current study was approved by Ethical committee of Shahid Sadoughi University of Medical Sciences.

RESULTS

In current study, 57 patients with bladder tumors were chosen in Shahid Rahnemoun hospital. Frequency of parameters including tumor type, grade and sex is shown in Table 1.

Table 1: Frequency of parameters with bladder cancer

Parameter	Classification	Frequency	Percent
Tumor	Malignant	40	70.2
	Benign	17	29.8
Grade	Low	22	55
	High	18	45
Sex	Women	12	21.1
	Men	45	78.9

As shown in Table 1, 70.2% of tumors were malignant. Moreover, frequency of patients in terms of gender showed that 80% of malignant tumors belonged to men and 20 % of them to women.

The parameters including tumor size, age, tumor size, EGFR, Bcl2 in patients with bladder cancer is shown in Table 2.

Table 2: The parameters in patients with bladder cancer

Parameters	Mean± SD	Min	Max	p-value
Age				
Malignant Benign	64.8±2.33	33	90	0.032
	55.53±3.5	28	79	
Tumor Size				
Malignant	1.85± 0.27	0.3	10	0.025
Benign	1. 51± 0.59	0.3	10	
EGFR				
Malignant	6.5± 3.23	0	95	0.840
Benign	8.23± 5.89	0	90	
Bcl2				
Malignant	7.07±2.8	0	80	0.119
Benign	22.06± 7.36	0	10	

Frequency distribution of patients in terms of EGFR showed that among 40 malignant tumors, eight (20%) were EGFR positive. In addition, among 17 benign tumors, two (11.7%) were EGFR positive. Totally, among 57 tumors, 10 tumors (17.4%) were EGFR positive.

Moreover, frequency distribution of EGFR showed that there was no significant difference between benign and malignant tumors in terms of EGFR expression (Chi-Square test) (P=0.830).

In addition, frequency distribution of patients in terms of bcl-2 showed that among 40 malignant tumors, 16 tumors (40%) were bcl-2 positive. In addition, among 17 benign tumors, 9 (52.9%) were bcl-2 positive. Totally, among 57 tumors, 25 tumors were bcl-2 positive (44%). No significant difference was seen between two groups (benign and malignant groups) regarding Bcl-2 (P=0.094).

Table 3: The expression of EGFR and Bcl2 in terms of grade

Bio-marker	Low grade Mean ± SD	High grade Mean ± SD	P-value
EGFR	4.13± 13.22	`7.69± 26.26	0.91
Bcl2	3.72± 12.81	13.31±26.14	0.511

The minimum and maximum of EGFR in low grade and high grade was 0-60 and 0-95, respectively. In addition, the minimum and maximum of Bcl2 in low grade and high grade was 0-60 and 0-80, respectively As shown in Table 3, no significant difference was seen between EGFR and Bcl2 expression in terms of grade (P>0.05).

DISCUSSION

The prevalence of bladder cancer is high in Iran (1) and studies regarding the role of EGFR and bcl2 expression in bladder cancer were controversial (14-19).

Moreover, the expression of EGFR and bcl2 in bladder cancer has not been studied in the region. Therefore, we evaluated expression of these biomarkers in benign and malignant tumors of bladder and observed that among 40 malignant tumors, eight tumors (20%) were EGFR positive. In addition, among 17 benign tumor, two (11.7%) were EGFR positive. Totally, among 57 tumors, 10 tumors (17.4%) were EGFR positive. Hashemi et al., evaluated the expression of EGFR in urinary bladder cancer and observed high expression of EGFR in 26.2% of cases (11). Hashemi et al. reported that EGFR was over-expressed in 26.2 % of bladder cancer tissue specimens (20). Enache et al., demonstrated immunoexpression of EGFR in 53.3% of cases of bladder cancer (15). The findings of various studies indicate different results.

In addition, there was no relation between EGFR expression and grade. Hashemi et al, assessed the prognostic value of EGFR in urinary bladder cancer and observed a significant relation between EGFR expression and grade of disease (11) which was inconsistent with our study. It seems that one reason of difference between two studies was due to sample size. Our study consisted of 40 bladder cancer patients, while Hashemi et al., considered 126 cases of bladder cancer. Railkar et al. showed a high amplification rate of EGFR in most bladder cancers. Therefore, they proposed treatment of EGFR expression in bladder cancer with EGFR Targeted Photoimmunotherapy (PIT). It is believed that this method may offer a selective and new therapy for non-muscle invasive bladder cancer (21). Rebouissou et al. reported that basal phenotype which is enriched with EGFR was found approximately in 25% of bladder tumors (22). It seems that tumors with EGFR expression can be candidate for cancer treatment.

Moreover in our study, expression of bcl-2 was observed in 44 % of patients with bladder cancer. In current study, there was no significant difference between two groups, considering bcl-2 expression. Korkolopoulou et al. evaluated expression of bcl-2 proteins in bladder carcinomas and observed that 44% of patients were bcl-2 positive (17) which is consistent with our findings. Krish et al. also demonstrated that Bcl-2 overexpression is rare in bladder carcinoma (16).

The finding of this study is inconsistent with our study. It seems that factors including gender, race, type of tumor, and geographical region are influential factors on different expression of bcl-2(16). Swellam et al. evaluated the incidence of bcl-2 expression in malignant bladder tumors (57 tumors with squamous cell carcinoma and 61 tumors with transitional cell carcinoma) and showed a potential role of bcl-2 expression in carcinogenesis. Moreover, they proposed that anti-bcl-2 therapy may be useful for these patients (14). Cho et al. reported that over-expression of bcl-2 protein in bladder cancer inhibited cisplatin-induced bax translocation (23).

In addition, we did not find any relation between grade and bcl-2 expression. Swellam et al., observed no relation between bcl-2 expression and histological grade which was consistent with our study (14). Korkolopoulou et al. reported that bcl-2 expression was not related to grade, in consistence with our study (17). Cook et al. achieved similar findings and reported no significant relation between tumor grade and bcl-2 expression (13). Therefore, most studies reported no relation between bcl-2 expression and grade; while Ricardo Gonzalez-Campora et al. found that bcl-2 over-expression was associated with low grade malignancy (24). Therefore, studies in this regard were controversial and need more research.

In addition, tumor size in patients with malignant bladder carcinoma was significantly greater than benign bladder carcinoma. Thompson et al. reported that the risk of malignancy tumors increases with tumor size (25). The finding of this study is consistent with our study. Cheng et al. reported that tumor size had important role for predicting metastasis free survival (26). The comparison of two groups in terms of age showed that the mean age of patients in malignant group was significantly higher than benign group. White et al. reported that cancer is an age-related disease because the occurrence of cancers increases with age. The finding was consistent with our study (27). The incidence of cancer in older ages poses unique challenges to attaining a high quality of life. Brandt et al. reported that age is a main risk factor for breast cancer (28).

Conclusion

According to results of current study, no significant difference was seen between benign and malignant tumors in terms of EGFR and bcl-2 expression. Thus, assessment of these two biomarkers is not proposed in bladder tumors. Moreover, no relation was seen between EGFR and Bcl2 expression with grade. Therefore, expression of these biomarkers didn't affect on grade of the tumor.

Conflict of interest

There is no conflict of interest

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