Annals of Medicine and Surgery 81 (2022) 104372

Contents lists available at ScienceDirect

Annals of Medicine and Surgery

journal homepage: www.elsevier.com/locate/amsu

Cross-sectional Study

The value of BCL2 and CK20 expression in predicting behavioral patterns of bladder cancer, a cross sectional study

Elham Pourebrahimi [a], Hedieh Moradi Tabriz [a], Seyed Amir Miratashi Yazdi [b], Elham Nazar [a,*], Sara Batmani [a]

[a] Department of Pathology, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Surgery, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Bladder cancer
BCL2
CK20
Prognosis

ABSTRACT

Background: Several biomarkers have been investigated to predict the biological behavior and prognosis of patients with bladder cancer. We evaluated the role of two important markers including BCL2 and CK20 in evaluating the biological behavior of bladder tumors.

Methods: This cross-sectional study was performed on 30 patients suffering from one of the neoplasms of the bladder. To evaluate the expression of BCL2 and CK20 markers, the neoplastic tissue sample was initially extracted and immunohistochemistry staining was employed.

Results: The positivity of CK20 and BCL2 in the patients' specimens was found to be 53.3% and 10.0%, respectively. There was no association between CK20 and BCL2 expressions and tumor size, tumor stage, or tumor-related vascular invasion, but BCL2 expression was shown to be higher in the low-grade specimens, while the expression rate of CK20 was found to be significantly higher in high grade samples.

Conclusion: Evaluation of the expression of CK20 and BCL2 markers can be very valuable in predicting bladder tumor grade.

1. Introduction

Bladder cancer is the most common malignant tumor in the urinary tract and is the ninth most common cancer in the world. More men than women and whites more than Africans are affected by this cancer [1]. The most common type is urothelial carcinoma, which accounts for about 90% of primary bladder tumors [2]. Other types of bladder cancer including squamous cell carcinoma and adenocarcinomas are less common. Like most cancers, the development and spread of bladder cancer depends on a combination of genetic and environmental factors. Among these factors, chemical factors are the most important that bladder tumors are more common in industrial areas and their incidence also increases with exposure to smoking and arylamines [3]. Age is also an important risk factor for this cancer, as most cases of urothelial carcinoma of the bladder occur in patients over the age of 55 years [4]. Gross or microscopic hematuria is the most common manifestation of the disease, followed by symptoms related to secondary urinary tract infection [5]. The majority (75%) of these tumors are well-differentiated and non-invasive (pTa and pT1), which can be controlled by transurethral resection of the bladder [6]. However, up to 70% of patients with superficial urothelial carcinoma will recur after resection, and 10–15% will progress to invasive urothelial carcinoma [7].

Bladder cancer development involves a multi-step process that includes various changes in the activity of genes regulating cell division and apoptosis. The B-cell lymphoma 2 (Bcl-2) protein family, a group of related proteins, and several other genes, including c-myc, Hras, ABL, Apo-1, and p53, play an important regulatory role in apoptosis [8,9]. Bcl-2 overexpression is associated with decreased tumor sensitivity to chemotherapy and radiotherapy [10]. Therefore, overexpression of Bcl-2 is of great importance for the pathogenesis, progression of bladder cancer and its response to therapeutic interventions.

Cytokeratin 20 (CK20) is a member of the cytokeratin family that shows a restricted pattern of expression in normal tissues. CK20 expression in the urothelium is restricted to superficial umbrella cells of the bladder even in the presence of severe inflammation [11]. Only malignancy induces a change in the expression pattern of CK20 [12]. The aim of this study was to investigate CK20 and BCL2 expression in patients with bladder tumor and its relationship with prognostic factors

* Corresponding author. Department of pathology, Tehran University of Medical sciences, Tehran, Iran.
E-mail address: enazar@sina.tums.ac.ir (E. Nazar).

https://doi.org/10.1016/j.amsu.2022.104372
Received 11 July 2022; Received in revised form 2 August 2022; Accepted 11 August 2022
Available online 23 August 2022

such as tumor stage and grade.

2. Materials and methods

This cross-sectional study was performed on patients (30) referred to Sina hospital from 2021 to 2022 suffering from one of the neoplasms of the bladder that the diagnosis of malignancy could be proven through histological assessments, and the evidence of malignancy could also be tracked in cystoscopy. Patients receiving chemotherapy (neoadjuvant) were excluded from the study.

All the patients had signed a written consent form before the intervention.

To evaluate the expression of study markers, the neoplastic tissue sample was initially extracted and fixed in 10% formalin and then blocked in paraffin and stained with H&E (Fig. 1). The samples were pathologically evaluated in terms of tumor grade and stage, tumor size, the presence of lymphatic vessels invasion, and tumor multiplicity. Also, immunohistochemistry staining for CK20 and BCL2 markers was performed on the prepared paraffin block to evaluate their expression. Finally, the expression level of each of CK20 and BCL2 markers in the group of patients with bladder neoplasms was evaluated and its association with tumor behaviors was also assessed. In assessment of CK20 expression, negative staining or staining restricted to superficial umbrella cells were considered to be normal and thus staining atypical cells or the entire thickness of the epithelium of the bladder was considered to be abnormal. In assessing BCL2 expression, nuclear staining less than 5% or cytoplasmic staining was considered normal, while nuclear staining more than 5% in urothelial cells was considered abnormal (Fig. 2).

This study was approved by the Research Ethics Board of Sina hospital, Tehran university of medical sciences, IR.TUMS.SINAHOSPITAL.REC.1399.110.

This cross-sectional study has been reported in line with the STROCSS Criteria [13].

2.1. Statistical analysis

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using *t*-test or Mann-Whitney test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. The categorical variables were compared using the Chi-Square test or Fisher's exact test if required. P values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for windows (IBM, Armonk, New York) was used.

3. Results

In this study and during two years of examining patients referred to Sinai Hospital in 2021 and 2022, 30 patients with bladder neoplasm were included in the study. The average age of patients was 66.6 ± 5.6 years ranged 46–87 years and 80.0% of participants were male. The most common surgical approach considered for the affected patients was *Trans*-urethral resection of bladder tumor (TUR-BT) (93.3%) followed by total cystectomy in 6.7%. In pathological assessment, vascular invasion was revealed in 10.0% of neoplastic samples. Regarding tumor size, 40.0% of tumors sized less than 2 cm, 46.6% sized 2–5 cm and 13.4% sized higher than 5 cm. In total, 53.3% of tumor samples were stratified as low grade and 46.7% as high grade. All tumors had single pattern without multi-focal feature. The baseline characteristics of study subjects are summarized in Table 1.

Overall, the positivity of CK20 and BCL2 in the patients' specimens was found to be 53.3% and 10.0% respectively. As indicated in Table 2, we showed no association of patients' age with expression of both study markers. The positivity for BCL2 was significantly higher in women than in men ($p = 0.006$), while CK20 expression was similar in both genders ($p = 0.372$). There was no association between CK20 and BCL2 expressions and types of surgeries scheduled for the patients. Also, we showed no association between CK20 and BCL2 expression and tumor-related vascular invasion (Table 2). Also, the expression of these two markers was completely independent of tumor size. As shown in Table 2, BCL2 expression was shown to be higher in the low-grade specimens ($p = 0.041$), while the expression rate of CK20 was found to be significantly higher in high grade samples ($p = 0.033$). We found no association of tumor stage with the expression of CK20 ($p = 0.032$) and BCL2 ($p = 0.09$) markers.

4. Discussion

Biomarkers along with pathological features are widely used to assess tumor behaviors including tumor grade and stage, neural and vascular invasions, distant metastasis, and response to therapeutic interventions. Due to the aggressiveness of histopathological evaluations as well as the limitations of some imaging methods, special attention has been paid to the evaluation of sensitive and especially specific biomarkers in the evaluation of the biological behavior of cancer and its differentiation from benign tissues. Regarding the evaluation of bladder cancer, several biomarkers have been tested, and strong evidence has been obtained that some of these markers are valuable in diagnosing the behavior of the tumor and its prognostic features. However, there is still no consensus on which of these markers can specifically predict the biological behavior of cancer or even be an alternative to the gold standard methods of pathology. The expression of Bcl-2 in different malignancies is variable and depends on the cell line. Bcl-2 overexpression is a poor prognostic factor in high-grade lymphoma, leukemia, neuroblastoma, and prostate cancer [14] while lung and breast cancer patients with Bcl-2 expression have a better chance of survival [15]. The CK20 immunohistochemical staining pattern is a useful adjunct in the diagnosis of urothelial dysplasia, as only malignant cells

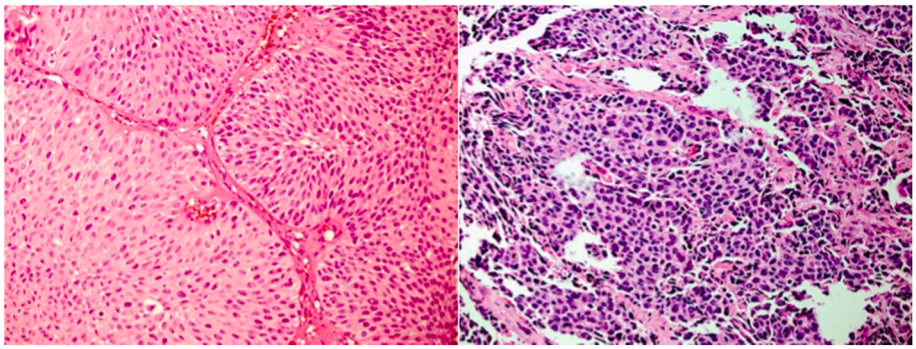

Fig. 1. Histopathological examination of bladder neoplasms including low grade (left) and high grade (right), H&E X100.

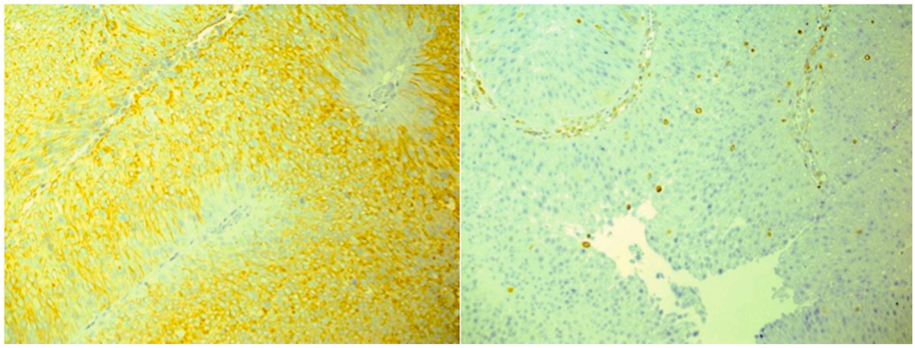

Fig. 2. Immunohistochemical staining show positive for CK20 (left) and BCL2 (right).

Table 1
Baseline characteristics of study population.

Gender, %	
Male	24 (80.0)
Female	6 (20.0)
Age subgroups, %	
<55 years	4 (13.4)
55–65 years	11 (36.6)
66–75 years	9 (30.0)
>75 years	6 (20.0)
Surgical approach	
TUR-BT	28 (93.3)
Total cystectomy	2 (6.7)
Vascular invasion, %	
Positive	3 (10.0)
Negative	27 (90.0)
Tumor size, cm	
<2 cm	12 (40.0)
2–5 cm	14 (46.6)
>5 cm	4 (13.4)
Tumor grade	
Low	16 (53.3)
High	14 (46.7)
Tumor stage	
Ta	10 (33.3)
T1	13 (43.3)
T2	6 (20.0)
T3	1 (3.4)
Tumor mutifocality	
Single	30 (100)
Multi-focal	0 (0.0)

show positive CK20 staining [12]. Abnormal cytokeratin expression has been found in various forms of neoplasia and other diseases and is associated with tumor recurrence [16]. In this regard, we evaluated the role of two important markers including BCL2 and CK20 in evaluating the biological behavior of bladder tumors. In the present observation, a notably high expression of CK20 (53.3%), but partially low expression of BCL2 (10.0%) was found in those with different types of bladder cancer. Naturally, none of these two markers have been specific for diagnosing bladder cancer. But regarding which markers had the ability to predict the biological behavior of the tumor, our study showed that lower expression of BCL2 and adversely higher expression of CK20 was in line with tumor higher grades. In other words, both markers were found to be prognosis for predicting tumor grading. Because tumor grade has an important and central role in predicting tumor-related prognosis, the value of both biomarkers in differentiating good and poorer prognosis can be proposed. Similar and also adverse results have been obtained in other studies. As shown by Fraile et al. [17], BCL2 and CK20 were not correlated with grade, stage and recurrence of the disease that was contrary to our survey with respect to predicting tumor grade. In a study by Nakopoulou et al. [18], BCL2 immunohistochemical positivity was observed in 52% of bladder cancers that was significantly higher than that reported in our study. In their study, tumor stage showed a significant inverse correlation with overall BCL2 positivity that was also in contrary to our study. In another survey by Bertz et al. [19], CK20 expression was significantly correlated with recurrence-free survival. Moreover, Wolf et al. [20] also indicated that BCL2 positivity was also associated with decreased tumor-free survival. It seems that the relationship between the expression of these two markers and the biological

Table 2
BCL2 and CK20 expression according to baseline characteristics.

Item	BCL2 expression		P value	CK20 expression		P value
	Positive (n = 3)	Negative (n = 27)		Positive (n = 16)	Negative (n = 14)	
Gender, %			0.006			0.37
Male	1 (33.3)	23 (85.2)		13 (81.2)	11 (78.6)	
Female	2 (66.7)	4 (14.8)		3 (18.8)	3 (21.4)	
Mean age, year	71.2 ± 7.7	68.6 ± 2.2	0.26	69.5 ± 1.9	68.8 ± 2.0	0.33
Surgical approach						
TUR-BT	3 (100)	25 (92.6)	0.31	14 (87.5)	14 (100)	0.09
Total cystectomy	0 (0.0)	2 (7.4)		2 (12.5)	0 (0.0)	
Vascular invasion, %			0.27			0.31
Positive	0 (0.0)	3 (11.1)		2 (12.5)	1 (7.1)	
Negative	3 (100)	24 (88.9)		14 (87.5)	13 (92.9)	
Mean tumor size, cm	2.6 ± 0.9	2.5 ± 1.0	0.29	2.7 ± 1.1	2.4 ± 0.8	0.31
Tumor grade			0.04			0.03
Low	3 (100)	13 (48.1)		6 (37.5)	10 (71.4)	
High	0 (0.0)	14 (51.9)		10 (62.5)	4 (28.6)	
Tumor stage			0.09			0.32
Ta	2 (66.7)	8 (29.6)		5 (31.3)	5 (35.7)	
T1	1 (33.3)	12 (44.4)		7 (43.7)	6 (42.9)	
T2	0 (0.0)	6 (22.2)		3 (18.7)	3 (21.4)	
T3	0 (0.0)	1 (3.8)		1 (6.3)	0 (0.0)	

behavior of cancer as well as its prognosis is very different in various societies, and therefore, regarding the value of each marker in predicting cancer behaviors, it should be completely based on the society. This may be due to patient characteristics or antibody specifications [21]. Our limitations were small and unicentric population who evaluated and we will require further studies with more sample size.

5. Conclusion

In summary, in our patients' population suffering bladder cancer, positivity of CK20 and BCL2 is predicted in 53.3% and 10.0% respectively. Both markers are prognostic for predicting tumor grading that lower expression of BCL2 and adversely higher expression of CK20 is in line with tumor higher grades. But, the expression of these markers is not associated with vascular invasion, size of tumor or its stage.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Sources of funding

None.

Ethical approval

All items of the project were approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.SINAHOSPITAL.REC.1400.042).

Consent

Informed consent was obtained from the patient and the family of the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Author contribution

SAMY: revise the paper and follow up. HMT: major contribution of the idea, study design, revises the paper, and follows up. EN: pathologist, and writing the paper EP & SB: data collection.

Registration of research studies

1. Name of the registry: Tehran University of Medical Sciences
2. Unique Identifying number or registration ID: (IR.TUMS.SINAHOSPITAL.REC.1400.042).
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): http://ethics.research.ac.ir/IR.TUMS.SINAHOSPITAL.REC.1400.042

Guarantor

Elham Nazar.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.104372.

References

[1] A.T. Lenis, P.M. Lec, K. Chamie, Bladder cancer: a review, Jama 324 (19) (2020) 1980–1991.
[2] C.L. Amling, Diagnosis and management of superficial bladder cancer, Current problems in cancer 25 (4) (2001) IN1–278.
[3] J. Dobruch, M. Oszczudłowski, Bladder cancer: current challenges and future directions, Medicina 57 (8) (2021) 749.
[4] V.K. Wong, D. Ganeshan, C.T. Jensen, C.E. Devine, Imaging and management of bladder cancer, Cancers 13 (6) (2021) 1396.
[5] A. Pham, L.K. Ballas, Trimodality therapy for bladder cancer: modern management and future directions, Current opinion in urology 29 (3) (2019) 210.
[6] D.T. Miyamoto, K.W. Mouw, F.Y. Feng, Shipley Wu, J.A. Efstathiou, Molecular biomarkers in bladder preservation therapy for muscle-invasive bladder cancer, The Lancet Oncology 19 (12) (2018) e683–e695.
[7] Recent developments in the treatment of advanced bladder cancer, in: J.L. Godwin, J. Hoffman-Censits, E. Plimack (Eds.), Urologic Oncology: Seminars and Original Investigations, Elsevier, 2018.
[8] F. Soria, L.-M. Krabbe, T. Todenhöfer, J. Dobruch, A.P. Mitra, B.A. Inman, et al., Molecular markers in bladder cancer, World journal of urology 37 (1) (2019) 31–40.
[9] E.J. Kirsh, D.A. Baunoch, W.M. Stadler, Expression of bcl-2 and bcl-X in bladder cancer, The Journal of urology 159 (4) (1998) 1348–1353.
[10] P. Cooke, N. James, R. Ganesan, A. Burton, L. Young, D. Wallace, Bcl-2 expression identifies patients with advanced bladder cancer treated by radiotherapy who benefit from neoadjuvant chemotherapy, BJU international 85 (7) (2000) 829–835.
[11] M.J. Gandhi, D. Ferriola, Y. Huang, J.L. Duke, D. Monos, Targeted next-generation sequencing for human leukocyte antigen typing in a clinical laboratory: metrics of relevance and considerations for its successful implementation, Archives of Pathology and Laboratory Medicine 141 (6) (2017) 806–812.
[12] J. Jiang, T.M. Ulbright, C. Younger, K. Sanchez, D.G. Bostwick, M.O. Koch, et al., Cytokeratin 7 and cytokeratin 20 in primary urinary bladder carcinoma and matched lymph node metastasis, Archives of pathology & laboratory medicine 125 (7) (2001) 921–923.
[13] G. Mathew, R. Agha, J. Albrecht, P. Goel, I. Mukherjee, P. Pai, et al., STROCSS 2021: strengthening the reporting of cohort, cross-sectional and case-control studies in surgery, International Journal of Surgery Open 37 (2021), 100430.
[14] M. Enache, C. Simionescu, L.C. Lascu, Ki67 and Bcl-2 immunoexpression in primitive urothelial bladder carcinoma, Rom J Morphol Embryol 53 (3) (2012) 521–525.
[15] A. Pollack, C.S. Wu, B. Czerniak, G.K. Zagars, W.F. Benedict, T.J. McDonnell, Abnormal bcl-2 and pRb expression are independent correlates of radiation response in muscle-invasive bladder cancer, Clinical cancer research: an official journal of the American Association for Cancer Research 3 (10) (1997) 1823–1829.
[16] S. Mumtaz, A.A. Hashmi, S.H. Hasan, M.M. Edhi, M. Khan, Diagnostic utility of p53 and CK20 immunohistochemical expression grading urothelial malignancies, International archives of medicine 7 (1) (2014) 1–8.
[17] P. San miguel fraile, I. Antón badiola, J. Ortiz rey, C. Álvarez álvarez, A. Fernández costas, M. Lago fernández, et al., Comparative analysis of p53, ki-67, bcl-2 and ck20 expression in superficial transitional cell carcinoma of urinary bladder: correlation with recurrence, histological grade and clinical stage, Actas Urologicas Espanolas 27 (8) (2003) 587–593.
[18] L. Nakopoulou, C. Vourlakou, A. Zervas, A. Tzonou, H. Gakiopoulou, M.-A. Dimopoulos, The prevalence of bcl-2, p53, and Ki-67 immunoreactivity in transitional cell bladder carcinomas and their clinicopathologic correlates, Human pathology 29 (2) (1998) 146–154.
[19] S. Bertz, W. Otto, S. Denzinger, W.F. Wieland, M. Burger, R. Stöhr, et al., Combination of CK20 and Ki-67 immunostaining analysis predicts recurrence, progression, and cancer-specific survival in pT1 urothelial bladder cancer, European urology 65 (1) (2014) 218–226.
[20] H. Wolf, C. Stöber, R. Hohenfellner, J. Leissner, Prognostic value of p53, p21/WAF1, Bcl-2, Bax, Bak and Ki-67 immunoreactivity in pT1 G3 urothelial bladder carcinomas, Tumor biology 22 (5) (2001) 328–336.
[21] H.M. Tabriz, E. Nazar, S.A. Ahmadi, E. Azimi, F. Majidi, Survivin and Her2 expressions in different grades of urothelial neoplasms of urinary bladder, Iranian Journal of Pathology 16 (2) (2021) 154.