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Evaluation of the prognostic value of CD117 and CK20 biomarkers in urinary bladder carcinoma

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Abstract

Background: Over-expression of CD117 and cytokeratin 20 (CK20) is seen in many malignancies. Given that few studies have been conducted regarding the role of these biomarkers in the etiology of bladder tumors, this study aimed to evaluate the prognostic value of CD117 and CK 20 biomarkers in the benign and malignant tumors of urinary bladder carcinoma.

Methods: This case-control study was conducted on 62 bladder tumors (17 benign tumors and 45 malignant tumors). The immunohistochemistry technique was used to assess CK20 and CD117 biomarkers in tumor samples.

Results: There is no significant difference between benign and malignant groups in terms of CD117 (p=0.094). No significant difference was observed between expression of CD117 in terms of grade (p=0.184). However, a significant difference was observed between benign and malignant groups in terms of CK20 expression (p=0.022). Moreover, a significant difference was observed between CK20 expression in terms of grade (p=0.009). In addition, a significant difference was observed between the age of patients considering grade (p<0.047).

Conclusion: According to these findings, it seems that CK20 play an important role in the carcinogenesis of urinary bladder carcinoma. Furthermore, these findings indicated that patients with higher age had higher grade. In addition, assessment of CD117 expression did not play a main role in tumor progression. Therefore, it is not recommended for bladder tumors.

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1. Introduction:

Bladder cancer is the fourth malignancy in men [1-3]. It is estimated that more than 560,000 patients with bladder cancer live in the United States. Recent study has shown the advanced bladder cancer is associated with mortality and short survival [4]. The rate of bladder cancer incidence in men is about twice as much as women and

increases sharply with age, so that 9 out of 10 patients diagnosed with bladder cancer are at age .
≥65 years [2]. The growing number of older people causes the number of incidence cases and deaths increases [2]. Risk factors for bladder cancer are certain chemotherapeutic agents, cigarette smoking [5-12], and exposure to polycyclic aromatic hydrocarbons, benzene products, and phenacetin. The most common type of bladder cancer is transitional cell carcinoma (urothelial carcinoma) [13]. Other types of bladder cancer are squamous carcinoma and adenocarcinoma [2].
Precise and susceptible detection of bladder carcinoma is critical to diagnose of disease at an early stage. Moreover, the assessment of prognosis, prediction of the answer to therapy and recurrence is necessary [2]. Although cytology of urine remains as the gold standard method, several new urinary biomarkers have been recognized for diagnosis, prognosis and prediction [14]. A trans-membrane tyrosine kinase receptor c-Kit (CD117) is encoded by proto-oncogene c-kit. It is involved in hematopoiesis and carcinogenesis [14]. The over-expression of c-Kit is seen in many malignancies [15] such as breast tumors, gastrointestinal stromal tumors, and small cell lung carcinoma [1]. CK20 belongs to the epithelial subgroup of intermediate filaments. It is a main tool to detect and recognize the type of cancer, including urothelial carcinoma. It may be as a potential biomarker of bladder tumour recurrence, and progression or answer to therapy [16]. Since metastatic adenocarcinoma from unknown primary sites is a main clinical problem, cytokeratin 20 (CK20) is suggested to recognize the primary sites [17]. Few studies assessed the concurrent expression of biomarkers, including CK20 and CD117 in tumors of patients with transitional bladder cancer, therefore this study aimed to examine the prognostic value of c-kit and CK20 expression in transitional cell

2. Materials and Methods

2.1 Sample selection

This case-control study was conducted on 62 patients with benign and malignant bladder tumors. The current study was approved by Ethics Committee of Shahid Sadoughi University of Medical Sciences (number:IR.SSU.MEDICINE.REC.1394.296). Seventeen patients were in the benign group and forty-six in the malignant group.

2.2 Histopathological analysis

Sixty-two paraffin embedded samples were taken from the pathology department of Shahid Rahnemoon Hospital, Yazd, Iran. The histopathological diagnosis was performed by criteria according to Lopez et al., 2004 [18]. Tumour grade was classified into three groups (grade I, grade II, grade III).

2.3 Immunohistochemical analysis of CD117 and CK20 expression

The immunohistochemistry technique was used for the diagnosis of c-Kit and CD117 biomarkers in tumour samples. The immunohistochemistry method was performed on poly-L-lysin coated slides and 3 µm thick histological sections were mounted on them. Slides were dewaxed at temperature of 60°C and rehydrated with decreasing concentration of alcohol. Endogenous peroxidase activity in tissue samples was blocked by 0.3% hydrogen peroxide. After washing with tris buffer salin (TBS) to prevent the creation of non-specific connections, a blocking buffer was used. Then, the sections were exposed with 50 µlit diluted primary antibody (Polyclonal rabbit antibody: abcam for 24 hours). After washing with TBS, slides were incubated by 50 µlit secondary antibody (abbit-mouse antibody) for 25 minutes. Then the sections were incubated with 50 µlit 3,3-diamino-benzidine tetrahydrochloride and 1000 µlit substrate for 10 min. Then slides were placed in hematoxylin solution for 1 minute and rinsed in tap water. Afterwards, the slide were immersed in graded alcohol, xylene and finally mount. The immunohistochemical staining of CD117 and CK 20 expression is shown in **Figure 1.**

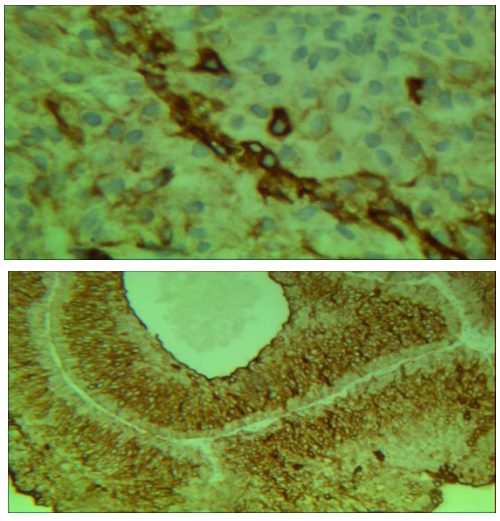

Figure 1: A) CD117 expression (15percent), B) CK 20 expression (70 percent)

2.4 Statistical analysis

In current study, data were entered SPSS version 22. Mann Whitney U Test and Chi Square were used for the analysis of data.

3. Result

In the present study among 62 patients, 17(27.4%) and 45(72.6%) patients had benign and neoplasm tumors, respectively. Moreover, 49 patients (79%) were men and 13 (21%) women.

Table 1 shows the classification of CD117 in benign and malignant groups.As shown in **Table 1**, no significant difference was observed between the benign and malignant groups in terms of CD117 (p>0.05).

Table 1. Classification of CD117 in two groups of patients

Groups	0	1	p-value Chi-Square test
Benign	17(100%)	0	0.094
Malignant	37(82.2%)	8(17.8%)	
Total	54(87.1%)	8(12.9%)	

0: No staining, 1: Staining

Table 2 shows the classification of CD117 in terms of grade in patients with bladder cancer. As shown in **Table 2**, no significant difference was observed between the expression of CD117 in terms of grade (p=0.184).

Table 2. The classification of CD117 in terms of grade in patients

CD117 expression	0	1	2	p-value Chi-Square test
0	16(29.6%)	23(42.6%)	15(27.8%)	0.184
1	0	4(50%)	4(50%)	
Total	16(25.8%)	27(43.5%)	19(30.6%)	

Table 3 shows the classification of CD117 with respect to gender in patients with bladder cancer. As shown in **Table 3**, there is no significant difference between the expression of CD117 with respect to gender (p=0.186).

Table 3. The classification of CD117 with respect to sex

CD117 expression	Men N(%)	Women N(%)	p-value Chi-Square test
0	41(75.9%)	13(24.1%)	0.186
1	8(100%)	0	
Total	49(79%)	13(21%)	

Table 4 shows the classification of patients in terms of CK20 expression in the benign and malignant groups. As shown in **Table 4**, there is a significant difference between the benign and malignant groups in terms of CK20 expression (p<0.05).

Table 4. **Classification of patients in terms of CK20 in benign and malignant group**

Groups	0	1	2	3	p-value Chi-Square test
Benign	10(58.8%)	3 (17.6%)	2 (11.8%)	2 (11.8%)	0.022
Malignant	11(24.4%)	5 (11.1%)	8(17.8%)	21 (46.7%)	
Total	21(33.9%)	8 (12.9%)	10 (16.1%)	23 (37.1%)	

Table 5 shows the classification of CK20 in terms of grade. As clear from **Table 5,** a significant difference was observed between CK20 expression in terms of grade (p<0.05).

Table 5. The classification of CK20 in terms of grade

CK20 expression	0	1	2	p-value Chi-Square test
0	9	5	7	0.009
1	3	5	0	
2	2	7	1	
3	2	10	11	
Total	16	27	19	

Table 6 shows the frequency distribution of age in terms of grade. As shown in **Table 6**, a significant difference was observed between patients age in terms of grade ($p<0.05$). It indicated that patients with higher age had higher grade.

Table 6. **Frequency distribution of age in terms of grade**

Grade	Less than 60	More than 60	p-value Chi-Square test
0	10(62.5%)	6 (37.5%)	0.047
1	11(40.7%)	16 (59.3%)	
2	4 (21.1%)	15 (78.9%)	
Total	25 (40.3%)	37 (59.7%)	

4. Discussion

The results of immunohistochemical staining in tumour tissue of bladder showed that CK20 over-expression was observed in 75.6% of malignant tumors. Moreover, a significant correlation was seen between CK20 expression and grade. Jiang et al., assessed CK20 in primary and matched lymph node metastasis of urinary bladder carcinoma. For this purpose, they studied 26 patients with primary urinary bladder carcinoma and evaluated the expression of CK20 in these patients. Positive CK20 immunoreactivity was observed in twelve cases (46%) of primary cancer and its matched lymph node metastasis [19]. Hammam et al., evaluated the expression of CK20 in 150 patients with urothelial carcinoma in urinary bladder cancer in Egypt [20] and used an imunohistochemical technique for the detection of CK20 biomarker. Grades I, II and III was observed in 20, 40 and 90 cases, respectively. Moreover, the over expression of CK20 was observed in 105 /150 patients (70%). They also showed the inverse relationship between the expression of CK20 and tumour grade [20], which was not consistent with our study. Buchumensky et al., assessed cytokeratin 20 as a marker for early detection in patients with bladder cell carcinoma [21]. The reverse transcriptase polymerase chain reaction technique was used for determining the CK20 expression in urine of patients with bladder carcinoma. The findings showed that CK20 was negative in the urine samples of the control group. Moreover, CK20 was positive in 131/144 patients with bladder cancer. They reported that CK20 was a potential biomarker for bladder cancer and was more sensitive than urinary cytology [21]. YK et al., evaluated the CK20 expression in normal and cancer bladder tissues. The results showed that the CK20 expression was expressed in 103/154 (66.9%) and 2/30 (6.67%) of cancer and normal bladder tissues, respectively. Pathologic results also demonstrated that the intensity of CK20 staining in cancer tissues was significantly associated with grade. The findings proposed that CK20 expression may be an important feature of bladder cancer and predict its prognosis [2] which was consistent with our study. Kein et al., measured CK20 expression in patients with bladder cancer and indicated that CK20 was an excellent biomarker for detecting bladder cancer [22]. Other studies also demonstrated that the presence of CK20 expression was a marker and predictor of urothelial neoplasia [23-25]. It seems that CK20 expression may be very helpful in determining prognosis and appropriate therapeutic strategies.

In our study, the evaluation of CD117 in malignant and normal tissues of bladder cancer showed that there is no significant difference between two groups in terms of CD117 expression. Moreover, no significant difference was seen between CD117 expressions in terms of grade. Aliza et al., evaluated the c-Kit expression in transitional cell bladder cancer and reported that strong expression and moderate expression were seen in 12 and 7 patients, respectively. CD117 expression was also higher in patients with higher grade [26]. Pan et al., evaluated c-Kit expression in small cell carcinoma of the urinary bladder and reported that among 52 patients, 14 (27%) were positive for c-Kit expression. No association was seen between expression of c-Kit and clinicopathologic parameters and survival [27]. Zigeuner et al., investigated CD117 expression in tumors of patients with transitional cell carcinomas and observed CD117 immunoreactivity in 9% of these tumors. They reported that CD117 immunoreactivity is infrequent in these patients and routine screening of CD117 in bladder tumour tissues seems cost-ineffective and cannot be recommended. Therefore, CD117 immunoreactivity does not provide a rationale to investigate imatinib mesylate therapy [28]. Tealeb et al., also assessed CD117 in bladder carcinoma by immunohistochemical method and concluded that CD117 expression could be assumed as a poor prognostic parameter in bladder carcinoma [14].

5. Conclusion

The results of the current study supported the role of CK20 in the carcinogenesis of urinary bladder carcinoma. Moreover, it seems that CK20 expression may be considered as a poor prognostic parameter in transitional bladder carcinoma. Furthermore, these findings indicated that patients with higher age had higher grade. In addition, the assessment of CD117 expression did not play a main role in tumour progression, therefore, it is not recommended for bladder tumors.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1.Metts M. Bladder cancer. A review of diagnosis and management. J National Medical association. 2000; 92(6): 285-294.

2.Ye F, Wang l, Castillo-Martin M, McBride R1, Galsky M. Biomarkers for bladder cancer management: present and future. Am J Clin Exp Urol. 2014; 2(1): 1-14.

3.Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014; 64: 9-29.

4.NouraniH. Low-dose Gemcitabine and Carboplatin as a Good Palliation for Local Symptoms in Advanced Transitional Cell Carcinoma of Bladder. Iranian J Blood Cancer. 2009; 1(3):119-120

5.Sanli O, Dobruch J, Knowles M, Burger M, , Alemozaffar M. Bladder cancer. 2017; 13-17.

6. Freedman N, Silverman D, R Hollenbeck A, Schatzkin A, Abnet Ch. Association between smoking and risk of bladder cancer among men and women. JAMA. 2011; 306(7): 737–745.

7.D'Avanzo B, Negri E, La Vecchia C, Gramenzi A, Bianchi C, Franceschi S, Boyle P. Cigarette smoking and bladder cancer. Eur J Cancer. 1990; 26(6): 714-8.

8.Neal D. Freedman, Debra T. Silverman, ScD, ScM; Albert R. Hollenbeck. Association Between Smoking and Risk of Bladder Cancer Among Men and Women. JAMA. 2011; 306(7): 737-745

9. Tobacco Smoke and Involuntary Smoking. In: IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Lyon, France: IARC; 2004. 83

10. Silverman DT, Devesa SS, Moore LE, Rothman N. Bladder cancer. In: Schottenfeld D, Fraumeni JF Jr, eds. Cancer Epidemiology and Prevention. 3rd ed. New York, NY: Oxford University Press; 2006:1101-1127

11. Masaoka Keitaro H, Hidemi M, Kenji I. Cigarette smoking and bladder cancer risk: an evaluation based on a systematic review of epidemiologic evidence in the Japanese population. Japanese J Clin Oncol. 2016; 46 (3): 273–283.

12.Cumberbatch MG, Rota M, Catto JW, La Vecchia C. The role of tobacco smoke in bladder and kidney carcinogenesis: a comparison of exposures and meta-analysis of incidence and mortality risks. Eur Urol Forthcoming. 2015; 1-10

13.Chung K. The etiology of bladder cancer and its prevention. J Cancer Sci Ther. 2013; 5(10): 346-361

14.Taleb S. C kit and Bcl2 in bladder carcinoma:An immunohistochemical study. AAMJ. 2013; 11(1): 214-227.

15.Shams T and Metawea M, Overexpression of C-KIT in Schistosomal Urinary Bladder Carcinoma. Med. J. Cairo Univ. 2011; 79(1): 449-455

16. Raheem S.A, Saied A.N, Shaer R, Mustafa O, Ali A. The Role of CK20, p53 and p63 in differentiation of some urothelial lesions of urinary bladder, immunohistochemical study. Open J Pathol. 2014; 181-193

17. Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in discriminating primary from metastatic adenocarcinoma. European J Cancer. 2002; 38(6): 758-763

18. Lopez-Beltran A, Sauter G, Gasser T, tumors of the urinary system. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA (eds), Pathology and genetics of tumours of the urinary system and male genital organs, World Health Organization Classification of Tumours, IARC Press, Lyon, 2004; 89– 154.

19. Jiang J, Ulbright TM, Younger C, Sanchez K, Bostwick DG, Koch MO, Eble JN, Cheng L. Cytokeratin 7 and cytokeratin 20 in primary urinary bladder carcinoma and matched lymph node metastasis. Arch Pathol Lab Med. 2001;125(7): 921-3.

20. Hammam O, Wishahiz M, Khalil H, El Ganzouri H, Badawy M, Elkhquly A, Elesaily K. Expression of cytokeratin 7, 20, 14 in urothelial carcinoma and squamous cell carcinoma of the Egyprian urinary bladder cancer. J Egypt Soc Parasitol. 2014; 44(3): 733-40.

21. Buchumensky V, Klein A, Zemer R, Kessler OJ, Zimlichman S, Nissenkorn I. Cytokeratin 20: a new marker for early detection of bladder cell carcinoma? J Urol. 1998.160(6): 1971-4.

22.Klein A, Zemer R, Buchumensky V, Klaper R, Nissenkorn I. Expression of cytokeratin 20 in urinary cytology of patients with bladder carcinoma. Cancer. 1998; 82: 349–54.

23.Han A. Coexpression of cytokeratins 7 and 20 confirms urothelial carcinoma presenting as an intrarenal tumour. Cancer. 2000; 4-9.

24. Burchill SA, Bradbury MF, Pittman K, Southgate J, Smith B, Selby P. Detection of epithelial cancer cells in peripheral blood by reverse transcriptase-polymerase chain reaction. Br J Cancer. 1995; 71: 278–81.

25. Harnden P, Allam A, Joyce AD, Patel A, Selby P, Southgate J. Cytokeratin 20 expression by non-invasive transitional cell carcinomas: potential for distinguishing recurrent from non-recurrent disease. Histopathology. 1995; 27: 169–74.

26. Aliza Z, Gad N, Yoram D, Alon H, Tuvia H, Gideon SY, Edward Y, Lea RW. Expression of C-KIT receptor in transitional cell carcinoma of urinary bladder and its correlation with clinicopathological variables Conexunimedicale 2009; 16.

27. Pan C, Yang XJ, Lopez-Beltran A, MacLennan GT, Eble GN, Koch MO, et al. kit expression in small cell carcinoma of the Urinary bladder: prognostic and therapeutic implications. Modern Pathol. 2005; 18: 320–323.

28. Zigeuner R, Ratschek M, Langer C. Kit (CD117) immunoreactivity is rare in renal cell and upper urinary tract transitional cell carcinomas. Bju International. 2005; 5 – 31.