The Retrospective Analysis of Prognostic Significance of Smoking Status in Bladder Cancer

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Purpose: This study aimed to evaluate the prognostic significance of smoking status in muscle invasive bladder cancer (MIBC) and non-MIBC in recurrence-free (RFS), progression-free (PFS), disease-free survival (DFS), and cancer-specific survival (CSS).

Materials and Methods: We retrospectively evaluated 541 patients with MIBC and non-MIBC who were surgically treated during 2002–2013. Smoking status was defined as never smokers (NS; n=160, 30%), former smokers (FS; smoking cessation for ≥ 1 year, n=176, 33%), and current smokers (CS; > 100 cigarettes, n=198, 37%). We statistically compared these groups' clinicopathological factors for the predictive factors for RFS and PFS for non-MIBC (NMIBC) and DFS for MIBC, and CSS using multivariate model.

Results: The CS, FS, and NS groups exhibited insignificantly different pathological staging, grades, and immunohistological characteristics ($p > 0.05$). Among the 441 patients with NMIBC, pathologic tumor size was a significant risk factor for RFS (1–3 cm: hazard ratio [HR], 1.88; > 3 cm: HR, 2.21; $p < 0.05$); age (HR, 1.06), intravesical therapy (HR, 0.25), and high-grade cancer (HR, 8.33) significant for PFS; and age (HR, 1.08), intravesical instillation (HR, 0.26), and smoking status (FS: HR, 0.40; CS: HR, 0.44) significant for CSS ($p < 0.05$). The 93 patients with MIBC had no significant risk factors for DFS, although their significant risk factors for CSS were age (HR, 1.05), female sex (HR, 2.64), and carcinoma *in situ* (HR, 4.72) ($p < 0.05$).

Conclusions: Smoking status only significantly affected CSS in patients with NMIBC. (Korean J Urol Oncol 2017;15:111-120)

Key Words: Bladder cancer • Muscles • Invasiveness • Smoking • Prognosis

INTRODUCTION

Cigarette smoking is a strong risk factor for the development of bladder cancer (BC). For example, smoking accounts for ap-

proximately one-half of the BC risk among men and one-third of this risk among women, and smokers have a 2–4 fold higher risk of developing BC, compared to nonsmokers.^{1,2} However, there are limited and unclear data regarding the smoking-related mechanism of carcinogenesis for BC. Furthermore, there are limited data regarding the effects of smoking on pathological staging, grading, treatment response, and prognosis among patients with BC.

Several studies have reported that smoking is associated with more invasiveness, more advanced stage and grade, higher recurrence and progression rates, shorter cancer-specific survival, and poorer therapeutic response among patients with BC.²⁻⁷

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Furthermore, smoking is a prognostic factor for clinical outcomes from BC after transurethral resection of the bladder (TURB) and radical cystectomy (RC).^{5,8-10} However, other studies have reported conflicting effects of smoking on these previously confirmed prognostic effects that low-graded tumors were associated with the smoking.¹¹⁻¹³ Therefore, the present study was designed to evaluate the prognostic significance of smoking status among clinicopathological, immunohistochemical in muscle invasive (MIBC) and nonmuscle invasive BC (NMIBC) treated with surgery. Furthermore, the effect of smoking status on postsurgical outcomes and the expression of biomarkers in the BC tissues were also analyzed statistically.

MATERIALS AND METHODS

1. Ethical Considerations

This study was approved by the Institutional Review Board (IRB) of the National Cancer Center (approval number: NCC-NCS-13-833), and conducted according to the principles expressed in the Declaration of Helsinki. The requirement for written informed consent by participant was waived, due to the retrospective design by the IRB. All patients' records/information was anonymized and deidentified prior to analysis.

2. Patient Population

We evaluated 541 patients with initially diagnosed BC who underwent TURB or RC with urinary diversion at the National Cancer Center (Goyang, Korea) during 2002–2013. The patients' clinicopathological characteristics were analyzed including smoking status (history, duration, and intensity) and the immunohistochemical expression of p53, retinoblastoma tumor suppressor (Rb), Bcl-2, phosphatase and tensin homolog (PTEN), and Ki-67. All pathology slides were retrospectively reviewed by a 15-year-old experienced senior genitourinary pathologist (WSP), who assigned the pathological stages and grades according to the World Health Organization's 1973 grading system and the American Joint Committee on Cancer's 2002 staging system.^{14,15}

3. Smoking Assessment

At the initial clinical visit, patients consulted with a trained nurse (SJJ) and physician (HKS), who recorded self-reported data regarding cigarette smoking status (never smokers [NS], former smokers [FS],¹⁶ and current smokers [CS]¹⁷) and the du-

ration and intensity of the smoking habit, which included the number of cigarette pack-years, the number of years of smoking, and the number of years since smoking cessation. The exposure variable was defined as pack-years, which was calculated as (cigarettes smoked per day/20) × (years smoked). The NS, FS, and CS groups were defined as patients who had never smoked or who had smoked <100 cigarettes during their lifetime, patients who quit smoking ≥1 year before their diagnosis, and patients who still smoked regularly (>100 cigarettes) at the time of diagnosis, respectively. However, no information regarding smoking style, product brand, and degree of inhalation were obtained.

4. TURB and Intravesical Instillation Therapy

All patients with proven primary urothelial carcinoma of the bladder underwent complete TURB according to guideline's recommendations.^{18,19} Patients without sufficient tissue or high graded BC to confirm the presence of MIBC repeatedly and routinely underwent TURB at the surgeons' discretion within 2–6 weeks after the initial treatment, based on the pathological and intraoperative findings. The surgeons' discretion was used to indicate early single-dose mitomycin C treatment and/or adjuvant treatment with Bacillus Calmette-Guérin (BCG), mitomycin C, epirubicin, or doxorubicin intravesical instillation therapy (IVT). The analyses included all patients with pure urothelial cell carcinomas, BC with mixed histological profiles that involved squamous cell and/or glandular differentiation, and all partial cystectomies for curative purposes. However, we excluded cases with any other histological variants, MIBC with clinical lymph node positivity and/or distant metastases, non-urothelial cell carcinomas, salvage or palliative cystectomies, and no medical follow-up data after the RC.

5. Follow-up and Subsequent Treatment

After treatment with TURB, RC, or partial cystectomy, patients were generally followed-up according to the guideline's recommendations.^{18,19} In cases of TURB for NMIBC, disease recurrence was defined as the first tumor recurrence in the bladder, regardless of tumor stage, and progression was defined as any tumor recurrence at stage T2 or higher in the bladder. The tumor was resected when disease recurrence was detected. When disease recurrence was not detected but the urine cytology results were positive, the patients underwent bladder and prostatic urethra biopsies in addition to an upper urinary tract

workup. In cases of RC or partial cystectomy for MIBC, recurrence was defined as local recurrence in the pelvic cavity or distant metastasis that was confirmed using imaging and biopsy (if indicated). In cases of death, the cause of death was

determined by the treating physicians, by a chart review that was corroborated by the death certificate, or by the death certificate alone. Tumor recurrence in the upper urinary tract was not considered tumor recurrence, but rather as a second primary

Table 1. Baseline demographic data from 534 patients with bladder cancer

Variable	Never smoker (n=160)	Former smoker (n=176)	Current smoker (n=198)	p-value*
Age (yr)	67.5 (36–91)	68 (36–91)	65 (24–87)	0.068
Sex				<0.001
Male	76 (17.4)	172 (39.3)	190 (43.4)	
Female	84 (87.5)	4 (4.2)	8 (8.3)	
pTstage				0.301
Tis, Ta, T1	132 (82.5)	143 (81.3)	172 (86.9)	
Over T2	28 (17.5)	33 (18.8)	26 (13.1)	
Grade				0.247
G1	33/158 (20.9)	24/174 (13.8)	28/198 (14.1)	
G2	64/158 (40.5)	81/174 (46.6)	99/198 (50.0)	
G3	61/158 (38.6)	69/174 (39.7)	71/198 (35.9)	
Grade (ISUP)				0.167
Low	62/156 (39.7)	62/172 (36.0)	86/188 (45.7)	
High	94/156 (60.3)	110/172 (64.0)	102/188 (54.3)	
P53				0.922
Negative	34/95 (35.8)	41/112 (36.6)	40/104 (38.5)	
Positive	61/95 (64.2)	71/112 (63.4)	64/104 (61.5)	
Rb				0.584
Negative	79/88 (89.8)	84/98 (85.7)	81/90 (90.0)	
Positive	9/88 (10.2)	14/98 (14.3)	9/90 (10.0)	
PTEN				0.117
Negative	74/88 (84.1)	72/97 (74.2)	63/88 (71.6)	
Positive	14/88 (15.9)	25/97 (25.8)	25/88 (28.4)	
Bcl-2				0.954
Negative	84/96 (87.5)	98/113 (86.7)	89/101 (88.1)	
Positive	12/96 (12.5)	15/113 (13.3)	12/101 (11.9)	
Ki-67				0.142
Negative	25/96 (26.0)	28/142 (25.0)	37/102 (36.3)	
Positive	71/96 (74.0)	84/142 (75.0)	65/102 (63.7)	
Smoking period (yr)	NA	30 (5–65)	42 (5–66)	<0.001 [†]
No. of cigarettes per day				0.505 [†]
1–9	NA	13/174 (7.5)	24/197 (12.2)	
10–19	NA	42/174 (24.1)	47/197 (23.9)	
20–29	NA	93/174 (53.4)	98/197 (49.7)	
≥30	NA	26/174 (14.9)	28/197 (14.2)	
Smoking amount (pack year)	NA	26 (2.5–100)	37 (2.5–110)	0.002 [†]
Cumulative smoking exposure				<0.001 [†]
Light short	NA	11/173 (6.4)	3/195 (1.5)	
Light long	NA	44/173 (25.4)	67/195 (34.4)	
Heavy short	NA	18/173 (10.4)	3/195 (1.5)	
Heavy long	NA	100/173 (57.8)	122/195 (62.6)	

Values are presented as median (range) or number (%).

ISUP: International Society of Urologic Pathologists, Rb: retinoblastoma tumor suppressor, PTEN: phosphatase and tensin homolog, NA: not available.

*Chi-square tests and Kruskal-Wallis tests as appropriate. [†]Former smoker vs. current smoker.

tumor.

6. Immunohistochemistry and Assessment of Tissue Microarrays

Paraffin-embedded sections of the excised tumors were subjected to immunohistochemical analysis to evaluate the expression of Rb, p53, Bcl-2, PTEN, and Ki-67. The formalin-fixed paraffin-embedded tissues were sectioned, mounted, deparaffinized, dehydrated, and stained with 5 antibodies according to the protocol from our previous study.²⁰ The immunohistochemical results were independently evaluated by 2 blinded pathologists under a light microscope, the results were classified as positive or negative, and were subsequently cross-checked. Positive Bcl-2 expression was defined as positive staining in >10% of the tumor cells. Tissues were considered positive for p53 overexpression when >10% of the nuclei exhibited positive staining, and the cutoff for Ki-67 overexpression was 20%. Loss of Rb was considered present when >10% of the tissue nuclei exhibited negative staining in the presence of normal epithelial and mesenchymal cell expression.

Loss of PTEN was considered present when >50% of the cells exhibited negative cytoplasmic staining.

7. Statistical Analysis

Baseline characteristics were evaluated using the chi-square test and Kruskal-Wallis test, as appropriate, according to smoking status (NS vs. FS vs. CS). RFS or DFS was calculated as the interval from surgery to the first documented clinical recurrence. PFS and CSS were measured from the date of treatment initiation to the date of BC progression or death. The Kaplan-Meier method, log-rank test, and a Cox proportional hazard model were used for the survival analyses. Only factors with a p-values of <0.10 in the univariate analyses were entered into the multivariable Cox regression model. We also excluded variables where >20% of the patients exhibited missing data. Statistical analyses were performed using Stata software ver. 13.1 (StataCorp., College Station, TX, USA), all analyses were 2-tailed, and differences were considered statistically significant at a p-value of <0.05.

Table 2. Analysis of recurrence-free survival (RFS), progression-free survival (PFS), and cancer-specific survival (CSS) in 441 patients with nonmuscle invasive bladder cancer according to smoking status

Variable	RFS (n=286)			PFS (n=296)			CSS (n=304)		
	HR	p-value	95% CI	HR	p-value	95% CI	HR	p-value	95% CI
Age				1.06	0.008	1.02-1.11	1.08	<0.001	1.05-1.12
Instillation therapy	0.65	0.149	0.36-1.17	0.25	0.001	0.11-0.59	0.26	0.001	0.12-0.58
Grade									
G1	1.00			1.00			1.00		
G2	1.17	0.632	0.62-2.21	0.49	0.490	0.07-3.65	0.75	0.580	0.28-2.05
G3	1.53	0.313	0.67-3.51	0.68	0.721	0.08-5.81	1.46	0.560	0.41-5.16
Grade									
Low (ISUP)	1.00			1.00			1.00		
High	1.03	0.910	0.62-1.72	8.33	0.007	1.79-38.68	1.04	0.937	0.42-2.56
Tumor number									
1	1.00			1.00			1.00		
2-7	0.90	0.701	0.53-1.54	0.88	0.789	0.35-2.22	1.02	0.955	0.44-2.37
>7	1.52	0.170	0.83-2.78	1.41	0.504	0.51-3.89	1.59	0.299	0.66-3.83
Tumor size (cm)									
<1	1.00								
1-3	1.88	0.012	1.15-3.07						
>3	2.21	0.003	1.31-3.73						
Lymphovascular invasion	0.89	0.760	0.44-1.82	1.17	0.750	0.45-3.06			
Never smoker	1.00			1.00			1.00		
Former smoker	1.14	0.632	0.67-1.93	0.96	0.928	0.38-2.44	0.40	0.013	0.2-0.82
Current smoker	1.33	0.265	0.81-2.19	1.40	0.491	0.54-3.62	0.44	0.026	0.21-0.90

HR: hazard ratio, CI: confidence interval, ISUP: International Society of Urologic Pathologists.

Fig. 1. Recurrence-free (A, B), progression-free (C, D), and cancer-specific survival (E, F) curves according the smoking status among patients with nonmuscle invasive bladder cancer. TUR: transurethral resection.

RESULTS

Among the 534 patients, we identified 441 patients (82.6%) with NMIBC and 93 patients (17.4%) with MIBC patients. These patients included 160 NS (30%), 176 FS (33%), and 198 CS (37%). When we compared the CS, FS, and NS groups, we did not observe any significant differences in their pathological staging, grades, and immunohistological findings (Table 1). The median smoking duration was 30 years (range, 5–65 years) for FS, and 42 years (range, 5–66 years) for CS. The smoking amounts were 26 pack-years (range, 2.5–100 pack/yr) for FS, and 37.0 pack-years (range, 2.5–110 pack-years) for CS.

Among the 441 patients with NMIBC, the significant risk factor for recurrence was pathologic tumor size (1–3 cm: hazard ratio [HR], 1.88; 95% confidence interval [CI], 1.15–3.07; >3 cm: HR, 2.21; 95% CI, 1.31–3.73; $p < 0.05$) (Table 2, Supplementary Table 1). The factors that were significantly associated with progression were age (HR, 1.06; 95% CI, 1.02–1.11), IVT (HR, 0.25; 95% CI, 0.11–0.59), and high-grade cancer (HR, 8.33; 95% CI, 1.79–38.68) (all, $p < 0.05$) (Table 2, Supplementary Table 2). The factors that were significantly associated with mortality were age (HR, 1.08; 95% CI, 1.05–1.12), IVT (HR, 0.26; 95% CI, 0.12–0.58), and smoking status (FS: HR, 0.40; 95% CI, 0.20–0.82; CS: HR, 0.44; 95% CI, 0.21–0.90) (all, $p < 0.05$) (Table 2, Supplementary Table 3). The log-rank test was used to compare the Kaplan-Meier survival curves according to smoking status, and revealed that NS exhibited significantly better CSS, compared to ever smokers ($p = 0.0298$) (Fig. 1, Table 2, and Supplementary Table 4).

Among the 93 patients with MIBC, there were no significant risk factors for DFS (Table 3, Supplementary Table 5). The sig-

nificant risk factors for mortality were age (HR, 1.05; 95% CI, 1.01–1.08), female sex (HR, 2.64; 95% CI, 1.04–6.70), and carcinoma *in situ* (HR, 4.72; 95% CI, 1.50–14.86) (all, $p < 0.05$) (Table 3). The log-rank test was used to compare the Kaplan-Meier survival curves, and revealed significantly different CSS according to smoking status ($p < 0.05$), although no differences were observed for DFS (Fig. 2, Table 3, Supplementary Table 6).

DISCUSSION

Cigarette smoking has known a leading cause of BC, due to the production of multiple carcinogenic substances that can induce sequential genetic and epigenetic changes and instabilities in the urothelium.^{21,22} These substances are cleared via excretion through the bladder, which leads to a risk of urothelial carcinoma that increases in proportion to the duration of exposure to these carcinogens in the urine.²³ A significant dose-response relationship between cumulative smoking exposure and clinical outcomes has been shown among patients with primary NMIBC.^{2,4} This relationship indicates that the interaction between genetic factors and smoking exposure might influence the development of more advanced and higher-grade tumors, recurrence, progression, and cancer-specific mortality due to BC.^{12,21}

However, as the characteristics of BC according to smoking status have not been sufficiently evaluated, this study evaluated the different prognostic effects of smoking status on BC according to muscle-invasiveness and found that only smoking status significantly affected CSS in NMIBC, but any significant differences in RFS and PFS were not observed. Previous prospective cohort study of 348,010 Korean participants inves-

Table 3. Analysis of disease-free survival (DFS) and cancer-specific survival (CSS) in 93 patients with muscle invasive bladder cancer according to smoking status

Variable	DFS (n=52)			CSS (n=92)		
	HR	p-value	95% CI	HR	p-value	95% CI
Age	-	-	-	1.05	0.006	1.01–1.08
Female sex	9.65	0.07	0.82–113.69	2.64	0.041	1.04–6.70
Carcinoma <i>in situ</i>	-	-	-	4.72	0.008	1.50–14.86
Never smoker	1.00	-	-	1.00	-	-
Former smoker	4.55	0.229	0.39–53.58	0.70	0.409	0.30–1.64
Current smoker	1.54	0.726	0.14–17.25	0.38	0.058	0.14–1.03

HR: hazard ratio, CI: confidence interval, ISUP: International Society of Urologic Pathologists.

Fig. 2. Disease-free (A, B) and cancer-specific survival (C, D) curves according to smoking status among patients with muscle invasive bladder cancer. TUR: transurethral resection.

tigated the relationship of smoking status with BC incidence and mortality, and found that CS exhibited a higher incidence and mortality in both sexes, although these factors were not affected by the amount and duration of smoking.²⁰ Another Korean study of 156 patients with BC investigated the effect of smoking on RFS and PFS in BC, and found no significant differences in the stages and grades among the CS, FS, and NS. Nevertheless, the authors reported a significant difference in the recurrence rate between the FS and NS groups ($p < 0.05$).²⁴

In the present study, we did not observe any differences in the aggressiveness and cancer-related immunohistochemical findings according to smoking status. Interestingly, the only significant difference according to smoking status was observed for CSS, and only smokers (CS or FS) with NMIBC exhibited

improved CSS outcomes. This finding may be related to the fact that smokers are more likely to visit hospitals and undergo rigorous evaluations for cardiovascular and pulmonary disease (vs. patients who have never smoked). Thus, physicians might be more aware of BC in these patients, who might be diagnosed and treated at lower-grade disease (vs. nonsmokers). Another explanation might be that unidentified genetic, ethnic, environmental, behavioral, or lifestyle factors might have influenced the association between smoking and BC prognosis, as various factors that we did not account for have been relevant in Western studies.²⁵

NMIBC develops from urothelial cells that have been damaged by the carcinogens from cigarette smoke. In CS or NS patients, their urothelial cells would have high levels of exposure to these carcinogens, which might lead to disease re-

currence, progression, and biological aggressiveness, which are important end points in the management of NMIBC.^{6-8,11,12} However, in this study, any significant differences in RFS and PFS were observed according to smoking status, although CS exhibited a significantly improved CSS. The absence of a significant association between smoking status and RFS in NMIBC might be due to IVT obscuring any differences in recurrence or mortality, as it significantly affected PFS and CSS in this study.^{26,27} Some reports have also suggested that smoking affects the efficacy of IVT and BCG by reducing cytokine activity, B- and T-cell responses, and natural killer cell activation.^{28,29} However, one recent study did not find that smoking affected BCG response,²⁹ and the present study's results also indicate that the efficacy of IVT in NMIBC was independently able to prevent disease progression to MIBC.

Another reason for the insignificant association between smoking status and BC prognosis might be that the influence of smoking status on recurrence was more relevant among high-risk patients during a long-term follow-up. However, this study did not classify the patients into risk groups, and the median follow-up time was not only 4 years not enough long to expect the prognostic influences of smoking status in BC. Therefore, longer follow-up and classification according to risk may be necessary to detect differences in recurrence according to smoking status.

Given the hypothetical interaction between carcinogens in the urine and detrimental genetic changes throughout the urothelium, it is possible that RC is an important prognostic factor in patients with MIBC. In this context, RC might result in complete removal of the damaged urothelial cells and elimination of carcinogen exposure, due to the urinary diversion. Several clinical studies also support the hypothesis that smokers with BC experience lower rates of new tumor occurrence, and worse survival and mortality outcomes, after urinary diversion procedures.⁹ Another Korean study found that NS tended to have poorer RFS, although there was no association between smoking and BC prognosis after RC,¹¹ similar to this study reporting smoking status not an independent risk factor for DFS in MIBC.

Lastly, the association between the expression of various common biomarkers (p53, Rb, PTEN, Bcl-2, and Ki-67) and disease recurrence or progression according to smoking status has been evaluated that any statistically significant associations were found in this study. In contrast, a recent study used similar

biomarkers (p53, p21, pRb, and p27) found that smoking was a significant prognostic factor in BC, and that these biomarkers predicted recurrence and mortality according to smoking status.³⁰ This discrepancy may be related to different methodologies in these studies.

This study contained several important limitations that warrant consideration such as a nonrandomized retrospective design with relatively short follow-up, and a possible recall bias from the self-reported smoking data. Further absence of considerations were also limited factors of this study about the patients' comorbidities, smoking habits (amount, duration, and time since cessation), forms of cigarette products, the effect of different intravesical chemotherapy and BCG agents. In addition, further consideration about the risk stratification including stage and grade might be helpful to explain the relationship between the smoking and the prognoses in patients with BC. Therefore, a larger prospective study with a long-term follow-up, as well as biochemical verification of smoking and hormonal status, is needed to identify sex-related differences and compensate for these limitations.

CONCLUSIONS

In the present study, no statistically significant differences of poor prognoses were observed according to smoking status among the patients' clinicopathological and prognostic characteristics in BC. Further larger prospective study with a long-term follow-up would be needed for the prognostic effect of smoking status in BC.

CONFLICT OF INTEREST

The authors claim no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Tables can be found via <https://doi.org/10.22465/kjuo.2017.15.3.111>.

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