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Article

Smoking's Negative Effect on Pathological Grade and Stage in Patients with Primary, Single, < 3cm Bladder Cancer

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Abstract: Introduction: We investigated the potential effect of smoking on pathological staging in clinically low-risk patients. Material-Methods: Data of 59 patients who were diagnosed with a bladder tumor for the first time and had a single lesion radiologically and endoscopically smaller than 3 cm were investigated, retrospectively. A total of 33 patients who currently smoke or smoked were classified as ever smokers group and 26 patients who did not ever smoke were classified as never smokers group. Pathological diagnoses of the patients in both groups were compared. Results: A total of 9 patients (27.3%) in ever smokers group and 18 patients (69.2%) in never smokers group had Ta disease ($p<0.05$). Morover, 19 patients (57.6%) in ever smokers group and 5 patients (19.2%) in never smokers group had T1 disease ($p<0.05$). The number of patients with low grade (LG) tumor were 8 (24.2%) and 19 (73.1%) in ever smoking and never smoking groups, respectively ($p<0.05$). The number of patients with high grade (HG) tumor were 25 (75.8%) and 7 (26.9%) in ever smoking and never smoking groups, respectively ($p<0.05$). Ta HG was detected in 9 (27.3%) patients in ever smoking group. In contrast, no patients in never smoking group had Ta HG disease ($p<0.05$). The number of patients with T1 HG was 17 (51.5%) in ever smoking group and 2 (7.69%) in never smoking group ($p<0.05$). Conclusion:Smoking seems to associate with pathologically worse stage and grade in patients with primary, single, < 3cm bladder cancer.

Keywords: smoking; pathologic stage; pathologic grade; low risk

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1. Introduction

Bladder cancer is the ninth most frequently-diagnosed cancer worldwide [1]. In bladder cancer, male/female ratio is 3.5:1 worldwide [2]. Association of the smoking habit with a bladder tumor has been well known. Smoking is the worst risk factor for bladder cancer and increases the risk of bladder cancer by 2-4 times [3]. The pathological stage and grade of the tumor determined by histopathological examination are important prognostic factors that determine the number and diameter of the tumor, prior recurrence rate, and carcinoma in situ for bladder cancer [4,5]. Bladder cancer in patients who smoke and have a non-muscle-invasive disease is associated with an advanced tumor stage and grade [6]. However, the studies investigating the effect of smoking on the stage and grade in clinically low-risk patients with bladder tumors that are smaller than 3 cm are limited. The aim of the present study was to discuss the effect of cigarette smoking on pathological staging in patients with clinically low-risk bladder cancer.

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2. Materials and Methods

The files of 154 patients who were diagnosed with bladder cancer for the first time between 2009 and 2013 were retrospectively reviewed. Among them, 59 first-time patients with a single lesion that was radiologically and endoscopically <3 cm in diameter were finally included in the study. European Association of Urology (EAU) guidelines for non-muscle invasive bladder cancer divided patients into risk groups. According to these data we consider as clinically low-risk patients the ones that have the primary and solitary tumors, < 3 cm, and no carcinoma in situ (CIS) characteristics [5,7]. Patients who were diagnosed with carcinoma in situ (CIS) in pathology specimens were excluded.

The age at first diagnosis, smoking status, stage, grade of the primary tumor, and tumor diameter and number were evaluated. Patients who had no data on the records about smoking habits were excluded from the study. Patients who actively smoke cigarettes or former smokers were designated as ever smokers group and lifetime non-smokers were designated as never smokers group. Ever smokers group consisted of 33 patients and never smokers group had 26 patients. Pathological diagnoses of the patients in both groups were compared.

The grading of the samples was performed according to the World Health Organization (WHO) system in 2004 and the staging was performed according to TNM classification approved by the Union International Contre le Cancer (UICC) in 2009.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.1.Statistical Analysis

Data obtained in this study were analyzed using the SPSS 20 (IBM SPSS Statistics; Armonk, NY, USA) package program. Continuous variables with non-Gaussian distribution were presented as a a median (25th and 75th percentiles). Statistical comparisons of two groups

were performed with the Mann–Whitney U test for data with a non-Gaussian distribution. Pearson's chi-square test and Fisher's exact test were used to compare categorical variables. Logistic regression analysis was used when the independent variables include nominal measures and the outcome variable was binary. This analysis was also used to interpret odds ratios with 95% confidence intervals. The statistical significance was accepted when $p<0.05$.

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3. Results

Epidemiologic and pathologic data are displayed in Tables 1 and 2.

Table 1. The differences between groups in terms of age values and gender frequency

	Ever smokers group (n = 33)	Never smokers group (n = 26)	p
Age (years)	69.5 (55.8 – 75.3)	66.0 (57.0 – 73.0)	= 0.427*
Male/Female (N/N)	33/0	21/4	= 0.013**

* p value calculated by Mann-Whitney U Test ** p value calculated by Fisher's exact test

The number of patients with the pathologic stage Ta was 9 (27.3%) in ever smokers group and 18 (69.2%) in never smokers group (p=0.003). In the never smokers group, the rate of detection of the Ta tumor was significantly higher while the rate of detection of the T1 tumor was significantly higher in ever smokers group (p=0.007). Pathological T2 tumor rates were equally distributed in both groups (p = 1). The number of patients with a LG tumor was 8 (24.2%) in ever smokers group and 19 (73.1%) in never smokers group (p <0.05). The number of patients with a HG tumor was 25 (75.8%) and 7 (26.9%) in ever smokers and never smokers groups, respectively (p <0.05). It was found that cigarette smoking associates with a higher tumor grade. When the pathological grade and stage distributions of the groups were evaluated together, the number of Ta LG patients was 11 (33.3%) and 15 (57.7%) in ever smokers and never smokers groups, respectively. There were 9 Ta HG patients (27.3%) in ever smokers group and zero in never smokers group. Smoking was not associated with a LG pathological stage (p = 0.108) and it was found to increase the risk of Ta HG (p <0.05). The number of patients with T1 HG tumors were 17 (51.5%) in ever smokers group and 2 (7.69%) patients in never smokers group (p <0.05). We found that smoking was associated with a HG in Ta and T1 stage tumors. It was determined that the association of smoking with the tumor stage and grade in muscle-invasive bladder tumors was similar to that of non-smokers group (p=1) (Table 2).

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123 **Table 2.** The relationship between the groups and the variables of the chi-square test
124 results and the odds ratios interpreted by logistic regression analysis

		Ever smokers group	Never smokers group	Total		
		n (%)	n (%)	n (%)	p	Odds Ratio (95% CI)
Ta	Absent	24 (72.7%)	8 (30.8%)	32 (54.2%)	**0.003***	0.17 (0.06-0.52)
	Exist	9 (27.3%)	18 (69.2%)	27 (45.8%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
T_1	Absent	14 (42.4%)	21 (80.8%)	35 (59.3%)	**0.007***	5.70 (1.73-18.80)
	Exist	19 (57.6%)	5 (19.2%)	24 (40.7%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
T_2	Absent	28 (84.9%)	23 (88.5%)	51 (86.4%)	1**	1.37 (0.29-6.25)
	Exist	5 (15.2%)	3 (11.5%)	8 (13.6%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
Grade	LG	8 (24.2%)	19 (73.1%)	27 (45.8%)	**0.001***	8.48 (2.62-27.52)
	HG	25 (75.8%)	7 (26.9%)	32 (54.2%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
Ta LG	Absent	22 (66.7%)	11 (42.3%)	33 (55.9%)	0.108*	0.37 (0.13-1.06)
	Exist	11 (33.3%)	15 (57.7%)	26 (44.1%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
Ta HG	Absent	24 (72.7%)	26 (100 %)	50 (84.7%)	**0.003****	**0**
	Exist	9 (27.3%)	0 (0%)	9 (15.3%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
T_1 LG	Absent	31 (93.9%)	23 (88.5%)	54 (91.5%)	0.646**	0.50 (0.08-3.21)
	Exist	2 (6.1%)	3 (11.5%)	5 (8.5%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
T_1 HG	Absent	16 (48.5%)	24 (92.3%)	40 (67.8%)	**0.001****	12.82 (2.58-62.50)
	Exist	17 (51.5%)	2 (7.7%)	19 (32.2%)		
	Total	33 (100%)	26 (100%)	59 (100%)		
T_2 LG	Absent	32 (97.0%)	25 (96,2%)	57 (96.6%)	1**	0.78 (0.05-13.16)
	Exist	1 (3.0%)	1 (3.9%)	2 (3.4%)		
	Total	33 (100%)	26 (100%)	59 (100%)		

T2 HG	Absent	29 (87,9%)	23 (88.5%)	52 (88.1%)	1**	1.04 (0.21-5.26)
	Exist	4 (12.1%)	3 (11.5%)	7 (11.9%)		
	Total	33 (100%)	26 (100%)	59 (100%)		

LG: low grade, **HG**: high grade, CI, confidence interval, * p value calculated by Pearson's chi-square test, ** p value calculated by Fisher's exact test.

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4. Discussion

Occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons and smoking are two important risk factors for developing bladder cancer. Tobacco contains more than 60 carcinogens including benzidine derivatives and aromatic amines. These substances have an important role in developing bladder cancer. In addition, studies have shown that high-grade bladder tumors develop more often in people with high-risk occupations such as industrial plants, which process dye, paint, metals, and petroleum products [8–10]. Moreover, cigarette smoking increases the risk of recurrence and progression of non-muscle-invasive bladder cancer (NMIBC) [11]. The association of cigarette smoking with bladder cancer has been known for 60 years and accounts for about 50% of cases [12].

Two main mutation pathways are responsible for bladder cancer development. One of these is the fibroblast growth factor receptor-3 (FBFR-3) mutation, which is significantly associated with lower grade tumor development [13].The other mutation is related to the p53-oncogene and associated with the development of high grade tumors. Smoking is associated with the development of higher grades of bladder cancer and causes this through mutations in both pathways. Therefore, the prevalence of aggressive tumors in smokers is higher than in non-smokers. In a study published recently, the rate of high grade tumors in smokers was 26% and in non-smokers was 13%. This difference was statistically significant ($p < 0.05$) [13]. In our study, the prevelance of high grade bladder cancer was significantly higher in patients who smoked than in never smokers (91% vs. 7.7%).

Age and gender are important risk factors for bladder cancer. Bladder cancer is detected more often in men. However, in women the prognosis is worse. Women who smoke have shown an increased risk of invasive bladder cancer when compared to men who smoke[14]. Bladder

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cancer affects people of middle and advanced ages. Approximately 90% of initially diagnosed bladder cancer patients are >60 years of age. Bladder cancer under 35 years of age is rare. Sturgeon et al. [15] showed that cigarette smoking increased the risk of muscle-invasive tumors in patients younger than 60 years. There was no statistically significant relationship between smoking and the stage of the tumor in patients >60 years old or older. The average age of our study was 66.4 years and 62.7 years in the ever smoker and never smoker groups, respectively. We did not detect any correlation between muscle-invasive tumors and cigarette smoking, which may be because there were no female patients in the smoker group and the mean age was more than60 years.

The relationship between cigarette smoking and the stage of the tumor at the initial diagnosis differs in various studies. Although some studies have reported that cigarette smoking does not affect the stage and grade of the tumor, some other studies have reported that cigarette smoking is associated with high grade tumors and some studies found that cigarette smoking is associated with low grade tumors [14–18]. In a recent study, Jiang et al. [14] reported that the incidence of advanced stage bladder tumors especially muscle-invasive bladder tumors was higher in smokers. The same study also reported that, as smoking duration and smoking intensity increased, high grade tumors and muscle-invasive tumors were detected at twice the rate than low grade tumors. Nevertheless, Sturgeon et al. [15] investigated the relationship between cigarette smoking and the grade of the bladder tumor. They found that smoking was strongly associated with low-grade bladder cancer. The authors did not explain the cause of this result.

Regarding tumor size, Fleshner et al. [16] reported that smoking did not affect the tumor grade and stage and showed that smoking significantly increases the risk of detecting a tumor regardless of its size. Recently, Carpenter [17] found that there was no significant difference in the tumor stage and grade in smokers but reported that recurrence was significantly higher.

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Su et al. [18] reported that tumors smaller than 3 cm tend to have a lower grade and stage than tumors larger than 3 cm in size. In our study, although the tumor size in both groups was smaller than 3 cm, it was found that high grade and stage tumors were more prevalent in the smoker group. These findings suggest that smoking increases the stage and grade of the tumor regardless of its size.

The risk of developing bladder cancer is directly related to the duration and intensity of cigarette smoking [19]. In a study showing the relationship between smoking and bladder cancer stages and grades, it was reported that active smokers have higher grades and stages of bladder cancer when compared to those who have never smoked and have quit smoking. Additionally, those who have quit smoking have higher stages and grades than those who never smoked. The duration of smoking and quitting cigarette smoking affects the risk of bladder cancer [20].

There are some limitations in the present study. The retrospective nature was the main limitation of the study. Additionally, the duration of smoking was not investigated due to the retrospective design of the study. The small number of patients and the small number of enrolled women are some other limitations of this study. Although there are several limitations mentioned, we believe that the findings are clinically useful.

5. Conclusions

Under the light of the data, smoking seems to be associated with worse pathological features (tumor stage, tumor grade) in patients with primary, single, <3cm, non-muscle-invasive bladder cancer. Despite the limitations of our study, we find this finding clinically useful.

222 **Author Contributions:** Ercan Öğreden and Ural Oğuz took part in the conception and
223 design. Orhan Yalçın provided administrative support. Ercan Öğreden, Ural Oğuz, Erhan
224 Demirelli, and Orhan Yalçın provided study material and/or patients. Ercan Öğreden and
225 Erhan Demirelli collected and assembled the data. Ercan Öğreden and Ural Oğuz analyzed
226 and interpreted the data. Ercan Öğreden wrote the manuscript. Ercan Öğreden approved the
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References

1. Antoni, S.; Ferlay, J.; Soerjomataram, I.; Znaor, A.; Jemal, A.; Bray, F. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. Eur Urol. 2017, 71, 96-108. https://doi: 10.1016/j.eururo.2016.06.010.

2. Horstman, M.; Witthuhn, R.; Falk, M.; Stenzl, A. Gender-specific differences in bladder cancer: a retrospective analysis. Gend Med. 2008,5, 385-394. https://doi: 10.1016/j.genm.2008.11.002.

3. vanOsch, F.H.; Jochems, S.H.; vanSchooten, F.J.; Bryan, R.T.; Zeegers, M.P. Quantified relations between exposure to tobacco smoking and bladder cancer risk: a meta-analysis of 89 observational studies. Int J Epidemiol. 2016, 857-870. https://doi: 10.1093/ije/dyw044.

4. Delclos, G.L.; Lerner, S.P. Occupational risk factors. Scand J UrolNephrolSuppl. 2008, 58-63. https://doi.org/10.1080/03008880802284423.

5. European Association of Urology Guideleines for Non-muscle-invasive bladder cancer 2018. Available at:https://uroweb.org/guideline/non-muscle-invasive-bladder-cancer/#6.

6. Mitra, A.P.; Cote, R.J. Molecular pathogenesis and diagnostics of bladder cancer. AnnuRevPathol. 2009, 4, 251-285. https://doi.org/10.1146/annurev.

7. Sylvester, R.J.; van der Meijden, A.P.; Oosterlinck, W.; Witjes, J.A.; Bouffioux, C.; Denis, L.; et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol. 2006, 49, 466-465; discussion 475-477. https://doi.org/10.1016/j.eururo.2005.12.031.

8. Brooks, D.R.; Geller, A.C.; Chang, J.; Miller, D.R. Occupation, smoking, and the risk of high-grade invasive bladder cancer in Missouri. Am J IndMed. 1992, 21, 699-713. https://doi.org/10.1002/ajim.4700210510.

9. Hecht, S.S. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. NatRevCancer. 2003, 3, 733-744. https://doi:10.1038/nrc1190.

10. Hayes, R.B.; Freidell, G.H.; HoarZahm, S.; Cole, P. Are the known bladder cancer risk factors associated with more advanced bladder cancer? CancerCauses Control. 1993, 4, 157-162. https://doi.org/10.1007/BF00053157.

11. Figueroa, J.D.; Middlebrooks, C.D.; Banday, A.R.; Ye, Y.; Garcia-Closas, M.; Chatterjee, N.; et al. Identification of a novel susceptibility locus at 13q34 and refinement of the 20p12.2 region as a multi-signal locus associated with bladder cancer risk in individuals of European ancestry. HumMolGenet. 2016, 25, 1203-1214. https://doi.org/10.1093/hmg/ddv492.

12. Chavan, S.; Bray, F.; Lortet-Tieulent, J.; Goodman, M.; Jemal, A. International variations in bladder cancer incidence and mortality. EurUrol. 2014, 66, 59-73. https://doi.org/10.1016/j.eururo.2013.10.001.

13. Pietzak, E.J.; Malkowicz, S.B. Does quantification of smoking history correlate with initial bladder tumor grade and stage? CurrUrolRep. 2014, 15, 416. https://doi.org/10.1007/s1193.

14. Jiang, X.; Castelao, J.E.; Yuan, J.M.; Stern, M.C.; Conti, D.V.; Cortessis, V.K.; et al. Cigarette smoking and subtypes of bladder cancer. Int J Cancer. 2012, 130, 896-901. https://doi.org/10.1002/ijc.26068.

15. Sturgeon, S.R.; Hartge, P.; Silverman, D.T.; Kantor, A.F.; Linehan, W.M.; Lynch, C.; et al. Associations between bladder cancer risk factors and tumor stage and grade at diagnosis. Epidemiology. 1994, 5, 218-225. http://www.jstor.org/stable/3702364.

16. Fleshner, N.; Garland, J.; Moadel, A.; Herr, H.; Ostroff, J.; Trambert, R.; et al. Influence of smoking status on the disease-related outcomes of patients with tobacco-associated superficial transitional cell carcinoma of the bladder. Cancer. 1999, 86, 2337-2345. https://doi.org/10.1002/(SICI)1097-0142(19991201).

17. Carpenter, A. Clinical experience with transitional cell carcinoma of the bladder with special reference to smoking. J Urol. 1989, 141, 527-528. https://doi.org/10.1016/S0022-5347(17)40880-9.

18. Su, X.; Fang, D.; Li, X.; Xiong, G.; Zhang, L.; Hao, H.; et al. The Influence of Tumor Size on Oncologic Outcomes for Patients with Upper Tract Urothelial Carcinoma after Radical Nephroureterectomy. BiomedResInt. 2016, 1-8. http://dx.doi.org/10.1155/2016/4368943.

19. Brennan, P.; Bogillot, O.; Cordier, S.; Greiser, E.; Schill, W.; Vineis, P.; et al. Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies. Int J Cancer. 2000, 86, 289-294. https://doi.org/10.1002/(SICI)1097-0215(20000415).

20. vanRoekel, E.H.; Cheng, K.K.; James, N.D.; Wallace, D.M.; Billingham, L.J.; Murray, P.G.; et al. Smoking is associated with lower age, higher grade, higher stage, and larger size of malignant bladder tumors at diagnosis. Int J Cancer. 2013, 133, 446-454. https://doi.org/10.1002/ijc.28017.