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The Effect of Transurethral Resection and BCG Therapy on Cytokine Levels in Non-Muscle Invasive Bladder Cancer

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What's known on the subject? and What does the study add?

In this study, we found that in non-muscle invasive bladder cancer cases, transurethral resection of bladder tumor decreased the tumor weight and accordingly, cytokine levels decreased.

Abstract

Objective: The present study investigated the effect of treatment on interleukin (IL)-1, IL-6, IL-8, and neopterin levels in patients with non-muscle invasive bladder cancer (NMIBC).

Materials and Methods: Thirty patients with NMIBC and 30 age-matched controls were included in the study. Preoperative, postoperative first control [at two weeks after second transurethral resection of bladder tumor (TURBT)] and the second control (at the end of intravesical immunotherapy) blood samples were analyzed using ELISA to determine IL-1, IL-6, IL-8, and neopterin levels. The mean cytokine levels of the patients were statistically compared and comparing the patients' and controls' levels.

Results: There were no statistically significant differences between the mean IL-1, IL-6, IL-8, and neopterin levels of the patient and control groups before initial TURBT. In the patient group, there were no statistically significant differences in the IL-6 and IL-8 levels after both TURBT and intravesical Bacillus Calmette-Guérin (BCG) therapy. The mean of preoperative IL-1 and neopterin levels significantly decreased after TURBT ($p<0.05$). However, this reduction does not continue after intravesical BCG instillation.

Conclusion: The findings of this study showed that the IL-1, IL-6, IL-8, and neopterin levels of the patients with NMIBC were similar to the levels of healthy controls. IL-1 and neopterin levels significantly decreased after TURBT. But these reduction did not continue after intravesical BCG instillation. These findings demonstrate that IL-1 and neopterin levels decrease after TURBT due to the reduction in tumor weight or tumor removal.

Keywords: Bladder cancer, IL-1, IL-6, IL-8, neopterin

Introduction

Bladder cancer is the seventh most commonly diagnosed cancer in men, in whom it is approximately four times more common than in women. At the time of diagnosis, approximately 75% of patients present with non-muscle-invasive bladder cancer (NMIBC), which are confined to the mucosa (Ta, carcinoma *in situ*) or submucosa (T1) (1). Many meta-analyses have confirmed that intravesical Bacillus Calmette-Guérin (BCG) immunotherapy after transurethral resection of bladder tumor (TURBT) is superior to TURBT alone for preventing the recurrence and/or progression of NMIBC (2-5). Therefore, the European Association of Urology (EAU) Guidelines on NMIBC recommend intravesical BCG immunotherapy after TURBT in patients with intermediate- or high-risk NMIBC (1).

Some studies have investigated the effect of intravesical BCG immunotherapy on some angiogenetic factors and cytokines

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such as interleukin (IL)-8 in patients with NMIBC (6,7). They suggested that these factors and cytokines might be used for follow-up after intravesical BCG immunotherapy in NMIBC. The antitumor features of intravesical BCG immunotherapy primarily depend on the BCG-induced inflammatory response (8) that is impaired in NMIBC as reflected by an imbalanced production of immuno-modulating cytokines (9). It is important to more fully understand the significance of these cytokines for predicting the outcome of intravesical BCG immunotherapy in NMIBC. Some studies have shown that proinflammatory cytokines, including IL-1, IL-6, IL-8 and neopterin played an important role in the active immune response in cancer (10-12). Therefore, these cytokine levels may decrease after treatment of cancer (surgical resection and medical therapy). The present study compared IL-1, IL-6, IL-8, and neopterin levels of the patients with NMIBC and healthy controls, and investigate the changes in these cytokines and neopterin levels after TURBT and intravesical BCG immunotherapy.

Materials and Methods

Study Population

Patients with newly diagnosed bladder cancer (n=41) who underwent initial TURBT and 30 age-matched controls were enrolled in the study. The inclusion criteria were newly diagnosed intermediate or high-risk NMIBC who received a second TURBT and 6 doses of BCG. Exclusion criteria were low-risk NMIBC, T2 and BCG toxicity. Three patients with low-risk NMIBC and 3 patients with MIBC (T2) after initial TURBT were excluded from the study. Thirty-five patients underwent the second TURBT at 4-6 weeks after the initial TURBT. Three patients with MIBC after the second TURBT were excluded from the study. Thirty-two patients received intravesical BCG therapy once a week for 6 weeks. Two patients were excluded from the study because of BCG toxicity. The patients' enrollment algorithm is illustrated in Figure 1. The study group comprised 30 patients with intermediate- or high-risk NMIBC and 30 age-matched controls. IL-1, IL-6, IL-8, and neopterin levels were measured by enzyme-linked immunosorbent assay (ELISA) in the blood samples of the patients before initial TURBT (preoperative group), at 2 weeks after the second TURBT (postoperative first control group) and at 2 weeks after the end of induction intravesical BCG immunotherapy (postoperative second control group). These cytokines were also measured by ELISA in the blood samples of controls. Informed consent was obtained from all the patients and controls who participated in the study and The Local Ethics Committee (Celal Bayar University Ethic Committee) approved the study protocol (decision number: 20478486/243).

Statistical Analysis

Statistical analysis was performed using "Statistical Package for Social Sciences 22.0 software (SPSS 22.0 for MAC)". Descriptive statistics were presented as mean ± standard deviation, frequency and percentages. The Shapiro-Wilk test was used to determine whether the data showed a normal distribution. It was observed that all parameters we examined conformed to a normal distribution. Student's t-test (t-test in independent groups) was used to compare normally distributed continuous variables between the control and patient groups before initial TURBT. Mann-Whitney U test was used to compare not normally distributed continuous variables between the control and patient groups before initial TURBT. The comparison between the patient and control groups for sex was performed using the chi-square test. In the patient group, repeated measures analysis of variance (ANOVA) was used to compare variables between the preoperative, postoperative first control and postoperative second control. When appropriate, a Bonferroni t-test was used as a Post-hoc test. $P<0.05$ was defined as the statistical significance level.

Results

The mean age of the patient (22 males and 8 females) and control (21 males and 9 females) groups were 57.3±7.8 and 55.8±9.0 years, respectively (p=0.33). There were no statistically significant differences between the mean IL-1, IL-6, IL-8, and neopterin levels of the patient and control groups before initial TURBT (Table 1). In the

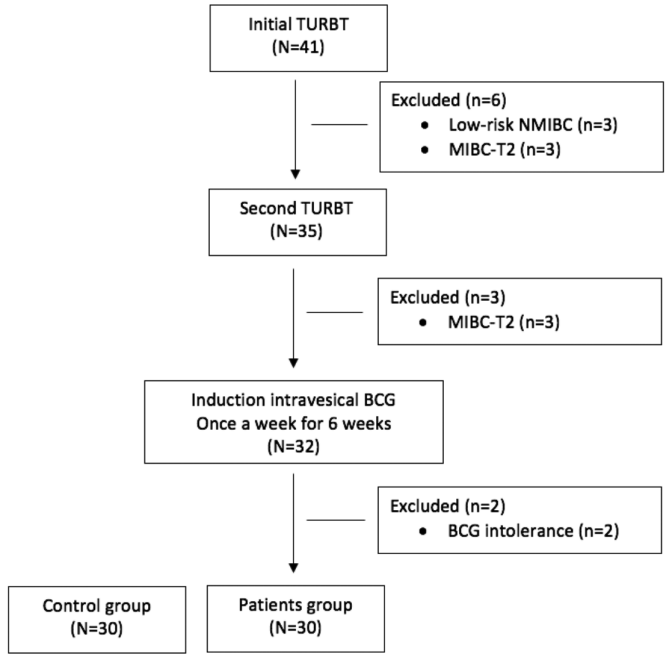

Figure 1. Flow diagram of the study participants

TURBT: Transurethral resection of bladder tumor, NMIBC: Non-muscle-invasive bladder cancer, MIBC: Muscle-invasive bladder cancer, BCG: Bacillus Calmette-Guérin

patient group, there were no statistically significant differences in the preoperative IL-6 and IL-8 levels after both TURBT and intravesical BCG therapy [respectively, $X^2(2)=1.333$, p=0.513, and $X^2(2)=2.778$, p=0.249]. The mean IL-1 levels of the preoperative, postoperative first control and postoperative second controls were 4.09±2.43, 3.99±2.49 and 3.95±2.30, respectively [$X^2(2)=10.500$, p=0.005]. In the pairwise comparison analysis, there was a significant decrease between the IL-1 levels in the preoperative and postoperative first controls (p<0.05) (Figure 2). The mean neopterin levels in the preoperative, postoperative first control and postoperative second controls were 3.31±1.18, 2.89±1.60 and 2.89±1.35, respectively [$X^2(2)=14.941$ p=0.001]. In the pairwise comparison analysis, there were decreases between neopterin levels in the preoperative and postoperative first controls (p<0.05), in the preoperative and postoperative second controls (p<0.05) (Figure 3).

Discussion

This study demonstrated that the IL-1 and neopterin levels of the patients with NMIBC significantly decreased after TURBT, however these decreases did not continue after intravesical BCG immunotherapy (Figures 2 and 3). Although the IL-1 levels of the patients were higher than the levels of the controls, this difference was not statistically significant. According to our best knowledge, there has been no study in literature to examine IL-1 levels in the blood samples of patients with NMIBC. Some studies have evaluated IL-1 levels in urine samples of patients with NMIBC in hours after intravesical BCG therapy (13,14). They found that the urine IL-1 levels increased after intravesical BCG therapy and reported that the result reflected the local inflammatory response to BCG. They also suggested further studies that would evaluate the possible role of IL-1 against NMIBC (14). We investigated the effect of treatment (TURBT and intravesical BCG) on the IL-1 levels in the blood of the patients with NMIBC and our results showed that TURBT caused a significant decrease in the IL-1 level. This finding is novel to the literature. This reduction may be related to the decrease in tumor weight or tumor removal.

Similar to the reduction of the IL-1 level, the neopterin levels of the patients with NMIBC decreased after TURBT in our study. According to the best of our knowledge, only one study examined neopterin levels in the blood samples of patients with NMIBC (15). In this study, the authors measured the neopterin levels before, at 4th, 24th, 48th and 96th hour after intravesical BCG and investigated the role in the immune response of neopterin after BCG. They found that the highest blood neopterin levels were found 48 hours after intravesical BCG therapy and were significantly higher than levels before BCG, 4 hours and 24 hours after BCG. They also suggested that neopterin in serum might be used as a parameter for monitoring the treatment course. We differently measured neopterin levels after TURBT. Similar to the decrease in IL-1, the reduction of neopterin levels after TURBT may be related to the decrease in tumor weight and tumor removal.

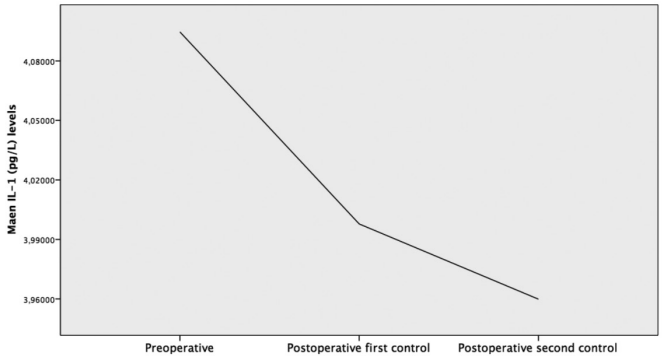

Figure 2. The maen IL-1 levels of the patients in preoperative, postoperative first control and postoperative second control

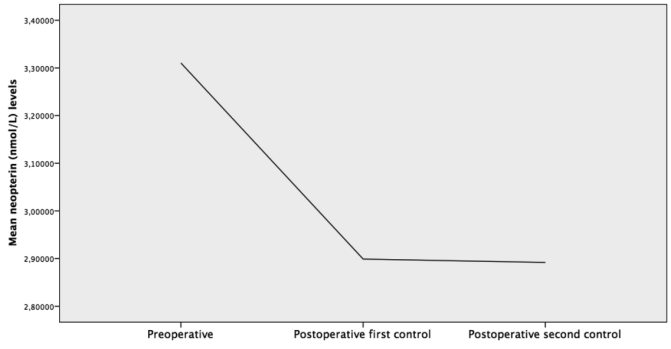

Figure 3. The mean neopterin levels of the patients in preoperative, postoperative first control and postoperative second control

Table 1. Mean IL-1, IL-6, IL-8 and neopterin levels of patient and control groups

	Patient group (preoperative) Mean ± SD	Control group Mean ± SD	p-value
IL-1 (pg/L)	4.09±2.43	3.65±1.30	0.96
IL-6 (ng/L)	4.13±2.13	3.82±1.24	0.58
IL-8 (ng/L)	5.37±2.81	4.32±1.50	0.05
Neopterin (nmol/L)	3.31±1.21	3.70±1.31	0.34

P<0.05 is defined a statistically significant, SD: Standard deviation, IL: Interleukin

There have been some studies that examined IL-6 levels in patients with bladder cancer (16-20). Only one (20) of these studies evaluated this cytokine in the blood samples of patients. In the other studies, it was measured either in urine samples (18,19) or in tumor issues (16,17). Kumari et al. (20) evaluated serum the IL-6 levels in 72 patients with bladder cancer (52 NMIBC and 20 MIBC). They divided the patients into 2 groups according to the presence of recurrence and found that the IL-6 levels of the patients with recurrent were higher than the patients with non-recurrent. They also reported the association of high concentrations of some cytokines, such as IL-6, with poor recurrence-free survival in the patients with bladder cancer. However, in their study, the IL-6 levels were not compared before and after the treatment of bladder cancer. Therefore, we do not know the change in IL-6 levels after the treatment in their study. We differently examined the IL-6 levels before and after TURBT and intravesical BCG instillation in only NMIBC. The results of our study showed that the before IL-6 levels before the treatment did not change statistically after both TURBT and intravesical BCG therapy. Therefore, according to our outcomes, IL-6 is not a proper biomarker to follow patients with NMIBC.

Similar to IL-6, there have been some studies that examined IL-8 levels in the urine of patients with NMIBC (19,20). The results of these studies showed that there was a significant relationship between high IL-8 levels and poor prognosis in the follow-up of NMIBC. However, they did not investigate the IL-8 levels in the blood of the patients. We compared the IL-8 levels in the blood of the patients with NMIBC with the healthy controls and found that there was no significant difference. We also compared the preoperative IL-8 levels with the IL-8 levels after TURBT and intravesical BCG therapy. We found that the preoperative IL-8 levels did not change after treatment with NMIBC. Therefore, although the previous studies suggested that urinary IL-8 levels in patients might be used to predict the prognosis of NMIBC, the findings of our study showed that serum IL-8 levels are not an appropriate cytokine to use in patients with NMIBC.

Study Limitations

There were some limitations to our study. The first one was that we did not follow the patients after intravesical BCG therapy. Therefore, we could not assess the progression and recurrence status of the patients. The other limitations were the small sample size and choice of cut-off times for blood sampling. The last limitation was that we measured the cytokines only in blood samples and did not perform urine tests.

Conclusion

The findings of this study showed that IL-1, IL-6, IL-8, and neopterin levels in the blood of patients with NMIBC were similar to the levels of healthy controls. Although the IL-6 and IL-8 levels did not change after TURBT and intravesical BCG instillation, the IL-1 and neopterin levels significantly decreased after TURBT. But these reductions in the IL-1 and neopterin levels did not continue after intravesical BCG instillation. In conclusion, our findings demonstrated that the IL-1 and neopterin levels decrease after TURBT due to the decrease in tumor weight or tumor removal. We suggest further studies that will investigate IL-1 and neopterin in long-term follow-up after TURBT.

Ethics

Ethics Committee Approval: The Local Ethics Committee (Celal Bayar University Ethic Committee) approved the study protocol (decision number: 20478486/243).

Informed Consent: Informed consent was obtained from all the patients and controls who participated in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: O.Ü., Z.A., F.K., Concept: O.Ü., T.M., Design: O.Ü., Data Collection or Processing: O.Ü., G.T., T.M., Z.A., F.K., Analysis or Interpretation: O.Ü., G.T., Literature Search: O.Ü., Writing: O.Ü.

Conflict of Interest: No conflict of interest was declared by the authors.

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