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Investigation of *TXNIP*, *VDR* and *hOGG1* gene expression patterns and potential therapeutic targets in bladder cancer patients

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ABSTRACT

Backround. The aim of this study was to examine the expression levels of the *Thioredoxin interacting protein (TXNIP),* Vitamin D receptor (*VDR),* Human 8-oxoguanine DNA N-glycosylase 1 *(hOGG1)* genes in bladder cancer patients, according to clinical staging and determine the levels of potential therapeutic targets in serum samples.

Material and Methods. Tissue and serum samples of patients who underwent transurethral resection (TUR) between 2017 and 2018 were obtained. Levels of *TXNIP*, *hOGG1*, and *VDR* genes were assessed using Real time-polymerase chain reaction (RT-PCR), while levels of Thioredoxin (Trx), 8-hydroxy-2' -deoxyguanosine (8-OHdG), and 1,25-dihydroxyvitamin D (25(OH)D_3) were evaluated using the enzyme-linked immunosorbant assay (ELISA) method. Selenium levels were also measured using Optical Emission Spectroscopy (ICP-OES) in both tissue and serum samples. The protein-protein interactions and molecular and biological function

of the proteins were assessed using Search Tool for the Retrieval of Interacting Genes/Proteins. Statistical analysis was conducted using IBM SPSS Statistics version 20.0.
Results. The *TXNIP* gene showed higher expression in low-grade bladder cancer patients up to stage T1, but decreased in high-grade T1 and T2 stages. Both *VDR* and *hOGG1* gene expressions were consistently lower across all clinical subgroups. No significant differences were found in serum 25(OH)D_3, 8-OHdG, Hypoxia Inducible Factor 1 Alpha (HIF-1α), selenium (Se), and tissue Se levels.
Conclusions. *TXNIP* mRNA expression was remarkably lower in advanced stages. VDR and hOGG1 expression were low in all bladder cancer subgroups. These parameters could serve as potential targets for preventing or treating bladder cancer.

Introduction

According to Global Cancer Observatory (GLOBOCAN), bladder cancer ranks among the most common types of cancer globally, with a reported 573,278 new cases in 2020 [1]. Bladder cancer can manifest in various forms, including non-muscle-invasive bladder cancer (NMIBC) characterized by Ta/T1 stages, muscle-invasive bladder cancer (MIBC) progressing from T2 to T4 stages, or metastatic bladder cancer. Each type is characterized by distinct molecular drivers. While the overall recurrence rate for NMIBC varies between 60% and 70%, the rate of progression to a higher stage or grade, and metastasis, ranges from 20% to 30%. [2]. With the help of large-scale gene expression and sequencing studies, our comprehension of bladder cancer biology has improved, and this has led to the development of targeted treatments and immunotherapies that are more effective in clinical settings.

Selenoproteins may play a crucial role in preventing redox imbalance in bladder cancer. They are suggested to contribute to the anticarcinogenic activity through mechanisms involving oxidative stress and redox regulation, encoded by selenium as the 21st amino acid, selenocysteine [3]. In recent years, the thioredoxin system has emerged as a key player in oxidative stress regulation. Comprising thioredoxin (Trx), thioredoxin reductase (TrxR), and nicotinamide adenine dinucleotide phosphate (NADPH), this system is selenium-dependent. A deficiency in selenium (Se) can lead to decreased Trx antioxidant activity, heightened oxidative stress, and reduced apoptosis [4]. Within the thioredoxin family, thioredoxin-interacting protein (*TXNIP*) stands out as a significant player. *TXNIP* exhibits pro-oxidant properties and directly modulates Trx's antioxidant function by binding to its active site, thereby inhibiting its disulfide reductase activity and earning the title of an endogenous inhibitor of Trx [5,6].

Limited epidemiological studies have pointed to the selenium level in plasma or serum as a potential risk factor for bladder cancer. High selenium levels have been associated with a significant reduction in bladder cancer development risk compared to other cancer types (33% lower risk, 95% CI: 3% to 45%) [7]. The role of selenoproteins in bladder cancer remains poorly explored, and their significance in this context is not yet fully understood [8].

The Vitamin D receptor (VDR) is an essential DNA-binding transcription factor responsible for regulating the activity of vitamin D and its metabolic enzymes. According to a study, the *VDR* signaling pathway is essential for the effective induction of cell death by the *TXNIP- ASK1-JNK1* pathway, which was demonstrated using cell lines with VDR-knockout induced by CRISPR/Cas9 or siRNA [9]. DNA damage and oxidative DNA damage are commonly observed in various pathologies, including cancer [10,11].

Measuring *hOGG1* amplification is a useful tool for assessing the risk associated with oxidative DNA damage-repair changes [12]. Additionally, 8-hydroxy-2'-deoxyguanosine (8-OHdG) measurement serves as a direct marker of oxidative DNA damage, commonly employed for this purpose [13].

The aim of this study was to determine the expression levels of the *TXNIP, VDR, hOGG1* genes and to examine the levels of 25(OH)D_3, Trx, TXNIP, Hypoxia Inducible Factor 1 Alpha (HIF-1α), 8-OHdG, and Selenium in bladder cancer patients according to clinical staging.

Materials and methods

The compliance of this study with ethical principles and standards was approved by Kocaeli University, Clinical Research Ethics Committee (KÜ GOAKEK2017 / 1.6).

Sample collection

Patients who presented to Kocaeli University Faculty of Medicine Urology Outpatient Clinic were included in the study. Volunteers aged 18 and older, diagnosed with or suspected of bladder cancer, and individuals undergoing transurethral resection of bladder tumor (TUR-BT) surgery, were included in the study. Individuals diagnosed with other types of cancer were excluded from the study. Urethelial carcinoma tissues of 61 patients and normal tissues from 12 controls in a total of 73 voluntarily were included. Informed consent had obtained from all patients.

Collection of tissue samples

To stabilize and preserve cellular RNA, all of the tissues (cancer and normal) obtained by transurethral resection (TUR-BT) were immediately transferred to the RNA-Later solution (Life Tech., USA) and delivered to the -80°C refrigerator in the laboratory as soon as possible. Tissues were stored at -80oC until the day of the experiment. At the same time, all of the TUR-BT materials were routinely sent to the Department of Pathology for the determination of tumor grades and stages.

Collection of serum samples

Prior to the TUR-BT operation, blood samples were collected from the patients. Additionally, control serum samples were obtained from individuals without bladder cancer. After centrifugation, all serum samples were preserved at -80°C for subsequent utilization in ELISA analysis.

The demographic and pathological information of voluntaries was obtained from the patient files.

Tissue RT-PCR

Bladder cancer patient group is divided into 5 groups. TA-low grade (n = 18), TA-high grade (n = 6), T1-low grade (n = 8), T1-high grade (n = 14), and T2-high grade (n = 15). Total RNA isolation from bladder tissue samples belonging to the patients and control group included in the study was performed with the help of a commercial kit (Thermo Scientific™ GeneJET RNA purification kit, K0732). Concentrations and purities of total RNA samples isolated from the patients and control groups were determined by spectrophotometric (NanoDrop, Thermo Sci., USA) method before cDNA synthesis. At the same time, RNA integrity was checked by agarose gel electrophoresis.

cDNA synthesis was performed with a commercial kit from the isolated total RNA samples (High-Capacity cDNA RT Kit, Applied Biosystems, Catalog Number 43688. *TXNIP* [14], *VDR* [15], *hOGG1* [16], and β-actin genes were analysed using RT-PCR system (Bionem is exicyclertm 96 RT-PCR System, Turkey) shown in **Table 1**. The RT-PCR process for the samples was carried out following optimized protocols, and the mRNA levels of all genes were calculated using the (Light-Cycler Relative Quantitation Software Program), enabling relative quantitative analysis.

Table 1. The specific primer sequences (5'-3') used in the mRNA expression analysis of *Txnip*, *VDR*, *hOGG1*, and β-actin.

	Primer sequences
***TXNIP* (14)**	
Forward	5'- AGATCAGGTCTAAGCAGCAGAACA -3'
Reverse	5'-CCATATAGCAGGGAGGAGCTTC -3';
***VDR* (15)**	
Forward	5'- TGTAGAACATCTTTTGTATCAGGA -3'
Reverse	5'- AATGTAAGAAGCTGTAGCAAT- 3'
***hOGG1* (16)**	
Forward	5'-GGAAGGTGCTTGGGGAAT-3'
Reverse	5'-ACTGTCACTAGTCTCACCAG-3'
β-aktin	
Forward	5'- GACCACACCTTCTACAATGAG -3'
Reverse	5'- GCATACCCCTCGTAGATGGG -3'

Examination of parameters in serum samples by ELISA method

Serum samples obtained from individuals who were in the cancer patients and control groups included in the study were studied by the ELISA method using commercial kits. Trx (Cat No: E-EL-H1727), 8-OHdG (Cat No: E-EL-0028), and HIF-1α (Cat No: E-EL-H1277) levels were measured with ELABSCIENCE USA ELISA kit. TXNIP (Cat No: DZE201122136) was measured with SUNRED Shangai ELISA kit. Vitamin 25(OH)D_3 levels were measured by the chemiluminescence measurement method using the IDS-iSYS 25-Hydroxy

Vitamin D kit (Reference number: IS-2700S), using the IDS-iSYS in vitro diagnostic analyzer (V 4.03, UK).

Determination of Selenium in Tissue and Serum Samples by ICP-OES

The analysis of tissue and serum samples was conducted using ICP-OES (ICAP-6000, Inductively Coupled Plasma Atomic Emission Spectroscopy – iCAP 6000 – Thermo) at the Department of Biophysics, Istanbul University. The ICP-OES emission spectrometer equipped with a plus autosampler was computer-controlled (Thermo Fisher Scientific Inc., Istanbul, Turkey). The ICP-OES system was operated under appropriate conditions, including the selection of suitable wavelengths for each element, such as Se at 196.090 nm. The plasma operating conditions were set at a sample flow rate and elution flow rate of 1.5 L/min, a plasma gas flow rate of 15 L/min, and an argon carrier flow rate of 0.5. The peristaltic pump was set to a speed of 100 rpm [17].

Bioinformatic and Statistical Analyses

The protein-protein interactions and molecular and biological function of the proteins were assessed using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, https://string-db.org) analyse tool. The data underwent statistical analysis using IBM SPSS 20.0 software. Data normality was assessed through Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics were used, presenting numerical variables as mean ± standard deviation or median (25–75%) based on distribution. Group comparisons utilized independent sample t-tests and one-way ANOVA for normally distributed variables, and Mann-Whitney U and Kruskal-Wallis tests for non-normally distributed ones. Multiple comparisons were performed via Tukey, Dunnett, and Dunn tests. Spearman's correlation analysis and binary logistic regression were employed. Significance was set at $p < 0.05$. Statistical power was assessed using G*Power 3.1.9.2 software, showing a power of 0.83 ($\alpha = 0.05$, effect size = 0.676).

Results

The patient group consisted of 61 individuals and was further subdivided into five groups based

Table 2. Descriptive analyses of the control and cancer groups.

	Cancer group	Control group
Patient (n)	61	12
Age (Mean – year)	67.7	64.8
Female (n, %)	4 (6.56%)	2 (16.66%)
Male (n, %)	57 (93.44%)	10 (83.34%)
Tumor Number		
Single focus **(n)**	29	
Many focuses **(n)**	32	
Tumor Size		
≤ 3 cm	23	
≥ 3 cm	38	
Histological Stage		
Ta	25 (12% Female, 88% Male)	
T1	21 (9.52% Female, 90.48% Male)	
T2	15 (6.66% Female, 93.34% Male)	
Histological Grade		
Low grade	25	
High grade	36	
Information of TUR-B		
First time	24	
2 and more than 2	37	
Lenf Node		
Ta – High grade	1	
T2 – High grade	1	

on tumor type and grade: TA low-grade (n = 18), TA high-grade (n = 6), T1 low-grade (n = 8), T1 high-grade (n = 14), and T2 high-grade (n = 15). Additionally, there was a control group of 12 individuals. The mean age of control group was 64.8 (n = 12), mean age of cancer group was 67.7 (n = 61). In the control group, the rate of males was 83.34% (n = 10), while that of females was 16.66% (n = 2). In contrast, in the cancer group, the rate of males was 93.4% (n = 57), and that of females was 6.56% (n = 4). **Table 2** presents the descriptive statistical data of the tumor tissues.

RT-PCR results of tissue samples

Bladder cancer tissue samples were divided into subgroups based on the grade specified in the pathology report, and these subgroups were compared with each other. Additionally, a control group was included, resulting in a total of 6 groups. Compared to the control group, the gene expression levels were significantly higher in the Ta-low grade, Ta-high grade, and T1-low grade groups. Conversely, the expression levels in the T1-high grade and T2-low grade groups were found to be significantly lower than those in the control, Ta-low grade, Ta-high grade, and T1-low grade groups (**Figure 1**).

Moreover, patient subgroups exhibited a statistically significant decrease in *VDR* gene expression levels ($p < 0.05$) (**Figure 2**).

Similarly, a statistically significant decrease was observed in patient subgroups' *hOGG1* gene expression levels ($p < 0.05$) (**Figure 3**).

Bioinformatic analyses

The STRING analyses indicated that two biological pathways predominantly emerged when analysing the *hOGG1, VDR* and *TXNIP*. The results showed that there was a direct relationship related with DNA repair between the *hOGG1 (OGG1)* and *VDR (CYP27B1)* protein. According to the result, the DNA repair mechanism activities were decreased during the carcinogenesis. In contrast, the *TXNIP*, play role in the oxidative stress mechanism, does not directly interact with *hOGG1 and VDR* and the mechanisms related to oxidative stress increase up to the low grade T1 stage during carcinogenesis. But after then, the level of *TXNIP* sharply decreased in the high grade T1 and T2 patients (**Figure 4**).

ELISA of serum samples

Serum samples from 12 control and 61 patient groups were used to measure the levels of TXNIP,

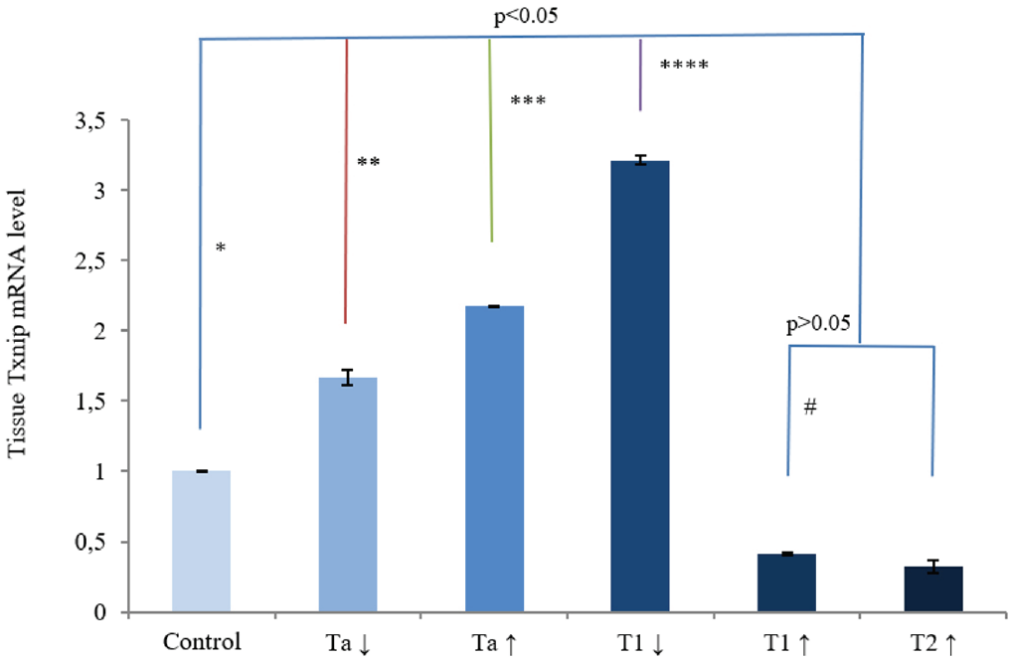

Figure 1. Tissue mRNA levels of *TXNIP gene* expressions according to tumor stage and grade. ↓, Low grade; ↑, High grade. Ta↓, Ta Low grade; Ta↑, Ta High grade; T1↓, T1 Low grade; T1↑, T1 High grade; T2↑, T2 High grade. * Control vs T1↑ and T2↑ ($p < 0.05$); ** Ta↓ vs T1↑ and T2↑ ($p < 0.05$); ***Ta↑ vs T1↑ and T2↑ ($p < 0.05$); ****T1↓ & T1↑ vs T2↑ ($p < 0.05$); # T1↑ vs T2↑ ($p > 0.05$).

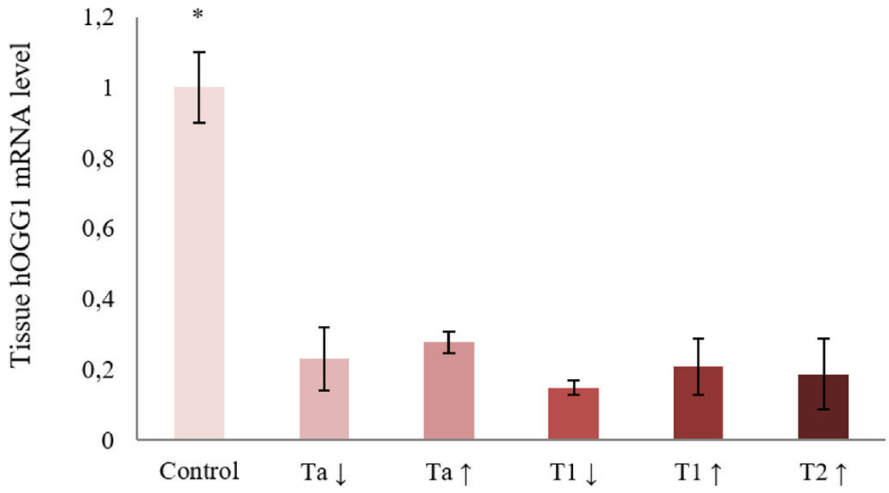

Figure 2. Tissue mRNA levels of *hOGG1 gene* expressions according to tumor stage and grade. ↓, Low grade; ↑, High grade. Ta↓, Ta Low grade; Ta↑, Ta High grade; T1↓, T1 Low grade; T1↑, T1 High grade; T2↑, T2 High grade. *Control vs Ta↓, Ta↑, T1↓, T1↑ and T2↑ ($p < 0.05$).

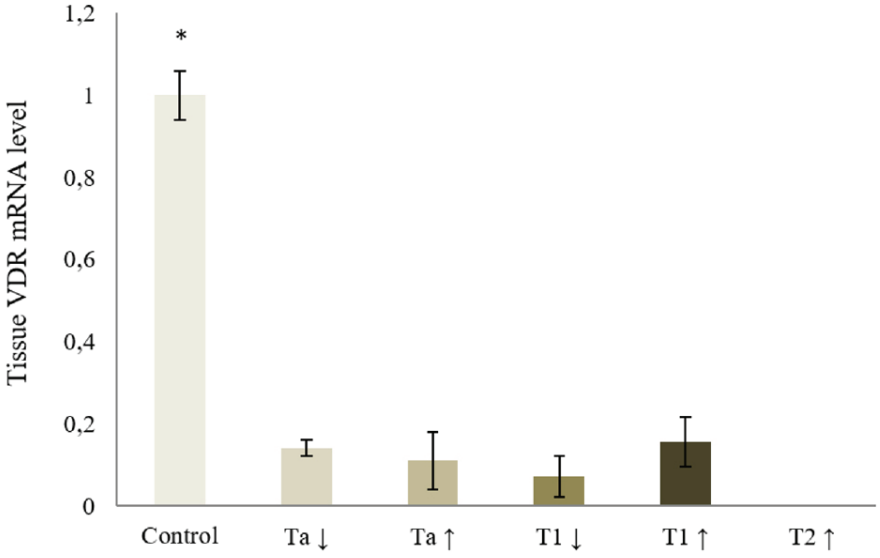

Figure 3. Tissue mRNA levels of *VDR gene* expressions according to tumor stage and grade. ↓, Low grade; ↑, High grade. Ta↓, Ta Low grade; Ta↑, Ta High grade; T1↓, T1 Low grade; T1↑, T1 High grade; T2↑, T2 High grade. *Control vs Ta↓, Ta↑, T1↓, T1↑ and T2↑ ($p < 0.05$).

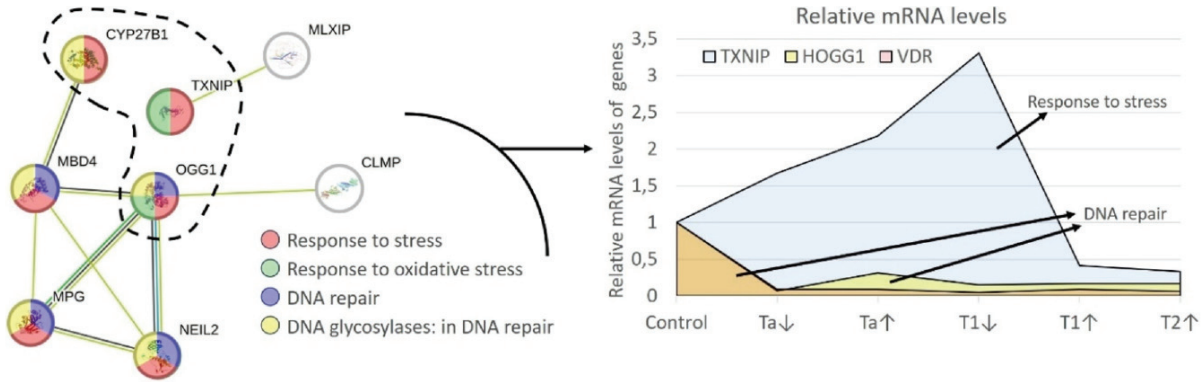

Figure 4. The illustration of the protein network revealed by STRING analysis, along with the mRNA expression of biological pathways. *VDR*; *CYP27B1*, Vitamin D Reseptor, *TXNIP*; Thioredoxin interacting protein, *OGG1*; 8-oxoguanine DNA N-glycosylase 1, MBD4; Methyl-CpG Binding Domain Protein 4, MPG; 3-Methyladenine DNA Glycosylase, NEIL2; Nei-like DNA Glycosylase 2.

Trx, 8-OHdG, HIF-1α, and 25(OH)D_3 using ELISA method (**Table 3**).

The control group had a mean age of 63.5 years (n = 12), while the patient group had a mean age of 67.2 years (n = 61). The control group consisted of 2 (16.7%) females and 10 (83.3%) males, whereas the patient group had 6 (11.11%) females and 48 (88.89%) males.

Table 3. The comparative statistical result of the serum ELISA results for the control and cancer groups.

	Control group (n = 12) Median (25–75 %)	Patient group (n = 61) Median (25–75 %)	p value
TXNIP (ng/mL)	18.45 (16.40–23.64)	14.7 (9.78–20.53)	0.100
Trx (ng/mL)	10.10 (7.45–13.55)	8.53 (3.72–13.75)	0.358
8-OHdG (ng/mL)	113.93 (103.64–120.57)	117.79 (112.45–123.29)	0.720
HIF-1α (ng/mL)	0.52 (0.33–1.16)	0.45 (0.26–0.75)	0.198
25(OH)D_3 (ng/mL)	16.6 (9.0–21.1)	14.70 (9.78–20.53)	0.861

*p < 0.05 significance level, Mann-Whitney U test was used.

Spearman's correlation test revealed that in the TA, G1 cancer group, a moderate positive correlation was observed between TXNIP and HIF-1α (r = 0.438, p = 0.036). In the TA, G3 cancer group, a strong positive correlation was found between 25(OH)D_3 and hif1a (r = 0.829, p = 0.042). In the T1-G3 cancer group, a moderate negative significance was found between HIF-1α and 25(OH)D_3 (r=-0.623, p = 0.03). No significant correlation was observed between the other parameters and subgroups (p ≥ 0.05).

Selenium level of serum and tissue

There were no differences in serum Se levels between both the control and patient groups, as well as among the different patient subgroups (p = 0.45). When tissue Se levels were compared to the control group [mean±SD: 19.042 ± 19.51, median: 11.17 (4.62–29.89 µg/ml)], no significance was found in the patient group [mean±SD: 18.07 ± 45.71, median: 3.86 (1.39–10.58) µg/ml] (p = 0.05). But there was no significant difference among the patient subgroups.

Discussion

Findings suggest that *TXNIP* mRNA gene expression is reduced in bladder cancer [18]. Research has convincingly demonstrated that the lack of *TXNIP* contributes to the progression of murine bladder cancer initiated by BBN in mice lacking *TXNIP*. Knock out TXNIP facilitates CXCR4-induced ERK phosphorylation, promoting bladder carcinogenesis. According to the same study on human bladder cancer tissues, TXNIP expression is suppressed in bladder cancers compared to normal urothelium. This suppression is particularly pronounced in high-grade and/or high-stage (pT1 or higher) cancers and could be inversely correlated to the grade and stage of bladder cancer [19]. A finding substantiated by in vitro experiments [20,21]. The decrease in gene expression in various cancer types has been documented in the literature [18,22]. Although we could not demonstrate a linear decrease between subgroups, we show that TXNIP gene expression is suppressed in the more advanced stages of bladder cancer (T1 and T2) (Figure 1). *TXNIP* levels show an increase up to T1 low-grade bladder cancer, but they significantly decrease in more advanced stages, such as T1 high-grade and T2 high-grade. Although not measured in this study, it has been reported in the literature that this induction leads to reduced Trx levels [23,24]. In recurrent bladder cancer, comprehensive gene expression profile analyses have also revealed that *TXNIP* is suppressed [25]. Bioinformatics analyses indicated that two main biological pathways emerged: stress response and DNA repair mechanisms. It has been shown that TXNIP, which plays a role in the oxidative stress mechanism, does not interact directly with hOGG1 and VDR. Additionally, this analysis revealed a direct relationship related to DNA repair between the hOGG1 (OGG1) and VDR (CYP27B1) proteins. The VDR and hOGG1 genes have been found to be directly associated with the Methyl-CpG Binding Domain Protein 4 (MBD4), 3-Methyladenine DNA Glycosylase (MPG), and Nei-like DNA Glycosylase 2 (NEIL2)

genes. According to these results, the activities of DNA repair mechanisms have decreased during the carcinogenesis process.

Based on the study findings, groups showed a deficiency in vitamin D levels. However, when examining gene expression in various cancer subgroups, the *VDR* gene expression was notably lower in the cancer groups (Figure 1). The T2 high-grade cancer subgroup exhibited the lowest *VDR* gene expression, almost negligible. In immunohistochemically examined bladder cancer cells, reduced VDR expression has clinically significant implications, as it may predict poorer prognosis, evidenced by lower overall survival in the study. VDR expression could serve as a potential prognostic marker in urothelial bladder cancer patients [26]. Given that vitamin D is a positive regulator of VDR expression, we cannot exclude the possibility that VDR levels in bladder cancer cells are related to and/or regulated by local and systemic vitamin D levels. Thus, lower VDR expression may reflect vitamin D deficiency, leading to poor outcomes in these bladder cancer patients [27,28].

The literature has reported that increased levels of circulating vitamin D_3 are associated with a reduced risk of various cancer types, including bladder, breast, colorectal, kidney, and prostate cancer [29]. According to the American Association of Cancer Research (AACR), vitamin D deficiency is common and has been associated with advanced stages of various cancer types. The Clinical Practice Guideline of The Endocrine Society establishes the definitions for vitamin D deficiency, insufficiency, and sufficiency, which rely on serum concentrations of 25(OH)D_3, the most commonly utilized biomarker to assess vitamin D status. According to the guideline, serum 25(OH) D_3 levels below 20 ng/mL are categorized as deficiency, levels ranging from 21 to 29 ng/mL are classified as insufficiency, and levels between 30 and 100 ng/mL are considered sufficient [30]. It has been shown that vitamin D_3 can regulate ROS levels in endometrial cancer cells by increasing TXNIP expression, resulting in the inhibition of human endometrial cancer cell proliferation [31]. In this study, serum vitamin D levels of the control and patient groups were defined as deficiency according to the guidelines. We think that the reason for the deficiency level in the control group is due to seasonal and regional factors.

One study found that the DNA repair enzyme of the Base Excision Repair (BER) pathway, *hOGG1*, which removes oxidatively damaged guanine (8-oxodG) from DNA, was reduced in leukocytes from bladder cancer patients. *OGG1* levels were examined in smokers and non-smokers [32]. It states that low OGG1 activity is associated with high levels of 8-oxoG in cancer types such as lung cancer and head and neck cancers [33–35].

On the other hand, it is noted that OGG1 activity is higher in colorectal cancer patients, which is linked to oxidative stress [36]. Additionally, another study found no significant correlation between OGG1 activity and protein levels in NMIBC tissues, suggesting that OGG1 activity can be influenced by other factors. Therefore, mRNA expression and protein levels may not reliably predict OGG1 activity [37]. It is important to emphasize that DNA repair capacity may decrease due to various factors such as smoking, obesity, etc [38]. Decreased *OGG1* expression is associated with tumor growth and progression because its ability to repair DNA can often contribute to genomic instability. In this study, decrease *hOGG1* expression was observed among all bladder cancer subgroups. We did not evaluate the risk factors in the study. Although the gene expression levels in the bladder tissue were found to be significantly low compared the control group, no difference was found in the serum levels of 8-OHdG between the control and patient group.

The level of selenium in the body has a significant impact on the growth and proliferation of cells, their mobility, development, and survival. Additionally, it is related to intercellular interactions and the redox regulation of intracellular signaling cascades involved in inflammation and apoptosis. TXNIP inactivates the anti-oxidative function of TRX by binding to the redox-active cysteine residues, causing TRX to be reversibly reduced via the actions of TRX reductase and NADPH [4]. In this context, selenium intake is necessary for the normal activity of selenoproteins, particularly GPX and TRXR, which play a role in redox regulation [39]. While the complete anticancer mechanism of Se remains unclear, it shows potential for involvement in various stages of the carcinogenic pathway [40].Although the meta-analysis of seven epidemiological studies, which included 1910 cases and 17,339 controls/

cohort members and examined the relationship between plasma or serum Se levels and bladder cancer risk, showed an inverse association between bladder cancer risk and Se levels, in this study, no significant difference was found between tissue and serum Se levels [41].

Hypoxic conditions influence factors such as angiogenesis and treatment resistance by increasing the expression of proteins, particularly HIF-1α, which are important in tumor adaptation [42,43]. In a study, it was found that the serum level of HIF-1α in the case group was significantly higher compared to the control group. When serum HIF-1α levels were compared with certain factors, such as the primary tumor, lymph node, and metastasis stages, they were found to be higher. Although serum HIF-1α was also observed to be higher in advanced urethelial bladder cancer grades, this difference was not statistically significant [44]. Similarly, in our study, no significant difference was found between the groups. When expression levels in tissue were examined, significant results were also reached in the literature [45].

Conclusion

This study highlights significant decreases in the expression of *TXNIP, VDR*, and *hOGG1* genes in bladder cancer patients, revealing their critical roles in tumor biology. Knowing the levels of Se, HIF-1α, and vitamin D contributes to the assessment of oxidative stress and cellular survival mechanisms. TXNIP is a thioredoxin-binding protein that plays an important role in regulating oxidative stress, inhibiting cell proliferation, and inducing apoptosis. Its expression is generally diminished in tumors, suggesting a tumor-suppressive function in various cancers, including bladder cancer, which has received less attention in the literature. The reduced expression of these genes is associated with the invasion of bladder cancer cells into deeper layers of the muscularis propria and the acquisition of metastatic potential. Additionally, the activity of BER proteins may serve as valuable biomarkers for prognosis, progression, and response to genotoxic therapies. Therefore, targeting *TXNIP, VDR*, and *hOGG1* is essential for improving the diagnosis and treatment of malignant tumors, particularly in bladder cancer, as it is crucial to better understand their roles and validate their potential as therapeutic targets.

Limitations

This study was conducted within a limited timeframe, which restricted the number of bladder cancer subgroups to achieve parity according to pathological results. A more detailed examination of protein levels could have been conducted.

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Conflict of interest statement
The authors declare no conflict of interest.

Author contribution
EA and MOD designed the experiments and collected data and draft manuscript preparation, visualization; EA, MS, HY, MY, FA and AME performed experiments and analysis and interpretation of Results; MOD and OD discussed critical revision or editing of the article; AME Supervised; EA, MOD, MS, HY, MY, FA, AME and OD Final approved of the version to be published.

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Ethical compliance
The compliance of this study with ethical principles and standards was approved by Kocaeli University, School of Medicine, Clinical Research Ethics Committee (KÜ GOAKEK2017 / 1.6). All participants included in this study provided their informed consent.

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