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Contents

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Review

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The Importance of STARD3 and Lipid Metabolism in Prostate Cancer

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Abstract

Prostate cancer is common in men and continues to pose a significant medical challenge. The primary feature of prostate cancer is hormone sensitivity. Prostate cancer cells become dependent on testicular androgen for survival. Steroidogenesis involves the synthesis of steroid hormones such as androgens from cholesterol. Tumor cells increase fatty acid production with androgens. Synthesized fatty acids contribute to membrane formation and support oncogenic signaling. Hormone-dependent tumors require cholesterol for proliferation. Cancer cells have higher intracellular cholesterol levels than non-tumor cells. STARD3, a member of the START protein family, is a transmembrane protein that facilitates cholesterol transport and resides in late endosomes. STARD3 stimulates steroidogenesis by inducing the motion of lysosomal cholesterol into mitochondria. High mitochondrial cholesterol levels can prevent apoptotic cell death in different cancer types, thereby triggering tumor progression. This review discusses recent studies on the relationship between cholesterol levels and prostate cancer risk, as well as the properties and activity of STARD3. To the best of our knowledge, this study is the first to comprehensively summarize the role and therapeutic potential of STARD3 in prostate cancer.

Keywords: Cancer, lipid, prostate, STARD3

Introduction

Prostate cancer is among the most prevalent malignancies affecting men worldwide. Approximately 1,6 million new cases and 366,000 deaths are reported annually (1). The majority of prostate cancers are identified through prostate-specific antigen (PSA) screening, subsequently confirmed by diagnostic biopsy, and further evaluated using imaging techniques to assess potential metastatic dissemination. Patients who cannot be treated with surgery or radiation are treated with drugs targeting the androgen receptor (AR), which is the main causative agent of prostate cancer, and androgen deprivation therapy. PSA testing was repeated after treatment to evaluate disease recurrence. However, only 25% of men with a PSA level >4.0 ng/mL are diagnosed with prostate cancer during biopsy. False negatives are also common (2). Despite recent advances, there are important medical problems in men due to the overtreatment of cancer and the undertreatment of metastatic prostate cancer (3).

The primary feature of prostate cancer is hormone sensitivity. Because the prostate cannot produce testosterone, prostate cancer cells become dependent on testicular androgens for survival. In steroidogenesis, steroid hormones such as androgens are synthesized from cholesterol (4). Androgens in prostate cells; promote proliferation, lipogenic enzyme expression, and differentiation but prevent apoptosis (5). Given the relationship between prostate cancer and androgens, tumor cells increase fatty acid production. Synthesized fatty acids contribute to membrane formation and support oncogenic signaling. Zadra et al. (6) reported that the simultaneous blockade of lipogenesis and AR signaling strongly reduced prostate cancer growth.

Several studies have established a relationship between dietary lipids and prostate cancer. Tamura et al. (7) demonstrated that prostate cancer cells utilize saturated very long-chain fatty acids as substrates for membrane synthesis and androgen production, thereby promoting tumor growth. Epidemiological data and preclinical animal studies further suggest that dietary

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fat intake is involved in the initiation and progression of prostate cancer (8). The presence of lipids in cancer cells supports the growth, spread, and survival of cancer. As tumors break down or metastasize, new lipid synthesis is triggered to prepare the tumors for future environmental conditions. Thus, the cycle of lipid synthesis and degradation increases tumor survival. Treatments targeting lipid synthesis and oxidation block AR synthesis inhibit prostate cancer growth (9).

Lipid and Prostate Cancer

Cholesterol is a fat-like substance necessary for cell membrane formation and hormone production. Intracellular cholesterol homeostasis is tightly regulated. This homeostasis is determined by the rate of cholesterol synthesis in the liver, its uptake, conversion to steroid hormones, and the rate at which it is excreted from the body as bile. Circulating cholesterol is transported by lipoproteins, which are primarily classified into two main categories: low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Elevated levels of cholesterol, LDL, and low HDL, contribute to cancer progression by increasing inflammation, proliferation, intratumoral steroidogenesis, and altering membrane lipid structure (10,11). LDL ensures the storage of cholesterol in cells that require repair as well as the release of cholesterol and triglycerides for metabolism (12). HDL, on the other hand, acts to remove cellular cholesterol and exerts antioxidant and anti-inflammatory effects. With these effects, HDL can reduce oxidative stress, inflammation, and cholesterol content in tumor cells, thereby affecting cancer cell proliferation (13). Cancer cells can synthesize cholesterol or take it up as LDL. Hormone-dependent tumors, such as prostate cancer, require cholesterol for proliferation. Cholesterol plays a precursor role in the production of androgens, which affect prostate cancer growth (14).

Several studies have investigated the relationship between cholesterol levels and prostate cancer risk. Some studies have suggested that high LDL-C levels contribute to the development and progression of prostate cancer, whereas high HDL-C levels may have a protective effect. In their study, Ossoli et al. (13) HDL was shown to inhibit cell proliferation induced by LDL by reducing cholesterol content in prostate cancer cell lines. After monitoring 53,944 men and 24,475 women diagnosed with cancer, elevated ≥240 mg/dL total cholesterol levels were positively associated with an increased risk of prostate and colon cancer in men, as well as breast cancer in women, compared with levels below 160 mg/dL (15). Jamnagerwalla et al. (16) demonstrated that elevated total serum cholesterol was correlated with an increased risk of high-grade prostate cancer. In contrast, no significant association was observed between serum LDL levels and the risk of overall, low-grade, or high-grade prostate cancer. Interestingly, elevated serum HDL levels were linked to a higher risk of both overall and high-grade prostate cancer (16). In prostate cancer patients with a Gleason score of 8-10, men with low levels of cholesterol have a lower risk of prostate cancer than men with high cholesterol (17). In a study analyzing 438 prostate cancer cases, it was observed that men with cholesterol levels below 240 mg/dL had a reduced risk of developing high-grade prostate cancer compared with those with cholesterol levels exceeding 240 mg/dL (18). Farwell et al. (19) identified a significant correlation between prostate cancer risk and total serum cholesterol, reporting a 45% increase in the overall risk of prostate cancer and a 204% increase in the risk of high-grade prostate cancer. Similarly, another research group found a positive association between cholesterol levels and the incidence of high-grade prostate cancer (Gleason score ≥8) in a cohort of 650 men diagnosed with the disease (20).

Collectively, these findings indicate that elevated cholesterol levels are associated with an increased risk of prostate cancer. Accordingly, statins, which inhibit cholesterol biosynthesis, reduce serum cholesterol levels, and suppress prostate cancer cell proliferation, have been linked to decreased cancer progression (21). Although the correlation between cholesterol levels and prostate cancer remains a topic of ongoing research, it is clear that cholesterol, especially HDL and LDL, plays a role in affecting prostate health. Maintaining a healthy lifestyle and managing cholesterol levels can help reduce the risk of prostate cancer. As research continues, a deeper understanding of these connections will pave the way for future targeted preventive strategies and interventions.

STARD3 and Prostate Cancer

STARD3 is one of the 15 members of the StART family. STARD3 acts as a lipid transfer protein that directs sterols to endosomes and regulates cholesterol accumulation. The overexpression of STARD3 also affects cholesterol trafficking, leading to increased mitochondrial cholesterol content. Some cancers are associated with the misuse of cholesterol derived from late endosomes (LE) or lysosomes. Cancer cells have higher intracellular cholesterol levels than non-tumor cells. Cholesterol accumulation contributes to the formation of membrane micro-domains rich in sphingolipids, stimulating the progression and migration of cancer cells. STARD3 is an important candidate for cancer therapy, and the identification of selective STARD3 inhibitors represents an interesting yet undiscovered area of research (22).

START proteins represent a family of lipid transfer proteins (LTPs) implicated in diverse cellular functions, including non-vesicular cholesterol transport. This family comprises 15 members categorized into six subfamilies based on their amino acid sequences and ligand-binding characteristics. STARD3 is distinguished by its C-terminal START domain, central FFAT domain, and N-terminal MENTAL domain, which collectively facilitate lipid trafficking. Through its FFAT motif, STARD3 interacts with VAP-A and VAP-B (vesicle-associated membrane protein-associated proteins) localized in the endoplasmic reticulum (ER), thereby establishing membrane contact sites between the ER and other organelles. Additionally, STARD3 acts as a LTP that directs sterols to endosomes and promotes membrane formation inside endosomes. In other words, it regulates cholesterol accumulation in endosomes and mediates distribution between organelles (23-25). High STARD3 expression increases the accumulation of free sterols in the endosomal region (26) (Figure 1, Table 1).

Cholesterol contributes to the tight packing of membrane lipids, resulting in membranes with reduced water permeability and increased thickness due to the straightening of lipid chains. Cholesterol is predominantly synthesized in the ER and

subsequently distributed to other cellular membranes, including the mitochondria, where it undergoes irreversible conversion into bile acids or steroid hormones. After entering the limiting membranes of LE and lysosomes, LDL cholesterol is transported either to the plasma membrane via vesicular recycling or to the ER through non-vesicular mechanisms. Certain cancers are associated with dysregulation of cholesterol trafficking originating from LE or lysosomes. The cancer-associated LTP STARD3 is frequently localized in proliferative regions. STARD3 contains transmembrane domains that anchor it to endosomal membranes, mediating lipid transfer between endosomes and the ER. Furthermore, STARD3 overexpression disrupts cholesterol trafficking, leading to elevated mitochondrial cholesterol levels (27).

The prostate, an androgen-regulated organ, is one of the most common sites of malignancy in men. Androgens in prostate cells stimulate cell proliferation and differentiation while inhibiting apoptosis (28). The MLN64 protein, which is encoded from chromosome 17, contains a C-terminal START domain homologous to the StAR protein. The START domain harbors a hydrophobic tunnel that can bind a single cholesterol molecule, a feature crucial for regulating steroid biosynthesis (29). MLN64 increases steroidogenesis and regulates the transfer and conversion of cholesterol into pregnenolone (30).

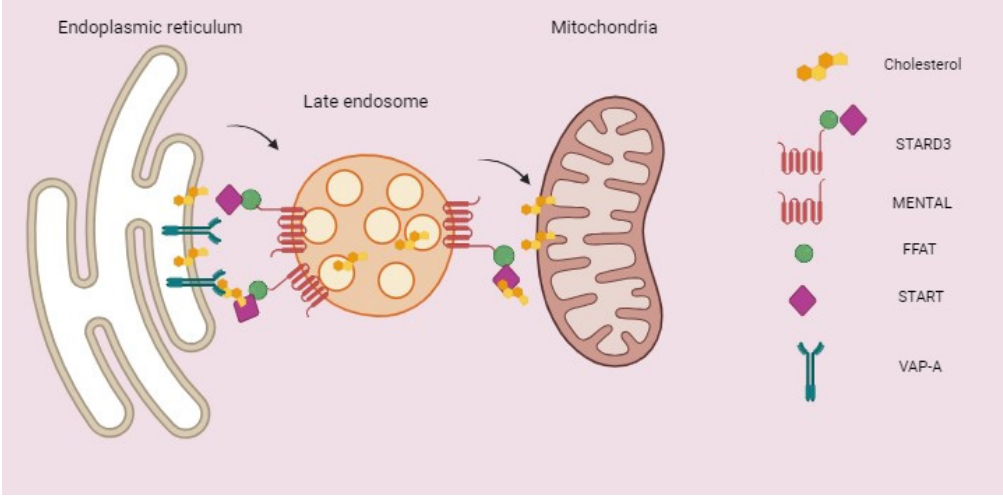

Figure 1. Cholesterol transport from the endoplasmic reticulum to late endosomes and mitochondria via STARD3

Table 1. Summary of the changes in the STARD3 protein and related molecules in diseases

No	Name	Disease	Function	Description	Year	Reference
1.	STARD1	Niemann-Pick type C	Cholesterol transport	The reexpression of ACDase in fibroblasts from patients with NPC disease lowers STARD1 expression and causes a decrease in mitochondrial cholesterol accumulation.	2021	(24)
2.	STARD3	Chronic obstructive pulmonary disease	Cholesterol transport	External cholesterol altered airway epithelial sensitivity of inflammation in response to cigarette smoke extract, through the regulation of STARD3-MFN2 pathway.	2022	(25)
3.	STARD3	HeLa cells	Cholesterol accumulation in endosomes	STARD3 is a cholesterol transporter scaffolding ER-endosome contacts and modulating cellular cholesterol repartition by delivering cholesterol to endosomes.	2017	(26)
4.	STARD3	Breast cancer	Over-expression of STARD3	The homology between the C-terminal part of STARD3 and the functional StAR domain (SHD) suggests that STARD3 and StAR may both play a role in steroidogenesis.	1997	(30)
5.	STARD3	Cell culture	Cholesterol transport	STARD3 participates in mobilization and utilization of lysosomal cholesterol by virtue of the START domain's role in cholesterol transport.	2002	(32)
6.	StAR	Cell culture	The ability of STARD3 to stimulate steroidogenesis	STARD3 stimulates steroidogenesis by virtue of its homology to StAR.	1997	(33)
7.	STARD3	Niemann-Pick type C	Mitochondrial cholesterol transport	A transport pathway for endosomal cholesterol to mitochondria requires STARD3 and that may be responsible for increased mitochondrial cholesterol in NPC disease.	2010	(34)

Table 1. Continued

No	Name	Disease	Function	Description	Year	Reference
8.	STARD3	Niemann-Pick type C	Over-expression of STARD3	STARD3 expression is increased in NPC cells and plays a key role in cholesterol transport into the mitochondria.	2017	(35)
9.	StAR	Hepatocellular carcinoma	Mitochondrial cholesterol transport	StAR silencing by siRNA resulted in the net decrease of mitochondrial cholesterol levels.	2008	(36)
10.	STARD3	Breast cancer	Over-expression of STARD3	StARD3 over-expression may contribute to breast cancer aggressiveness by increasing membrane cholesterol and enhancing oncogenic signaling.	2015	(37)
12.	STARD3	Prostate cancer	Over-expression of STARD3	STARD3 expression seems to be correlated with high stage, high Gleason score and short relapse-free time in the prostate cancer patients	2007	(38)

Hormone-dependent tumors, such as prostate cancer, induce intratumoral steroidogenesis independently of circulating androgen. Cytochrome P450c17 plays a role in androgen biosynthesis, acting as both 17 α-hydroxylase and 17,20-lyase in steroid biosynthesis (28). The precursors of potent androgens facilitate the enzymatic conversion of progesterone and pregnenolone into the androgens androstenedione and dehydroepiandrosterone, respectively (31). The expression of the CYP17 and MLN64 genes, which encode key enzymes involved in androgen biosynthesis, is known to be upregulated in neoplastic tissues. Elevated MLN64 and CYP17 activity in prostate cancer tissues stimulates steroidogenesis by facilitating continuous cholesterol transport to mitochondria and enhances androgen production via the catalytic function of cytochrome CYP17. Prostate cancer is strongly affected by steroid hormones, and the regulation of these genes can significantly reduce the risk of developing cancer (28).

STARD3 is a transmembrane protein that plays a crucial role in cholesterol transport and is predominantly localized in LE. Additionally, STARD3 promotes steroidogenesis by facilitating the transfer of lysosomal cholesterol to mitochondria (32-35). Several studies have shown that high mitochondrial cholesterol levels can inhibit apoptotic cell death in different types of cancer, thereby triggering tumor progression (36,37). The overexpression of STARD3 in cancer potentially stimulates independent steroidogenesis, favoring the development of hormone-dependent cancers, such as prostate cancer. Elevated STARD3 levels in prostate cancer patients are associated with metastasis, local recurrence, and reduced overall survival. Consequently, STARD3 is a potential oncogene, for which the first inhibitor has recently been identified (22). In prostate cancer, a linear relationship has been identified between the expression of STARD3 and CYP17, which are integral to the steroid biosynthesis pathway. The co-expression of STARD3 and CYP17 in prostate cancer promotes steroidogenesis by facilitating continuous cholesterol transfer to mitochondria and enhancing androgen biosynthesis via the catalytic activity of cytochrome CYP17. Consequently, dysregulated STARD3 and CYP17 expression is linked to poor prognosis in prostate cancer patients (38). Although the precise molecular mechanism remains unclear, these findings imply that elevated STARD3 expression contributes to membrane cholesterol accumulation, potentially driving increased cancer aggressiveness (22). The application of the STARD3 inhibitor VS1 in breast and colon cancer cell lines has been shown to specifically target STARD3 and trigger its degradation (39). Investigations in this area are expected to advance the development of STARD3-targeted in silico approaches. Furthermore, the design of anti-STARD3 therapies has significant potential as a therapeutic strategy against cancer.

Conclusion

Some cancers are linked to the misuse of cholesterol derived from LE or lysosomes. Cancer cells exhibit higher intracellular cholesterol levels than non-tumor cells. This cholesterol accumulation contributes to the formation of sphingolipid-enriched membrane microdomains, which promote cancer cell proliferation, progression, and migration. StAR-associated MLN64 and CYP17 in prostate cancer tissue increase the activation of steroidogenesis and androgen biosynthesis through cholesterol transfer to the mitochondria. Prostate cancer is strongly affected by steroid hormones, and it is considered that regulating these genes can significantly reduce the risk of developing cancer. Within this context, it remains to be determined whether STARD3 alone or in combination with AR can trigger progression to prostate cancer. As a cholesterol-specific START protein, STARD3 is considered a molecular target for therapeutic interventions aimed at modulating the lipid metabolism of neoplastic cells by either correcting its dysregulated function or silencing its expression in cancer cells.

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Original Article

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The Prognostic Importance of Expressions of FBLN2 and Microsatellit Instability (MSH2, MSH6, MLH1, PMS2) Immunhistochemical Biomarkers in Upper Urinary Tract Urothelial Cell Carcinomas

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Abstract

Objective: In our study, we aimed to investigate the effect of fibulin 2 (FBLN2) expression, which is one of the extracellular matrix components, and microsatellite instability (MSI) status on the prognosis of the disease in the tumor tissues of patients with upper-tract urothelial carcinoma and to determine the possibility of predicting metastasis and recurrence during follow-up.

Materials and Methods: Fifty five patients who underwent radical nephroureterectomy in our clinic between 2008 and 2021 and whose data we could access were included in the study. The materials of our patients were accessed from the pathology archive of our institution, and materials containing cancer tissue were prepared. FBLN2 and MSI immunohistochemistry staining was performed. FBLN2 staining was reported according to severity and extent. FBLN2 staining intensity was categorized as unstained or stained. The extent of FBLN2 was grouped as 0-50% staining beneath the tumor area and 50% and above staining. MSI, on the other hand, was categorized into 2 groups of protein loss or not.

Results: No significant correlation was found between the severity and prevalence of FBLN2 and the clinicopathological data of the patients by statistical analysis. Additionally, no statistically significant correlation was found between FBLN2 expression and prevalence and both disease-free and overall survival. Although some significant results were obtained in the MSI analysis, we considered it insignificant due to the small number of patients.

Conclusion: Prospective, multicentre, randomized studies with a large number of patients are required to achieve more significant results.

Keywords: Urothelial carcinoma, FBLN2 (fibulin 2), MSI (microsatellite instability)

Introduction

Urinary system-transforming epithelial tumors are among the malignancies that are seen at a fourth frequency in men and an eighth frequency in women worldwide, and their frequency is gradually increasing. Although 95% of these tumors originate from the bladder, approximately 5% originate from the upper urinary system, and standard treatment requires surgical treatments, such as radical nephroureterectomy (RNU) (1). Although new modalities such as endoscopic or kidney-sparing local treatments, neoadjuvant chemotherapy protocols, and lymph node dissection have been tried to contribute to survival, particularly in the last 20 years, in low-risk tumors (2), 5-year survival is below 50% in pT2/pT3 patients and below 10% in pT4 patients (3). All of these factors make it mandatory to thoroughly perform risk assessments for upper-tract urothelial carcinomas (UTUC) to contribute to prognosis.

Pathological features are mostly used to evaluate the prognosis of UTUCs, and even cancer-specific survival is predicted using various nomograms. Recent studies have shown that the extracellular matrix (ECM) also plays an important role in tumor development. The ECM is a complex network of macromolecules such as fibrous proteins, proteoglycans, glycosaminoglycans and glycoproteins with different properties. FBLN2, which is controlled by fibulin 2 (FBLN2) gene located in the ECM and basement membranes and located in chromosome 3p25.1, plays

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a role in the stabilization of the matrix by forming homodimeric complexes (4). Recent studies have suggested that FBLN2 plays a role as a precancerous factor in lung and pancreatic cancers and as a tumor suppressor in breast cancer and nasopharyngeal carcinoma (5-8). Although there are very few studies on the role of FBLN2 in urothelial carcinoma, FBLN2 overexpression in tumor tissue is thought to be related to tumor invasiveness and metastaticity (9).

In addition, microsatellite instability (MSI) biomarkers (MSH2, MSH6, MLH1, PMS2) are known to have prognostic significance in many tumors (colorectal cancers and endometrial cancers) and are now routinely used (10,11). Although it has been observed that the MSI status in tumor tissue has prognostic significance for urothelial cancers in the studies conducted in this regard, it is emphasized that more studies are needed for their routine use (12).

The aim of this study was to retrospectively investigate the effects of the immunohistochemical expression of FBLN2 and microsatellite instability (MSH2, MSH6, MLH1, PMS2) biomarkers on the prognosis of UTUC in pathological archive materials from patients who underwent RNU due to UTUC.

Materials and Methods

This study was approved by decision number 314 dated March 24, 2021, from the Gazi University clinical research ethics committee and the Gazi University Scientific Research Projects Coordination Unit supported by project number TTU-2021-7269.

From July 2008 to July 2021, 105 patients who underwent RNU due to UTUC at our clinic were screened. A total of 55 patients with available clinical and pathological data were included in the study. Patients were retrospectively scanned using the patient information management system and oncology files kept for each patient in our department during the operation period. Demographic, clinical, and pathological data were collected on SPSS file. Paraffin blocks were obtained from the pathology archive of the patients included in the study, and samples were prepared and stained by the Department of Pathology (Figures 1,2). Then, the samples were examined by a single pathologist that was a faculty member of the Department of Pathology, and the results were listed in a file. The data were coded and collected in SPSS files.

Statistical Analysis

The relationships between FBLN2 expression and various clinicopathological features were evaluated using Pearson's chi-square test and Mann-Whitney U test. As endpoints, disease-free and overall survival times were evaluated. Kaplan-Meier test. The SPSS Statistics V.20.0 (Statistical package for the Social Sciences) software was used for statistical analyses. Statistical significance was determined as $p<0.05$.

Results

Of the 55 patients included in the study, 41 (74.5%) were male and 14 (25.5%) were female, with a median age of 65.0 (37.0-83.0) years. None of the patients received chemotherapy or radiotherapy before surgery. The materials prepared from paraffin blocks obtained from the pathology archive were stained with FBLN2 and MSI antibodies and evaluated by a pathology department pathologist. Patients were grouped as no expression and with expression. 32 (58.2%) patients showed FBLN2 expression, whereas 23 (41.8%) patients did not show FBLN2 expression. The prevalence of FBLN2 was expressed as percentages. In the statistical analysis, those stained 50% (including unstained) and those stained 50% were evaluated in 2 groups. No significant correlation was found in the comparative analysis of FBLN2 staining intensity and the clinicopathological data of the patients (Table 1). When analyzed only according to patient age, FBLN2 was expressed at higher ages ($p<0.009$). The median age of patients with FBLN2 expression was 69.0 years (49.0-83.0), whereas the median age of patients without expression was 63.0 years (37.0-72.0). The comparative analysis of the prevalence of FBLN2 staining in tumor tissues with clinicopathological variables revealed no significant correlation (Table 2). As in the case of FBLN2 staining intensity, only the age variable showed an increase in the staining prevalence with increasing age. The median age of patients with an FBLN2 prevalence of 0 and below 50% was 63.0 (37.0-74.0), whereas the median age of patients with a prevalence of 50% and above was 71.0 (49.0-83.0) ($p<0.003$).

The Kaplan-Meier method and log-rank test were used to analyze the effect of both FBLN2 staining intensity and FBLN2 prevalence on disease-free and overall survival, and no significant relationship was found. In the present study, MMR protein

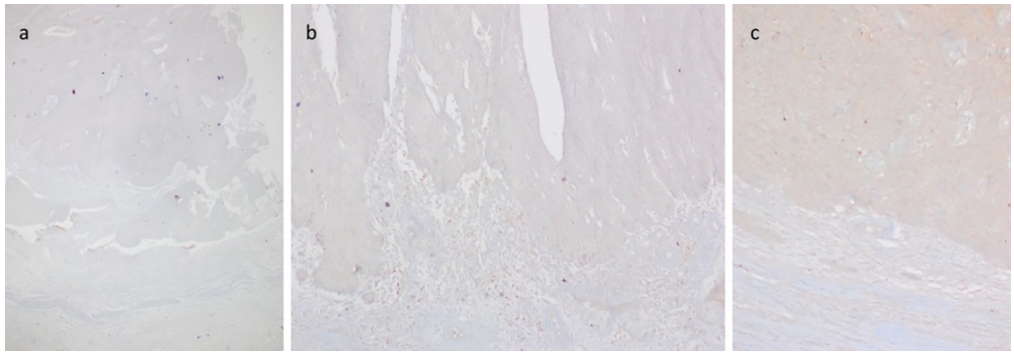

Figure 1. Fibulin 2 expression in tumor tissues

a. No fibulin 2 expression was observed, (score 0). b. Fibulin 2 expression was observed in tumor cells (score 1). c. Severe fibulin 2 expression was observed in tumor cells (Gazi University Faculty of Medicine, Department of Pathology)

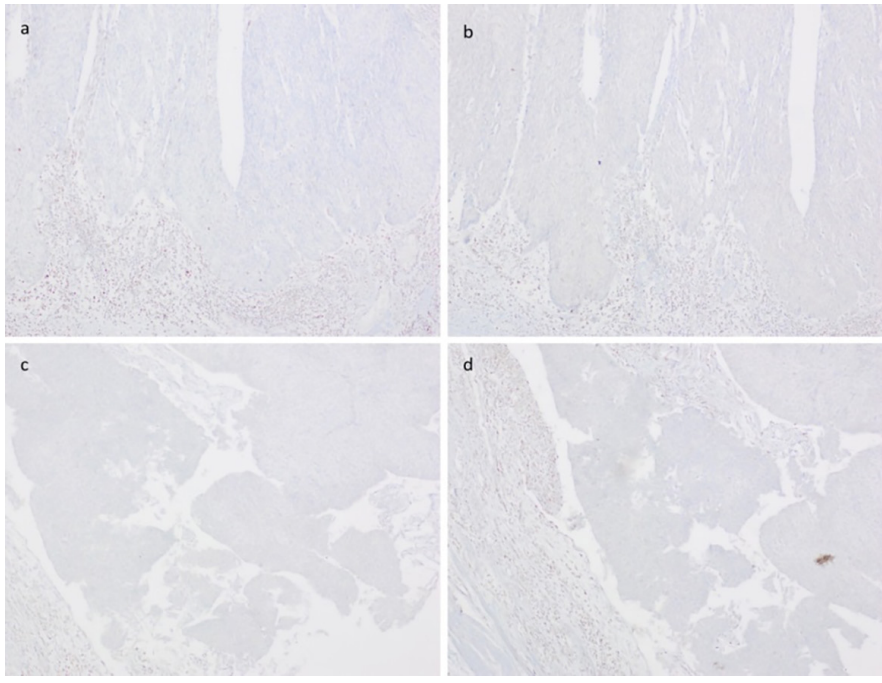

Figure 2. Microsatellite instability protein expression loss in tumor tissues

a. Loss of MLH1 in tumor cells. Nuclear expression was observed in lymphocytes around tumor cells but not in tumor cells. b. Loss of PMS2 in the same case. c. Loss of MSH2 in tumor cells. d. MSH6 loss in the same case

(Gazi University Faculty of Medicine, Department of Pathology)

Table 1. Relationship of FBLN2 expression with variables

	No expression 0 (n=23) (41.8%)	**Has expression 1 (n=32) (58.2%)**	**Total (n=55)**	**p-value**
Gender				
Female	5 (21.7%)	9 (28.1%)	14 (25.5%)	0.592
Male	18 (78.3%)	23 (71.9%)	41 (74.5%)	
Age				
Median (range)	63.0 (37.0-72.0)	69.0 (49.0-83.0)	65.0 (37.0-83.0)	0.009
Tumor location				
Renal pelvis	12 (52.2%)	22 (68.8%)	34 (61.8%)	0.374
Ureter	7 (30.4%)	5 (15.6%)	12 (21.8%)	
Pelvis + ureter	4 (17.4%)	5 (15.6%)	9 (16.4%)	
Multifocality				
Only	17 (73.9%)	27 (84.4%)	44 (80.0%)	0.339
Multiple	6 (26.1%)	5 (15.6%)	11 (20.0%)	
Tumor pathology				
Ta	9 (39.1%)	7 (21.9%)	16 (29.1%)	0.285
T1	3 (13.0%)	3 (9.4%)	6 (10.9%)	
T2-4	11 (47.8%)	22 (68.8%)	33 (60.0%)	
Tumor grade				
Low	13 (56.5%)	10 (31.2%)	23 (41.8%)	0.061
High	10 (43.5%)	22 (68.8%)	32 (58.2%)	
LVI				
No	18 (78.3%)	22 (68.8%)	40 (72.7%)	0.435
Yes	5 (21.7%)	10 (31.2%)	15 (27.3%)	

Table 1. Continued				
	No expression 0 (n=23) (41.8%)	**Has expression 1 (n=32) (58.2%)**	**Total (n=55)**	**p-value**
PNI				
No	19 (82.6%)	24 (75.0%)	43 (78.2%)	0.500
Yes	4 (17.4%)	8 (25.0%)	12 (21.8%)	
Tumor size cm				
Median (range)	3.5 (1.5-12.5) cm	5.4 (1.5-22) cm	5.0 (1.5-22.0) cm	0.191
Nodal metastasis				
No	21 (91.3%)	26 (81.2%)	47 (85.5%)	0.297
Yes	2 (8.7%)	6 (18.8%)	8 (14.5%)	

Table 2. Relationship of FBLN2 prevalence to variables				
	Stained below 50% 1 (n=28) (50.9%)	**Stained 50% and above 2 (n=27) (49.1%)**	**Total (n=55)**	**p-value**
Gender				
Female	5 (17.9%)	9 (33.3%)	14 (25.5%)	0.188
Male	23 (82.1%)	18 (66.7%)	41 (74.5%)	
Age				
Median (range)	63.0 (37.0-74.0)	71.0 (49.0-83.0)	65.0 (37.0-83.0)	0.003
Tumor location				
Renal pelvis	14 (50.0%)	20 (74.1%)	34 (61.8%)	0.185
Ureter	8 (28.6%)	4 (14.8%)	12 (21.8%)	
Pelvis + ureter	6 (21.4%)	3 (11.1%)	9 (16.4%)	
Multifocality				
Only	20 (71.4%)	24 (88.9%)	44 (80.0%)	0.106
Multiple	8 (28.6%)	3 (11.1%)	11 (20.0%)	
Tumor pathology				
Ta	9 (32.1%)	7 (25.9%)	16 (29.1%)	0.557
T1	4 (14.3%)	2 (7.4%)	6 (10.9%)	
T2-4	15 (53.6%)	18 (66.7%)	33 (60.0%)	
Tumor grade				
Low	14 (50.0%)	9 (33.3%)	23 (41.8%)	0.210
High	14 (50.0%)	18 (66.7%)	32 (58.2%)	
LVI				
No	22 (78.6%)	18 (66.7%)	40 (72.7%)	0.322
Yes	6 (21.4%)	9 (33.3%)	15 (27.3%)	
PNI				
No	23 (82.1%)	20 (74.1%)	43 (78.2%)	0.469
Yes	5 (17.9%)	7 (25.9%)	12 (21.8%)	
Tumor size cm				
Median (range)	5.15 (1.5-15)	5.0 (1.9-22)	5.0 (1.5-22.0)	0.433
Nodal metastasis				
No	25 (89.3%)	22 (81.5%)	47 (85.5%)	0.412
Yes	3 (10.7%)	5 (18.5%)	8 (14.5%)	

PNI: Perineural invasion, LVI: Lymphovascular invasion

expression loss was observed in only 3 (5.4%) of the 55 patients. Loss of MLH1/PMS2 protein pair was observed in two patients. In another patient, loss of the MSH2/MSH6 protein pair was observed. Because protein expression loss was observed in only three patients, although some results were significant in the analysis of the relationship between the clinicopathological data of the patients and protein expression loss, these results were not considered significant due to the small number of patients.

Discussion

Upper urinary tract urothelial cell carcinomas constitute 5% of all urothelial carcinomas and have a 5-year survival rate of 50% for pTa and 10% for pT2/pT3 (1,3,13). Although UTUC has a low prognosis, studies assessing its prognosis are extremely limited in the literature. Clinically, patient age, tumor shape, cytology positivity, biopsy tumor grade and presence of hydronephrosis, and pathologically, tumor stage, tumor grade, presence of CIS, presence of LVI and presence of LNI are the most important prognostic predictive factors, but the search for new biomarkers continues (14). In addition, there is not much information about genetic markers in UTUCs. Although markers such as microsatellite instability, promoter hypermethylation, fibroblast growth factor receptor 3, and PD-L1 are available, these markers do not provide sufficient prognostic prediction. In this study, we aimed to investigate the role of MSH2, MSH6, MLH1, PMS2, and FBLN2, which have been shown to have prognostic value especially in colorectal cancers, in UTUCs and to identify a new marker that will give hope in predicting survival.

FBLN2 was previously studied in lung, nasopharyngeal, and breast cancers. Baird et al. (5) 46 showed that in primary lung adenocarcinomas, FBLN2 can promote malignant progression and tumor cell adhesion to collagen cross-links. All 46 samples expressed FBLN2, and these samples were also compared with normal lung tissue lacking FBLN2 *in vitro*. As a result, it was observed that cells proliferated less, formed fewer colonies, and cell migration was less in normal tissue cultures lacking FBLN2 compared with tumor tissues (5). In nasopharyngeal cancers, Law et al. (6) found that FBLN2 is a tumor suppressor with antiangiogenic properties. In this study, 14 out of 30 nasopharyngeal biopsy examinations (46.7%) showed that FBLN2 was expressed at a very low level in tumor tissues compared with normal tissues. They also concluded that FBLN2 inhibited cell migration, proliferation, and angiogenesis in their experiments with in vitro cell cultures (6). Tan et al. (7) In the study including 23 female patients with breast cancer, 46 biopsies were taken with 2 samples from each woman. In pathological samples, FBLN2 was expressed in normal tissues and decreased in breast cancer tissues. As a result of the research, the authors concluded that FBLN2 in breast tissue prevents the spread of cancer cells together with the basement membrane. In another study conducted in in vitro breast cancer cell culture at the University of Nebraska, decreased FBLN2 expression was shown to facilitate cancer infiltration and cell migration. In this study, we observed that cell migration and invasion decreased when FBLN2 was re-introduced into cell cultures lacking FBLN2, but no change was observed in cell growth and adhesion properties (7,8).

On the other hand, although FBLN1, FBLN3, and FBLN5 from the fibulin family have been investigated in UCs in several studies (15-17), there is only one publication on FBLN2 in the literature. In this study, Li et al. (9) conducted a study in Taiwan and observed high FBLN2 immunoactivity in the pathological materials of 340 urothelial cell carcinoma (UCC) and 295 bladder UCC patients. They demonstrated that high FBLN2 immunoexpression was significantly associated with aggressive features, such as high tumor stage and grade, perineural invasion (PNI), lymphovascular invasion (LVI), lymph node metastasis, and high mitotic rate, as well as disease progression and metastasis development. In this study, 29.4% of patients with high FBLN2-expressing tumors and only 6.5% of patients with low FBLN2-expressing tumors died from UCC. Furthermore, 32.9% of patients with high FBLN2-expressing tumors developed metastasis, whereas only 8.2% of patients with low FBLN2-expressing tumors developed metastasis. Kaplan-Meier analysis showed that high FBLN2 expression was significantly associated with worse disease-free survival ($p<0.0001$) and worse metastasis-free survival ($p<0.0001$). As a result, the authors concluded that these findings may help inform early radical surgery, systemic chemotherapy, or immunotherapy and the aggressive management of UC (9).

Similarly, we investigated the relationship between FBLN2 immunoexpression and aggressive UCC characteristics, such as tumor stage and grade, PNI, LVI, lymph node metastasis, tumor location, multiplicity, and diameter. At the end of the study, we found that FBLN2 expression in tumor tissues and the extent of staining in pathology material were not associated with overall and disease-free survival. There may be various reasons for our different results from those of other studies in the literature. The first is that the number of patients in the other study was 635, and 340 of them were UCC patients, whereas the number of UCC patients in our study was limited to 55. This difference in the number of patients may have affected the statistical results. Another point is that the Biorbyt-branded Orb69091 coded kit was used in the study by Li et al., (9) whereas the ABCAM-branded ab234993 coded kit was used in our study. There are no other studies on urothelial carcinomas treated with this brand. Different kits may have caused different staining results. Again, while monoclonal antibody was used in another study, a polyclonal antibody was used in our study, which may have a direct effect on the staining results.

Microsatellites are repetitive DNA sequences that comprise approximately 3% of the human genome. A normal tissue DNA repair system called mismatch repair (MMR) can correct DNA replication errors. The MMR system includes MLH1, PMS1, PMS2, MSH2, MLH3, and MSH6 proteins (18). In eukaryotes, replication errors are first detected by MSH2/MSH6 (MutSα) and MSH2/MSH3 (MutSβ) heterodimers and then corrected by the DNA MMR system with the MLH1/PMS2 complex. It degrades the mutated DNA and initiates re-synthesis (10). However, the possibility of gene mutations increases in tumor cells because of the absence of MMR genes or defects in the replication repair process. The incidence of this disorder varies between approximately 4% and 27%, according to the literature. This mechanism was first described in tumors of patients with

hereditary non-polyposis colorectal carcinoma (HNPCC). These tumors are almost always found with high MSI. MLH1, MSH2, MSH6, and PMS2 are responsible for 95% of known Lynch syndrome-associated mutations. In addition, non-colorectal tumors are frequently observed in patients with this genetic mutation (11,19-21). It has been observed that the incidence of UCC is higher in patients with HNPCC than in normal patients (22-24). UCC may be observed in approximately 5% of patients with HNPCC. Studies conducted in family members of these patients have shown that the incidence of UUSCC increases 14 to 22 times compared with the general population and may develop 10-15 years earlier than normal (22). In a study conducted by Harper et al. (23) in 2016, 214 patients were analyzed, and MSI was observed in 14 (7%) patients. The loss of MSH2 and MSH6 was observed in 12 patients (86%), and isolated MSH6 loss was observed in 2 patients (14%). None of the patients experienced loss of MLH1 or PMS2. It was observed that 9 (64%) of these patients were associated with Lynch syndrome. In addition, endometrial cancer and colorectal carcinoma were observed in 2 patients and colorectal carcinoma in one patient. In 2003, Roupret et al. (24) observed gene loss in 27 (16%) of 164 patients diagnosed with UCC. These patients were subjected to additional genetic analysis, and MSH2 mutation was found in 3 patients. As a result, they showed that the MSI test should be performed in all patients, and it was positive in approximately 40% of the cases. They emphasized that hereditary cancer may be suspected especially if the MSI level is high, as in 16% of their cases, the patient is younger than 60 years, and there is a family history of HNPCC-related cancer.

With the demonstration of this relationship between HNPCC and UCC, MSI might be effective in the prognosis of UCC, and various studies were performed in this regard (12,25,26). Among these, Urakami et al. (25) screened 143 UCC patients for MSI and found gene loss in a total of 7 (5%) patients. MLH1/PMS2 loss was observed in one case, MSH2/MSH6 loss in five patients, and isolated MSH6 loss in one patient. Two patients with MSH2/MSH6 loss were subjected to further genetic analysis, and both patients were found to have an MSH2 germline mutation. In a study of 139 patients with urothelial carcinoma, Sobrino-Reig et al. (12) observed MSI in approximately 10.3% (13 patients). In this study, the likelihood of MSI was high in male patients, those with a tumor located in the bladder or ureter at the time of diagnosis, those with a papillary histological pattern that did not infiltrate the lamina propria, and those with perivesical tissues in cases of infiltrating tumor. The authors concluded that the combination of clinical data and histopathological features may allow early identification of patients with high probability of MSI.

In our study, loss of MMR protein expression was observed in only 3 patients. The loss of MSH2/MSH6 pair protein expression was observed in one patient and the loss of MLH1/PMS2 pair protein expression was observed in 2 patients. All 3 patients were male with a history of smoking. Although the presence of MSI has shown significant results in UCCs in other studies, we believe that the small number of our patients prevented us from showing this significant relationship.

Study Limitations

Many studies have shown that the ECM plays an important role in regulating organogenesis and tissue hemostasis. It is also known that high or low FBLN2 expression in cancer tissue has a supportive or inhibitory effect on cancer invasiveness and metastasis development. Similarly, MSI status has been shown to significantly affect cancer prognosis.

Although FBLN2 and MSI were found to be significant in determining cancer prognosis in many studies, no significant relationship was found in our study. In conclusion, we could not obtain a clear predictive prediction due to various limiting factors in our study, such as the lack of a standard immunostaining and scoring system for FBLN2 expression and the small number of patients. However, we believe that there is a need to identify new biomarkers to prolong survival in these highly invasive patients and to conduct multicentre, prospective, randomized studies with a high number of patients. Although this study could not show an association between the above biomarkers and the prognosis of upper urinary tract urothelial cell carcinomas, it may help to conduct more quality, prospective, multicentre, randomized studies in the future. In addition, prospective studies may provide adequate prognostic information with optimal surgical follow-up.

Conclusion

In conclusion, although we could not find a significant relationship between biomarkers such as FBLN2, MSH2, MSH6, MLH1, and PMS2 in UCCs in this study, we think that studies with a higher number of patients and including other biomarkers are needed due to the lack of sufficient data on prognosis in these tumors.

Ethics

Ethics Committee Approval: This study was approved by decision number 314 dated March 24, 2021, from the Gazi University clinical research Ethics Committee.

Informed Consent: Retrospectively study.

Acknowledgements

Publication: The results of the study were not published in full or in part in form of abstracts.

Contribution: There is not any contributors who may not be listed as authors.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.A., A.B.K., M.Y.K., B.C.Ö., İ.I.G., Concept: S.A., A.B.K., İ.I.G., Design: S.A., A.B.K., İ.I.G., Data Collection or Processing: S.A., A.B.K., B.C.Ö., Analysis or Interpretation: S.A., A.B.K., M.Y.K., İ.I.G., Literature Search: S.A., A.B.K., Writing: S.A., A.B.K., M.Y.K., B.C.Ö., İ.I.G.

Conflict of Interest: One author of this article, (Murat Yavuz Koparal) is a member of the Editorial Board of the Bulletin of

Urooncology. However, he did not take part in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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Original Article

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Effects of Melatonin on Different Stages of Bladder Cancer Survival

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Abstract

Objective: This study investigated the impact of melatonin on bladder cancer cells, focusing on its influence on cell survival and apoptosis at various stages, and evaluated its potential as a therapeutic agent.
Materials and Methods: The research involved the use of the bladder cancer cell lines RT-112 (grade 1, non-invasive) and HTB-9 (grade 2, invasive) cultured in RPMI-1640 medium. The XTT assay was used to assess cell viability after treating cells with different concentrations of melatonin for various durations. Apoptotic activity was examined via annexin V/PI staining, followed by flow cytometry to quantify cell death. To explore the apoptotic mechanisms involved, Caspase 3/7 activity was also measured. Statistical evaluations were performed using GraphPad Prism, and two-way analysis of variance and Tukey's test were used to determine significance ($p<0.05$).
Results: Melatonin demonstrated a marked reduction in cell viability for both RT-112 and HTB-9 bladder cancer cell lines, with IC50 values determined at 1.115 mM for RT-112 and 2.111 mM for HTB-9. After 24 h of melatonin treatment, the rates of apoptotic cells increased to 47.85% for RT-112 and 38% for HTB-9, both showing statistical significance compared with the control groups ($p<0.0001$). Additionally, cells treated with melatonin showed increased caspase 3/7 activity, indicating the strong induction of apoptosis. Specifically, caspase 3/7 activities were recorded at 46.4% for RT-112 and 38.9% for HTB-9, both significantly exceeding those of control cells ($p<0.0001$). These findings suggest that melatonin effectively inhibits bladder cancer cell proliferation by enhancing apoptosis.
Conclusion: In summary, melatonin is a promising therapeutic agent for bladder cancer because it lowers cell viability and triggers apoptosis in cancer cell lines. The influence of caspase 3/7 activity underscores the importance of activating apoptotic pathways, opening new research possibilities for clinical applications.
Keywords: Annexin V/PI, caspase 3/7, HTB-9 (5637), melatonin, RT-112

Introduction

Bladder cancer ranks as the tenth most prevalent cancer globally, with the incidence of this malignancy steadily rising over recent decades (1). Despite improvements in diagnostic and treatment approaches, the high rates of recurrence and progression pose a substantial health challenge (2,3). Conventional treatment options for bladder tumors, such as transurethral resection, chemotherapy, and immunotherapy, often come with significant side effects and limitations, highlighting the need for new therapeutic agents (4,5). Recently, melatonin -a hormone mainly secreted by the pineal gland- has attracted interest for its potential anticancer properties (6). Apart from its established role in managing circadian rhythms and sleep-wake cycles, melatonin exhibits antioxidant, anti-inflammatory, and immunomodulatory characteristics, making it a promising contender in oncology (7-9).

Research has increasingly emphasized melatonin's capacity to influence multiple biological mechanisms that play a role in cancer progression, including oxidative stress, apoptosis, and the regulation of the cell cycle (10-13). In the context of bladder cancer, oxidative stress is a significant contributor to both carcinogenesis and tumor progression. Melatonin acts as an effective free radical scavenger, potentially mitigating these effects by lowering the levels of reactive oxygen species and bolstering antioxidant enzyme activity (14,15). Furthermore, melatonin has been shown to promote apoptosis and inhibit

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the proliferation of various cancer cell lines, particularly those originating from bladder tissue (16,17). Its engagement with melatonin receptors (MT1 and MT2) in cancer cells indicates its involvement in cell signaling pathways that may affect tumor dynamics (16,18).

Additionally, disturbances in circadian rhythms, commonly seen in shift workers or individuals with inconsistent sleep schedules, have been linked to an elevated risk of developing bladder cancer (19,20). Melatonin's role in preserving circadian balance suggests a potential preventive function in bladder carcinogenesis (14,21). Given these diverse actions, a deeper understanding of the relationship between melatonin and bladder cancer could pave the way for innovative therapeutic strategies. This study aimed to analyze existing knowledge regarding melatonin's impact on bladder cancer cells, specifically focusing on its effects on cell survival and apoptosis across different stages of the disease.

Materials and Methods

This study employed cell lines; thus, ethical approval was not deemed necessary. The bladder cancer cell lines RT-112 (ATCC) and HTB-9 (ATCC) were obtained commercially.

Cell Culture

RT-112 (grade 1, non-invasive) and HTB-9 (grade 2, invasive) bladder cancer cells were maintained in RPMI-1640 (Capricorn Scientific) medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Capricorn Scientific) at 37 °C using standard cell culture techniques in an incubator with %5 CO_2 and 95% humidity.

Cell Viability Assay

Cell viability for RT-112 and HTB-9 was assessed using the XTT (Biotium) assay to evaluate the cytotoxic effects of melatonin (22). Briefly, 1×10^4 cells were seeded in 96-well plates and allowed to attach overnight. Cells were then treated with various concentrations of melatonin (0, 0.5, 1, 2, and 5 mM) for 12, 24, or 48 h. Following treatment, an XTT solution was added, and the mixture was incubated for 3 h at 37 °C. Cell viability was measured using a microplate reader at an absorbance wavelength of 460 nm. Each experiment was repeated three times.

Annexin V/PI Analysis

To evaluate apoptosis, RT-112 and HTB-9 bladder cancer cells were plated in 6-well dishes. The cell lines were treated with melatonin at concentrations of 1.077 mM for RT-112 and 2.111 mM for HTB-9. After a 24-h incubation period, all cells were collected using trypsin-EDTA, washed with PBS, and the concentration was adjusted to 1×10^5 cells in 100 µL. The resulting cell suspension was transferred to 12x75 mm polystyrene tubes, and 5 µL of Annexin V-FITC and 5 µL of propidium iodide were added to 500 µL of 1X Annexin Binding Buffer. After incubating at room temperature for 15 min, cells were analyzed using an ACEA NovoCyte flow cytometer (Agilent). Based on flow cytometric measurements, cells were analyzed as described in our previous study (23).

Caspase 3/7 Analysis

As in our previous studies, caspase 3/7 analysis was performed to identify the apoptotic pathways utilized by RT-112 and HTB-9 cells (24). Cells were treated with the IC50 of melatonin and incubated overnight. After 24 h of treatment, the cells were trypsinized, collected, and assessed for caspase 3/7 activity using the Cell Meter™ Caspase 3/7 flow cytometry assay kit on an ACEA NovoCyte flow cytometer (Agilent). All measurements were repeated three times, and the average standard deviation was calculated for the data.

Statistical Analysis

Statistical evaluations were performed using GraphPad Prism version 8.4.2. The two-way ANOVA test was used for intergroup comparisons, with differences in means compared with the control group assessed using the Tukey test. Results are expressed as mean ± standard deviation, and a significance level of $p<0.05$ was established.

Results

Melatonin Reduces the Viability of Bladder Cancer Cells

The effects of melatonin were assessed at concentrations of 0, 0.25, 0.50, 1, 2, and 5 mM on the RT-112 and HTB-9 human bladder cancer cell lines, with treatment durations of 12, 24, and 48 h. As illustrated in Figure 1, melatonin exerted significant cytotoxic effects on the bladder cancer cell lines in a time-dependent manner, with IC50 values of 1.115 mM for RT-112 and 2.111 mM for HTB-9 after 24 h. The XTT assay results revealed that the viability of RT-112 cells after 24 h was 100%, 79.68%, 69.24%, 49%, 36.07%, and 20.50%. Similarly, for HTB-9 cells, the viability percentages were 100%, 88.6%, 82.80%, 63.32%, 50.30%, and 32.70% at the same concentrations (Figure 1). These findings indicate that melatonin reduces the viability of bladder cancer cells in a dose-dependent manner, suggesting its potential to inhibit cell proliferation. Increased melatonin concentrations correlated with a more pronounced decrease in cell viability for both RT-112 and HTB-9 cells.

Melatonin Induces Apoptosis in RT-112 and HTB-9 Cells

Treatment with melatonin at concentrations of 1.115 mM for RT-112 and 2.111 mM for HTB-9 for 24 h resulted in higher rates of apoptosis, with 47.85% and 38% of cells, respectively, undergoing apoptotic changes compared with the control group. This finding is consistent with the cytotoxicity data (Figure 2). The results confirmed that melatonin effectively inhibited the growth of RT-112 and HTB-9 bladder cancer cells by promoting apoptosis. Additionally, melatonin-treated RT-112 and HTB-9 cells exhibited a reduction in viable cell populations of 51.2% and 59.25%, respectively, compared with controls. Notably, non-invasive RT-112 cells exhibited a higher apoptosis rate than invasive HTB-9 cells.

Melatonin Promotes Caspase 3/7 Activity in RT-112 and HTB-9 Cells

To elucidate the mechanism by which melatonin induces apoptosis, we measured the levels of caspase 3/7, a known

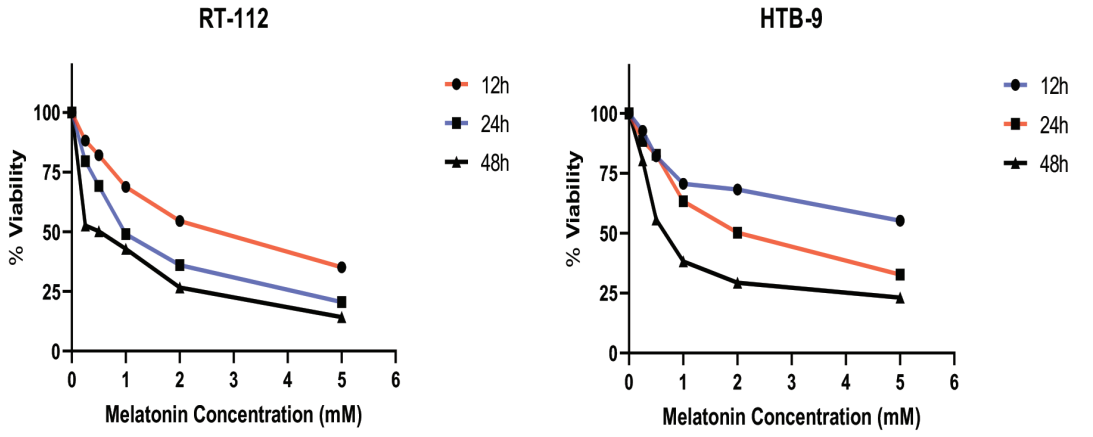

Figure 1. XTT analysis demonstrating the cell viability of RT-112 and HTB-9 cells following treatment with increasing concentrations of melatonin over 12, 24, and 48 h

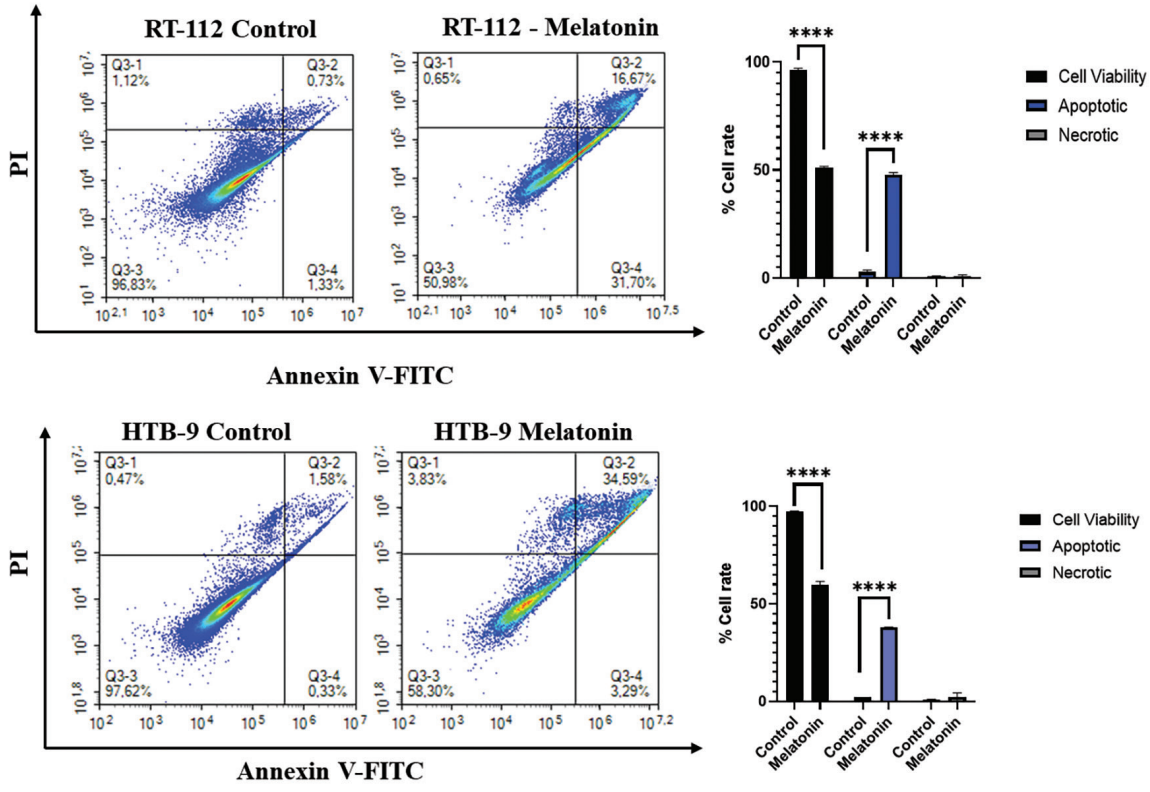

Figure 2. Representative dot plot graphs show RT-112 and HTB-9 cells treated with melatonin at their IC50 concentrations for 24 h, used to assess apoptotic cell death through Annexin V/FITC-PI staining. Additionally, the percentages of viable, apoptotic, and necrotic cells were analyzed statistically, with values representing three independent experiments (****p<0.0001)

marker of apoptotic activity, using flow cytometry. The results showed a significant increase in caspase 3/7 activation in both RT-112 and HTB-9 cells treated with melatonin compared with controls after 24 h at IC50 concentrations (p<0.0001) (Figure 3). Specifically, caspase 3/7 activity was 46.4% in RT-112 cells and 38.9% in HTB-9 cells in the melatonin-treated group, which was significantly higher than that in the control group (p<0.0001). These findings corroborate the apoptosis assay results.

Discussion

Bladder cancer remains a significant health challenge because of its increasing incidence and high rates of recurrence and

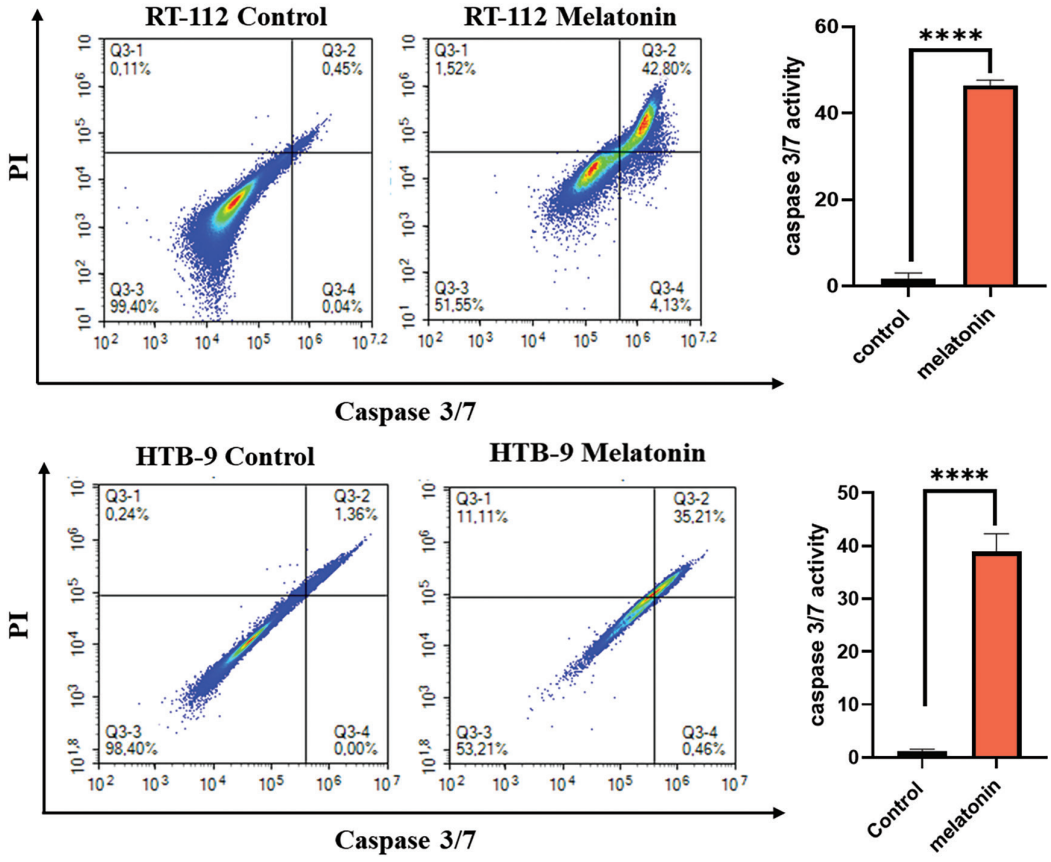

Figure 3. Caspase 3/7 activity measured by flow cytometry in RT-112 and HTB-9 cells treated with IC50 melatonin for 24 h. Bar graphs show the comparison of melatonin treated cells and control group of caspase 3/7 activity. Data are displayed as the mean ± standard deviation. Significant differences are shown compared with control and theobromine-treated cells (****p<0.0001)

progression. In this study, we examined the effects of melatonin on the bladder cancer cell lines RT-112 (non-invasive) and HTB-9 (invasive), specifically focusing on its influence on cell survival and apoptosis.

Our results indicate that melatonin effectively decreases the viability of bladder cancer cells in a dose-dependent manner. The IC50 values of RT-112 and HTB-9 after 24 h of treatment were 1.115 and 2.111 mM, respectively. These outcomes are consistent with previous studies that documented the cytotoxic properties of melatonin across various cancer cell types (25). Prior studies have also suggested that melatonin can hinder cancer cell proliferation by inducing apoptosis, a finding that aligns with our observations of significant apoptosis rates of 47.85% in RT-112 cells and 38% in HTB-9 cells (26).

The mechanism by which melatonin exerts its effects appears to be associated with its capacity to activate apoptotic pathways. The results of the caspase 3/7 assay revealed a substantial increase in caspase activity in both cell lines following treatment with melatonin, indicating that melatonin not only diminishes cell viability but also facilitates apoptotic cell death (26). This result is in agreement with prior reports that highlighted the role of melatonin in augmenting caspase activity in cancer cells (27).

Additionally, our findings highlight the differential responses of bladder cancer cell lines to melatonin, with noninvasive RT-112 cells demonstrating a higher rate of apoptosis compared with invasive HTB-9 cells. These findings suggest a potential role for melatonin as a therapeutic agent specifically targeting early-stage bladder cancer although further research is necessary to clarify the underlying mechanisms. The existing literature suggests that the invasive characteristics of cancer cells may contribute to a certain degree of resistance to apoptosis, potentially accounting for the lower apoptosis rate observed in HTB-9 cells (2,21).

Another crucial factor in our discussion was the relationship between circadian rhythms and cancer progression. Alterations in circadian rhythms have been associated with an increased risk of bladder cancer (19). Melatonin's role in maintaining circadian homeostasis might play a part in its preventive effects against bladder carcinogenesis, as highlighted in recent studies emphasizing the significance of circadian regulation in tumor biology (20). Therefore, incorporating melatonin into treatment protocols could provide dual advantages by directly targeting cancer cells and restoring circadian rhythms.

Our findings indicate that melatonin has considerable antitumor effects against bladder cancer cells. The pronounced apoptotic

response in RT-112 cells suggests the potential of melatonin as a therapeutic agent for early-stage bladder cancer treatment. These results demonstrate that melatonin inhibits cancer cell survival and activates apoptotic pathways. The reduced apoptotic response in HTB-9 cells, which can be attributed to their invasive traits, supports the idea that the aggressive nature of certain cancer cells may contribute to their treatment resistance.

Study Limitations

Although our study underscores the promise of melatonin in bladder cancer management, it is crucial to acknowledge its limitations. The findings are derived from in vitro studies, necessitating further research, including in vivo studies and clinical trials, to validate our results and evaluate the therapeutic efficacy of melatonin in patients with bladder cancer.

Conclusion

In conclusion, our study provides compelling evidence of the antiproliferative effects of melatonin in bladder cancer cell lines. The ability of melatonin to reduce cell viability, induce apoptosis, and modulate oxidative stress positions makes it a potential novel therapeutic agent for bladder cancer management. These findings add to the growing body of literature advocating for the integration of melatonin into cancer treatment strategies, particularly for individuals at elevated risk of developing bladder cancer.

Ethics

Ethics Committee Approval: This study employed cell lines; thus, ethical approval was not deemed necessary.

Informed Consent: Patient consent is not required.

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Contribution: There is not any contributors who may not be listed as authors.

Footnotes

Authorship Contributions

Concept: T.O-S., A.S., E.A., A.N.K., Design: T.O-S., A.S., E.A., A.N.K., Data Collection or Processing: T.O-S., A.S., E.A., A.N.K., Analysis or Interpretation: T.O-S., A.S., E.A., A.N.K., Literature Search: T.O-S., A.S., E.A., A.N.K., Writing: T.O-S., A.S., E.A., A.N.K. .

Conflict of Interest: One author of this article, (Ahmet Nihat Karakoyunlu) is a member of the Editorial Board of the Bulletin of Urooncology. However, he did not take part in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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Original Article

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Importance of Transrectal Povidone-iodine Activity in Reducing Infections After Prostate Biopsy

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Abstract

Objective: To investigate the effectiveness of transrectal povidone-iodine antiseptic solution in minimizing infections following transrectal ultrasound-guided prostate biopsy.

Materials and Methods: We retrospectively analyzed the medical records of 240 patients who underwent TRUS-guided biopsy at our clinic between January 2016 and December 2020. All patients received antibiotic prophylaxis prior to the procedure. The patients were categorized into three groups: Group 1, which served as the control, received only antibiotic prophylaxis; Group 2, which consisted of patients administered transrectal povidone-iodine via a catheter syringe; and Group 3, which underwent povidone-iodine rectal cleansing. The primary objective was to compare infection rates and complications among the three groups, with a particular emphasis on the combined effect of povidone-iodine and prophylactic antibiotics versus antibiotics alone.

Results: Infection rates were notably reduced in patients who received povidone-iodine interventions ($p<0.05$). Febrile infections occurred in 10 cases (4.25%) patients in Group 1, in whom ciprofloxacin alone was administered. In contrast, febrile complications were observed in 3 cases in Group 2, and only 2 cases in Group 3, corresponding to rates of 1.3% and 0.8%, respectively.

Conclusion: The combination of transrectal povidone-iodine and prophylactic antibiotics demonstrated significant efficacy in minimizing febrile infectious complications associated with TRUS-guided biopsy.

Keywords: Antibiotic prophylaxis, prostate biopsy, rectal suppository, povidone-iodine, gavage injector

Introduction

Prostate cancer (PCa) is the most frequently diagnosed malignancy in men worldwide. Since the pivotal study by Stamey et al., (1) prostate-specific antigen (PSA) has become the most significant and widely utilized biomarker for detecting PCa. The diagnosis of PCa has seen notable improvements with the introduction of transrectal ultrasound-guided prostate biopsy (TRUS-Bx), which remains the preferred diagnostic technique for suspected cases. Annually, approximately 400,000 new PCa cases are reported across Europe (2).

Despite negative biopsy results, numerous TRUS-Bx procedures are still performed. The biopsy process involves accessing the prostate through the rectum, which is an area rich in blood vessels and bacterial flora. This area increases the risk of infection. Urological infections following biopsies primarily originate from bacterial contamination in the rectum. Despite being generally safe and well-tolerated, prostate biopsies can result in adverse effects, including sexual dysfunction due to psychological stress, rectal bleeding, urinary retention, hematospermia, hematuria, and post-biopsy pain.

Acute urinary tract infections, prostatitis, and epididymitis, as well as rare but severe complications like urosepsis with life-threatening outcomes may also ocur (3). Among the most frequently observed complications post-TRUS-Bx are hematospermia (5.7-89%) and hematuria (14.4-84%), followed by rectal bleeding (1.3-39.6%). Dysuria occurs in 7-7.2% of cases, whereas urinary tract infections and sepsis are reported at rates of 6.1% and 0.5%, respectively (4).

To mitigate these infectious complications, various preventive measures have been introduced, with antibiotic prophylaxis being the primary approach. Antibiotic prophylaxis aims to limit bacterial colonization at the biopsy site. Another preventive method involves antiseptic measures, such as rectal povidone-iodine cleansing, to minimize bacterial contamination at the biopsy needle entry point. Additional preventive strategies include rectal cleansing using enemas and the use of smaller, calibrated needles (5).

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Our study focused on evaluating the effectiveness of these preventive approaches, particularly comparing the rates of infectious complications among patients undergoing TRUS-Bx using different prophylactic strategies.

Materials and Methods

The records of patients who underwent transrectal ultrasound-guided biopsy (TRUS-Bx) at our clinic from January 2016 to December 2020 were retrospectively analyzed. This study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki. All participants received information regarding the use of their data for scientific purposes, and written consent was obtained from each participant. Ethical clearance for this research was obtained from the Ethics Committee of Tokat Gaziosmanpaşa University Medical Faculty (approval number: 83116987-031, date: 05.01.2023).

Operation Techniques

Two hundred forty patients who underwent TRUS-guided biopsy (TRUS-Bx) were randomly assigned to three equally sized groups. The criteria for performing biopsies included PSA levels >2.5 ng/mL and/or abnormalities detected during digital rectal examination.

Exclusion criteria comprised patients on anticoagulant therapy, individuals with chronic liver disease, renal failure, a history of PCa or repeated biopsies, bleeding disorders, malignancies in other pelvic organs, and concurrent rectal conditions, such as hemorrhoids, polyps, strictures, or fissures.

All patients received a total dose of 1500 mg of ciprofloxacin (750 mg twice daily) for 5 days, beginning 2 days before the procedure. Sodium phosphate enema (19 g monobasic sodium phosphate and 7 g dibasic sodium phosphate, libalax) was administered 2-4 h prior to biopsy. Urine culture negativity was verified before biopsy. During the procedure, the participants were positioned in the left lateral decubitus position with their left knee flexed.

After carefully applying the inclusion and exclusion criteria, the first 80 patients who met the specified time intervals were assigned to Group 1, the next 80 to Group 2, and the remaining 80 to Group 3. Statistical analysis was conducted to compare these groups.

In Group 1, only antibiotic prophylaxis. Group 2 received 30 cc of povidone-iodine via a 50 mL gavage syringe into the rectum (Figure 1). In Group 3, the rectal wall was cleaned for 2 min using gauze soaked in povidone-iodine (Figure 2).

For all patients, the biopsy method was selected randomly, and a standard 12-core biopsy procedure was performed using an 18 G biopsy needle (Geotek Medikal, Turkey) with an automatic biopsy gun. If any suspicious area was identified during TRUS-Bx, one or two additional biopsies were collected.

After biopsy, patients were transported to the hospital ward and discharged on the same day. Patients were informed of potential complications, such as dysuria, rectal bleeding, hematuria, and anal pain, which typically resolve spontaneously. Patients presenting with a body temperature exceeding 37.8 °C and symptoms like chills, urinary urgency, frequency, or dysuria within 3 days after the procedure were advised to seek emergency care.

Those with a body temperature of 37.8 °C or higher were hospitalized, and further evaluations, including urinalysis, urinary culture, and blood culture, were conducted for all admitted patients.

Statistical Analysis

Statistical evaluations included group numbers, medians, and 25th (Q1) and 75th (Q3) percentiles. Analyses were conducted using the MedCalc software (version 20.009). To assess normality within the groups, the Kolmogorov-Smirnov test was applied.

Because the groups did not meet the assumptions of a normal distribution, the Kruskal-Wallis test was applied. Bonferroni correction was used for multiple comparisons across groups. Statistical significance was determined at the $p<0.05$ threshold.

Results

A retrospective analysis was conducted on patient records, which included prostate volume, demographic information, PSA levels, underlying diabetes mellitus, and infectious complications. The groups exhibited similarities in prostate volume, average age, PSA levels, PCa rates, and diabetes mellitus incidence (Tables 1, 2).

Infection occurred in 10 cases (4.2%) patients receiving ciprofloxacin antibiotic prophylaxis alone (Group 1). Febrile infections were identified in 3 cases in Group 2 and only 2 cases in Group 3, corresponding to infection rates of 1.3% and 0.8%, respectively. Groups receiving povidone-iodine treatment exhibited a significant decrease in infection rates ($p<0.05$).

Blood and urine culture analyses were performed for all hospitalized patients due to infection. The most common microorganisms isolated were *Escherichia coli* (80%), *Klebsiella pneumoniae* (10%), and *Staphylococcus* spp. (10%). Following

Table 1. Demographic characteristics, PSA level and prostate volume of patients

	Groups									p-value
	Group 1 (n=80)			Group 2 (n=80)			Group 3 (n=80)			
	Median	25.P	75.P	Median	25.P	75.P	Median	25.P	75.P	
Age	61	58	63.5	61	58	63.5	61	58	63.5	ns
PSA level (ng/mL)	9.9	7.85	11.5	9.9	7.8	11.5	9.9	7.85	11.5	ns
Prostate volume (cc)	62	53.5	76.5	61.5	54.5	76.5	62	54.5	76.5	ns

ns: No signifance, PSA: Prostate-specific antigen, *significant difference at the level of <0.001 with Kruskal-Wallis test

Table 2. Incidence of infectious complications, diabetes mellitus and prostate cancer rates								
		Groups						p-value
		Group 1 (n=80)		Group 2 (n=80)		Group 3 (n=80)		
		n	%	n	%	n	%	
Diabetes mellitus	Yes	9	3.7	10	4.2	11	4.6	ns
	No	71	29.6	70	29.2	69	28.7	
Prostate cancer	Yes	13	5.4	14	5.8	14	5.8	ns
	No	67	27.9	66	27.5	66	27.5	
Infectious complications	Yes	10	4.2	3	1.3	2	0.8	<0.05*
	No	70	29.2	77	32.1	78	32.5	
ns: No signifance, *significant difference at the level of <0.05 with chi-square test								

successful medical treatment, all patients were discharged after an average hospitalization period of 7 days, achieving full recovery.

Discussion

TRUS-Bx remains the primary diagnostic modality for PCa. However, complications related to biopsy can result in significant morbidity and mortality, including susceptibility to severe infections, such as sepsis, hospitalization, and, in rare cases, mortality. This makes infection a critical concern in the procedure (4).

To address the rising resistance to quinolones, research over the past decade has focused on exploring alternative strategies. These include modifying antibiotic regimens, incorporating preoperative rectal swab cultures, employing targeted antibiotic prophylaxis, disinfecting biopsy needles with formalin, and using mucosal antisepsis methods like povidone-iodine before transrectal biopsy. Additionally, alternative approaches, such as the transperineal biopsy method, have been investigated (6). In this study, we utilized mucosal antisepsis and povidone-iodine prior to biopsy.

Guidelines from both the European Urology Association and the American Urology Association suggest the use of oral or intravenous fluoroquinolone prophylaxis to reduce infectious complications prior to TRUS-Bx (7). Nevertheless, the optimal choice of antibiotics and the appropriate duration for prophylaxis remain subjects of debate. Among the available fluoroquinolones, ciprofloxacin is commonly favored for TRUS-Bx because of its superior penetration into prostate tissue and potent activity against intestinal flora and coliform bacteria (8).

Although ciprofloxacin has a metabolic rate of 50-70% in urine, it is significantly more active than norfloxacin. Nonetheless, recent studies have identified a concerning rise in quinolone resistance, contributing to increased hospitalization rates after prostate biopsy, with some studies reporting up to 50% resistance rates (8). Despite ciprofloxacin's widespread use, our study revealed higher postoperative infection rates (4.2%) in Group 1. In comparison, infection rates in Groups 2 and 3 decreased significantly to 1.3% and 0.8%, respectively.

Rectal swab culture-based prophylaxis has been thoroughly investigated; however, uncertainty persists regarding its routine use among patients receiving TRUS-Bx. Recent research has indicated that targeted prophylaxis does not significantly reduce severe infectious complications. Studies comparing targeted and empirical antibiotic prophylaxis to prevent sepsis following transrectal prostate biopsy found no significant differences in sepsis rates across large patient cohorts (9).

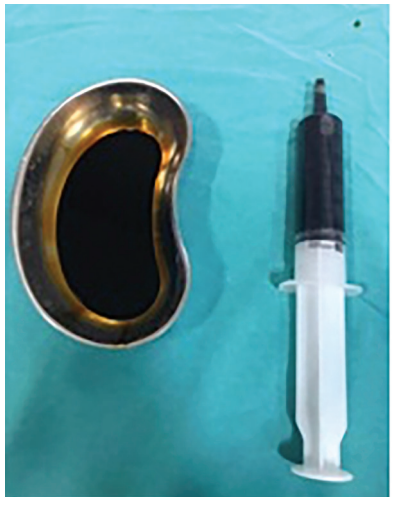

Figure 1. 30 cc of povidone-iodine in a 50 mL gavage syringe

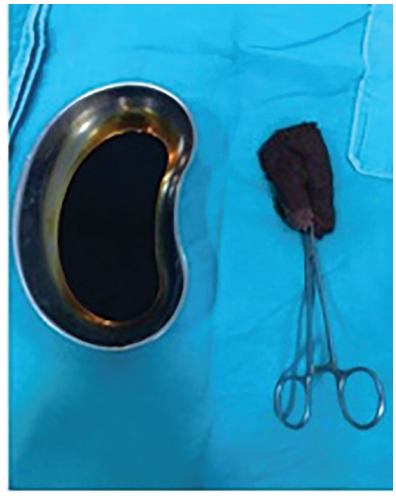

Figure 2. Gauze patch soaked in povidone-iodine

Large-scale studies have also highlighted the limitations of targeted prophylaxis, including high costs, the need for multiple clinic visits, insufficient support from microbiology laboratories, and specific requirements for culture media. These factors have cast doubt on the practicality of targeted prophylaxis in routine clinical settings. Overall, its benefits appear to be limited.

It has been reported that cleaning biopsy injection tips with 10% formalin between procedures effectively reduces infectious complications after TRUS-Bx (10). Although statistical significance has not been firmly established, experiments have demonstrated formalin's potential to inhibit bacterial growth, particularly bacteria resistant to fluoroquinolones. However, the lack of prospective, randomized controlled studies on formalin disinfection limits its clinical application. Comprehensive clinical trials are required to validate the effectiveness of formalin disinfection and to promote its wider implementation in clinical settings.

Multiple studies comparing transrectal and transperineal prostate biopsies suggest that the transperineal method is equally effective as TRUS-Bx for diagnosing PCa (11). The transperineal technique has shown a lower rate of infectious complications when compared to the transrectal approach. Nevertheless, although the transperineal biopsy has a safer profile, it comes with certain drawbacks, including the need for general anesthesia, increased costs, longer procedure times, and specialized equipment requirements (12). However, its lower infection rates highlight the potential of transperineal biopsy as a viable technique to TRUS-Bx.

As discussed earlier, various strategies have been employed in clinical practice to minimize complications following prostate biopsy. In this study, we evaluated the efficacy of povidone-iodine through two distinct methods and observed a significant reduction in infection rates.

Povidone-iodine, widely recognized for its ability to reduce infection risks in colorectal surgery and wound care, was combined with prophylactic antibiotics before TRUS-Bx to further mitigate infection risks (13). Prior studies have demonstrated the superior effectiveness of povidone-iodine when used alongside antibiotics for infection prevention (14).

It is well-established that enema use alone is insufficient for preventing infections during TRUS-Bx (15). According to EAU guidelines, rectal disinfection with povidone-iodine before TRUS-Bx is recommended (16). In our study, we observed that the combined use of povidone-iodine and enema significantly lowered infection rates following biopsy procedures.

Previous studies have explored various preoperative rectal cleansing methods and povidone-iodine applications. Ghafoori et al. (17) showed that administration of a povidone-iodine solution into the rectum effectively decreased the rate of infectious complications after TRUS-Bx. Similarly, Park et al. (18) reported that compared with povidone-iodine enemas, povidone-iodine suppositories were more effective in minimizing infections.

Additional studies have shown that direct cleansing of the rectal dome and perianal region with povidone-iodine reduces post-biopsy infection risks by limiting rectal microbial colonization (19). Chen et al. (20) introduced a rectal cleansing technique using povidone-iodine-soaked gauze to target the prostate area, which led to a 9.5% decrease in post-procedure infection rates.

Research evaluating povidone-iodine rectal cleansing for prebiopsy preparation has strongly supported its role in reducing infections. A meta-analysis further confirmed that combining antibiotics with povidone-iodine disinfection significantly lowers overall infection rates (21).

In a retrospective analysis conducted at a Korean hospital, Hwang et al., (22) reported that povidone-iodine enemas notably decreased. Our study similarly evaluated two rectal applications of povidone-iodine and found a substantial reduction in infection rates, which is consistent with prior research.

In contrast, Abughosh et al. (23) reported a 42% relative reduction in infection rates using povidone-iodine rectal cleansing in a large-scale study involving 865 patients, although this finding was not statistically significant. Conversely, Ryu et al. (24) found that povidone-iodine suppositories had no significant impact on complication rates.

Additionally, our study included evaluations using the International Prostate Symptom Score and the Sexual Health Inventory for Men questionnaires. No significant differences were observed in lower urinary tract symptoms or sexual function. However, our research exclusively focused on infectious complications, and no quality of life assessments were performed (24).

Study Limitations

Several limitations of our study should be acknowledged. First, its retrospective design, which relies on data extracted from medical records and TRUS-Bx procedure notes, introduces inherent constraints, such as limited data availability and potential bias. Additionally, although urine analysis was performed for all patients, more comprehensive assessments, including blood culture, urine culture, and other laboratory tests, were selectively conducted for hospitalized individuals presenting with febrile symptoms.

Moreover, asymptomatic or mildly symptomatic outpatients may have been excluded from our analysis. Additionally, the sample size was relatively small, and its single-center design restricted the generalizability of the findings. To overcome these limitations and ensure more robust conclusions, future studies should include larger-scale, prospective, and randomized clinical trials.

Conclusion

Our findings demonstrate that combining transrectal 10% povidone-iodine injection administered via a gavage syringe with antibiotic prophylaxis, as well as performing povidone-iodine rectal cleansing, is an effective, affordable, and practical approach for reducing infectious complications associated with TRUS-Bx.

Ethics

Ethics Committee Approval: Ethical clearance for this research was obtained from the Ethics Committee of Tokat Gaziosmanpaşa University Medical Faculty (approval number: 83116987-031, date: 05.01.2023).

Informed Consent: Written consent was obtained from each participant.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: F.F., K.Y., Concept: F.F., K.Y., Design: F.F., K.Y., Data Collection or Processing: F.F., K.Y., Analysis or Interpretation: F.F., K.Y., Literature Search: F.F., K.Y., Writing: F.F., K.Y.

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Original Article

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Combining Multiparametric MRI and PSA Density for Improved Diagnostic Accuracy in Prostate Cancer

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Abstract

Objective: The objective of this research was to investigate the combined use of multiparametric magnetic resonance imaging (Mp-MRI) and prostate-specific antigen density (PSAD) to increase diagnostic accuracy in detecting prostate cancer (PCa) and to reduce unnecessary biopsies.
Materials and Methods: This retrospective analysis included 399 patients who underwent prostate biopsy at Ankara City Hospital between 2021 and 2022, primarily due to clinical indications suggestive of PCa. The patient cohort was categorized into distinct groups according to their PSAD, with a defined threshold of 0.15 ng/mL/cc, and their respective prostate imaging reporting and data system (PI-RADS) scores; subsequently, the diagnostic performance metrics, including sensitivity, specificity, and predictive values for determining Pca, were meticulously evaluated across different combinations of PI-RADS classifications and PSAD levels.
Results: Among the 399 patients, 37.6% had PCa and 16.8% had clinically significant PCa (csPca). Patients who exhibited PI-RADS scores of 3 or higher combined with a PSAD score of at least 0.15 ng/mL/cc exhibited the greatest positive predictive value, achieving 74.1% for overall PCa and 39.3% for csPca. The integration of PI-RADS assessment with PSAD thresholds notably enhanced diagnostic accuracy, leading to improved detection rates of clinically significant cases while concurrently minimizing the frequency of unnecessary biopsy procedures.
Conclusion: The simultaneous application of Mp-MRI and PSAD enhances the precision of Pca diagnosis and serves as a valuable tool for reducing the need for unnecessary biopsies, especially in patients with PI-RADS scores of 3 or above accompanied by elevated PSAD levels.
Keywords: Mp-MRI, PI-RADS, PSAD, prostate cancer

Introduction

Prostate cancer (PCa) accounts for 15% of all cancers and is the most frequently diagnosed malignancy in men (1). One in eight men is at risk of developing PCa during their lifetime (2). PCa is mostly asymptomatic in the early stages, except for a few cases, which results in a higher number of undiagnosed cases compared with those diagnosed. Autopsy studies have found a PCa prevalence of 3-8% in men under the age of 30 (2), with this rate increasing approximately 1.7-fold each year (3). After the age of 79, the prevalence of tyrosine phthalate increases dramatically, reaching 48-71%. PCa is diagnosed in only 2% of patients aged below 50 years. Therefore, routine PCa screening is recommended for men aged >50 years worldwide (3).

Epidemiological data on PCa have varied over time due to etiological factors and The utilization of prostate-specific antigen (PSA) testing. Since the late 1980s, PSA levels, along with digital rectal examination (DRE), have been used for PCa screening (4). However, serum PSA levels are specific to the prostate but not to cancer itself. Thus, they can vary due to factors like age, ethnicity, and prostate volume, even in healthy men. Furthermore, PSA levels can also be elevated in non-malignant conditions, including infections, benign prostatic hyperplasia, physical trauma, and following transurethral procedures (5). These factors can lead to unnecessary decisions for transrectal ultrasound-guided biopsy based on elevated PSA levels (6). Hence, additional tests were introduced to assess PSA levels in diagnosing and monitoring PCa.

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Originally, a PSA limit value of 4.0 ng/mL was introduced as a criterion for the detection of PCa (7). At this threshold, PSA sensitivity and specificity were 20% and specificity 60% (7). Lowering the PSA threshold resulted in reduced specificity but decreased sensitivity. Therefore, no standard PSA threshold has been determined (8). The primary goal of PCa screening is to detect aggressive and potentially fatal tumors early. Effective screening should aim to reduce the mortality of PCa. Based on this understanding, it is clear that lower PSA levels should be considered as thresholds, although, as outlined in the guidelines of the European Association of Urology, more data are required to support this recommendation. However, biopsy is now widely accepted as necessary for PSA levels exceeding 25 ng/mL. Several adjustments to serum PSA measurements have been investigated to improve the clarity of PSA in the early detection of PCa (7).

In current urological practice, PSA, PSA density (PSAD), and DRE are used together to assess patients before deciding on a biopsy (8). Additionally, multiparametric magnetic resonance imaging (Mp-MRI) has been utilized since the 1980s to assess the anatomical structures of the prostate gland and surrounding tissues (9). With advances in MRI technology, the test reliability of Mp-MRI for detecting PCa has increased (10). PSAD, which is determined by severing the serum PSA level by the prostate volume gained through transrectal ultrasound, aids in distinguishing malignant tumors from benign prostatic enlargement, particularly in cases where PSA values ranged from 4 to 10 ng/mL (11). PSAD has been shown to be twice as discriminative as PSA alone, offering higher specificity and sensitivity (12).

In recent years, the use of Mp-MRI has become more widespread, especially after the development of the T2- weighted multiparametric prostate MRI protocol, which includes dynamic contrast-enhanced sequences that provide both anatomical and functional imaging (13).

The present study aimed to assess the combined specificity and sensitivity of Mp-MRI and PSAD for diagnosing PCa and preventing unnecessary prostate biopsies.

Materials and Methods

Our study adopted a retrospective design, initiated after obtaining approval from the Ankara City Hospital Ethics Committee (approval number: E1-22-3002, date: 02.11.2022). A total of 399 patients who underwent clinical evaluation for Pca and underwent prostate biopsy between 2021 and 2022 at Ankara City Hospital were retrospectively evaluated. The exclusion criteria were: patients without tissue diagnosis within six months of Mp-MRI, patients who had undergone previous transurethral resection, patients without PSA levels measured within 1 month before or after Mp-MRI, and patients whose Mp-MRI was performed without contrast due to renal dysfunction, as well as those with nodules, firmness, or fixation identified during DRE. For patients who underwent biopsy, serum PSA, DRE, prostate size, and PSAD were recorded. The patients were between 42 and 84 years old, with an average age of 64.3±6.9 years.

PSA levels and prostate volumes, measured using transrectal ultrasound, were noted, and PSAD was determined using the following formula: total PSA prostate size. The cutoff value for PSAD was 0.15 ng/mL/cc. Patients were grouped into two categories according to PSAD: Group 1 included those with a PSAD of 0.15, and Group 2 included those with a PSAD of 0.15. The patients' Mp-MRI results were obtained from both external centers and our own institution and classified according to the prostate imaging reporting and data system (PI-RADS) into PI-RADS scores of 1, 2, 3, 4, and 5.

Regardless of the PSAD grouping, patients were divided into two groups based on their PI-RADS scores: patients with PI-RADS scores less than 3, and those with PI-RADS scores of 3 or higher. The total and free PSA values were recorded for all patients.

For the patients included in our study, systematic 12-core biopsies were performed, and in some cases, additional targeted biopsies were performed. Prostate biopsy was performed in the left lateral decubitus orientation, with DRE findings recorded prior to the procedure. Prostate dimensions were measured, and the prostate size was calculated using the ellipsoid formula (transverse diameter × anteroposterior diameter × craniocaudal diameter × $\pi/2$), and the results were recorded. Patients diagnosed with PCa were classified according to the ISUP classification system. Patients with ISUP grade ≥2 were classified as having clinically significant PCa (csPCa).

Statistical Analysis

Data coding and statistical evaluations were conducted using SPSS 22 software (IBM SPSS Statistics, IBM Corporation, Chicago, IL). The Shapiro-Wilk test was applied to examine the normality of data distribution. Depending on the distribution pattern, data were expressed either as mean ± standard deviation (SD) or as median (range: minimum-maximum). Univariate analysis was performed to identify potential risk factors associated with PCa. Patients were stratified into four groups according to their PI-RADS scores and PSAD levels. For each group, the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were calculated. A p-value 0.05 was considered statistically significant.

Results

The average age of the 399 patients enrolled in the study was 64.3±6.9 years, with a median PSA value of

6.9 ng/mL (range: 1.1-49). PCa was detected in 150 patients (37.6%), and csPca was detected in 67 patients (16.8%). The detection rate of PCa was 11.2% in the PI-RADS <3 group, and 52.3% in the PI-RADS ≥3 group. In the PSAD <0.15 ng/mL/cc group, 23.6% of patients were identified as having Pca, whereas in the PSAD ≥0.15 ng/mL/cc group, the detection rate was 62.1%.

The patients were categorized into four distinct groups based on the combination of their PI-RADS scores and PSAD levels: PI-RADS <3 with PSAD <0.15 ng/mL/cc, PI-RADS <3 with PSAD ≥0.15 ng/mL/cc, PI-RADS ≥3 with PSAD <0.15 ng/mL/cc, and PI-RADS ≥3 with PSAD ≥0.15 ng/mL/cc. For each group, sensitivity, specificity, NPV, and PPV were determined. The highest PPV for both Pca and csPCa was found in the PI-RADS ≥3 + PSAD ≥0.15

ng/mL/cc group, with a PPV of 74.1% for Pca and 39.3% for csPCa (Table 1).

Univariate logistic regression analysis showed that PI-RADS ≥3 [odds ratio (OR) =8.718; 95% confidence interval (CI) =4.906-15.492; p<0.001], PSAD ≥0.15 ng/mL/cc (OR =5.291; 95% CI =3.397-8.241; p<0.001), and the combination of PI-RADS ≥3 and PSAD ≥0.15 ng/mL/cc (OR =9.398; 95% CI =5.68-15.549; p<0.001) were strong determinants of Pca (Table 2).

The integration of PI-RADS scores and PSAD significantly improved the detection of PCa. In particular, in patients with PI-RADS scores of ≥3 and PSAD values ≥0.15 ng/mL/cc, the PPV for Pca was 74.1%, and for csPca, it was 39.3%. The results revealed that patients with higher PI-RADS scores and PSAD levels should undergo biopsy, which can help reduce unnecessary biopsies. The results demonstrated that combining PI-RADS and PSAD offered more accurate diagnostic outcomes and improved the identification of csPca (Table 3).

Discussion

The utilization of Mp-MRI to enhance the precision of PCa diagnosis is increasing (14). The main expectation from this imaging modality is to accurately identify patients at risk of malignancy without the need for invasive procedures.

Table 1. PI-RADS score and prostate-specific antigen levels on multiparametric magnetic resonance imaging

	n=399	PI-RADS <3 (n=143), 35.8%	PI-RADS ≥3 (n=256), 64.2%	PSA <0.15 (n=254), 63.7%
Age (years) (mean ± SD)	64.3±6.9	64.5±6.9	64.2±6.9	64.5±6.9
Digital prostate examination				
Symptom no	n (%) 325 (81.5)	129 (90.2)	196 (76.6)	223 (87.8)
Symptom yes	n (%) 74 (18.5)	14 (9.8)	60 (23.4)	31 (12.2)
PSA (ng/mL) [median (min-max)]	6.9 (1.1-49)	5.7 (2.7-40)	7.5 (1.1-49)	5.8 (1.1-22.5)
Prostate volume (cc) [median (min-max)]	54 (20-220)	56 (20-220)	50.5 (20-210)	62.5 (25-210)
Prostate cancer stage, n (%)	150 (37.6)	16 (11.2)	134 (52.3)	60 (23.6)
ISUP stage 1	83	12	71	38
ISUP stage 2	28	1	27	12
ISUP stage 3	13	1	12	5
ISUP stage 4	19	2	17	4
ISUP stage 5	7	0	7	1
csPCa, n (%)	67 (16.8)	4 (2.8)	63 (24.6)	22 (8.7)

SD: Standard deviation, PSA: Prostate-specific antigen, PSAD: Prostate-specific antigen density, PI-RADS: Prostate imaging reporting and data system, ISUP: International Society of Urological Pathology, csPCa: Clinically significant prostate cancer

Table 2. Evaluation of the relationship between PI-RADS score, prostate-specific antigen density, and prostate cancer detection using univariate and multivariate regression analyses

Parameter	OR (%95 CI)	p-value	OR (%95 CI)	p-value
PI-RADS ≥3	8.718 (4.906-15.492)	**<0.001**	7.777 (4.272-14.158)	**<0.001**
PSAD ≥0.15 ng/mL/cc	5.291 (3.397-8.241)	**<0.001**	4.674 (2.891-7.557)	**<0.001**
PI-RADS ≥3 + PSAD ≥0.15 ng/mL/cc	9.398 (5.68-15.549)	**<0.001**	1.727 (0.517-5.775)	0.375

CI: Confidence interval, PSAD: Prostate specific antigen density, PI-RADS: Prostate imaging-reporting and data system. Bold p-values indicate clinical significance

Table 3. Effectiveness of the PI-RADS score and prostate-specific antigen density combination in the diagnosis of prostate cancer

Combinations of PI-RADS score and PSAD	Sensitivity (%)		Specifity (%)		Negative predictive value (%)		Positive predictive value (%)	
	PCa	csPCa	PCa	csPCa	PCa	csPCa	PCa	csPCa
PI-RADS <3 + PSAD <0.15 ng/mL/cc, n=110	6%	4.5%	59.4%	67.8%	51.2%	77.9%	8.2%	2.7%
PI-RADS <3 + PSAD ≥0.15 ng/mL/cc, n=33	4.7%	1.5%	89.6%	90.4%	60.9%	82%	21.2%	0.3%
PI-RADS ≥3+ PSAD <0.15 ng/mL/cc, n=144	34%	28.4%	62.6%	62.3%	61.2%	81.2%	35.4%	13.2%
PI-RADS ≥3+ PSAD ≥0.15 ng/mL/cc, n=112	55.3%	65.7%	80.5%	79.5%	41.8%	92%	74.1%	39.3%

PSAD: Prostate-specific antigen density, PI-RADS: PI-RADS: prostate imaging reporting and data system, PCa: Prostate cancer, csPCa: Clinically significant prostate cancer

In a study by Thompson et al., (14) even in healthy controls, PCa was detected at very low PSA levels. The detection rates in individuals with PSA levels ≤0.5 / 0.6-1 / 1.1-2 / 2.1-3 / 3.1-4 ng/mL were found to be 6.6%, 10.1%, 17%, 23.9%, and 26.9%, respectively (15). In a study of 288 patients by Washino et al., (15) the PI-RADS score and PSAD were analyzed in relation to Mp-MRI. CsPca was identified in 76-97% of patients with a PSAD ≥0.15 and PI-RADS score ≥4 or in patients with a PI-RADS rating of 3 and PSAD ≥0.3. In cases in which both the PI-RADS score and PSAD were elevated, biopsies were performed in patients who initially had negative biopsy results, and Pca was later determined in 22% of those patients (16). In our study, 74.1% (n=83) of the 112 patients with a PI-RADS score ≥3 and PSAD ≥0.15 were determined to have Pca, whereas 39.3% (n=44) were identified as having csPca. In contrast, only 8.2% (n=9) of the 110 patients with a PSAD <0.15 and PI-RADS <3 had Pca, with csPca detected in only 2.7% (n=3) (Table 1). The csPca values of the three patients with a PI-RADS score <3 and PSAD <0.15 suggests that more careful consideration is necessary when making biopsy decisions for this group.

To prevent redundant biopsies in the diagnosis of Pca, recent studies have employed a combination of Mp- MRI and PSAD. In a meta-analysis of 3006 patients by Schoots et al., (16) the relationship between Mp- MRI results, PSAD levels, and clinically significant disease was evaluated. The study found that patients with low-risk PSAD and negative Mp-MRI results could avoid biopsies, whereas those with high-risk PSAD still required biopsy. In particular, in patients with PI-RADS 3 lesions, PSAD plays a crucial role in biopsy decision-making. Mp-MRI-positive patients (PI-RADS 4-5) should undergo biopsy, regardless of PSAD risk categories (17). In our study, the PPV of Pca in patients with PI-RADS ≥3 and PSAD ≥0.15 was 74%, whereas that of csPca was 39%. These results indicate that patients with these characteristics should undergo biopsy. In a retrospective study by Luis Rico et al. (17), which included 99 patients who underwent transperineal prostate biopsy between 2015 and 2020, the role of lesion volume and PSAD in determining csPca in patients with PI-RADS 3 lesions was examined. The study concluded that using lesion volume and PSAD together could lead to more accurate biopsy decisions and help avoid unnecessary biopsies. No csPca was identified in the PI-RADS 3a group (lesion volume <0.5 mL), but in the PI-RADS 3b group (lesion volume >0.5 mL), 18% had csPca. In patients with PI-RADS 3b lesions and PSAD >0.15, the rate of csPca increased to 62.5%, whereas no cancer was detected in those with PSAD <0.15, suggesting that biopsies could be prevented in these patients (18).

Felker et al. (18) identified effective factors for predicting csPca in patients with PI-RADS 3 lesions. In their study of 90 patients, PSAD was found to be the most important factor. CsPca was observed in 60% of patients with a PSAD ≥0.15 ng/mL2. Based on this criterion, biopsies would have been performed only in high-risk patients, thereby reducing unnecessary biopsies by 90% (19). A study by Albert et al. (19) indicated that the integration of Mp-MRI and PSAD could accurately identify low-risk patients and prevent unnecessary biopsies. Using PSAD and Mp-MRI results, low-risk patients could be identified who do not require biopsy, whereas high-risk patients could be prioritized for biopsy (20). In our study, the probability of detecting PCa and csPca was higher in patients with a PI-RADS score ≥3 and PSAD >0.15 ng/mL/cm^3. Ghafoori et al. (20) found that PSAD improved the effectiveness of PSA in determining Pca in a group of 330 patients who underwent transrectal biopsy, with Pca diagnosed in 121 patients (36.7%) (21). Lotfi et al. (21) showed that PSAD, especially in men with PSA values between 4 and 10 ng/mL, was more important than total PSA levels (22). Bazinet et al. (22) established the most appropriate cut-off value for PSAD of 15% for detecting Pca in men with standard DRE observation and PSA values between 4 and 10 ng/mL (23). Kefi et al. (23) reported that 15% PSAD limit 15% resulted in 44% sensitivity and 76% specificity for detecting cancer (24). Catalona et al. (24) discovered that using a PSAD limit of 15% led to approximately 50% of PCa patients being missed (25). Benson et al. (25) concluded that PSAD was more valuable than PSA alone in determining Pca (26). In our study, the average PSA level among the 399 individuals who underwent biopsy was 6.9 ng/mL. The Pca identification rate was 23.6% in the PSAD <0.15 ng/mL/cc group and 62.1% in the PSAD ≥0.15 ng/mL/cc group. CsPca was detected in 8.7% (n=22) of patients in Group 1 (PSAD <0.15) and 31% (n=45) of patients in Group 2 (PSAD ≥0.15).

Study Limitations

Although our institution has an Mp-MRI machine, we do not have an Mp-MRI fusion biopsy system, which allowed us to perform cognitive biopsies under transrectal ultrasound guidance. These factors may affect the generalizability and applicability of our findings.

Conclusion

The findings of our study highlight the importance of PSAD and Mp-MRI in the diagnosis of Pca. The combination of these two parameters helps improve the accuracy of biopsy decisions, preventing unnecessary biopsies and enabling better detection of csPca.

Ethics

Ethics Committee Approval: Our study adopted a retrospective design, initiated after obtaining approval from the Ankara City Hospital Ethics Committee (approval number: E1-22-3002, date: 02.11.2022)..

Informed Consent: Retrospective study.

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Authorship Contributions

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Case Report

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Bull Urooncol 2024;23(4):116-118

Xanthoma of the Bladder - A Rare Entity

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Abstract

Xanthoma is a non-neoplastic lesion caused by an abnormal focal aggregation of lipid-containing histiocytes in tissues. Typically associated with hyperlipidemia, it can also be observed in patients with normal lipid levels. Bladder xanthoma is a rare lesion. It is usually asymptomatic and is usually encountered incidentally. It may be isolated or associated with urothelial carcinoma of the urinary bladder. In this case report, we present an incidental case of bladder xanthoma in a 64-year-old patient who underwent surgical treatment due to lower urinary tract symptoms and an investigation of the etiology of hematuria.
Keywords: Xanthoma, bladder xanthoma, cystoscopy, hyperlipidemia, case report

Introduction

Xanthoma is an abnormal focal accumulation of lipid-containing histiocytes in tissues. It is not a true neoplasia but a benign condition characterized by reactive histiocytic proliferation secondary to serum lipid abnormalities, which can be observed in patients with primary or secondary hyperlipidemia (1). Although evidence shows that it is generally associated with a high lipid profile, it may also be observed in patients with a normal blood lipid profile and as part of the inflammatory process secondary to surgery or trauma (2). Xanthomas are most commonly seen in the skin, subcutaneous tissue, tendons, and gastrointestinal system. Bladder localization of xanthomas is a rare condition (2,3). In this case report, we aimed to present a rare case of bladder xanthoma in the light of the literature and to contribute to the literature.

Case Report

A 64-year-old male patient was admitted to our urology outpatient clinic with complaints of decreased urinary flow, frequent urination, and intermittent hematuria for approximately 6 months. In his history, it was learned that he had previously received different long-term medical treatments in other centers for these complaints. The patient had no other chronic diseases or medications. The routine urologic examination of the patient was unremarkable except for a smoothly circumscribed enlarged prostate tissue on the finger rectal examination. Laboratory values revealed high serum total prostate-specific antigen (PSA) value (33.7 ng/mL) and erythrocyte cell counts on urinalysis. The lipid profile of the patient was within normal limits as triglyceride: 131 mg/dL, cholesterol: 154 mg/dL, high density lipoprotein: 36 mg/dL, low density lipoprotein: 92 mg/dL. In the uroflowmetry evaluation, the maximum urine flow rate was 3.7 mL/sec, and the amount of post-mictional residual urine was 400 mL. Urinary ultrasonography revealed no pathological features except for enlarged prostate tissue infante to the bladder. The patient was foley catheterized. Multiparametric prostate magnetic resonance imaging (MRI) was planned as further imaging evaluation due to the high PSA value. MRI showed approximately 155 cc prostate tissue indented to the base of the bladder. Peripheral and transitional zone evaluations revealed PIRADS 2 and 3 lesions in some locations. The bladder lumen and surrounding tissues have no pathological appearance. Cognitive fusion biopsy was performed from suspicious prostate areas according to MRI. Prostate tissue biopsy pathological results showed benign prostatic hypertrophy/chronic inflammation foci. Surgical intervention was planned for patients with high International Prostate Symptom Score and lower urinary tract symptoms (LUTS). On cystourethroscopy, the prostatic lobe appeared as a closed trilobe. In the bladder, a yellow-white soft tissue structure with a total load of approximately 15 mm was observed 4 cm superolateral to the right ureteral orifice (Figure 1). Transurethral resection was performed on these foci, and specimens were sent for pathological examination. The patient

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was discharged on postoperative day 2. Histopathological examination revealed foamy cytoplasm infiltrating into the lamina propria under normal urothelium. The final pathological diagnosis was bladder xanthoma (Figure 2). Patient consent was obtained for the case reports to be published for academic purposes.

Discussion

Bladder xanthoma is a very rare, non-neoplastic lesion. Since the first description by Miliauskas (1), very few cases have been reported. Most patients with bladder xanthoma do not present with prominent symptoms and are usually detected incidentally on imaging examinations or cystoscopic examinations performed on complaints of hematuria, suprapubic pain, and LUTS (2). It may be associated with a bladder tumor or found in isolation (2). In the clinical analysis of multicentric cases in a single series by Li et al. (3), the male-to-female ratio was 2.1:1, the mean age was 60.8 years, and the mean tumor diameter was 21 mm. Lipid profile disorder was noted in approximately 46% of patients. According to the literature, concomitant urothelial carcinoma is present in 50% of cases. In these cases, low-grade urothelial carcinoma is usually observed (3). However, cases of high-grade urothelial carcinoma have also been reported (4,5). On cystoscopic examination, they are generally described as velvety yellow-white lesions with pedicles (6,7). Histopathological differentiation of bladder natoma from other conditions is important for correct diagnosis and treatment planning. In the differential diagnosis, xanthogranulomatous cystitis, malakoplakia, and stony ring cell carcinoma should be considered (3,8). Treatment of lipid metabolism abnormalities, if any, is recommended. Considering the clinical symptoms related to the lesion in the urinary bladder and the possibility of urothelial carcinoma, surgical resection is recommended as both diagnostic and therapeutic according to the literature (3). Nishimura et al. (6) and Chitale et al. (7) made the diagnosis using punch biopsies in their study, and there was no change in the shape or size of the lesions in control cystoscopy performed 6 months later. No recurrence was observed in patients who underwent transurethral resection and in those who underwent control cystoscopy. Recurrence or increase in lesion size was not reported in cases associated with bladder tumors (2,3). Patient-based cystoscopic follow-up can be performed considering whether xanthoma is isolated or associated with urothelial carcinoma, the size of the lesion and the treatment applied. Xanthomas are not premalignant lesions, and long-term follow-up is not recommended (9,10). In our case, there was no lipid metabolism disorder. This was an isolated bladder xanthoma, and the data were compatible with the literature in terms of age, gender, and lesion size. Complete transurethral resection was performed in accordance with the literature. No recurrence or new tumor formation was detected in the 3rd month control cystoscopy.

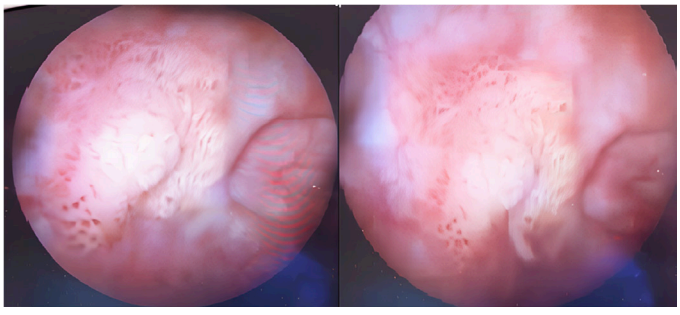

Figure 1. Images of the lesion during cystoscopy

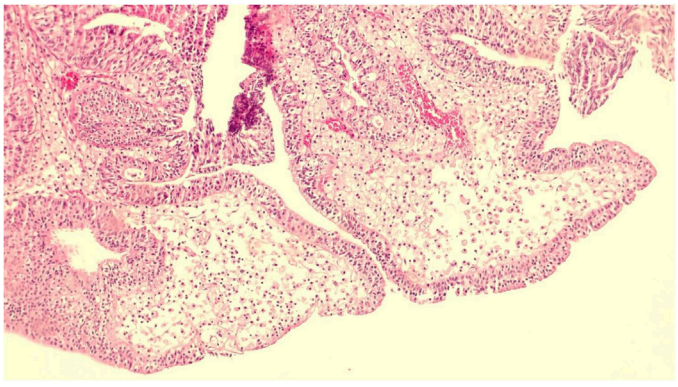

Figure 2. Under the benign urothelial epithelium, dense foamy histiocytes are observed in the lamina propria (hematoxylin and eosin stain x100)

Conclusion

Xanthomas of the urinary bladder are very rare benign lesions. They are usually detected incidentally. Patients with xanthomas should be evaluated for underlying disorders of lipid metabolism. Transurethral resection is both diagnostic and therapeutic because half of the patients have a concomitant bladder neoplasm. There are no premalignant lesions, and long-term follow-up is not required. In the differential diagnosis, xanthogranulomatous cystitis, malakoplakia, and signet ring cell carcinoma should be examined immunohistochemically.

Ethics

Informed Consent: Patient consent was obtained for the case reports to be published for academic purposes.

Acknowledgements

Publication: The results of the study were not published in full or in part in form of abstracts.

Contribution: There is not any contributors who may not be listed as authors.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.A.D., H.S., Ş.K., S.A., A.F.V.,

Concept: M.A.D., A.F.V., Design: M.A.D., Data Collection or Processing: M.A.D., H.S., A.F.V., Analysis or Interpretation: M.A.D., Ş.K., S.A., Literature Search: M.A.D., H.S., Writing: M.A.D., H.S., A.F.V.

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Case Report

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Simultaneous Partial Nephrectomy and Radiofrequency Ablation in a Solitary Kidney Patient

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Abstract

Renal cell carcinomas, which comprise the majority of primary kidney tumors, present diverse challenges in treatment planning. This case report explores the application of simultaneous partial nephrectomy and radiofrequency (RF) ablation in a patient with a solitary kidney, emphasizing the significance of specific interventions. A 52-year-old female patient who underwent radical nephrectomy presented with right flank pain and hematuria. Contrast-enhanced computed tomography revealed exophytic and calyceal system-extending lesions in the right kidney. A multidisciplinary approach involving interventional radiology and nephrology facilitated preoperative preparation. The patient underwent simultaneous partial nephrectomy and RF ablation in a single session, resulting in favorable outcomes. This meta-analysis highlighted radical nephrectomy's association with chronic kidney disease, emphasizing the need for specialized treatment approaches. Ablative treatment was associated with superior perioperative outcomes, whereas partial nephrectomy exhibited higher urological complication rates. Minimally invasive techniques are crucial, especially for solitary kidney cases and small renal tumors. Simultaneous partial nephrectomy and RF ablation are effective for managing exophytic and calyceal system-extending masses in solitary kidneys. The preservation of renal function is of paramount importance, prompting consideration of ablative treatment alongside partial nephrectomy. Despite limited evidence, ablative therapies offer a viable alternative for frail and comorbid patients, ensuring long-term oncological durability and superior preservation of renal function.

Keywords: Ablation, partial nephrectomy, radical nephrectomy, radiofrequency ablation, renal cell carcinoma, renal tumor, tumor

Introduction

Renal cell carcinomas (RCC) are tumors originating from the renal cortex, constituting approximately 80-85% of primary kidney tumors (1). The prognosis of RCC is primarily dependent on its anatomical spread, number, histopathology, molecular characteristics of the tumor, and clinical factors in patients (2). Radical nephrectomy is the gold standard treatment for patients with more than one tumor detected in RCC. However, for solitary kidney masses, those with inadequate kidney reserve, patients deemed high-risk for radical surgery, and small kidney masses, partial nephrectomy and ablative treatments can be considered (3). In this case, our goal was to apply simultaneous partial nephrectomy and radiofrequency (RF) ablation treatment in the same session.

Case Report

A 52-year-old female patient presented to our clinic with right flank pain and hematuria. Eleven years ago, the patient underwent right robotic radical nephrectomy due to the detection of a mass in the right kidney during investigations. Contrast-enhanced computed tomography (CT) of the entire abdomen revealed the absence of the left kidney. In the right kidney upper pole, an exophytic extension measuring 4 x 3.5 x 3 cm from the cortex and a growth from the para-pelvic area toward the calyces measuring 3.5 x 2.5 x 2 cm were observed. Both of these lesions were malignant in nature, with heterogeneous, dense cystic lesions and significant contrast uptake (Figure 1). The patient was evaluated with interventional radiology and nephrology in the preoperative period, and all preoperative preparations were completed considering the

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patient's anephric status. Subsequently, the patient underwent partial nephrectomy for a 4 cm exophytic mass in the upper pole and interventional radiology, including RF, for a 3.5 cm mass. All procedures were performed during the same operative session (Figure 2).

The duration of the operation was 130 minutes, and the ischemia time was 18 minutes. No complications occurred during the peri- and early postoperative periods. The patient had a preoperative creatinine level of 1.2 and demonstrated normal serum creatinine values in the early postoperative period, which were within the normal limits (creatinine 0.9). The patient was discharged on the 5th postoperative day. The patient was scheduled for a follow-up appointment in the first postoperative month and underwent a control contrast-enhanced whole abdominal CT scan. Although no distinct residual findings were discernible at the cortex-located lesion, the approximately 17 x 13 mm segment of the lesion located in the para-pelvic region was interpreted as residual tissue showing contrast enhancement (Figure 3). The pathological diagnosis was classic clear cell RCC. Based on the follow-up imaging results, a decision was made to perform re-RF ablation. Informed consent was obtained from the patient.

Discussion

In a meta-analysis published in 2016, radical nephrectomy was associated with the highest incidence of chronic kidney disease and the largest decrease in the estimated glomerular filtration rate among renal tumor treatment options. Ablative treatment was associated with the most favorable perioperative outcomes.

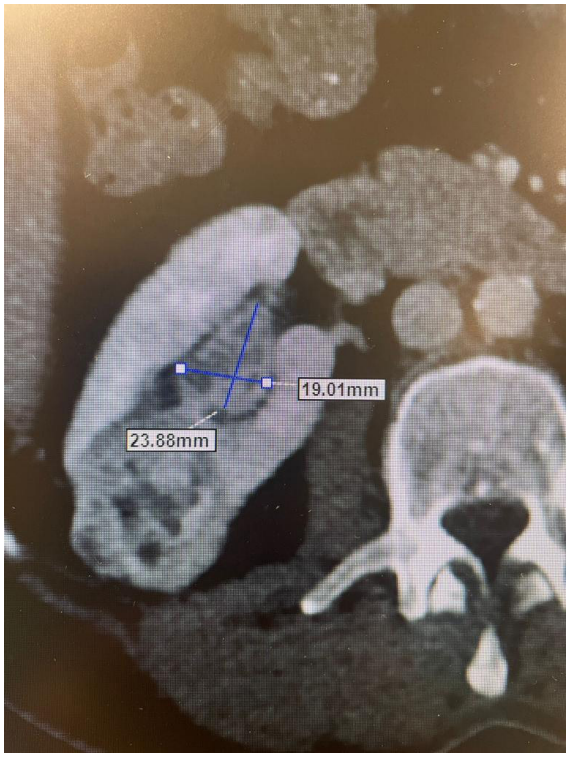

Figure 1. Pre-operative computed tomography images in the coronal section showing two malignant masses in the right kidney

Partial nephrectomy showed the highest rates of urologic complications, although overall minor/major complication rates were similar among interventions (4). It is necessary to consider minimally invasive techniques that preserve the kidney, especially in patients with a single kidney and small renal tumors.

Although the indications for ablative surgery in small renal tumors are not clearly defined, it can be categorized based on patient and tumor-specific characteristics. The fact that ablative methods can be applied multiple times without the need for renal ischemia is an important advantage (5). The other important group is patients with bilateral and multiple tumors or patients with syndromes such as von Hippel-Lindau and Birt-Hogg-Dubé, which are characterized by frequent tumor recurrence and multifocality. In patients requiring multiple interventions, partial nephrectomy has a negative impact on kidney function because of increased ischemia time (6).

Ablative therapies have similar long-term oncological durability, lower complication rates, and superior preservation of renal function compared with partial nephrectomy. Despite the low quality of evidence, ablative therapies are a reasonable alternative to partial nephrectomy in frail and comorbid patients (7).

In this case, we opted for this method to preserve the solitary kidney, considering the patient's age and comorbidities in accordance with the literature. One of the advantages of this

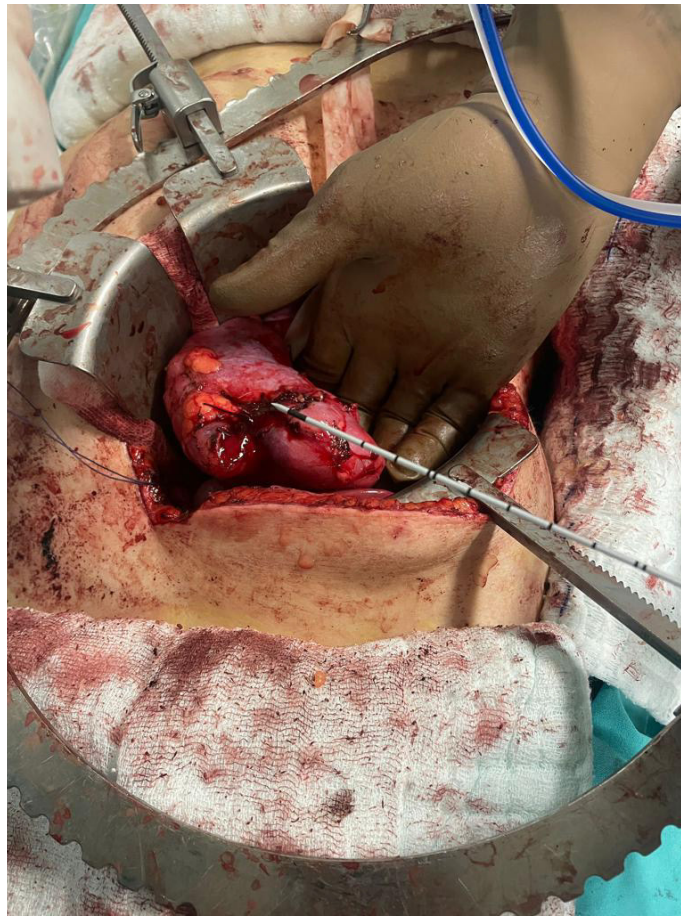

Figure 2. Intraoperative image of partial nephrectomy and radiofrequency ablation of the endophytic mass in the same session

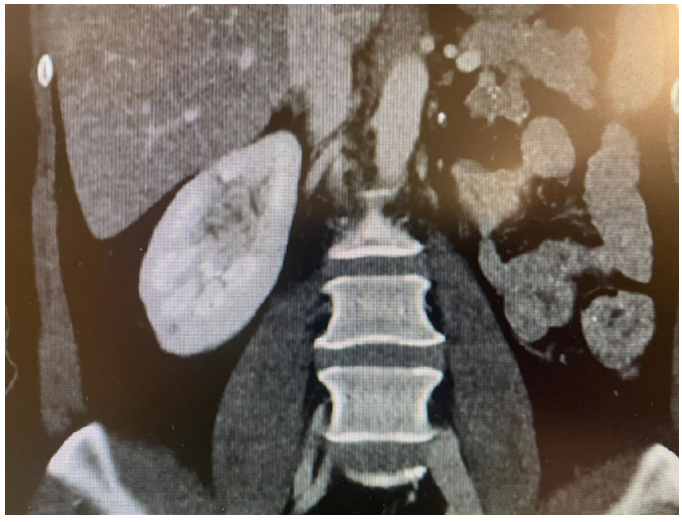

Figure 3. Postoperative first-month computed tomography images of the coronal section of the right kidney

procedure is the reproducibility of the RF technique. At the first-month follow-up, CT scan was performed again to detect an intraparenchymal residual mass. The mass size was reduced by approximately 85%. Consequently, the RF was applied again. During the patient's final evaluation, we observed that he was completely free of tumors.

Partial nephrectomy and RF ablation can be performed in the same session for both the exophytic and calyceal systems extending masses in the solitary kidney that require close follow-up. The goal is to avoid anephric status and the need for dialysis; therefore, it is beneficial to consider other ablative treatment options along with partial nephrectomy, if possible, with the aim of preserving renal function.

Ethics

Informed Consent: Informed consent was obtained from the patient.

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Authorship Contributions

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

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

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Case Report

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A Rare Cause of Macroscopic Hematuria: Primary Bladder Paraganglioma: A Case Report on Clinical, Imaging, and Treatment Approaches

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Abstract

Pheochromocytomas are tumors originating from the chromaffin cells of the adrenal medulla that can cause the release of catecholamines. Pheochromocytomas located outside the adrenal glands are referred to as paragangliomas and account for 10-15% of all pheochromocytoma cases. This case report presents a 30-year-old female patient diagnosed with primary bladder paraganglioma who presented to our clinic with the sole complaint of hematuria. After the detection of a mass in the bladder via abdominopelvic ultrasound to investigate the etiology of hematuria, further advanced investigations and histopathological examinations led to the diagnosis of the exceedingly rare primary bladder paraganglioma. By examining this disease clinically, radiologically, and histopathologically, we aim to expand our knowledge of this condition.

Keywords: Bladder, hematuria, paraganglioma

Introduction

Pheochromocytomas are frequently benign, non-epithelial tumors originating from the chromaffin cells of the adrenal medulla, causing catecholamine discharge. Pheochromocytomas located extra-adrenally are also referred to as paraganglioma, accounting for 10-15% of all cases (1). Primary bladder paraganglioma are exceedingly rare, accounting for only 0.06% of cases. They do not exhibit sex predilection, and the most common symptoms in these patients are hypertension, headache, palpitations, and hematuria (2). In addition to these symptoms, radiological imaging, such as ultrasound (US) and computed tomography (CT), may reveal a mass in the bladder, raising suspicion of a diagnosis of bladder cancer. However, a definitive diagnosis requires cystoscopic imaging and histopathological examination of a biopsy taken from the mass.

Currently, the treatment of primary bladder paraganglioma often involves transurethral resection of the bladder tumor (TURBT) or partial cystectomy, both of which typically result in curative outcomes (3). Nevertheless, because of the potential for local recurrence or metastasis even during long-term follow-up, especially in cases of malignant transformation, lifelong clinical monitoring is crucial.

Herein, we present the case of a 30-year-old female patient diagnosed with primary bladder paraganglioma following presentation to the urology outpatient clinic with macroscopic hematuria. Through clinical, radiological, and histopathological evaluation of this exceptionally rare condition, we aim to contribute to the existing literature.

Case Report

A 30-year-old female patient presented to our urology clinic with the sole complaint of macroscopic hematuria. Laboratory investigations revealed 2358 erythrocytes and 1 leukocyte in a complete urinalysis, while other biochemical parameters, such as complete blood count, renal function tests, and liver function tests, were within normal limits. Abdominopelvic US showing a hypoechoic mass measuring 25x35 mm with intraluminal extension in the right posterolateral wall of the bladder. Under

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local anesthesia, the patient underwent a diagnostic cystoscopy, revealing a solid mass approximately 2.5 cm in size with a polypoid extension on the right lateral wall of the bladder (Figure 1). Due to the patient's allergy to contrast medium, contrast-enhanced abdominopelvic CT could not be performed. Instead, 18-FDG-PET [2-deoxy-2-(fluorine-18) fluoro-D-glucose positron emission tomography] was performed, and the patient was preoperatively hospitalized for TURBT. The 18-FDG-PET scan revealed increased FDG uptake (SUV_{max}: 14) in a 28x36 mm soft tissue mass in the right posterolateral bladder wall, with physiological FDG uptake and distribution observed in other areas (Figure 2). Following preoperative verbal and written informed consent, TURBT was performed, achieving complete tumor resection along with its base (Figure 3).

Histopathological examination of the resected material revealed a tumor composed of nest structures (zellballen pattern) separated by fine fibrovascular septa, infiltrating the muscle layer beneath the urothelium-covered tissue samples. Tumor cells typically exhibit vesicular nuclei, occasionally large hyperchromatic nuclei, and extensive granular or clear cytoplasm. Necrosis and nuclear debris were observed in the center of the nests, leading to a definitive histopathological diagnosis of paraganglioma (Figure 4). The patient was discharged on postoperative day 2 without any complications after removal of the urethral foley catheter. Written and verbal informed consent was obtained from the patient prior to study participation.

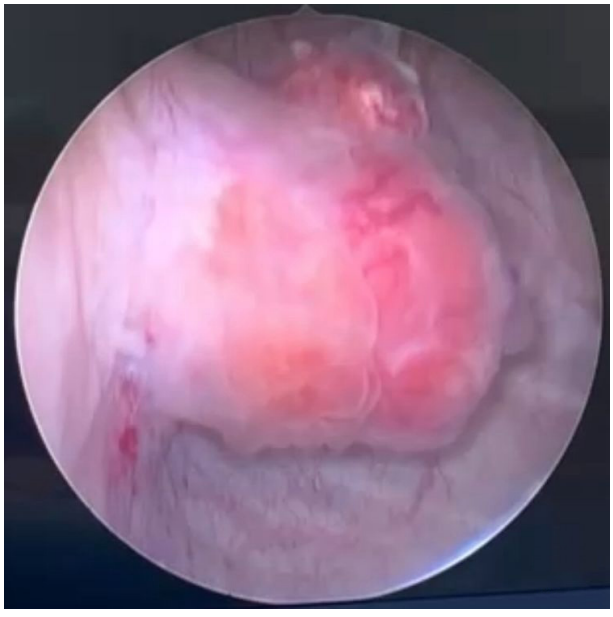

Figure 1. Cystoscopic view of the tumor on the right lateral wall of the bladder

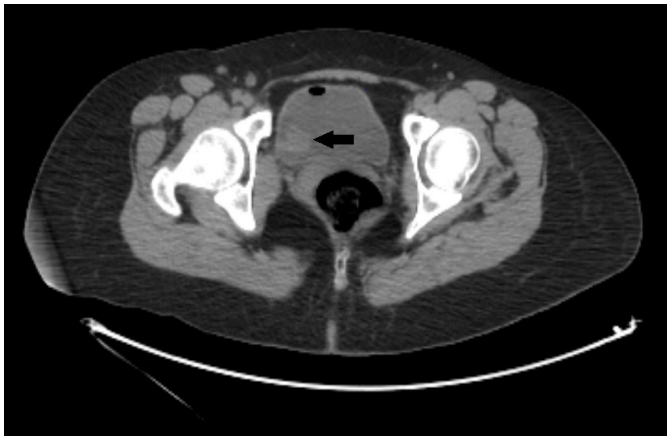

Figure 2. The mass showing increased FDG uptake in the soft tissue, measuring 28 x 36 mm in size, located in the right posterolateral aspect of the bladder, is indicated by the arrow (SUV_{max}: 14)

Discussion

Bladder paraganglioma are rare, non-epithelial neuroendocrine tumors originating from chromaffin cells (4). They constitute 0.05-0.06% of all bladder tumors and typically present between the ages of 43 and 50, with no gender predilection (2). The most common site of paraganglioma in the genitourinary system is the bladder (79.2%), followed by the urethra (12.7%), pelvis (4.9%), and ureter (3.2%) (5). The most frequent locations within the bladder are the dome and trigone. Bladder paraganglioma are submucosal in 45% and intramural in 42% of cases, with an average size of 2.5 cm (6). These tumors can present a wide range of clinical symptoms due to catecholamine release, including flushing, hypertension, palpitations, tremors, and hematuria (5,7).

Diagnostic imaging techniques for bladder paraganglioma include US, CT, and magnetic resonance imaging (MRI). On US, they appear as hypoechoic lesions with intraluminal extension (60%) and hypervascularity on Doppler (1,7). CT has a sensitivity of 91%, with the tumor appearing as a hyperdense, round, homogeneous lesion with prominent peritumoral vessels during the arterial phase; calcifications are seen in 10% of cases (2). MRI is more sensitive than CT and provides excellent soft-tissue resolution for detecting the tumor's location within the bladder

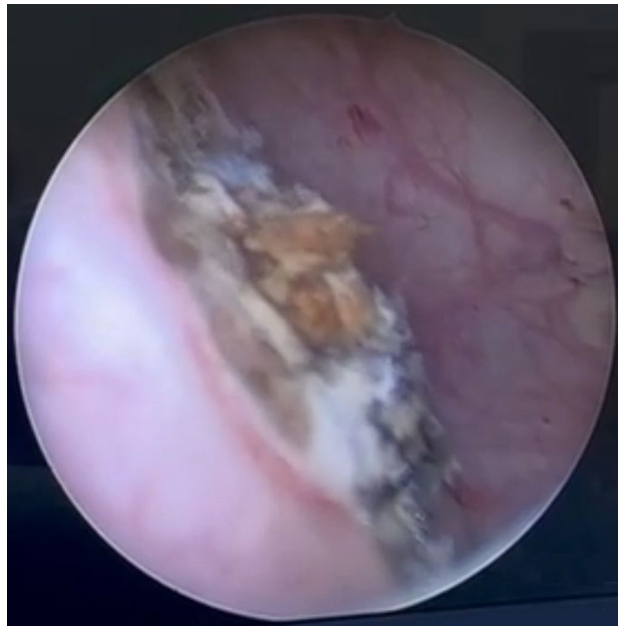

Figure 3. Cystoscopic appearance of the tumor bed after complete resection

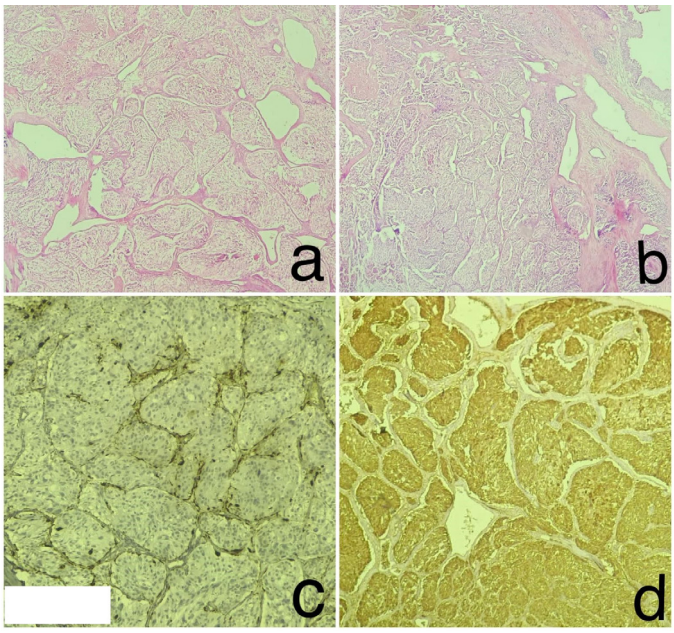

Figure 4. a) Tumor cells with large hyperchromatic nuclei, occasionally with a wide granular or clear cytoplasm, and visible in a vesicular appearance (H&E x100). **b)** Tumor cells with large hyperchromatic nuclei, occasionally with a wide granular or clear cytoplasm, and visible in a vesicular appearance (H&E x40). **c)** Nest structures, separated by fine fibrovascular septa, are believed to infiltrate the muscle layer from beneath the surface epithelium, as seen in tissue samples covered with urothelium, displaying the "Zellballen pattern" (S-100). **d.** Positive staining of sustentacular cells with synaptophysin and chromogranin

layers. Bladder paraganglioma typically appear hyperintense compared with the muscularis propria on T1- and T2-weighted images and often exhibit diffusion restriction (1,2,8).

Other imaging modalities, such as nuclear medicine studies, are particularly useful for identifying metastatic disease. These imaging techniques (Gallium-68 DOTATATE, 18F-FDG, and 18F-DOPA) have higher sensitivity and specificity for the imaging of abdominopelvic paraganglioma (2). However, in our case, due to the patient's contrast medium allergy, we used 18-FDG-PET along with US for imaging before obtaining a histopathological diagnosis. Additionally, screening with iodine-131 metaiodobenzylguanidine (MIBG) is highly sensitive and specific for detecting pheochromocytomas (2).

Approximately 10-26% of bladder paraganglioma undergo malignant transformation, potentially leading to lymph node involvement or distant metastasis (7,9). Staging is classified as T2 for muscle invasion, T3 for peripheral fat extension, and T4 for invasion into adjacent organs or muscles. Bladder paraganglioma do not have a T1 stage. N1 indicates pelvic lymph node involvement, and metastasis is considered when non-adrenal, non-parasympathetic ganglia are affected, with common sites being lymph nodes, bones, liver, and lungs (2).

Histologically, there are no definitive features that distinguish benign from malignant paraganglioma. The best-defined pathological indicators include positive immunohistochemical staining for synaptophysin and chromogranin, with the "Zellballen pattern" highlighted by S-100 in sustentacular cells.

The treatment options for paraganglioma vary depending on the tumor stage and include catecholamine blockade, surgery, chemotherapy, and radiotherapy. Surgical treatments, such as TURBT or partial cystectomy, are effective for localized or locally advanced bladder paraganglioma (2,10). Even after resections in which no tumor is observed at the surgical margins, approximately 15% of cases experience local recurrence, underscoring the importance of regular imaging follow-ups, ideally annually with MRI (2). Tsai et al. (11) recommended postoperative follow-up protocols including annual cystoscopy, plasma or urine catecholamine analyses, and MIBG scan, whereas Young et al. (12) considered urinary and serum vanillylmandelic acid levels as the best tools for detecting clinical recurrence or distant metastasis. In our case, we evaluated the postoperative follow-ups at 1 month using urine and serum catecholamine levels, supplemented with MIBG. Both urine and serum catecholamine levels were within the normal reference range, and no pathological findings were observed during the MIBG scan. During the 6th and 12th-month follow-ups, we only measured serum and urine catecholamine levels and, given their normality, recommended annual follow-up. The initial postoperative cystoscopy was performed after 3 months after the surgery. Because we did not observe any pathology in the bladder during the 3-month cystoscopy, we scheduled the subsequent cystoscopy for 6 months later.

There are significant differences and similarities between the various case reports in the literature and our case. In the case presented by Orsini et al. (13) the patient suffered from hypertension and post-micturition tachycardia attacks and was treated with robotic partial cystectomy. In the case reported by Pérez Barón et al., (14) the patient had complaints of hypertension, vertigo during micturition, and macroscopic hematuria; therefore, laparoscopic partial cystectomy and robot-assisted pelvic lymphadenectomy were chosen as treatment.

Hajji et al. (15) performed laparoscopic partial cystectomy in a patient with asymptomatic and incidentally detected paraganglioma. In the study by Üre et al. (16) a patient with post-micturition tachycardia and headache attacks underwent partial cystectomy.

Our case differs significantly from those of the literature. Our patient had only macroscopic hematuria as a symptom, and TURBT, a less-invasive method, was preferred for treatment. No recurrence was observed during follow-up. This approach is similar to the case presented by Doorn et al. (17) in which a paraganglioma incidentally detected during control cystoscopy in a patient who underwent nephroureterectomy for urothelial carcinoma was treated with TURBT.

The series of 10 cases by Qin et al. (8) focused on imaging methods and diagnostic stages, whereas our study comprehensively addressed all clinical, radiological, and histopathological stages from diagnosis to treatment. Although the ten-case study by Zhang et al. (18) showed similarities with our case in terms of patient selection and treatment methods, it differed in follow-up periods.

Conclusion

In conclusion, although bladder paraganglioma are rare, recognizing and clinically suspecting them is crucial. Accurate diagnosis, appropriate use of characteristic imaging techniques, and proper planning of interventions are vital to avoid unnecessary emergency surgeries or misdiagnoses inconsistent with the tumor's nature.

Ethics

Informed Consent: Verbal and written informed consent was obtained from the patients before surgery.

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Publication: The results of the study were not published in full or in part in form of abstracts.

Contribution: There is not any contributors who may not be listed as authors.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.N., Ö.I., Concept: B.S., Design: A.N., Data Collection or Processing: Ö.I., Analysis or Interpretation: B.S., Literature Search: A.N., Ö.I., Writing: A.N., Ö.I.

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