JJBS

Volume 18, Number 1,March 2025
ISSN 1995-6673
Pages 33 – 39
https://doi.org/10.54319/jjbs/180104

Jordan Journal of Biological Sciences

Association between Sex hormone-binding Globulin Levels and Thyroid Function in Bladder Cancer

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Received: *April 19, 2024; Revised: August 1, 2024; Accepted: August 23, 2024*

Abstract

Previous research has associated thyroid hormones with cancer development and progression, highlighting their role in synthesizing sex hormone-binding globulin (SHBG), which is implicated in cancer growth. However, the specific influence of these hormones and proteins on bladder cancer remains unexplored. This study aims to investigate potential correlations between free triiodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH), thyroxine-binding globulin (TBG), SHBG, and bladder cancer in Jordanian patients diagnosed with this malignancy. A cohort of 30 bladder cancer patients and 30 matched controls were recruited. Serum levels of fT3, fT4, TSH, TBG, and SHBG were measured using ELISA. Elevated concentrations of fT3 (4.64 ± 0.16 pmol/L), fT4 (14.86 ± 0.37 pmol/L), and SHBG (1227 ± 102.2 nmol/L) were observed in patients compared to healthy individuals ($p = 0.006$, $p = 0.002$, and $p < 0.0001$, respectively). However, TSH (1.47 ± 0.15 mIU/L) and TBG (4.3 ± 0.64 mg/L) levels showed no significant differences ($p = 0.319$, $p = 0.455$, respectively) between the groups. This study confirms a link between higher serum levels of fT3, fT4, and SHBG and the presence of bladder cancer, suggesting these elevated levels may indicate an increased risk. SHBG, in particular, stands out as a potential biomarker for predicting susceptibility to bladder cancer, warranting further investigation.

Keywords: Bladder cancer, thyroxine, thyroid-stimulating hormone, thyroxine-binding globulin, sex hormone binding globulin.

1. Introduction

Bladder cancer, a prevalent malignancy affecting the urinary bladder, poses a significant health burden globally, with various histological subtypes such as transitional cell carcinoma, squamous cell carcinoma, and adenocarcinoma (Babjuk, *et al.*, 2020). Urothelial (transitional cell) carcinoma represents the predominant subtype, comprising approximately 90% of cases (Clark, *et al.*, 2013 Comperat, *et al.*, 2022). Despite advancements in treatment modalities, bladder cancer remains a formidable challenge, ranking as the 10th most common cancer worldwide with an estimated 212,536 deaths and 573,278 new cases in 2020, according to Globocan (Sung, *et al.*, 2021). Bladder cancer is classified into muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC) based on the extent of tumor invasion. NMIBC is confined to the urothelium or lamina propria and often recurs but is less likely to progress to a life-threatening stage, whereas MIBC, which invades the detrusor muscle, requires aggressive treatment (Lopez-Beltran, *et al.*, 2024 de Jong, *et al.*, 2023). Smoking remains a well-established risk factor, contributing significantly to the incidence of bladder cancer (Sung, *et al.*, 2021 Zhao, *et al.*, 2022 Jubber, *et al.*, 2023).

Recent work has underscored the significance of sex hormone receptor signaling in the pathogenesis of urothelial cancer (Zhu, *et al.*, 2023). Studies have demonstrated that the activation of androgen and estrogen receptors play a pivotal role in initiating various cellular cascades and pathways linked to urothelial tumorigenesis, including the AKT/ERK pathway (Zheng, *et al.*, 2011).

Thyroxine (T4), a key hormone essential for normal growth, metabolism, and development, has been extensively studied regarding various types of cancer. Studies have revealed the intricate associations between cancer and thyroid hormones (Moeller, *et al.*, 2013). In the last two decades, the understanding of mechanisms by which thyroid hormones exert their effects has notably advanced. Thyroid hormones utilize a non-genomic pathway for their activity, involving a plasma membrane integrin called αvβ3 as a membrane receptor (Davis, *et al.*, 2016). Importantly, this receptor possesses two distinct hormone binding sites, S1 and S2, each initiating distinct signaling cascades (Freindorf, *et al.*, 2012). The S1 site selectively binds physiological levels of triiodothyronine (T3), activating phosphatidylinositol-3-kinase, subsequently stimulating the transcription factor hypoxia-inducible factor 1 (HIF1) (Moeller, *et al.*, 2013). The expression of HIF1 target genes is closely linked to tumor initiation, progression, invasion, and metastasis (Moeller,

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** **Abbreviations**: fT3 Free triiodothyronine; fT4 Free thyroxine; TSH Thyroid-stimulating hormone; TBG Thyroxine-binding globulin; SHBG Sex hormone-binding globulin; HIF1Hypoxia-inducible factor 1

et al., 2013). In contrast, the second site, S2, exhibits reduced affinity for T4 compared to T3, leading to activation of the ERK1/2 pathway (Lin, *et al.*, 2009).

Thyroid hormones, facilitated by the αvβ3 integrin, promote the proliferation of both blood vessel cells and cancer cells (Davis, *et al.*, 2016). Clinical studies have revealed that hyperthyroidism may be associated with an increased risk of certain solid tumors, while malignancies may progress more slowly or less aggressively in cases of spontaneous hypothyroidism (Hercbergs, *et al.*, 2010). These findings have been reported for various cancers, including lung (Khan, *et al.*, 2016), breast (Tran, *et al.*, 2023), prostate (Chan, *et al.*, 2017), ovary (Ness, *et al.*, 2000), colorectal (Gagliardi, *et al.*, 2023), central nervous system (Mellemgaard, *et al.*, 1998), esophageal (Turkyilmaz, *et al.*, 2010), hematologic cancer (Ghalaut, *et al.*, 2012), and overall solid cancer (Khan, *et al.*, 2016). However, the role of thyroid hormones in bladder cancer remains unexplored.

Emerging research has also delved into the intricate relationship between thyroid hormones and sex hormones, highlighting the significance of thyroid hormone in the synthesis of Sex Hormone-Binding Globulin (SHBG) (Selva, *et al.*, 2009). It is well-established that thyroid hormones facilitate SHBG synthesis (Selva, *et al.*, 2009), regulating the transport and binding of estrogen and androgen hormones to their respective receptors. Moreover, estrogen has been shown to elevate thyroxine-binding globulin (TBG) levels (Robbins, *et al.*, 1978), whereas androgens tend to reduce TBG levels (Tahboub, *et al.*, 2009), with TBG primarily responsible for transporting thyroid hormones to target tissues.

Considering the growing body of evidence linking sex hormones to bladder cancer, coupled with the intricate interplay between sex hormones and thyroid hormones, and the acknowledged role of thyroid hormones in cancer pathogenesis, we aimed to investigate the potential involvement of thyroid hormones in bladder cancer. Our study sought to explore possible associations between thyroid-stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), TBG, SHBG, and bladder cancer among Jordanian individuals diagnosed with this malignancy. We hypothesize that these hormones and proteins may exhibit correlations with an elevated risk and prevalence of bladder cancer. This research endeavor aspires to contribute to the improved management and understanding of bladder cancer.

2. Materials and Methods

2.1. Study Subjects and Sample Collection

This study was a case-control study conducted at the Urology Clinic at Princess Basma Teaching Hospital and the University Hospital of J.U.S.T in Irbid Province, located in the north of Jordan. The study included 30 patients diagnosed with early invasion (stage PT1) bladder cancer. Among these patients, there were 26 males and 4 females, ranging in age from 47 to 83 years. All patients had been diagnosed within 1 year of enrollment, had confirmed histology of bladder cancer, and had not received any prior chemotherapy or radiotherapy treatments. The study did not impose any age or gender restrictions.

Among the participants, 12 individuals had hypertension and were using angiotensin-converting enzyme inhibitors, four had type I diabetes and were using insulin, and five had type 2 diabetes and were using metformin. Additionally, 30 healthy volunteers of similar age (within 5 years) and gender, with no history of cancer, thyroid disorders, diabetes, high blood pressure, recent surgery, or use of vitamin supplements, were recruited as controls for comparison.

Individuals who had received any form of hormone therapy in the past, as well as those with chronic conditions such as chronic kidney disease, heart disease, uncontrolled diabetes mellitus, cerebrovascular disease history, or a history of any malignancy, were excluded from participating in the study.

It is important to note that this study is designed to investigate the association between specific factors (such as thyroid hormones) and bladder cancer risk in a specific population. The inclusion of controls and the careful consideration of confounding variables, such as age, gender, and medical conditions, helps to ensure reliable and meaningful research findings.

All blood samples were collected during the period from November 2022 to May 2023. Blood Samples were collected from patients and the control group in a plain tube without additives in the early morning without fasting requirements.

2.2. Ethical Approval

In the study, the aims and analysis of the research were transparently and honestly explained to all individuals who were recruited as subjects. Before enrollment, each participant provided written informed consent, signifying their voluntary decision to take part in the study. Additionally, a questionnaire was administered to gather relevant information from the participants. Furthermore, the study protocol and procedures received approval from the Institutional Review Board (IRB) Committee, with the assigned identification number 558-2022, at Jordan University of Science and Technology located in Irbid, Jordan. The IRB's approval signifies that the study design, ethical considerations, and protection of participants' rights were carefully reviewed and deemed acceptable.

2.3. Experimental Design and Sample Preparation

During the study, blood samples were collected from participants in the morning between 9:00 and 11:00 AM. Plain tubes without anticoagulants were used to collect the blood samples. After approximately 20 minutes to allow for clotting, the samples were centrifuged for 5 minutes at a speed of 4000 rpm. This centrifugation process helped to separate the serum from other components of the blood.

Following centrifugation, the serum was carefully separated from each specimen. Each sample was then divided into smaller aliquots and stored at a temperature of -83 °C for future use. We were concerned about the stability of thyroid hormones in frozen samples because we collected them over several months. But based on a recent study, thyroid hormone concentrations are stable even after years when are kept frozen at -25° (Mannisto, *et al.*, 2010). This freezing temperature helped to maintain

the integrity and stability of the samples until they were ready for measurement.

On the day of measurement, the samples were thawed at room temperature and gently mixed by inverting the tubes in a gentle manner. This ensured that the samples were well-mixed and representative of the subsequent measurements and analyses to be conducted. The described process of blood collection, serum separation, and storage followed standard procedures to ensure the quality and consistency of the samples for accurate measurements in the study.

2.4. Biochemical Measurements

Serum concentrations of fT3, fT4, TSH, TBG, and SHBG were determined using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. The TSH, fT4, and fT3 ELISA kits were purchased from Monobind Inc. (Lake Forest, CA 92630, USA), the TBG ELISA kit was purchased from Fine Test (cat no. EH0833; Wuhan Fine Biotech, China), and the SHBG ELISA kit was purchased from Cloud-clone Corp. (cat no. SEA396Hu; USA). To assess SHBG levels, serum samples underwent a 1,000-fold dilution in phosphate-buffered saline supplemented with 0.1% bovine serum albumin (cat no. P3688; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Absorbance readings were obtained using an ELx800 microplate reader (Bio Tek Instruments, Inc., Winooski, VT, USA) at a wavelength of 450 nm. Each assay was measured in duplicate, and then the mean was calculated. All these tests were conducted manually in the biochemistry research laboratory at Jordan University of Science and Technology.

2.5. Statistical Analysis

In this investigation, analysis was conducted employing GraphPad Prism version 12 (GraphPad Software Incorporated, San Diego, U.S.A.). To compare samples and controls with normally distributed data, a student's *t*-test was employed. Statistical significance was defined as a "p" value < 0.05. Normality of the data was assessed using the D'Agostino-Pearson and Shapiro-Wilk tests.

3. Results

Table 1 presents the comparison of baseline characteristics between the bladder cancer group (n=30) and the control group (n=30). The parameters analyzed include age, BMI, gender, and medical history. The p-values are above 0.05, suggesting no statistically significant differences between the two groups for these parameters.

Table 1. Baseline characteristics of the study participants.

Parameter	Bladder cancer (n=30)	Control (n=30)	p-value
Age, years ± SD	64.3 ± 9.8	61 ± 8.7	0.3
BMI, value ± SD	26.5 ± 3.4	25.9 ± 3.7	0.5
Male, n (%)	26 (86.7)	26 (86.7)	_
Female, n (%)	4 (13.3)	4 (13.3)	_
Medical history, n (%)			
Smoking	25 (83.3)	23 (76.7)	0.5
Hypertension	12 (40)	13 (43.3)	0.8
Diabetes	9 (30)	7 (23.3)	0.6

The fT4 average concentration in the bladder cancer patient group and the control group are illustrated in Figure 1. As demonstrated in the figure, the mean concentration of fT4 was significantly higher ($p = 0.002$) in the patient group (14.86 ± 0.37) compared to the control group (13.35 ± 0.24).

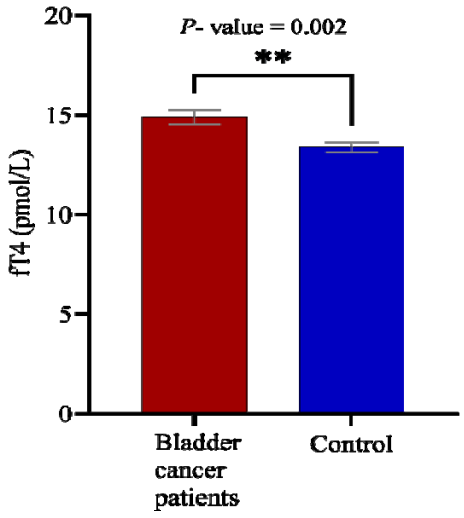

Figure 1. The concentration of free T4 in bladder cancer patients (n = 30) and control (n = 30). The mean concentration of fT4 was significantly higher in the patient group compared to the control group. Data represents the mean value ± S.E.M. (**p < 0.01).

Figure 2 depicts the average concentration of fT3 in the bladder cancer patient group and the control group. As illustrated in the figure, the mean concentration of fT3 was significantly higher ($p = 0.006$) in the patient group (4.64 ± 0.16) compared to the control group (4.09 ± 0.1).

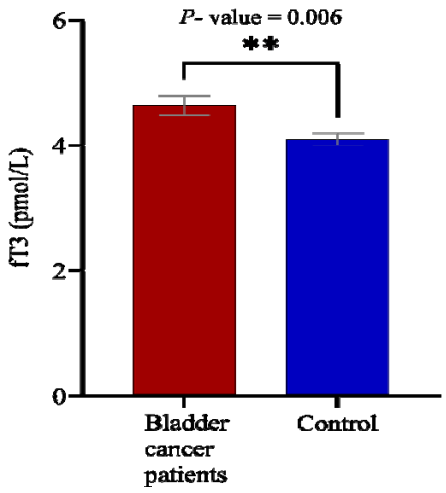

Figure 2. The concentration of free T3 in bladder cancer patients (n=30) and control (n=30). The mean concentration of fT3 was significantly higher in the patient group compared to the control group. Data represents the mean value ± S.E.M. (**p < 0.01).

The average concentration of TSH in the bladder cancer patient group and the control group is represented in Figure 3. As can be seen in the figure, the mean concentration of TSH decreased in the patients group compared to the control, but it did not reach a significant difference (p = 0.319). The mean ± SEM values of TSH in the patient group and control group were (1.47 ± 0.15) and (1.68 ± 0.14), respectively.

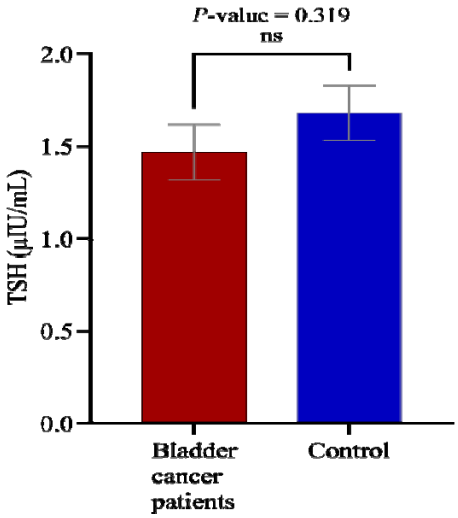

Figure 3. The concentration of free TSH in bladder cancer patients (n=30) and control (n=30). The mean concentration of TSH decreased in the patient group compared to the control group, but the difference was not statistically significant. Data represents the mean value ± S.E.M.

Figure 4 depicts the average concentration of TBG in the bladder cancer patient group and the control group. As illustrated in the figure, the mean concentration of TBG was not significantly different (p = 0.455) between the patient group (4.3 ± 0.64) and the control group (3.8 ± 0.62). The normal range for TBG is 10 – 25 µg/mL.

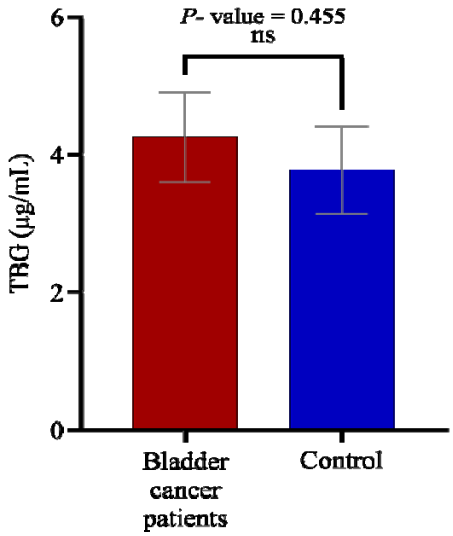

Figure 4. The concentration of TBG in bladder cancer patients (n=30) and control (n=30). The mean concentration of TBG was not significantly different between the patient group and the control group, with a p-value higher than 0.5. Data represents the mean value ± S.E.M.

Figure 5 demonstrates the average concentration of SHBG in the bladder cancer patient group and the control group. As illustrated in the figure, the mean concentration of SHBG was significantly higher (p < 0.0001) in the patient group (1227 ± 102.2) compared to the control group (642.7 ± 69.9).

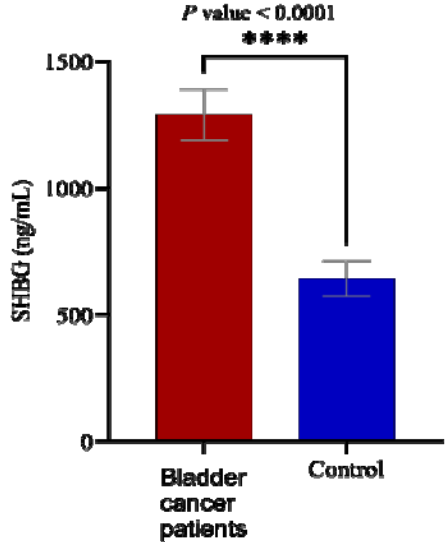

Figure 5. The concentration of SHBG in bladder cancer patients (n=30) and control (n=30). The mean concentration of SHBG was significantly higher in the patient group compared to the control group. Data represents the mean value ± S.E.M. (****p < .0001).

Figure 6 depicts Pearson's correlation between SHBG and thyroid hormones in bladder cancer patients. As illustrated in the figure, there was a strongly positive correlation between SHBG and fT4 concentrations (A: r^2 = 0.868, p < 0.0001) and between SHBG and fT3 concentrations (B: r^2 = 0.893, p < 0.0001).

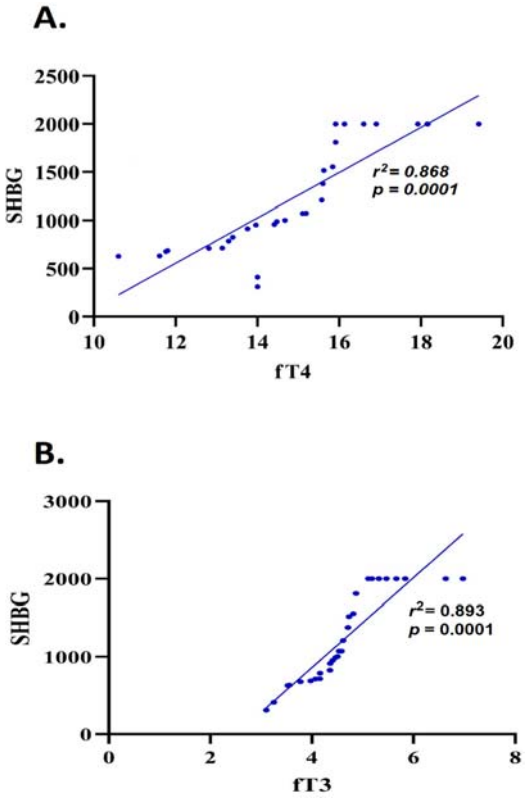

Figure 6: Pearson's correlation scatter plot between SHBG (ng/mL) and fT4 (pmol/L) (**A**), and fT3 (pmol/L) (**B**). There was a strong positive correlation between SHBG and fT4 concentrations and between SHBG and fT3 concentrations.

4. Discussion

Numerous studies have confirmed an association between thyroid hormones and almost all types of cancer, with the exception of bladder cancer (Krashin, *et al.*, 2019 Ma, *et al.*, 2023). Until now, no study has definitively clarified the relationship between thyroid hormones and bladder cancer. Therefore, it was crucial to investigate the connection between thyroid hormones and bladder cancer, which is precisely what we aimed to accomplish in this study.

In this case-control study of untreated early-stage bladder cancer patients, we observed significantly higher levels of fT4 and fT3 in bladder cancer patients compared to healthy controls, while the measurements were still within the known normal range. Conversely, we found that TSH levels decreased in bladder cancer patients when compared to healthy controls; however, this decrease did not reach statistical significance.

We attempted to provide a logical explanation for these findings. Considering that the average age of the study population is 64 years old, it is possible that age-related changes in the hypothalamus-pituitary-thyroid axis could contribute to the observed differences in TSH levels. Previous research has indicated that serum TSH levels tend to increase with age, while fT4 levels remain relatively stable. This discrepancy between TSH and fT4 levels in an elderly population could explain the lack of correlation observed in our study (Gesing, *et al.*, 2012). Another possible reason is the small sample size that caused the "p" value to not reach the significance level in the TSH test.

To the best of our knowledge, this is the inaugural case-control study that examines the connection between the complete spectrum of thyroid function and bladder cancer, both in Jordan and worldwide. Our research reveals that elevated levels of fT3 and fT4 may be linked to a higher occurrence and risk of bladder cancer. Interestingly, certain aspects of our findings align with the results from Khan et al.'s study, a prospective cohort study encompassing nearly 10,318 participants which demonstrated a significant association between higher levels of FT4 and an increased risk of various types of solid, lung, and breast cancers, while no correlation was observed with TSH levels (Khan, *et al.*, 2016).

In the current study, no difference was observed in TBG concentration between bladder cancer patients and healthy controls. These results validate the accuracy of the thyroid function test outcomes without any interferences. Furthermore, they suggest that individuals with bladder cancer may exhibit elevated levels of total T4 and T3 as well. Additionally, the findings indicate that the observed increases in fT4 and fT3 levels are indeed accurate and meaningful in relation to bladder cancer.

To date, there have been no studies that have definitively established the role of SHBG in bladder cancer. Therefore, in this study, we conducted measurements of SHBG protein concentration in bladder cancer patients for the first time. Our aim was to explore whether these results could provide insights into the potential association between SHBG and the observed differences in incidence rates between males and females.

In this study, SHBG concentration was much higher in bladder cancer patients compared to healthy controls. The increased concentration of SHBG may be attributed to the rise in thyroid hormone levels. Previous studies have shown that high levels of T3 and T4 can stimulate the liver to produce SHBG (Selva, *et al.*, 2009 Rosner, *et al.*, 1984). Consequently, our findings are in line with this relationship. These results could potentially provide an explanation for the high bladder cancer incidence rates in males.

According to Global Cancer Statistics, around 77% of bladder cancer cases occur in men (Sung, *et al.*, 2021). The potential mechanism underlying the impact of SHBG on this disparity in incidence rates can be explained as follows: Numerous studies have demonstrated that SHBG has the ability to stimulate the nongenomic action of androgens. This is attributed to the fact that the binding of androgens by SHBG can activate the cyclic adenosine monophosphate and protein kinase A pathways (Heinlein, *et al.*, 2002). This mechanism may have the potential to influence the transcriptional activation of the nuclear androgen receptor (AR) (Heinlein, *et al.*, 2002).

Moreover, previous studies have revealed that the transcriptional activity of the AR is augmented through protein kinase A stimulation, even in the presence of minimal androgen levels (Sadar, 1999 Nazareth, *et al.*, 1996 Ikonen, *et al.*, 1994). Regarding this mechanism, studies have identified that the proliferation of prostate cells in prostate cancer and the transcriptional activation of AR could be intensified by interactions between

dihydrotestosterone and SHBG through the induction of signal transduction cascades (Nakhla, *et al.*, 1996). Furthermore, weak adrenal androgens or estradiol might contribute to AR transcriptional activity by stimulating SHBG signaling (Nakhla, *et al.*, 1997)cl

In conclusion, this study reveals that there is an association between elevated serum levels of fT4, fT3, and SHBG and bladder cancer. These results suggest that fT4, fT3, and SHBG are positively associated with an increased incidence and risk of bladder cancer. SHBG also emerges as a potential candidate for serving as a valuable biochemical marker to predict susceptibility to bladder cancer. These findings indicate that monitoring and controlling T3, T4, and SHBG levels in bladder cancer patients may have implications for disease management and prognosis.

Nevertheless, it is crucial to emphasize the necessity for validating these findings through larger cohort studies with extended follow-up periods, encompassing a more substantial participant pool, and including gender-based analyses. Moreover, additional research is warranted to clarify the precise involvement of thyroid hormones in bladder cancer patients and delve into their potential impact on cancer progression and onset. Besides, investigating the dynamic interplay between SHBG and other factors contributing to bladder cancer progression could potentially cover the way for the development of targeted diagnostic approaches in the field.

5. Funding

This work was supported by Deanship of Research at Jordan University of Science and Technology.

6. Data availability

The data utilized in this study was generated by Department of Medical Laboratory Sciences at Jordan University of Science and Technology. While the data is not currently available online, the authors are willing to provide access to the data upon reasonable request from the editors.

Declarations

Conflict of interest

The authors declare no competing financial or non-financial interests.

Ethical approval

This study received approval from the Jordan University of Science and Technology (IRB 558-2022) and adhered to the ethical principles outlined in the 1964 Helsinki Declaration and its subsequent amendments.

Informed consent

All participants provided written informed consent for the publication of their data.

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