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Association of Triiodothyronine Levels With Prostate Cancer Histopathological Differentiation and Tumor Stage

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Abstract. *Background/Aim: The aim of this study was to determine the association between total triiodothyronine (T3), free fraction of thyroxin (FT4), and thyrotropin (TSH) levels with prostate cancer histopathological features. Patients and Methods: Blood samples from 140 patients with prostate cancer were analyzed preoperatively and stratified according to postoperative histopathological differentiation. The first group (N=62) included patients with prostate cancer Grade Groups (GG) 1-2, while the second group (N=63) included patients with prostate cancer GG 3-5. Results: T3 levels were significantly higher in patients with prostate cancer GG 3-5 (p=0.047). There was no significant difference in the FT4 and TSH levels between the two groups (p=0.680 and 0.801, respectively). T3 levels were positively correlated with tumor percentage involvement (TPI) (p=0.002), and pT stage (p=0.047) on definitive pathology. Conclusion: Higher T3 levels are associated with several indicators of prostate cancer histopathological aggressiveness.*

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Key Words: Prostate cancer, thyroid hormones, triiodothyronine, Grade Groups, prostatectomy, pathological T stage, tumor percentage involvement.

Thyroid hormones (TH) have major impact in metabolism, growth (1), differentiation (2), and apoptosis (3) and are essential for numerous basic body functions. Various studies have indicated that TH have an important role in cancer development (4), in the process of metastasis (5) and in subsequent cancer-related death (6). These observations have been confirmed in different neoplasms such as thyroid cancer (7), renal cancer (8), breast cancer (9), and prostate cancer (10).

Mondul *et al.* in a prospective, randomized controlled study revealed that hypothyroid patients have a decreased risk of developing prostate cancer compared to euthyroid ones [odds ratio (OR)=0.48, 95% confidence interval (CI)=0.28-0.81, p=0.006] (11). In addition, Lehrer *et al.* showed that T3 levels are significantly higher in prostate cancer patients than in age-matched healthy controls (12).

Despite these observations, there is a gap in knowledge addressing the underlying biological association between thyroid hormones and prostate cancer. Prostate cancer is the most common cancer in men and exhibits a significant heterogeneity in clinical behaviour (13, 14). Few studies investigated the role of thyroid hormones in prostate cancer development. *In vitro* studies have shown different effects of T3 and T4 hormones on prostate cancer cells. In a study

by Tsui *et al*., T3 suppressed the expression of anti-proliferative protein BTG2, and stimulated proliferation of androgen-dependent cells (15). Delgado-González *et al*. showed that T4 stimulates neuroendocrine differentiation, vascular endothelial growth factor secretion and invasiveness in low-invasive prostate cancer cells (16). In contrast, the same study demonstrated that T3 alone does not have any effect on the same cells, but it prevents isoproterenol-stimulated neuroendocrine differentiation and invasiveness. Zhang *et al*. demonstrated that T4 stimulates migration and inhibits detachment-induced apoptosis in prostate cancer cells (17). However, there is a gap in knowledge regarding potential association between TH and tumor histopathological differentiation grade. Therefore, the aim of the present study was to determine whether an association exists between levels of T3, FT4, and TSH and prostate cancer differentiation assessed by GG, a widely adopted and validated prostate cancer histopathological grading system (18, 19).

Patients and Methods

Patient selection. Prospectively, we enrolled 140 patients from two tertiary urology institutions in Zagreb, Croatia. They were recruited between July 2016 and February 2018. Sample size was calculated according to distribution of T3, FT4 and TSH in general population (20). However, our definitive sample size calculations were primarily focused on T3 levels, our primary endpoint. According to literature data, T3 levels are, at least hypothetically, the key driver of cancer aggressiveness (10). We estimated a 0.2 nmol/l difference in mean T3 levels between the two groups (null hypothesis). All patients gave written informed consent and the study was approved by the respective research ethics boards of both participating institutions.

Definitions. Prostate cancer differentiation was determined by Grade Groups, a validated histopathological grading system which was adopted by the World Health Organization in 2016 (19).

Based on the final prostatectomy pathology report, patients with prostate cancer GG 1-2, including patients with well-differentiated prostate cancer, were defined as group 1, while patients with prostate cancer GG 3-5, less differentiated prostate cancer, were defined as group 2. This grouping was made according to the results of a large study that included 20.845 men with clinically localized prostate cancer who underwent radical prostatectomy (21). According to the their results, the 5-year biochemical progression-free (BPF) probabilities for GG 1, GG 2, GG 3, GG 4, and GG 5 were 96%, 88%, 63%, 48%, and 26%, respectively. Due to the large drop in BPF probability between GG 2 and GG 3, we chose a dichotomization border between GG 1-2 and GG 3-5.

The stratification by the TPI on definitive pathology was ≤10%, >10-20%, >20%, as defined previously in literature (22).

Inclusion/exclusion criteria. The inclusion criteria for enrollment were patients aged 60-70 years, with biopsy-proven localized prostate cancer, who were scheduled for radical prostatectomy. Narrow age group was chosen as thyroid hormone levels show a considerable dependence on age (20).

Table I. *Patient characteristics stratified by two Grade Group study cohorts*.

	Groups				*p*-Value*
	1 (well-differentiated)		2 (less differentiated)		
	N	%	N	%	
Gleason score					
GS6(3+3)	9	14.5%	0	0.0%	**<0.001**
GS7(3+4)	53	85.5%	0	0.0%	
GS7(4+3)	0	0.0%	44	69.8%	
GS8(3+5)	0	0.0%	2	3.2%	
GS8(4+4)	0	0.0%	7	11.1%	
GS8(5+3)	0	0.0%	1	1.6%	
GS9(4+5)	0	0.0%	8	12.7%	
GS9(5+4)	0	0.0%	1	1.6%	
GS10(5+5)	0	0.0%	0	0.0%	
Gleason score					
6	9	14.5%	0	0.0%	**<0.001**
7	53	85.5%	44	69.8%	
8	0	0.0%	10	15.9%	
9	0	0.0%	9	14.3%	
Grade Group					
1	9	14.5%	0	0.0%	**<0.001**
2	53	85.5%	0	0.0%	
3	0	0.0%	44	69.8%	
4	0	0.0%	10	15.9%	
5	0	0.0%	9	14.3%	
pT stage					
2	56	90.3%	34	54.0%	**<0.001**
3	6	9.7%	29	46.0%	
Tumor percentage involvement (TPI)					
≤10%	25	40.3%	9	14.3%	**0.003**
>10-20%	22	35.5%	27	42.9%	
>20%	15	24.2%	27	42.9%	
Seminal vesicles					
Negative	60	96.8%	43	68.3%	**<0.001**
Positive	2	3.2%	20	31.7%	
Surgical margins					
Negative	49	79.0%	32	50.8%	**0.001**
Positive	13	21.0%	31	49.2%	
Extracapsular extension					
No	51	82.3%	31	49.2%	**<0.001**
Yes	11	17.7%	32	50.8%	

*Fisher-Freeman-Halton test. Statistically significant *p*-values are shown in bold.

Exclusion criteria were the presence of distant metastasis, a history of thyroid disorders or a newly detected elevation of anti-thyroid peroxidase antibodies (TPOAb) ≥34 kIU/l, and/or anti-thyroglobulin antibodies (TgAb) ≥115 kIU/l, and/or decreased/elevated TSH (<0.4 mIU/l or >4 mIU/l), having undergone a radiological procedure with iodinated contrast agent within one year of blood sampling, intake of medication known to affect thyroid function (*e.g.* amiodarone, lithium, any hormone

Table II. *Key clinical variables and thyroid hormones levels stratified by level of prostate cancer histopathological differentiation.*

Groups		N	Median	IQR	*p*-Value*
Age (years)	1 (well-differentiated)	62	64.50	62.00-66.25	0.171
	2 (less differentiated)	63	66.00	63.00-67.00	
BMI (kg/m^2)	1 (well-differentiated)	62	28.08	25.15-30.90	0.648
	2 (less differentiated)	63	27.70	25.20-30.25	
Tumor percentage involvement (TPI)	1 (well-differentiated)	62	15.00	10.00-21.25	**<0.001**
	2 (less differentiated)	63	20.00	15.00-35.00	
PSA (ng/ml)	1 (well-differentiated)	62	7.44	4.90-11.19	**<0.001**
	2 (less differentiated)	63	11.53	7.65-18.48	
T3 (nmol/l)	1 (well-differentiated)	62	1.40	1.20-1.50	**0.047**
	2 (less differentiated)	63	1.40	1.30-1.80	
FT4 (pmol/l)	1 (well-differentiated)	62	14.25	12.90-15.38	0.680
	2 (less differentiated)	63	14.50	12.80-15.80	
TSH (mIU/l)	1 (well-differentiated)	62	1.35	0.98-2.02	0.801
	2 (less differentiated)	63	1.46	0.87-1.86	

IQR, Interquartile range; BMI, body mass index; PSA, prostate-specific antigen; T3, triiodothyronine; FT4, free fraction of thyroxin; TSH, thyrotropin. *Mann-Whitney *U*-test. Statistically significant *p*-values are shown in bold.

therapy, agonists and antagonists of dopamine, anticonvulsants, neuroleptics, interferon) and a history of neck irradiation.

The presence of distant metastases was evaluated according to PSA and prostate biopsy results. If patients had PSA >20 ng/ml or GG 4/5 on biopsy, clinical staging was mandatory as per departmental standards, primarily using bone scan and computed tomography (CT) of the abdomen and pelvis without iodine contrast, and additionally pelvic magnetic resonance imaging, ^{18}F-fluorocholine PET/low-dose CT or any combination of these diagnostic procedures as decided on a case-by-case basis.

Laboratory analyses. Levels of T3, FT4, TSH, TPOAb, and TgAb were screened in preoperative blood samples. T3, FT4 and TSH were analyzed using an automated chemiluminescent immunoassay (Immulite 2000 Xpi Siemens, Siemens original kits, Siemens Healthcare Diagnostics, Llanberis, UK), while TPOAb and TgAb were measured using an automated electrochemiluminescent immunoassay (COBAS e411 Roche, Roche original kits, Roche Diagnostics, Mannheim, Germany).

As described above, patients with TSH values <0.4 or >4.0 mIU/l were excluded from the study in accordance to laboratory reference values. Likewise, also in accordance with the respective reference values, patients with TPOAb levels ≥34 kIU/l, and TgAb levels ≥115 kIU/l were excluded in order to eliminate influence of thyroid autoimmunity.

Statistical analysis. The Kolmogorov-Smirnov test was used to analyze normality of distribution of continuous variables. It revealed a non-parametric distribution of all continuous variables. Patient characteristics between the two GG cohorts were compared using the Mann-Whitney test and Fishers exact test for continuous and categorical variables, respectively. Spearman's rho correlation coefficients were used to analyze the association between individual clinical values of prostate cancer and thyroid function indicators. All *p*-values lower than 0.05 were considered statistically significant. All analyses were performed using IBM SPSS Statistics software version 25.0 (IBM Corp, Armonk, NY, USA).

Results

The total number of screened patients was 140. Fifteen patients (10.7%) were excluded from the study for different reasons. In 3 cases surgery was cancelled, while in 2 cases radical prostatectomy was abandoned due to intraoperative findings of inoperable cancer. Ten patients were excluded based on blood sample eligibility criteria, since elevated TPOAb and/or TgA, and/or decreased/elevated TSH were found. The final study population consisted of 125 patients. Table I lists patient characteristics stratified by two GG study cohorts.

The patients were divided into two predefined groups based on final pathology report. The number of patients in the first group (patients with well-differentiated prostate cancer - GG 1 and 2) and second group (patients with less differentiated prostate cancer - GG 3-5) was 63 and 62, respectively. Fifty-three patients (42.4%) had Gleason score (GS) 7 (3+4) or GG 2 prostate cancer. The majority of patients (72.0%) had pT2 stage disease. Positive seminal vesicles, positive surgical margins, and extracapsular extension were found in 17.6%, 35.2%, and 34.4% patients, respectively. Forty-two patients (33.6%) were found to have more than 20% of TPI in the prostatectomy specimen.

Hormones (T3, fT4, TSH) and prostate cancer. Statistically significant differences were observed in the T3 levels between the two GG cohorts (Table II, Mann-Whitney *U*-test). Patients in group 2 (*i.e.* GG 3-5) compared to patients in group 1 (*i.e.* GG 1-2) had higher baseline T3 levels (p=0.047).

A difference of 0.2 nmol/l was found between the mean T3 levels in group 1 and group 2 (mean T3: 1.4 and 1.6 nmol/l,

Table III. *Spearman correlation analysis between clinical variables and hormone levels on whole study population (N=125).*

		Tumor percentage involvement (TPI)	PSA (ng/ml)	T3 (nmol/l)	FT4 (pmol/l)	TSH (mIU/l)	Age (years)	BMI (kg/m^2)	Grade group	pT stage	Gleason score
Tumor percentage involvement (TPI)	Rho	1.000	0.427	0.277	–0.036	0.011	–0.027	0.194	0.451	0.433	0.423
	p-Value		**<0.001**	**0.002**	0.689	0.901	0.767	**0.030**	**<0.001**	**<0.001**	**<0.001**
PSA (ng/ml)	Rho	0.427	1.000	–0.057	0.066	0.017	0.026	–0.048	0.378	0.411	0.300
	p-Value	**<0.001**		0.525	0.466	0.855	0.770	0.595	**<0.001**	**<0.001**	**0.001**
T3 (nmol/l)	Rho	0.277	–0.057	1.000	–0.031	0.159	–0.060	0.103	0.209	0.178	0.188
	p-Value	**0.002**	0.525		0.728	0.076	0.507	0.253	**0.019**	**0.047**	**0.036**
FT4 (pmol/l)	Rho	–0.036	0.066	–0.031	1.000	–0.117	–0.170	0.161	0.004	–0.046	–0.056
	p-Value	0.689	0.466	0.728		0.195	0.058	0.073	0.965	0.609	0.533
TSH (mIU/l)	Rho	0.011	0.017	0.159	–0.117	1.000	0.146	0.113	–0.035	0.053	–0.047
	p-Value	0.901	0.855	0.076	0.195		0.104	0.209	0.700	0.557	0.603
Age (years)	Rho	–0.017	0.035	–0.054	–0.132	0.151	1.000	0.009	0.193	0.029	0.239
	p-Value	0.851	0.700	0.547	0.143	0.092		0.924	**0.031**	0.748	**0.007**
BMI (kg/m2)	Rho	0.194	–0.048	0.103	0.161	0.113	–0.016	1.000	–0.010	0.084	0.045
	p-Value	**0.030**	0.595	0.253	0.073	0.209	0.857		0.910	0.353	0.621
Grade Group	Rho	0.451	0.378	0.209	0.004	–0.035	0.167	–0.010	1.000	0.468	0.778
	p-Value	**<0.001**	**<0.001**	**0.019**	0.965	0.700	0.062	0.910		**<0.001**	**<0.001**
pTstage	Rho	0.433	0.411	0.178	–0.046	0.053	0.012	0.084	0.468	1.000	0.404
	p-Value	**<0.001**	**<0.001**	**0.047**	0.609	0.557	0.891	0.353	**<0.001**		**<0.001**
Gleason score	Rho	0.423	0.300	0.188	–0.056	–0.047	0.203	0.045	0.778	0.404	1.000
	p-Value	**<0.001**	**0.001**	**0.036**	0.533	0.603	**0.024**	0.621	**<0.001**	**<0.001**	

PSA, prostate-specific antigen; T3, triiodothyronine; FT4, free fraction of thyroxin; TSH, thyrotropin; BMI, body mass index. Statistically significant *p*-values are shown in bold.

respectively, with range 0.8-2 nmol/l and 1.0-3.7 nmol/l, respectively), while the median T3 levels were the same in group 1 and group 2, but IQR showed significant difference (median T3=1.4 and 1.4 nmol/l, respectively, with IQR=1.2-1.5 nmol/l and 1.3-1.8 nmol/l, respectively). No significant differences were observed in FT4 and TSH levels between the two GG cohorts (p=0.68 and 0.801, respectively).

Spearman correlation analysis between clinical variables and hormone levels on whole study population (N=125) is displayed in Table III. Among the thyroid-related parameters, the statistically significant positive correlation was observed between T3 levels and GS (Rho=0.188; p=0.036), GG (Rho=0.209; p=0.019), pT stage (Rho=0.178; p=0.047), and TPI (Rho=0.277; p=0.002). These positive correlations indicate that higher T3 values are significantly associated with higher GS, higher GG, higher pT stage and higher TPI. No significant correlation was found between other hormone parameters (*i.e.* TSH, FT4) and clinical variables. Also, we did not detect any positive correlations between T3, FT4, and TSH levels and initial preoperative prostate specific antigen (iPSA) levels.

Other relevant prostate cancer metrics. Stage pT3 was more prevalent in the group of patients with less differentiated tumors (p<0.001), as well as positive seminal vesicles (p<0.001), presence of positive surgical margins (p=0.001) and presence of extracapsular extension (p<0.001) (Table I, Fisher-Freeman-Halton test).

Statistically significant differences were observed in the TPI on prostatectomy, and PSA, between the two GG cohorts (Table II, Mann-Whitney U test). Patients with less differentiated prostate cancer (*i.e.* GG 3-5) compared to patients with well-differentiated prostate cancer (*i.e.* GG 1-2) had higher TPI (p<0.001) and higher baseline PSA levels (p<0.001). Spearman correlation analysis showed (Table III) an interesting positive correlation between body mass index (BMI) and TPI (p=0.030), indicating that higher BMI is significantly associated with higher TPI.

Discussion

The key finding of our study is that patients with less differentiated prostate cancer compared to the patients with well-differentiated prostate cancer have significantly higher levels of T3. However, no significant differences in the FT4 and TSH levels were observed among the two groups. Thyroid hormones, T3 and T4, act through their nuclear (23) and membrane (24) receptors, throughout all body systems. Increasing evidence, from *in vitro* (25-27), *in vivo* (28, 29), and population studies (30, 31), have suggested that thyroid

hormones are associated with many neoplasms, including the prostate cancer (32, 33). T3 is known to be far more potent than T4, so any consequent action on prostate cancer cells is more likely to be caused mainly by T3. TSH, on the other hand, acts indirectly *via* thyroid hormones. This might be the reason why no significant differences in the FT4 and TSH were detected among the two studied groups.

T3 is known to act *via* the TRα1, TRβ1, and TRβ2 nuclear receptors (34), binding up to 15 times more strongly than T4 (1). Binding of T3 to TRβ receptors activates the oncogenic phosphatidylinositol-3-kinase (PI3K) pathway (35). Similar results have been demonstrated for TRα receptors (36). Besides that, T3 also acts *via* the S1 and S2 subunits of the membrane receptor, αvβ3 integrin (37). Through binding to the S1 subunit, T3 leads to activation of PI3K pathway, while *via* the S2 subunit it activates the extracellular signal-regulated kinase (ERK1/2) pathway (37). The oncogenic PI3K pathway stimulates cell growth and inhibits apoptosis at the same time (38), while activation of ERK1/2 pathway leads to induction of fibroblast growth factor 2 and subsequent angiogenesis (27). Hence, T3, by exhibiting multiple actions through nuclear and membrane receptors may play a significant role in tumor development.

In a prospective study that included 29,691 patients, Hellevik *et al*. found that individuals suffering from hyperthyroidism have a higher overall risk of developing cancer than euthyroid or hypothyroid patients (30). Furthermore, individuals with hyperthyroidism had a significantly higher risk of developing prostate cancer than those with euthyroidism (HR=1.97, 95% CI=1.04-3.76). In addition, a dose-dependent correlation was established; individuals with overt hyperthyroidism had a higher risk for developing cancer than those with subclinical hyperthyroidism. Moreover, in a prospective population-based study in Western Australia, Chan *et al*. showed that lower serum concentrations of TSH and higher concentrations of FT4 are associated with an increased risk of prostate cancer (33). The study involved 1,623 men from 1994/1995 Busselton Health Survey who had archived sera and were monitored during a period of 20 years. Participants were 25-84 years old, without history of cancer, and were not taking therapy for thyroid disorders at baseline. One hundred twenty-six (7.8%) participants were diagnosed with prostate cancer. Higher TSH concentrations were associated with a lower risk of prostate cancer (HR=0.70 per 1 mIU/l increase in TSH, 95% CI=0.55-0.90, p=0.005). Also, higher concentrations of FT4 were positively correlated with risk of developing prostate cancer (HR=1.11 per 1 pmol/l increase in FT4, 95% CI=1.03-1.19, p=0.009).

Herein, we detected significant positive correlations of T3 values with GS (p=0.036) and GG (p=0.019) in the total study sample (N=125), indicating that higher serum T3 concentrations are associated with higher GS and higher GG. Lehrer *et al*. found higher T3 levels in the high-risk group compared with the intermediate- and low-risk group in patients with prostate cancer undergoing permanent seed brachytherapy (10). Patients were stratified to risk groups according to clinical T stage, GS and PSA.

We also found that the TPI is significantly higher in the patients with less differentiated prostate cancer. This finding confirms previously reported association of TPI with level of prostate cancer differentiation and consequential patient outcome. Uhlman *et al*. in a study of 3,528 patients found that the TPI was a statistically significant predictor of biochemical recurrence (p<0.05) (22). Ramos *et al*. also showed, in a study of 1,850 patients, that the TPI was associated with biochemical relapse (39). Patients with TPI ≥10% had a recurrence rate of 10%, while the patients with TPI <10% had a recurrence rate of less than 5% (p=0.001).

Androgen activity is a hallmark of prostate cancer. However, little is known about potential interplay between androgen signalling and thyroid hormones. Esquenet *et al*. in their *in vitro* work demonstrated that T3 modulates proliferation and concentration of androgen receptors in the prostate cancer cell lines LNCaP (40). It is plausible that T3 has a role in prostate cancer response to androgen suppression by modulating castration resistance with direct impact on biological aggressiveness of disease and therapeutic response (41).

In our study, we also observed significant positive correlations between T3 values and the TPI (p=0.002). Analogously, significant positive correlations of T3 with pT stage were also detected (p=0.047), indicating that higher serum T3 concentrations were also associated with higher pT stage of prostate cancer. We also found significant positive correlation between BMI and TPI, while there were no positive correlations of BMI with other clinical variables or hormone levels. However, in a larger retrospective study by Tomaszewski *et al*., including 2,500 patients after primary treatment with radical prostatectomy, no association was found between BMI and positive surgical margins, GS, extracapsular extension, positive seminal vesicles, or TPI (42).

Some limitations of our study need to be acknowledged. First, the potential inherent bias in our study cannot be ascertained due to possible subclinical effect of prostate cancer on thyroid and thyrotropin hormone levels. To amend this, we recommend in further larger studies follow-up of healthy subjects at least 2 years after baseline hormone levels in order to reduce this possible bias. Secondly, given by the nature of our study which primarily focused on histopathological outcome, we did not capture clinical outcome data (PSA recurrence-free survival, metastasis-free survival) for our cohort as this was outside of the scope of the study. However, in the future, we plan to investigate the association of TH levels with clinical outcomes, using a longer follow-up period, to further elucidate the role of T3 in natural history of screen-detected prostate cancer.

In conclusion, T3 was associated with less differentiated prostate cancer, higher pT stage of disease, and higher TPI, thus confirming our hypothesis. Our findings corroborate mounting evidence on T3 as potential biomarker, and possibly even contributing cause, of prostate cancer aggressiveness.

Conflicts of Interest

All Authors declare no conflicts of interest regarding this study.

Authors' Contributions

PPO, JM, SO, BK and TJ designed the study. PPO, SO, MBB, ŠŠ, BR and BM coordinated the selection of patients and shipment of the patients' samples. DK conducted the analysis of blood samples. BK and DJ performed histological examination and interpreted patients' data. MM conducted the statistical analysis. PPO, JM and FAV drafted the manuscript. AF, MF, BK, and TJ revised the manuscript. All Authors approved the final version of the manuscript.

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