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ORIGINAL ARTICLE

Are endogenously lower serum thyroid hormones new predictors for thyroid malignancy in addition to higher serum thyrotropin?

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Abstract It is well known that TSH plays a major role in the secretion of thyroid hormones, maintenance of thyroid specific gene expression, and gland growth. In this study, we aimed to evaluate association between tests of thyroid functions (fT3, fT4, TSH) and differentiated thyroid carcinoma. 441 patients operated for nodular goiter between 2005 and 2008 were analyzed. Thyroid functions were studied in the period of 1–30 days prior to surgery. In postoperative histopathological examination, differentiated thyroid carcinoma and benign thyroid disease were detected in 166 (37.6%) and 275 (62.4%) patients, respectively. Patients with thyroid malignancy had significantly lower serum fT3 ($P = 0.001$), lower fT4 ($P = 0.022$), and higher TSH levels ($P < 0.001$) compared to patients with benign disease, although all analytes were within the normal range. We subdivided by quartile serum fT3, fT4, and TSH in normal limits into three groups. The odds ratio (ORs) for the risk of thyroid cancer with a serum TSH between 0.63 and 1.67 μIU/ml and 1.68–4.00 μIU/ml, compared with a serum TSH between 0.40 and 0.62 μIU/ml were calculated as 2.60 (95% CIs 1.49–4.54) and 6.50 (95% CIs 3.51–12.03), respectively. There was also a greater risk of thyroid cancer in patients with fT3 levels of 1.57–3.00 pg/ml, compared with patients with fT3 levels of 3.89–4.71 pg/ml (OR 2.95, 95% CIs 1.68–5.20). For fT4, OR for the risk of thyroid cancer between 0.85 and 1.17 ng/dl compared with 1.48–1.78 ng/dl was 2.14 (95% CIs 1.22–3.74). In conclusion, lower fT3, fT4, and higher TSH concentrations within normal limits were related with increased thyroid cancer independent from sex and nodule type. Particularly, the association between lower fT3, fT4 levels and a diagnosis of thyroid cancer is a novel finding.

Keywords TSH · fT3 · fT4 · Differentiated thyroid cancer · Nodular goiter

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Introduction

Thyroid nodules are common in the adult population and increasingly detected by widespread use of ultrasound (US) examination or other scanning techniques. According to the population studied and the methods used to detect nodules, prevalence of nodular thyroid disease may vary. It was reported to range between 2 and 6% with palpation and 19–67% with US [1]. Approximately 5–10% of nodules are malignant [2, 3]. Differentiated thyroid cancer which includes papillary and follicular cancer comprises the vast majority (80–95%) of all thyroid cancers [4].

It is important to discriminate malignant and benign nodules in patients with nodular goiter. For this reason, many risk factors (age, sex, exposure to radiation, etc.) are defined and many imaging modalities are available. However, fine needle aspiration biopsy (FNAB) is considered to be the "gold standard" in the management of thyroid nodules and selection of patients for surgery with regard to its simplicity, safety, and cost-effectiveness [3]. Recently it has been reported that in patients with nodular thyroid disease, the risk of malignancy increases with serum thyrotropin (TSH) concentrations and, even within normal ranges, higher TSH values are associated with a significantly greater likelihood of thyroid cancer [5–10]. TSH levels have also been found higher in patients with advanced stage of thyroid cancer and it has been suggested that TSH may play a central role in cancer development and progression [8, 10]. It is well documented that TSH is involved in the regulation of thyroid functions such as secretion of thyroid hormones, maintenance of thyroid specific gene expression (differentiation), and gland growth. Experimental studies and clinical data have demonstrated the proliferative effect of TSH on thyroid cells in vitro and responsiveness of well-differentiated thyroid cancers to TSH. These observations provide the rationale for TSH suppression in patients with differentiated thyroid cancer [11]. It was shown that an undetectable TSH value was associated with longer relapse-free survival, and that TSH suppression was an independent predictor of recurrence [12]. The favorable impact of TSH suppression on thyroid cancer outcomes is likely to be due to the fact that TSH serves as one of several growth factors for thyroid tissue [13, 14].

In this study, we aimed to evaluate the association between thyroid functions and differentiated thyroid carcinoma in nodular goiter patients with normal free T3 (fT3), free T4 (fT4), and TSH levels and with histopathologically confirmed malignant or benign disease.

Patients and methods

Data from 441 patients operated for nodular goiter between 2005 and 2008 were retrospectively analyzed. Benign nodular disease or differentiated thyroid carcinoma (papillary, follicular, or hurthle cell cancer) were diagnosed histopathologically in all patients. We included patients with normal serum fT3, fT4, and TSH and with available preoperative serum anti-thyroid peroxidase antibody (Anti-TPOAb), anti-thyroglobulin antibody (Anti-TgAb) and patients who were detected to have hypoactive or normoactive nodule in preoperative thyroid scan. Exclusion criteria's were previous thyroidectomy, history of radioactive iodine treatment or head and neck radiation exposure, clinical or subclinical hypothyroidism or hyperthyroidism and positive serum TSH receptor antibody. To rule out possible factors that may affect TSH levels, we did not include patients receiving antithyroid drugs, L-thyroxine replacement or suppression treatment or any medication that may cause change in thyroid functions (estrogen, androgen, lithium, glucocorticoid, etc.). Also patients with hot or hot–cold nodules visualized in thyroid scan, even though serum TSH was in normal limits, were not recruited. Patients with positive thyroid antibodies and histopathologically detected Hashimoto thyroiditis (HT) were withdrawn from the study, however, patients with merely positive antibodies having no HT histopathologically were taken into analysis.

Imaging and thyroid fine needle aspiration biopsy

Thyroid US was performed in all cases (Esaote Technos-MPX and LA523 13-4 MHz probe; Geneva, Italy). Informed written consent was taken from all cases after explanation of the FNAB procedure. FNAB was carried out under US guidance (Logic Pro 200 GE and 7.5 MHz probe; Kyunggigo, Korea). All nodules >1 cm and nodules ≤1 cm with at least one of the US findings anticipating malignancy such as hypoechogenicity, microcalcification, margin irregularity and absence of halo were evaluated with FNAB. Cytologic diagnoses of FNAB were classified as benign, suspicious for malignancy, nondiagnostic, and malignant. Thyroid scintigraphy with Technetium-99m was executed.

Indications for operation

Patients with malignant or suspicious cytology results of FNAB underwent surgery. In nodules with nondiagnostic cytology, biopsies were repeated. If still nondiagnostic, surgery was performed for nodules that were clinically and ultrasonographically suspicious. Large goiter with or without symptoms of tracheal and/or esophageal compression was another indication for surgery.

Histopathologic results

Histopathologic results of operated patients were grouped as malignant (papillary carcinoma, follicular carcinoma, and hurthle cell carcinoma) or benign (nodular hyperplasia, colloidal goiter, follicular adenoma, and hurthle cell adenoma) thyroid disease.

Laboratory

Thyroid functions were evaluated in the period of 1–30 days prior to surgery. The preoperative values of serum TSH (normal range 0.4–4 μIU/ml, BIO-DPC Immulite

2000 Third generation kit, Los Angeles, USA), serum fT3 (normal range 1.57–4.71 pg/ml, BIO-DPC Immulite 2000 competitive chemiluminescent enzyme immunoassay, Los Angeles, USA), fT4 (normal range 0.85–1.78 ng/dl, BIO-DPC Immulite 2000 competitive chemiluminescent enzyme immunoassay, Los Angeles, USA), serum Anti-TPOAb (normal range 0–35 IU/ml, BIO-DPC Immulite 2000 chemiluminescent sequential immunometric assay Los Angeles, USA), serum Anti-TgAb (normal range 0–40 IU/ml, BIO-DPC Immulite 2000 chemiluminescent sequential immunometric assay Los Angeles, USA), and TSH receptor antibody (normal range 0–1U/l, BECKMAN and COULTER, Immunotec, radioimmunoassay, France) were obtained from the records. The thyroid antibodies levels over the upper range were accepted as positive.

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS Inc., Chicago, IL, United States). Continuous variables were shown as mean ± standard deviation. First, using the descriptive statistics, we obtained the quartiles of the distribution of fT3, fT4, and TSH, respectively. Data were separated into three individual groups based on each component of thyroid functions by using weighted average. Less than or equal to 25th percentile was assumed as the first group (low), the second one was defined as greater than 25th and lower than 75th percentile (medium), the last group was considered as greater than or equal to 75th percentile (high). The upper quartile of both fT3 and fT4 were accepted as the reference groups, whereas the lowest quartile was considered as the reference group for TSH. Univariate analyses were performed by applying Logistic Regression Analysis. According to reference categories, odds ratio (OR) and 95% confidence intervals (CIs) were calculated. Whether the meaningful effects of thyroid function on malignancy risk were evident or not was determined by Multiple Logistic Regression Analyses after adjustment for age, sex, and nodule type (solitary or multiple thyroid nodule). Any variable whose univariable test had a P value <0.25 was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cytology were calculated for determining the diagnostic performance. A P value <0.05 was considered statistically significant.

Results

Preoperative cytology results were malignant in 85 (19.3%), suspicious in 132 (29.9%), nondiagnostic in 18 (4.1%), and benign in 206 (46.7%) patients. Differentiated thyroid carcinoma was detected in 166 (37.6%) patients and benign thyroid disease was detected in 275 (62.4%) patients in postoperative histopathological examination. There were 147 patients with papillary thyroid carcinoma (88.6%), 13 with follicular thyroid carcinoma (7.8%) and six with hurthle cell carcinoma (3.6%). Thyroid cancer was the final diagnosis in 13 of 206 patients operated for benign cytology (6.3%) and 61 of 132 patients operated for suspicious cytology (46.2%). Eight of 18 patients with nondiagnostic cytology (44.4%) and 84 of 85 patients with malignant cytology (98.8%) were also diagnosed to have thyroid cancer. Accordingly, sensitivity of FNAB was 86.6%, while specificity of it was 99.5%. PPV, NPV, and accuracy of FNAB were calculated as 98.8, 93.7, and 95.2%, respectively.

Comparison of age, sex, and nodule type between patients with malignant thyroid disease and benign thyroid disease

We used final histopathologic diagnosis for statistical analysis. Of 441 patients included in the study, 347 were female (78.7%) and 94 were male (21.3%). There were 31 male patients (18.7%) among 166 malignant cases, while there were 63 male patients (22.9%) among 275 benign cases and the difference was not significant ($P = 0.29$). Mean age of patients with thyroid cancer was found to be 47.0 ± 12.6 years and with benign disease 44.6 ± 12.4 years, with no significant difference [$P = 0.055$, OR 1.02 (95% CIs 1.00–1.03)]. When we grouped age of patients as younger than 30 ($n = 40$), between 30 and 60 ($n = 348$) and older than 60 ($n = 53$), we saw that being older than 60 years was a risk factor for thyroid carcinoma compared to being younger than 30 years old [$P < 0.001$, OR 3.36 (95% CIs 1.37–8.23)]. Multiple thyroid nodule was present in 349 (79.1%) and solitary nodule was present in 92 (20.9%) patients in preoperative US examination. Although, solitary nodule was a feature in 25.3% of malignant cases and 18.2% of benign cases, thyroid cancer risk was not statistically significant in patients with solitary and multiple nodules [$P = 0.075$, OR 1.52 (95% CIs 0.96–2.43)].

Comparison of fT3, fT4, and TSH between patients with malignant thyroid disease and benign thyroid disease

In thyroid carcinoma patients, preoperative mean serum fT3 was 3.32 ± 0.60 pg/ml, mean serum fT4 was 1.29 ± 0.24 ng/dl, and mean serum TSH was 1.66 ± 0.96 μIU/ml. Patients with benign thyroid disease had a mean serum fT3 of 3.54 ± 0.68 pg/ml, fT4 of 1.34 ± 0.21 ng/dl, and TSH of 1.09 ± 0.73 μIU/ml. Within normal limits, serum fT3

and fT4 were significantly lower ($P = 0.001$ and $P = 0.022$, respectively), and serum TSH was significantly higher ($P < 0.001$) in patients with thyroid malignancy compared to patients with benign disease. As explained in statistical analysis part, we subdivided serum fT3, fT4, and TSH in normal limits into three groups (low, medium, and high). Ranges and number of patients in each group as well as distribution of preoperative serum fT3, fT4, and TSH groups in patients with thyroid cancer and benign thyroid disease and their OR for the risk of thyroid cancer are shown in Table 1.

Compared to patients with TSH level between 0.40 and 0.62 μIU/ml, thyroid cancer risk was 2.6- and 6.5-fold higher when TSH was between 0.63–1.67 μIU/ml and 1.68–4.00 μIU/ml, respectively. The risk was increased approximately 3-fold when fT3 was between 1.57 and 3.00 pg/ml in comparison to between 3.89 and 4.71 pg/ml. Similarly, having fT4 between 0.85 and 1.17 ng/dl yielded a twofold increase in thyroid cancer risk compared to having fT4 between 1.48 and 1.78 ng/dl.

We also calculated sensitivity, specificity, PPV, and NPV of higher TSH alone or in combination with lower fT3 and fT4 to determine diagnostic value of these parameters to predict thyroid malignancy. The results are summarized in Table 2. Combinations resulted with increased specificity while sensitivity was decreased. Sensitivity and NPV of these hormonal parameters were considerably low when compared with FNAB.

Table 2 Diagnostic value of different combinations of thyroid functions in discriminating benign and malignant thyroid diseases

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FNAB	86.6	99.5	98.8	93.7
TSH	39.2	83.6	78.9	45.3
TSH + fT3	30.7	88.7	81.8	43.5
TSH + fT3 + fT4	37.3	85.5	81.0	45.1

FNAB fine needle aspiration biopsy, TSH = 1.68–4.00 μIU/ml, fT3 = 1.57–3.00 pg/ml, fT4 = 0.85–1.17 ng/dl. *PPV* positive predictive value, *NPV* negative predictive value

Thyroid antibodies

Positive Anti-TPOAb and Anti-TgAb were detected in 10.2% ($n = 45$) and 12.7% ($n = 56$) of patients included in the study, respectively. These patients did not have HT in histopathological examination. No significant difference was observed in patients with benign and malignant disease in terms of AntiTPOAb (9.1% vs. 12%) and anti-TgAb (11.4% vs. 15.2%) positivity ($P = 0.32$, $P = 0.25$, respectively). We also compared distribution of fT3, fT4, and TSH groups in patients with positive and negative antibodies and detected no significant difference.

Regression analysis

In univariate analysis, fT3: 3.89–4.71 pg/ml (high), fT4: 1.48–1.78 ng/dl (high), TSH: 0.40–0.62 μIU/ml (low), multiple thyroid nodules, and male sex were considered as reference categories for malignancy. Logistic regression analyses for fT3, fT4, and TSH individually showed that low fT3 and low fT4 were related with thyroid cancer independent from sex, while TSH was related with malignancy independent from nodule type and sex (Table 3).

Table 1 Concentration ranges and number of patients in each groups of fT3, fT4, and TSH and distribution of these groups in patients with malignant and benign thyroid diseases

Variables	*n*	Benign ($n = 275$)	Malignant ($n = 166$)	*P* value	OR (95% CIs)
fT3 (pg/ml)					
3.89–4.71 (high)	110	81 (29.5%)	29 (17.5%)	–	1.00
3.01–3.88 (medium)	222	141 (51.3%)	81 (48.8%)	0.066	1.61 (0.97–2.66)
1.57–3.00 (low)	109	53 (19.3%)	56 (33.7%)	**<0.001**	2.95 (1.68–5.20)
fT4 (ng/dl)					
1.48–1.78 (high)	107	75 (27.3%)	32 (19.3%)	–	1.00
1.18–1.47 (medium)	225	143 (52.0%)	82 (49.4%)	0.242	1.34 (0.82–2.21)
0.85–1.17 (low)	109	57 (20.7%)	52 (31.3%)	**0.008**	2.14 (1.22–3.74)
TSH (μIU/ml)					
0.40–0.62 (low)	110	90 (32.7%)	20 (12.0%)	–	1.00
0.63–1.67 (medium)	221	140 (50.9%)	81 (48.8%)	**<0.001**	2.60 (1.49–4.54)
1.68–4.00 (high)	110	45 (16.4%)	65 (39.2%)	**<0.001**	6.50 (3.51–12.03)

fT3: 3.89–4.71 pg/ml (high), fT4: 1.48–1.78 ng/dl (high), and TSH: 0.40–0.62 μIU/ml (low) were considered as reference categories for malignancy. *OR* odds ratio, *CIs* confidence interval, *n* number of patients in each group

Table 3 Effects of fT3, fT4, and TSH groups on thyroid malignancy after adjusting for age, sex, and nodule type

Variables	OR	*P* value	95% CIs
>60 years old	2.10	**0.015**	1.16–3.82
Sex	1.20	0.474	0.73–1.98
Solitary nodule	1.86	**0.012**	1.15–3.03
Low fT3	3.00	**<0.001**	1.67–5.37
Medium fT3	1.54	0.098	0.92–2.58
>60 years old	2.16	**0.011**	1.19–3.89
Sex	1.29	0.323	0.78–2.11
Solitary nodule	1.67	**0.036**	1.03–2.69
Low fT4	2.07	**0.013**	1.17–3.65
Medium fT4	1.39	0.206	0.84–2.30
>60 years old	1.99	**0.028**	1.08–3.68
Sex	1.19	0.511	0.71–1.97
Solitary nodule	1.37	0.216	0.83–2.24
Medium TSH	2.51	**<0.001**	1.43–4.41
High TSH	5.95	**<0.001**	3.19–11.13

Low fT3: 1.57–3.00 pg/ml, medium fT3: 3.01–3.88 pg/ml, low fT4: 0.85–1.17 ng/dl, medium fT4: 1.18–1.47 ng/dl, medium TSH: 0.63–1.67 μIU/ml, high TSH: 1.68–4.00 μIU/ml. *OR* odds ratio, *CIs* confidence interval

Multiple logistic regression analyses including age, sex, nodule type, fT3, fT4, and TSH together were performed to determine which factors are independent risk predictors for thyroid malignancy. Accordingly, age, low fT3, low fT4, and medium/high TSH were found to be predictors for thyroid cancer independent from sex and nodule type (Table 4). In another words, effect of fT3, fT4, or TSH on thyroid malignancy was not independent from each other or age. In addition, fT3 and fT4 were related with thyroid cancer when TSH was in medium or high group.

Table 4 Independent predictors of thyroid malignancy, defined by multiple logistic regression analyses including sex, age, nodule type and fT3, fT4, and TSH groups

Variables	OR	*P* value	95% CIs
>60 years old	1.89	**0.047**	1.01–3.52
Sex	0.98	0.929	0.58–1.66
Solitary nodule	1.53	0.101	0.92–2.55
Low fT3	3.02	**<0.001**	1.63–5.58
Medium fT3	1.64	0.073	0.96–2.81
Low fT4	1.84	**0.050**	1.01–3.38
Medium fT4	1.43	0.188	0.84–2.44
Medium TSH	2.37	**0.003**	1.34–4.19
High TSH	5.74	**<0.001**	3.03–10.89

Low fT3: 1.57–3.00 pg/ml, medium fT3: 3.01–3.88 pg/ml, low fT4: 0.85–1.17 ng/dl, medium fT4: 1.18–1.47 ng/dl, medium TSH: 0.63–1.67 μIU/ml, high TSH: 1.68–4.00 μIU/ml. *OR* odds ratio, *CIs* confidence interval

Evaluation of fT3, fT4, and TSH according to cytologic results

We have used postoperative histopathologic diagnoses while making all the analysis above. We evaluated cytologic results as well. First, we compared serum fT3, fT4, and TSH levels in patients with malignant ($n = 85$) and benign ($n = 206$) cytologies. In patients with benign cytology, mean serum fT3, fT4, and TSH were 3.50 ± 0.69 pg/ml, 1.32 ± 0.21 ng/dl, and 1.04 ± 0.74 μIU/ml, respectively. In patients operated for malignant cytology, we found a mean serum fT3 of 3.33 ± 0.60 pg/ml, fT4 of 1.28 ± 0.23 ng/dl, and TSH of 1.76 ± 0.99 μIU/ml. There was a significant difference in terms of fT3 and TSH between patients with benign and malignant cytologies ($P = 0.034$, $P < 0.001$, respectively) revealing lower fT3 and higher TSH as risk factors for cytologically diagnosed malignancy. Identical fT4 levels were detected in two groups of patients ($P = 0.240$).

Among patients with preoperative suspicious cytology, 61 had thyroid cancer and 71 had benign thyroid disease postoperatively. When we compared mean serum fT3, fT4, and TSH levels in these patients, we saw that patients with malignant disease had lower fT3 (3.33 ± 0.59 pg/ml vs. 3.58 ± 0.66 pg/ml, $P = 0.028$) and higher TSH (1.58 ± 0.92 μIU/ml vs. 1.28 ± 0.76 μIU/ml, $P = 0.047$) compared to patients with benign disease. Of patients operated for suspicious cytology, fT4 did not differ between ones with final diagnoses of benign nodular disease and thyroid malignancy (1.37 ± 0.21 ng/dl vs. 1.29 ± 0.24 ng/dl, $P = 0.077$).

Discussion

Measurement of serum TSH, which is a highly sensitive determinant of thyroid dysfunction, is the recommended biochemical test in the initial evaluation of patients presenting with thyroid enlargement [3]. In the literature, there are six reports that have identified baseline serum TSH concentration as a predictor of the diagnosis of malignancy in patients with thyroid nodules [5–10]. In the present study, we showed that, even in normal ranges, lower serum fT3 and fT4, in addition to higher serum TSH, are risk factors for predicting thyroid cancer independent from sex and nodule type. Experimental studies have confirmed the expression and functional activity of TSH receptors in differentiated thyroid carcinoma and the proliferative effects of TSH on thyroid carcinoma cells in vitro [15, 16]. Serum TSH concentrations was reported to be positively correlated with risk for thyroid carcinoma-related death and relapse [17]. Also, endogenous higher TSH levels were shown to be associated with more advanced cancer stage

and extrathyroidal spread [8, 10]. There is clear evidence of improved survival with aggressive thyroid hormone suppression in high-risk cancer patients and improved survival with modest thyroid hormone suppression in stage II thyroid cancer patients [18].

Our study has some differences in terms of both patient selection and inclusion criteria and results. The association between lower fT3, fT4 levels and a diagnosis of thyroid cancer is a novel finding. In the literature, Jonklaas et al. found an association between low total T3 and thyroid cancer. However, the patient number in that study was comparatively limited, including 17 malignant cases in total [6]. Fiore et al. did not demonstrate an association between serum thyroid hormones and malignant diseases of the thyroid [10]. Contrary to this finding, we showed that, lower fT3, lower fT4, and higher TSH although in normal ranges were associated with thyroid cancer, as though a relative hypothyroid state was present in normal limits in carcinoma patients. The inverse logarithmic relationship between fT4 and TSH means that small changes in the serum fT4 concentration can, in turn, lead to much more dramatic changes in serum TSH [19]. The bioavailability of thyroid hormone is regulated by three iodothyronine deiodinases (D) [type I (D1), type II (D2), and type III (D3)]. D1 and D2 catalyze prohormone T4 to the receptor-active T3 and D3 catalyzes the conversion of T4 to reverse T3 and T3 to 3,3′-diiodothyronine, thus inactivating these hormones [20]. D1 and D2 levels are reported to be low in papillary thyroid carcinoma. This may be associated with dedifferentiation or malignant transformation of thyroid follicular cells. It is also possible that lower fT3 levels might be an indication of cellular dedifferentiation [21, 22]. It has been demonstrated recently that severe hypothyroidism is induced by high levels of D3 expression in vascular tumors, a condition that is referred to as consumptive hypothyroidism [23]. Also, in nonthyroidal illness, increased D3 expression may contribute to the decreased serum T3 [24]. According to our findings, we can speculate that decrease in D1 and D2 activity and increase in D3 activity may be responsible for low fT3 and fT4 levels in thyroid carcinoma patients. In addition, in recent years, probable direct effects of thyroid hormones on thyroid proliferation, independent from TSH, are being discussed. T3 receptor has been reported to control thyroid fibroblast growth factor expression via acting on its promoter. Also it has been shown in a mouse model that mutated forms of T3 receptor may increase phosphatidylinositol 3-kinase (PI3K) signaling and induce thyroid carcinomas [25]. Iodine deficiency causes a reduction in the level of circulating thyroid hormones associated with a consequent rise in serum TSH concentrations and chronic iodine deficiency is a well established risk factor for the development of goiter and differentiated thyroid carcinoma [26]. Several studies have shown that iodine intake may be associated with a higher prevalence of subclinical hypothyroidism in the population [27]. Our country is an iodine deficiency region and iodine supplementation program is ongoing [28]. Hence, iodine-related environmental factors may have contributed hormonal profile of our patients also. Still, a more direct role of fT3 and fT4 in the process of carcinogenesis cannot be entirely excluded.

Some trials investigating relation between thyroid functions and thyroid malignancy used palpation method to diagnose nodule type and performed FNAB only for dominant palpable nodule without US guidance [5, 7]. We have performed FNAB for all nodules >1 cm and for smaller nodules with suspicious US findings. Again, in previous studies, the nodules' benignity in the final diagnostic outcome were not confirmed histologically in all patients [5, 7, 10]. However, we used final histopathological results for diagnostic criteria in the current study. Also, in contrast to some of these studies [5, 7] in which different thyroid malignancies that have never been reported to be TSH dependent such as medullary or anaplastic cancers or thyroid lymphomas were grouped, we included only patients with differentiated thyroid cancer.

Thyroid autonomy and autoimmunity are two important issues that should be mentioned while considering relation between TSH and thyroid cancer. We perform thyroid scintigraphy for all patients with thyroid nodule/nodules [29]. In studies similar to ours, not all patients had been evaluated with thyroid scan [5, 7], or patients revealing both hot and cold nodules were included in the ones assessing scintigraphy [10]. It is obvious that patients with lower TSH concentrations may already have or be progressing toward the development of hot nodules. Presence of one or more hot nodules in radionuclide scanning is associated with a lower risk of malignancy than the finding of cold nodules [30]. In contrary to most previous studies [5, 7, 8, 10], in the present study we did not include patients with subclinical hyperthyroidism. Besides, we also excluded patients with hot or coexistent hot–cold nodules even though serum TSH was in normal ranges. Thereby, possible effects of thyroid autonomy on thyroid functions and malignancy incidence were eliminated. Accordingly, we can suggest that TSH, fT3, and fT4 are related with thyroid cancer without any effect of thyroid autonomy.

In a meta-analysis, Singh et al. reported that the prevalence of thyroid cancer in patients with HT is significantly higher than patients without HT [31]. Haymart et al. demonstrated significant association between histopathologic HT and higher TSH levels [9]. Other studies [6, 8] evaluating TSH and thyroid malignancy did not exclude patients with HT. However, in our study, patients with high serum antibody titer and histopathologically proven HT were not included so as to avoid potential influence of

thyroid autoimmunity on TSH and thyroid cancer. Yet, patients with merely positive antibody titers were included and no association between thyroid antibodies and serum TSH, fT3, and fT4 was detected. In addition, in our study, we did not include patients with subclinical hypothyroidism, while most other studies did [5, 7, 10]. Our findings suggest an association between thyroid cancer and serum TSH, fT3, and fT4 in the absence of thyroid autoimmunity.

We also assessed relation between preoperative cytologic results and serum TSH, fT3, and fT4. Patients with malignant cytology had significantly higher TSH and lower fT3 compared to patients with benign cytology. In addition, among patients operated for suspicious cytology, the ones with a final diagnose of malignancy had lower fT3 and higher TSH than the ones with a final diagnosis of benign disease. Experts advocate thyroidectomy, regardless of cytology results in subjects with a high clinical suspicion of malignancy [32]. Haymart et al. evaluated 18 patients with suspicious cytology and reported a trend for higher TSH with malignancy versus benign final pathology, although the difference was not significant ($P = 0.2$) [8]. However, Boelaert et al. demonstrated a significantly higher TSH in patients with suspicious cytology results [5]. According to our results we may suppose that, when a suspicious cytology result is obtained, serum TSH and fT3 may be additional useful parameters in the prediction of probability of underlying malignancy.

It is noteworthy that measurements of serum TSH alone or in combination with fT3 and fT4 are poor predictive test for thyroid cancer (see Table 3). Thus, serum TSH, fT3, and fT4 would better serve as one of several predictors of thyroid cancer risk that could be combined with other patient characteristics in order to aid clinical decision-making. Such an approach was recently suggested by Boelaert et al. who derived a formula using patient age, gender, goiter type, and serum TSH that they proposed could be employed to determine an individual's risk of thyroid cancer [5]. However, FNAB still remains the main tool in predicting malignancy; the above mentioned biochemical parameters are not reliable markers for thyroid cancer detection because of the high false negative results.

In conclusion, in the absence of thyroid autonomy and autoimmunity, within the normal ranges, lower serum fT3 and fT4 and higher serum TSH were associated with both cytologically and histopathologically detected malignancy in patients with thyroid nodules. Also, we think thyroid functions may be useful in predicting the risk of malignancy in patients with suspicious cytology. Particularly, lower fT3 and fT4 in thyroid malignancy is a novel finding. Large-scale trials are needed to evaluate possible additional benefit of serum TSH, fT3, and fT4 measurements to determine risk of malignancy in thyroid nodules.

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