Endogenous Thyrotropin and Triiodothyronine Concentrations in Individuals with Thyroid Cancer

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Background: Thyroid hormone suppression therapy is associated with decreased recurrence rates and improved survival in patients with differentiated thyroid cancer. Recently higher baseline thyrotropin (TSH) levels have been found to be associated with a postoperative diagnosis of differentiated thyroid cancer. Our objective was to confirm whether preoperative TSH levels were higher in patients who were diagnosed with differentiated thyroid cancer after undergoing thyroidectomy, compared with patients who were found to have benign disease. We also sought to determine whether thyroid hormone levels were lower in the patients with malignancy.

Methods: The study was a retrospective analysis of a prospective study. The study setting was the General Clinical Research Center of an Academic Medical Center. Participants were 50 euthyroid patients undergoing thyroidectomy. Thyroxine, triiodothyronine (T₃), and TSH levels were documented in patients prior to their scheduled thyroidectomy. Following thyroidectomy, patients were divided into those with a histologic diagnosis of either differentiated thyroid cancer or benign disease. Preoperative thyroid profiles were correlated with patients' postoperative diagnoses.

Results: All patients had a normal serum TSH concentration preoperatively. One-third of the group was diagnosed with thyroid cancer as a result of their thyroidectomy. These patients had a higher serum TSH level (mean = 1.50 mIU/L, CI 1.22–1.78 mIU/L) than patients with benign disease (mean = 1.01 mIU/mL, CI 0.84–1.18 mIU/L). There was a greater risk of having thyroid cancer in patients with TSH levels in the upper three quartiles of TSH values, compared with patients with TSH concentrations in the lowest quartile of TSH values (odd ratio = 8.7, CI 2.2–33.7). Patients with a thyroid cancer diagnosis also had lower T₃ concentrations measured by liquid chromatography tandem mass spectrometry (mean = 112.6 ng/dL, CI 103.8–121.4 ng/dL) than did patients with a benign diagnosis (mean 129.9 ng/dL, CI 121.4–138.4 ng/dL).

Conclusion: These data confirm that higher TSH concentrations, even within the normal range, are associated with a subsequent diagnosis of thyroid cancer in individuals with thyroid abnormalities. This further supports the hypothesis that TSH stimulates the growth or development of thyroid malignancy during its early or preclinical phase. We also show for the first time that patients with thyroid cancer also have lower T₃ levels than patients with benign disease.

Introduction

THYROXINE THERAPY has long been known to positively affect outcomes in differentiated thyroid cancer (1). Not only can thyroid hormone be used to control the spread of metastatic thyroid cancer (2,3), but it also decreases recurrence rates in thyroid cancer patients, compared with rates in those who do not receive thyroid hormone (1,3–7). Furthermore, use of thyroxine improves overall survival in high-risk patients (8). A retrospective study has shown that an undetectable thyrotropin (TSH) value was associated with longer relapse-free survival, and that TSH suppression was an in-

dependent predictor of recurrence (9). A tendency for a greater degree of TSH suppression to be associated with less disease progression was seen in a cohort of high-risk patients (10), although the difference did not reach significance. A meta-analysis that quantified adverse clinical outcomes from ten studies of patients on TSH suppression also suggested a benefit of this approach (11). However, some authors conclude that the benefit of TSH suppression is limited to patients with persistent or recurrent disease (12).

More recent data from a prospectively followed, but non-randomized cohort of patients with thyroid cancer has shown improved overall and disease-specific survival with TSH

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suppression in those stratified as having high-risk disease (13). Furthermore, suppression of TSH to an undetectable limit was associated with the greatest benefit. There was also improved overall survival associated with a subnormal TSH in patients classified by the National Thyroid Cancer Treatment Cooperative Study Group staging system (14) as having stage II disease. Moreover, more aggressive TSH suppression to yield an undetectable TSH did not further improve survival in these lower risk patients (13). Similarly, in another European cohort of patients with thyroid cancer, higher serum TSH levels were found to be associated with increased rates of thyroid carcinoma-related death and relapse (15).

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 (16,17). This benefit may be limited by changes in TSH receptors that occur in thyroid cancer cells (18–21). Such decreased TSH responsiveness could render some thyroid cancer cells less susceptible to manipulation of TSH concentrations.

A European study including patients with subclinical thyroid disease raised the possibility that endogenous TSH levels may also be a predictor of a subsequent thyroid cancer diagnosis (22). This report found that baseline serum TSH levels appeared to be a predictor of the risk of malignancy in patients who underwent a thyroidectomy for goiter, thyroid nodules, and indeterminate or malignant fine-needle aspiration cytology. The authors derived a formula for risk of malignancy based on serum TSH and other patient characteristics. Recently an additional study (23) showed a similar stepwise increase in thyroid cancer risk with increasing serum TSH levels in patients who had been subjected to thyroidectomy. Higher TSH levels also seemed to predict more advanced tumor stage. In our present analysis, we examined the preoperative serum TSH and thyroid hormone levels in a group of 50 euthyroid patients who were operated on for various thyroid diseases. Thyroid analyte levels were correlated with the patients' postoperative diagnoses.

Methods

Study hypothesis

This study was a secondary analysis of a prospective study designed to compare the circulating levels of thyroxine (T_4) and triiodothyronine (T_3) produced by the normally functioning thyroid gland with those resulting from standard thyroid hormone replacement in the same patient after thyroidectomy (24). This prior study sought to document whether levothyroxine replacement resulted in lower serum T_3 concentrations within individual patients. The present analysis examines the preoperative thyroid analytes in these previously studied 50 patients who underwent thyroidectomy. Analysis was performed to determine whether the concentrations of any of these analytes differed according to whether the patient's histologic diagnosis was benign thyroid disease or thyroid cancer.

Study overview

The study and its informed consent form were approved by the Georgetown University Institutional Review Board. The study was conducted in the General Clinical Research Center

(GCRC). Written informed consent was obtained and participants' medications were then reviewed at the initial study visit. Subject age, sex, and self-reported race and ethnicity were recorded. A medical history was obtained. Parameters documented during the study physical examination included weight, blood pressure, heart rate, thyroid examination, and neurological examination. Participants had thyroid profiles drawn on two separate occasions prior to their thyroidectomy. The study was conducted over a 42-month period.

Study subjects

Subjects aged 18–65 years were recruited from patients referred to the Department of Otolaryngology-Head and Neck Surgery Department for total or near-total thyroidectomy. Patients over age 65 years were not recruited in order to minimize the likelihood that medical conditions or prescribed medications might affect the concentrations of thyroid analytes. One hundred forty-two euthyroid patients who were already committed to a total or near-total thyroidectomy were approached about study participation in advance of their surgery. Fifty-three subjects agreed to participate, three of whom were withdrawn from the study after they did not undergo a total thyroidectomy. Preoperative diagnoses were goiter, nodular thyroid disease, suspected thyroid cancer, or known thyroid cancer. Ultrasound evaluation of the thyroid was not required as part of the study protocol. However, 90% of patients did, in fact, have such evaluations. Patients who had a coexistent diagnosis of hyperthyroidism or hypothyroidism or were being managed with thyroid hormone suppression therapy were excluded from the study. Patients were required to have been on a stable dose of any medications known to affect thyroid hormone protein binding in the 6 weeks prior to the study. Pregnant or lactating patients or those taking estrogen replacement therapy were not recruited. Patients with chronic, serious diseases such as cardiac, pulmonary, and renal disease, or patients who required steroid therapy were not eligible for study participation. Patients whose first or second preoperative thyroid profiles were abnormal were excluded from the study.

Thyroid profiles

Preoperative blood tests were obtained approximately one week and one day prior to thyroidectomy between 8:00 and 10:30 A.M. with the patient in a fasting state. Each thyroid profile consisted of a serum TSH, free T_4 (FT_4), and total T_3 (TT_3). Two separate presurgical profiles were included for each patient in order to minimize the effect of day-to-day fluctuations in thyroid hormone concentrations, TSH levels, and laboratory assays. The mean of these two measurements for each patient was reported in the study. All thyroid profiles were drawn in the GCRC and were analyzed by Quest Diagnostics (Madison, NJ), LabCorp (Burlington, NC), or Georgetown University Laboratories (Washington, DC). Both thyroid profiles for each patient were analyzed by the same laboratory in order to minimize interassay variation. In addition, an aliquot of each blood sample was analyzed in the GCRC Bioanalytic Core Laboratory for TT_3 measured by liquid chromatography tandem mass spectrometry (LC/MS/

MS) and TSH measured using the Dade Behring Inc. RxL Dimension Clinical Chemistry Analyzer (Newark, DE).

TSH assays used by the clinical laboratories employed a third generation ultra-sensitive immunochemiluminometric assay with a sensitivity of 0.01 mIU/L (laboratory reference ranges approximately 0.4–4.5 mIU/L). FT₄ and TT₃ levels were measured by the clinical laboratories using chemiluminescent immunoassays also. Over the period of this study reference ranges were approximately 0.8–1.80 ng/dL for FT₄ and 80–200 ng/dL for TT₃. Details of the LC/MS/MS assay are fully described in a recent publication (25). The manufacturer's reference range for TSH on the Dade Behring RxL Dimension Analyzer was 0.34–4.82 mIU/L.

Thyroid surgery and postoperative care

Thyroidectomies were performed at Georgetown University Hospital by an experienced head and neck surgeon. Decisions regarding the extent of thyroid surgery were made by the patient's referring physician and surgeon, without regard for study involvement. The usual practice for the surgeons whose patients participated in the study is to perform total or near-total thyroidectomy for patients with thyroid cancer, or those pursuing thyroidectomy for bilateral nodular disease. Only subjects who underwent a near-total or total thyroidectomy were included in this analysis. Peri-operative decisions, postoperative surgical care, treatment of hypocalcemia, and management of any other postoperative complications were uninfluenced by study participation.

The histologic diagnosis of each patient was recorded postoperatively.

Statistical analysis

This was a secondary analysis of a study originally designed to determine whether postoperative TT₃ concentrations were lower than preoperative TT₃ concentrations (24). The secondary analysis determined whether preoperative TSH concentrations and the concentrations of other analytes were different in the study population according to their postoperative diagnosis of benign thyroid disease or thyroid cancer.

Statistical services were provided by the GCRC Biostatistics Core using SAS version 9.1, 2002–2003 (SAS Institute Inc, Cary, NC). Statistical comparison was performed to determine whether there were differences in TSH, FT₄, and TT₃ levels between individuals diagnosed with benign thyroid disease compared with those diagnosed with thyroid cancer. The null hypothesis was that histologic diagnosis did not affect TSH, TT₃, or FT₄ levels. TSH, TT₃, and FT₄ levels in the two groups were compared using the *t* test or the Wilcoxon Rank-sum test. The Wilcoxon Rank-sum test was used for analytes that violated the normality assumption.

Post hoc covariates considered were sex (male, female), female gonadal status (premenopausal, menopausal), age, race (African American, White, Asian, Hispanic), clinical laboratory performing the assays (LabCorp, Quest, Georgetown), and year of entry into the study (2004, 2005, 2006, 2007). Multiple regression analysis was used to determine if analyte

TABLE 1. CHARACTERISTICS OF STUDY PARTICIPANTS

Characteristic		Both diagnoses		Benign		Thyroid cancer	
		Number (n = 50)	Percentage (100%)	Number (n = 33)	Percentage (%)	Number (n = 17)	Percentage (%)
Age	< 45	19	38	10	20	9	18
	≥ 45	31	62	23	46	8	16
Sex	Male	13	26	8	16	5	10
	Female	37	74	25	50	12	24
Race	White	34	68	22	44	12	24
	African American	9	18	8	16	1	2
	Hispanic	2	4	1	2	1	2
	Asian	5	10	2	4	3	6
Gonadal status (female)	Premenopausal	19	51	12	24	7	14
	Menopausal	18	49	13	26	5	10
	Menopausal on hormone therapy	0	0	0	0	0	0
Gonadal status (male)	No	13	100	8	16	5	10
	exogenous testosterone						
	Exogenous testosterone	0	0	0	0	0	0
Laboratory performing immunoassay	LabCorp	23	46	15	30	8	16
	Quest	12	24	8	16	4	8
	Georgetown	15	30	10	20	5	10
Year of entry into study	2004	12	24	11	22	1	2
	2005	10	20	7	14	3	6
	2006	21	42	11	22	10	10
	2007	7	14	4	8	3	6

differences associated with a benign or malignant diagnosis were affected by any of these covariates.

Finally, serum TSH values were broken down into quartiles as follows: 0.34–1.1 mIU/L, 1.2–2.0 mIU/L, 2.1–2.8 mIU/L, and 2.9–3.7 mIU/L. The performance of a serum TSH in the upper three quartiles as a screening test for differentiated thyroid cancer was calculated. The odds ratio in favor of having thyroid cancer for patients with a TSH in the upper three quartiles, compared with those in the lowest quartile, was also determined.

Results

The baseline characteristics of the studied patients are shown in Table 1. The mean age of the group was 49 years. Of the 50 patients in this analysis, 33 were found to have benign thyroid disease and 17 were found to have thyroid cancer. Thirty-four percent of the entire group therefore had a diagnosis of thyroid cancer. Further details regarding the patients with a diagnosis of thyroid cancer are given in Table 2. The histologic diagnoses for those with benign thyroid disease were as follows: 22 patients with multinodular goiter, six patients with solitary adenomatoid nodule, three patients with follicular adenoma, and two patients with Hürthle cell adenoma. The two patients with follicular thyroid cancer had stage I and stage III disease respectively. The breakdown of the remaining individuals with papillary thyroid cancer was as follows: seven patients with stage I disease, four patients with stage II disease, and four patients with stage III disease. One patient with a benign diagnosis and one patient with thyroid cancer had some histologic findings suggestive of Hashimoto's thyroiditis. Neither of these two individuals had been documented to have an elevated titer of thyroid peroxidase antibodies or an abnormal serum TSH.

The preoperative laboratory evaluation for study participants is shown in Table 3. The values displayed for each analyte represent the mean of two values. The patients underwent phlebotomy approximately one week (range 6–21 days) and one day (range 1–14 days) prior to undergoing thyroidectomy. The mean value for each analyte is shown separately for the entire patient group, the group with benign thyroid disease, and the group with thyroid malignancy. The serum TSH concentrations measured by the clinical laboratories were significantly higher ($p=0.0017$) in the group of patients with thyroid cancer (mean = 1.50 mIU/L, CI 1.22–1.78 mIU/L) than in the group of patients with benign disease (mean = 1.01 mIU/mL, CI 0.84–1.18 mIU/L). TSH concentrations measured by the Dade Behring RxL Dimension Analyzer were also significantly higher in the group of patients with thyroid cancer ($p=0.0021$). In addition, serum TT₃ concentrations measured by LC/MS/MS were significantly lower ($p=0.015$) in the group with thyroid cancer (mean = 112.6 ng/dL, CI 103.8–121.4 ng/dL) than in the group with benign disease (mean = 129.9 ng/dL, CI 121.4–138.4 ng/dL). TT₃ levels measured by immunoassay and FT₄ levels did not differ between the groups (see Table 3). The TSH, TT₃, and FT₄ levels are also displayed as box plots in Fig. 1 with separate box plots calculated for each histologic group. Figure 1a shows TSH concentrations, Fig. 1b shows TT₃ concentrations determined by LC/MS/MS, Fig. 1c shows TT₃ concentrations determined by immunoassay, and Fig. 1d shows FT₄ concentrations.

Figure 2 shows the breakdown of TSH values measured by the clinical laboratories into quartiles (0.34–1.1 mIU/L, 1.2–2.0 mIU/L, 2.1–2.8 mIU/L, 2.9–3.7 mIU/L). The number and percentage of TSH values that fall within each quartile are displayed according to the histologic diagnosis. Most (73%) of the patients with benign disease had TSH values within the

TABLE 2. DETAILS OF STAGING IN THE PATIENTS WITH DTC (DIFFERENTIATED THYROID CANCER)

Patient ID	Age/sex (M/F)	Histology (FTC, PTC)	Primary tumor size (cm)	Lymph node involvement	Invasion, multifocality	Distant metastases	Stage ^b	Mean serum TSH (clinical lab)	DTC known pre-op?
1	44/M	FTC	2	No	No	No	I	1.50	Yes
2	36/F	PTC	1.6	No	Yes	No	I	1.69	Yes
3	45/F	PTC ^a	0.8	No	No	No	I	3.20	No
4	38/M	PTC	2.5	No	No	No	I	1.16	Yes
5	39/F	PTC	3.5	Yes	Yes	No	I	1.40	Yes
6	25/F	PTC	0.5	No	Yes	No	I	1.35	No
7	44/F	PTC	1.7	No	Yes	No	I	0.98	Yes
8	51/M	PTC	0.6	No	No	No	I	0.74	Yes
9	34/F	PTC	3	Yes	Yes	No	II	2.11	Yes
10	37/M	PTC	3	Yes	Yes	No	II	2.30	Yes
11	36/F	PTC	1	Yes	Yes	No	II	1.30	Yes
12	22/M	PTC	1.6	Yes	Yes	No	II	1.13	Yes
13	61/F	PTC	0.7	Yes	Yes	No	III	1.80	Yes
14	61/F	PTC	1.4	No	Yes	No	III	1.38	Yes
15	65/F	FTC	5	Yes	Yes	No	III	1.19	Yes
16	50/F	PTC	1.0	Yes	No	No	III	1.18	Yes
17	59/F	PTC	0.9	Yes	No	No	III	1.15	Yes

FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; TSH, thyrotropin.

^aHistology also showed evidence of Hashimoto's thyroiditis.

^bStaging system is outlined in table 1 of Jonklaas *et al.* (13).

TABLE 3. CONCENTRATIONS OF THYROTROPIN (TSH), FREE THYROXINE (FT₄), AND TOTAL TRIIODOTHYRONINE (TT₃) VALUES IN PATIENTS WITH BENIGN DISEASE AND THYROID CANCER

Analytes	Preoperative time points		
	Mean of 1 and 2		
Patients (n)	50	33	17
Diagnosis	Both	Benign	Thyroid cancer
TSH (mIU/L) measured by clinical laboratory			
Mean	1.18	1.01	1.50
SD	0.58	0.51	0.59
CI	1.02–1.34	0.84–1.18	1.22–1.78
Significance ^a	$p = 0.0017$		
TSH (mIU/L) measured by GCRC laboratory			
Mean	1.12	0.99	1.38
SD	0.43	0.39	0.40
CI	1.00–1.24	0.86–1.12	1.19–1.57
Significance ^b	$p = 0.0021$		
FT ₄ (ng/dL)			
Mean	1.05	1.06	1.04
SD	0.19	0.19	0.17
CI	1.00–1.10	0.95–1.17	0.96–1.12
Significance ^b	$p = 0.78$		
TT ₃ by IA (ng/dL)			
Mean	129.3	133.9	120.5
SD	26.7	25.3	27.8
CI	121.9–136.7	125.4–142.4	107.3–133.7
Significance ^b	$p = 0.094$		
TT ₃ by LC/MS/MS (ng/dL)			
Mean	124.0	129.9	112.6
SD	24.3	24.9	18.6
CI	117.3–130.7	121.4–138.4	103.8–121.4
Significance ^b	$p = 0.015$		
Ratio (FT ₄ ×100/TT ₃)			
Mean	0.85	0.82	0.91
SD	0.22	0.21	0.25
CI	0.79–0.91	0.75–0.89	0.79–1.03
Significance ^b	$p = 0.80$		

^aComparing group with benign disease to group with thyroid cancer using Wilcoxon Rank-sum test (data not normally distributed).

^bComparing group with benign disease to group with thyroid cancer using *t* test (data normally distributed)

lowest quartile, whereas most (59%) of the patients with thyroid cancer had TSH values in the second quartile. However, a substantial proportion (21%) of patients with benign disease also had TSH values in the second quartile. No patients with benign disease had TSH values in the highest quartile. If a serum TSH falling within the highest three quartiles was used as a screening test for thyroid cancer (see Table 4), it can be seen that this test performed poorly with only fair sensitivity and specificity and poor positive predictive value. However, the negative predictive value of this test was acceptable (86%). The odds ratio in favor of having thyroid cancer with a serum TSH within the upper three quartiles, compared with having a serum TSH within the lowest quartile was calculated as 8.7 (CI 2.2–33.7) (see Table 5). The odds ratio for the risk of thyroid cancer in quartile 2 compared with quartile 1 was 8.6 (CI 2.0–35.9). The odds ratio for the risk of thyroid cancer in quartile 3 compared with quartile 1 was

6.0 (CI 0.6–55.7). This confidence interval crossed unity, and the number of patients in quartile 3 was small. There were too few patients in quartile 4 to calculate an odds ratio.

Figure 3 shows the mean TSH values for all 50 patients arranged in ascending order and coded by diagnosis. Figure 3a shows the values determined when TSH measurements were made by the clinical laboratories employed in the study. Figure 3b shows the values determined in the GCRC Bioanalytic Core laboratory using the Dade Behring RxL Dimension analyzer. It can be clearly seen that, although the range of TSH values is similar for both diagnoses, patients with thyroid cancer generally have higher TSH values across the entire series. However, all TSH values for each patient (including the mean of the preoperative determinations and the two individual preoperative determinations) fell within the normal laboratory reference range. The highest mean TSH values measured by immunoassay or Dade Behring RxL Dimension analyzer were all in the thyroid cancer group. The highest individual TSH values measured by immunoassay (prior to calculating the preoperative mean) were 2.5, 2.7 and 3.7 mIU/L in the thyroid cancer group, and 2.3, 2.4, and 2.7 mIU/L in the benign group. All remaining values were 2.2 mIU/L or less.

Based on the data from their 2006 study Boelaert *et al.* (22) developed a formula using patient age, sex, goiter type determined by palpation, and serum TSH that they proposed could be used to predict an individual's risk of thyroid cancer. This formula was applied to each of the 50 patients in our study. However, goiter type was determined by a combination of palpation and ultrasound examination. The mean calculated risk of thyroid cancer for the patients with a final diagnosis of benign disease was 5.4% (CI 4.03–6.75%). The mean calculated risk for patients who were diagnosed with thyroid cancer was 9.4% (CI 7.03–11.79%). The mean for these two groups was significantly different ($p = 0.03$).

Discussion

Our data show that having a high-normal serum TSH concentration is a risk factor for a subsequent diagnosis of thyroid cancer in individuals with abnormal thyroid examinations. Only patients who were euthyroid were recruited for this study, so all patients had serum TSH levels within the normal range. Thus, the increased risk of thyroid cancer was associated with higher TSH values within the normal range. Clearly our patient group was small, and only 17 patients within this group had a diagnosis of thyroid cancer. In addition, the number of patients who had serum TSH concentrations in the upper two quartiles was small, comprising only five patients out of the 50-patient group. This was also a secondary analysis of a prospective study designed to document changes in serum TT₃ concentrations after thyroidectomy. Nevertheless, within this limited group, our data confirm the role of TSH as one of the, presumably many, stimulants of thyroid cancer development. Although our patient numbers are quite small and our range of TSH values is narrow, we were still able to demonstrate a relationship between risk of malignancy and higher TSH values, perhaps indicating a powerful physiologic underpinning for this phenomenon.

The increased risk of having thyroid cancer with higher TSH levels that remain in the normal range has been reported

FIG. 1. (A) Serum thyrotropin (TSH) concentrations (measured by the clinical laboratories) in patients with benign disease and differentiated thyroid cancer (DTC). (B) Serum triiodothyronine (T_3) concentrations by liquid chromatography tandem mass spectrometry (LC/MS/MS) in patients with benign disease and DTC. (C) Serum T_3 concentrations in patients with benign disease and DTC. (D) Serum free thyroxine (FT_4) in patients with benign disease and DTC. In all panels, the top, bottom, and line through the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers extend from the 10th percentile to the 90th percentile. The filled square indicates the arithmetic mean.

previously in a larger European study examining a group of 1183 patients, 92 of whom were found to have thyroid cancer (22). This earlier study, in fact, documented an adjusted odds ratio for malignancy of 11 in patients with serum TSH concentrations above 5.5 mIU/L. Odds ratios for TSH values of 1.8–5.5 mIU/L and 1.0–1.7 mIU/L were 3.88 and 2.72 respectively. A recent study of individuals undergoing thyroid surgery at a single institution in the United States showed an odds ratio for malignancy increasing from 1.65 in the group of individuals with a low TSH to 4.56 in the group of individuals with a high TSH (23). Furthermore TSH levels were higher in individuals with more advanced stage disease.

Our data confirm the association between higher TSH values and malignancy in a small group of patients with thyroid abnormalities studied in the United States. Our patient group was too small to examine the association between serum TSH and thyroid cancer stage (see Table 2). Our data differ from these prior studies in that we did not study any

patients with abnormal TSH values, whereas the previous studies included patients with subclinical hypothyroidism and subclinical hyperthyroidism. We thus extend their findings to an entirely euthyroid population with an extremely narrow range of TSH values between 0.44–3.2 mIU/L. In contrast to the study by Boelaert and coauthors (22), in which thyroid function may have been documented several years prior to thyroidectomy, our study and that by Haymart *et al.* (23) documented thyroid function in close temporal proximity to thyroidectomy. Serum TSH was documented approximately a week prior to thyroidectomy in our study and within 3 months of thyroidectomy in the Haymart study.

There are several differences between our study and the two previously reported studies. Although our analysis was retrospective, the data was prospectively collected for another study (24). We obtained two preoperative TSH values for each patient, perhaps yielding a more accurate assessment of baseline TSH levels. In addition, we were also able to examine

FIG. 2. (A) The number of thyrotropin (TSH) values in each TSH quartile divided according to diagnosis of benign disease and differentiated thyroid cancer. (B) The percentage of TSH values in each quartile divided according to diagnosis. (Quartile 1 = 0.34–1.1, quartile 2 = 1.2–2.0, quartile 3 = 2.1–2.8, quartile 4 = 2.9–3.7). TSH values used are those from the clinical laboratories.

FT₄ and TT₃ levels in our patients. TT₃ levels measured by LC/MS/MS, but not immunoassay, were significantly lower in patients with thyroid cancer. The association between lower TT₃ levels and a diagnosis of thyroid cancer is a novel finding. It would appear logical to assume that serum T₃ is an "innocent bystander," and that the causative association is between TSH and thyroid cancer development. However, T₃, via its action on nuclear receptors, is a powerful regulator of gene expression (26). It is also possible that lower T₃ levels might be an indication of cellular dedifferentiation (27). Therefore, a more direct role of T₃ in the process of carcinogenesis cannot be entirely excluded.

TABLE 4. PERFORMANCE OF A SERUM THYROTROPIN (TSH) VALUE THAT FALLS WITHIN QUARTILES 2–4 (1.3–3.7 mIU/L) AS A SCREENING TEST FOR THYROID CANCER (TSH MEASURED BY CLINICAL LABORATORY)

	Thyroid cancer	Benign disease	Total
Positive test (TSH quartile 2–4)	13	9	22
Negative test (TSH quartile 1)	4	24	28
Total	17	35	50

Sensitivity = $13/(13 + 4) = 77\%$
 Specificity = $24/(24 + 9) = 73\%$
 Positive predictive value = $13/(13 + 9) = 59\%$
 Negative predictive value = $24/(24 + 4) = 86\%$

It has previously been suggested that the finding of a lower risk of malignancy in individuals with lower TSH values could be due to their harboring autonomous nodules that therefore have a lower risk of malignancy (28). Although all our patients were biochemically and historically euthyroid, none of them underwent studies such as thyroid scanning during administration of thyroid hormone in order to exclude such autonomy. The mean serum TSH in our patients with benign disease was 1.01 mIU/L. This is lower than the mean or median serum TSH values reported in large studies of the United States population (29–31). For example, the mean TSH in those individuals without any thyroid abnormalities was 1.48 mIU/L in the recent study by Hamilton and coworkers (31). This compares more closely to the mean TSH of 1.50 mIU/L seen in our group of participants with thyroid cancer. Thus, it is not clear whether our patients with thyroid cancer have higher TSH values than the entire group, or whether patients with benign disease have lower TSH values than the group as a whole.

Individuals with Hashimoto's thyroiditis are thought to have a higher risk of papillary thyroid cancer than patients without this diagnosis (32–35). It could be hypothesized that our observations are due to Hashimoto's thyroiditis in the

TABLE 5. ODDS RATIO IN FAVOR OF HAVING THYROID CANCER WITH SERUM THYROTROPIN (TSH) WITHIN QUARTILES 2 (1.2–2.0 mIU/L), 3 (2.1–2.8 mIU/L), OR 2–4 (1.2–3.7 mIU/L), COMPARED WITH QUARTILE 1 (0.34–1.1 mIU/L)^a

	Thyroid cancer	Benign disease
TSH quartile 2	10	7
TSH quartile 1	4	24
Odds ratio = $(10 \times 24)/(7 \times 4) = 8.6$ (CI 2.0–35.9)		
TSH quartile 3	2	2
TSH quartile 1	4	24
Odds ratio = $(2 \times 24)/(2 \times 4) = 6$ (CI = 0.6–55.7)		
TSH quartile 2–4	13	9
TSH quartile 1	4	24
Odds ratio = $(13 \times 24)/(9 \times 4) = 8.7$ (CI 2.2–33.7)		

^aTSH measured by clinical laboratory.

FIG. 3. (A) Thyrotropin (TSH) concentrations measured by clinical laboratory arranged in ascending order and divided by diagnosis (benign vs. differentiated thyroid cancer [DTC]). (B) TSH concentrations measured by Dade Behring RxL Dimension analyzer arranged in ascending order and divided by diagnosis (benign vs. DTC).

individuals with higher TSH concentrations. Certainly, we did not routinely document thyroid peroxidase antibodies in our patients preoperatively. However, we did not recruit patients with a history of either hypothyroidism or hyperthyroidism. Furthermore, this hypothesis is made untenable by the finding that only one of the 17 thyroid cancer patients was also noted to have histologic changes associated with Hashimoto's thyroiditis. If there is an increased risk of differentiated thyroid cancer associated with Hashimoto's thyroiditis, we hypothesize that this occurs by a separate mechanism other than by TSH stimulation. For example, such a mechanism has been suggested to be activation of the PI3k/Akt pathway (36). However, it is possible that stimulation by elevated TSH concentrations could be a second additional means of promoting tumor formation in patients with Hashimoto's thyroiditis.

In our patients it is possible that higher TSH levels *per se* are the causative factor, or alternatively, that prevailing TSH levels are associated with the causative factor. One alternative

causative factor would be low T_3 levels. However, the latter hypothesis would appear less likely given the extensive data showing the favorable effect of TSH suppression in patients with thyroid cancer (9,10,13,15), which can be achieved without causing elevated T_3 levels (24). Our study does not provide any additional data that would help determine the etiology of this finding of risk associated with upper normal TSH concentrations. It is possible that higher TSH levels, albeit within the normal range, are sufficient to promote the growth of pre-existing microcarcinomas. Alternatively, higher TSH levels could be associated with the initiation of carcinogenesis.

Using our data, the odds ratio of having thyroid cancer with TSH values in the upper three quartiles (1.2–3.7 mIU/L) compared with TSH values in the lowest quartile (TSH 0.35–1.1 mIU/L) was 8.7 (CI 2.2–33.7) (see Table 5.). Clearly, this estimation of risk must be cautiously interpreted in light of the fact that these patients were preselected. All patients were scheduled for a total thyroidectomy for thyroid abnormalities.

In other words, this risk would not be applicable to an individual with a serum TSH of, for example, 3 mIU/L and a normal thyroid examination. Given that our patient group is small and so highly selected, it is possible these data may not even be applicable to other populations with abnormal thyroid examinations. In addition, the absolute risk of having thyroid cancer needs to be taken into account in interpreting the fold-increase in risk.

Clearly, measurement of serum TSH alone is a poor predictive test for thyroid cancer (see Table 4). If a TSH level within the highest 3 quartiles is evaluated as a screening test, its performance is unsatisfactory. Although TSH levels are generally higher in our group of patients with thyroid cancer, the finding is neither sufficiently sensitive (77%) nor adequately specific (73%), and it does not allow discrimination of patients with benign and malignant diagnoses. Thus, serum TSH 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.* (22). They 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. When applied to the patients in our study, this formula yielded risks for individual patients that overlapped between the group with benign and malignant diagnoses, thus not providing discrimination. The mean risk of the two groups was different. However, our goiter classification was by a combination of palpation and ultrasound imaging, compared with classification by palpation in the European study. Fewer patients in our group actually had solitary nodules (22%), compared with 57% in the European study. Thus, this formula probably would require modification to incorporate goiter type determined by imaging also.

In summary, we believe these data, in aggregate with previous data, make a compelling case to support that higher TSH concentrations within the normal range are a risk factor for thyroid cancer in patients who have thyroid abnormalities, either by being associated with the development or progression of malignancy. Future studies may determine whether TSH levels could be used in a clinical situation, along with other diagnostic factors, to predict whether patients with thyroid abnormalities may have thyroid cancer. Further studies are also indicated to exclude the possibility that the adverse impact of high TSH concentrations on thyroid cancer risk is actually exerted via a mechanism involving low T₃ levels.

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