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Autoimmunity and thyrotropin level in developing thyroid malignancy

S. MEDENICA[1], N. RADOJEVIC[2], M. STOJKOVIC[3], B. NEDELJKOVIC-BELESLIN[3,4], S. SAVIC[3], J. CIRIC[3,4], B. TRBOJEVIC[3,4], M. ZARKOVIC[3,4]

[1]Department of Endocrinology, Clinical Center of Montenegro, Faculty of Medicine University of Montenegro, Podgorica, Montenegro
[2]Center for Pathology and Forensic Medicine, Clinical Center of Montenegro, Faculty of Medicine University of Montenegro, Podgorica, Montenegro
[3]Department of Thyroid Disease, Clinic for Endocrinology, Diabetes and Metabolic Disease, Belgrade, Serbia
[4]School of Medicine, University of Belgrade, Serbia

Abstract. – OBJECTIVE: Malignancies and autoimmune thyroid disease are still controversial, but recent studies prove that a long lasting thyroid disease may be linked with malignancy, e.g. papillary thyroid carcinoma in patients with Hashimoto thyroiditis. Having in mind that thyrotropin is a thyroid growth factor, the relationship between its serum values, as well as the levels of anti-peroxidase and anti-thyroglobulin antibodies and thyroid malignancy in patients with nodular thyroid goiter was examined.

PATIENTS AND METHODS: Six-hundred-thirty-seven medical records, which included the thyroid fine-needle aspiration cytology were retrospectively evaluated. Patients were grouped regarding the levels of thyrotropin, anti-peroxidase and anti-thyroglobulin antibodies (in or out of the reference ranges) and compared with cytology findings for establishing their prognostic potential for malignancy.

RESULTS: Elevated serum thyrotropin (≥ 4.5 mIU/L) was found in 27.3% of patients with thyroid malignancy compared with 10.8% with benign and 16.1% with unspecified cytology finding ($p < 0.01$). In the group of patients with malignant cytology findings 7.0% of them had elevated anti-peroxidase antibodies level, and 1.4% had anti-peroxidase antibodies level in reference range. In the group of patients with malignant cytology findings 4.2% of them had elevated anti-thyroglobulin antibodies level, and 1.4% had anti-thyroglobulin antibodies level in reference range.

CONCLUSIONS: In patients with elevated serum thyrotropin concentration and/or chronic thyroiditis the occurrence of thyroid malignancy is increased.

Key Words:
Nodular goiter, Thyrotropin, thyroid autoimmunity, Anti-peroxidase antibodies, Anti-thyroglobulin antibodies.

Introduction

Nodular goiters (NGs) are clinically recognizable enlargements of the thyroid gland[1]. Numerous studies suggest a prevalence of 2-6% with palpation, 19-35% with ultrasound, and 8-65% in autopsy data[2,3]. A solitary thyroid nodule exists within a thyroid gland of normal dimensions and morphology, whereas a dominant thyroid nodule exists within a diffuse or multinodular goiter[4,5]. Most of the lesions are benign, but the principal problem facing the clinician is that of identifying the malignant nodule requiring surgery[6].

There are a number of well-established predictors of malignancy in thyroid nodules, including the finding of hard and fixed lesions on clinical examination, rapid growth of nodules, associated hoarseness, dysphagia or lymphadenopathy, although all of these symptoms and signs are relatively uncommon at diagnosis[7]. Since clinical findings cannot distinguish benign from malignant process, a fine-needle aspiration (FNA) is considered to be an essential tool in providing a rational approach to the clinical management of these nodules[8]. Diagnostic categories of thyroid FNA can be qualified as "malignant", "benign", "unspecified", and "unsuccessful".

Thyrotropin (TSH) is known as thyroid growth factor, as well as for thyroid nodules, but the pathogenic role of TSH in thyroid oncogenesis is unclear[9]. Suppression of serum TSH concentrations by administering exogenous thyroid hormone may inhibit the growth of established nodules as well as the development of new thyroid nodules[10,11]. There is mounting evidence that the serum concentration of TSH is an independent

Corresponding Author: Nemanja Radojevic, MD; e-mail: com_nr@yahoo.com

predictor for the diagnosis of thyroid malignancy in patients with nodular thyroid disease[10]. It is believed that this hormone plays a role in oncogenesis and the progression of malignancy since the TSH concentration increased in patients with more aggressive tumors, that is why patients with high serum TSH concentration and borderline cytology results require more detailed tests and more aggressive treatment.

On the other side, important clinical markers for autoimmune thyroid disease are antibodies to thyroid peroxidase (anti TPO-Ab) and thyroglobulin (anti Tg-Ab). Link between malignancy and autoimmune thyroid disease (AITD) is less controversial then in the past, since many recent studies dealt with it. Kim et al[12] showed an increased risk for papillary thyroid carcinoma (PTC) in patients with Hashimoto thyroiditis (HT), indicating that a long lasting thyroid disease may be a basis for developing malignancy. A meta-analysis of ten studies[13] showed a 2.77-fold increased incidence of thyroid cancer in patients with HT, compared with control populations, confirmed in recent studies[14,15] and also the association between Graves' disease and thyroid cancer was found[16]. Haymart et al[9] found a significant association between pathological HT and higher TSH levels. Fiore et al[17] shown that the frequency of thyroid cancer was not significantly different between antibody-positive and antibody-negative patients, and higher serum TSH concentration was found in those with PTC when compared with subjects with benign disease. The coexistence of PTC with HT is associated with good prognostic factors, and P2X7R expression in PTC was correlated with poor prognostic factors and the absence of HT[18].

The aim of the study is to analyze the relationship of serum TSH concentration with thyroid malignancies in patients with nodular thyroid goiter, as well as correlation between levels of anti TPO-Ab and anti Tg-Ab as markers of AITD and thyroid malignancies.

Patients and Methods

Six-hundred-thirty-seven medical records which included a thyroid FNA cytology obtained by the patients with the nodular thyroid goiter were retrospectively reviewed. FNA was followed by physical examination, medical history review, laboratory tests and thyroid ultrasound in all patients. FNA samples were divided in four diagnostic categories: "benign", "malignant", "unspecified", and "unsuccessful". The category "unspecified" refers to indefinite lesions which, based on cytological appearance, could not be classified with certainty into one of the "benign" or "malignant" group; these include follicular, Hurthle cell and suspicious findings. The category "unsuccessful" was related to those samples, which did not have enough material or the same material was not adequate for diagnosis.

Serum TSH was measured by an immunoluminometric assay on a random-access analyzer (Architect; Abbott Diagnostics Division, North, Chicago, IL, USA). All patients were classified on the basis of the serum TSH concentration into three categories: TSH less than normal (< 0.5 mU /l), TSH in reference range (0.51-4.5 mU / l), and TSH above normal (> 4.51 mU/l).

Anti TPO-Ab and anti Tg-Ab were measured with a fluorescence immunoenzymometric assay for the quantitative measurement of the IgG class of these antibodies (AxSYM; Abbott Diagnostics Division). The reference value for both is < 30 IU/ml (data provided by manufacturer), so the patients were grouped by those criteria: first group – in reference range, and the second one – above reference range.

Blood for TSH and antibodies testing were sampled in a range from one to fifteen days before FNA.

Statistical Analysis

Accompanying descriptive statistics, the analytical statistics (ANOVA and Kruskal-Wallis test) and correlation analysis (Spearman correlation) were used to evaluate data. $p < 0,05$ was considered statistically significant.

Results

Out of 637 patients, 581 were females and 56 males (mean age 52.5 ± 13.9). FNA cytology revealed 583 benign lesions, 22 malignant lesions, while 30 were unclassified, and in one case the FNA was unsuccessful. In the benign group the average value of TSH was 3.12 ± 12.70 mU/l with median 1.15 mU/l, the average value of TSH in the malignant diagnostic category group of patients was 9.83 ± 17.48 mU/l with a median of 3.31 mU/l, while the average value of TSH in those patients with indeterminate diagnosis was 3.16 ± 3.80 mU/l with a median of 2 mU/l. A significant difference between the four diagnostic categories by TSH level groups was found (χ^2 =

Table I. Diagnostic categories depending on the serum thyrotropin (TSH) concentration.

Fine-needle aspiration cytology result	TSH (mU/l)		
	≤ 0.5	0.51-4.5	≥ 4.51
Benign	22%	67.2%	10.8%
Malignant	9.1%	63.6%	27.3%
Unspecified	9.7%	74.2%	16.1%
Unsuccessful	100.0%	0 %	0%

8.136, $p = 0.017$) by Kruskal-Wallis tests, but very high standard deviation in malignant group indicates nonhomogeneity of that group.

Considering the serum TSH levels, 134 were below the reference range, 429 were in reference range, and 74 were above it. A distribution of FNA diagnostic categories depending on the serum TSH levels are given in Table I.

The level of anti TPO-Ab was in reference range in 380 (59.7%) of patients and for the other 40.3% it was elevated. Very similar results were obtained for anti Tg-Abs: elevated in 41%, and 59% in reference range. The level of antibodies was examined depending on FNA diagnostic categories, and the data were presented in Table II. The key fact is that 7% of patients with malignant cytological findings had increased level of anti TPO-Ab, while only 1.4% had its value in reference range. Regarding anti Tg-At, 4.2% of patients with malignancies had elevated levels, since in only 1.4% of them the levels were in reference range.

Discussion

Among the patients with elevated serum TSH concentration was a relatively large proportion [27.3% (6/22)] of those with malignant cytopathological findings. It can be assumed that the risk for developing thyroid malignancy increases in patients with elevated or in the upper third of referent range of serum TSH concentrations. Another explanation would be that patients with low concentrations of TSH develop an autonomous function, which is associated with a low risk of thyroid malignancy.

Fiore et al[17] found a significant age-dependent development of thyroid autonomy (TSH < 0.4 mU/ml) in patients with benign thyroid disease, but this reduction of TSH with age was less evident in those with papillary thyroid carcinoma (PTC). There is a possibility that even after PTC required total thyroidectomy, ^{131}I-whole-body-scan remains negative followed by detectible Tg indicating micrometastasis[19]. Furthermore, in patients with multinodular goiter, the frequency of thyroid autonomy was higher and the risk of PTC was lower than in those with solitary nodules[17]. Ichikawa et al[20] pointed the expression of TSH receptor on the cell membrane of the thyrocyte in benign and malignant tumors, and Carayon et al[21] shown that TSH increases the production of adenylate cyclase leading to cyclic adenosine monophosphate (cAMP) production and cell growth through the stimulation of these receptors. Independent of age, thyroid cancer incidence correlates with higher TSH. Higher TSH is associated with extrathyroidal extension of disease[22].

In a study on 1,500 patients, Boelaert et al[23] showed that more of those with serum concentra-

Table II. Diagnostic fine-needle aspiration categories depending on the levels of anti-peroxidase antibodies (anti TPO-Ab), anti-thyroglobulin antibodies (anti Tg-Ab).

		Fine-needle aspiration cytology result			
		Benign	Malignant	Unspecified	Unsuccessful
Anti TPO-Ab (IU/ml)	≤ 30	94.6%	1.4%	4.0%	0%
	> 30.1	83.0%	7.0%	9.0%	1.0%
Anti Tg-Ab (IU/ml)	≤ 30	91.3%	1.4%	7.2%	0.1%
	> 30.1	87.5%	4.2%	8.3%	0%

tion of TSH over 0.9 mIU/l are associated with a risk of thyroid malignancy diagnosis. Patients with subclinical hyperthyroidism (TSH < 0.4 mIU/l) had the lowest risk of malignancy, and patients with subclinical hypothyroidism (TSH > 5.5 mIU / l) had the highest risk.

Haymart et al[9] examined 843 patients who required surgical treatment and their preoperative serum concentration of TSH. The likelihood of malignancy was 16% when TSH was < 0.06 mIU/l, 25% for TSH between 0.40 and 1.39 mIU/l, 35% for TSH between 1.40 and 4.99 mIU/l and 52% in those with TSH of 5.0 mIU/l or greater. The risk of malignancy in thyroid nodules increases in parallel with TSH concentrations within the normal range.

There are plenty of arguments claiming otherwise. The development and progression of thyroid tumors are associated with phenotype-specific mutations of genes involved in growth control[25]. Derwahl et al[26] have shown in their *in vitro* studies that other factors such as insulin-like growth factor IGF-1 are important in stimulating a growth of thyroid cancer, while Kimura et al[27] hypothesized that collaboration of TSH with insulin and IGF-1 exerted its proliferative effect. TSH via cAMP, and various growth factors, in cooperation with insulin or insulin growth factor 1 (IGF-I) stimulate cell cycle progression and proliferation in various thyrocyte culture systems, including rat thyroid cell lines (FRTL-5, WRT, PC Cl3) and primary cultures of rat, dog, sheep and human thyroid. Shi et al[28] found that there is an inverse relationship between TSH receptor mRNA levels and cancer aggressiveness. These results indicate that decreased TSH receptor and increased c-myc gene expression levels are associated with thyroid cell de-differentiation. It is believed that thyroid cancer often occurs in the contralateral lobe compared to hyperfunctional nodule where TSH is suppressed. Studies dealing exploration of the genome have shown that serum TSH concentrations were lower in patients who are carriers of one of the two alleles that are associated with an increased risk of papillary and follicular carcinoma.

Autoimmune thyroid diseases (AITD) comprise Graves disease and Hashimoto thyroiditis[29], characterized by lymphocytic infiltration and auto-reactivity against thyroid autoantigenes, producing anti TPO Ab and anti Tg Ab.

Hashimoto's thyroiditis leads to hypothyroidism and increase of serum TSH concentration, while Graves' disease increases serum TSH concentration through TSH receptors stimulation. It could be assumed from the above that a connection between humoral thyroid autoimmunity and thyroid malignity is made through TSH receptors, or a structure of the thyroid gland is changed leading to development of thyroid malignancy.

Our findings have shown a significantly higher proportion of patients with malignant cytological findings and increased level of anti TPO Ab and anti Tg Ab, but also like it was expected, increased levels of these antibodies were noticed in patients with higher serum TSH concentration.

A meta analysis of 10 studies by Singh et al[13] has shown that patients with Hashimoto's thyroiditis have even a 2.77 times higher risk for thyroid malignancy compared with the control group. Boelaert et al[23] have shown in a 2006 study a significantly higher frequency of cancer in patients with present anti TPO Ab in comparison with patients where antibodies were not present. The results in this series of patients do not support the claim that thyroid cancer is more aggressive in Graves' disease patients than in euthyroid patients[16].

Contrary to already specified, Rebuffat et al[31] study has shown that anti TPO antibodies achieve cell-mediated cytotoxicity and antiproliferative activity in papillary thyroid carcinoma cell and not found higher risk for thyroid malignancy in patients with Hashimoto thyroiditis.

Kim et al[31] suggested that a positive serum TgAb test was an independent predictor for thyroid malignancy in thyroid nodules along with serum TSH levels regardless of the presence of AIT. Of the 1638 patients, malignant nodules had a higher rate of positive TgAb (30.8% vs. 19.6%; $p < 0.001$) and elevated TSH levels (2.5 ± 2.8 mIU/L vs. 2.1 ± 2.0 mIU/L; $p = 0.021$) than benign nodules[31]. Co-occurrences of chronic lymphocytic thyroiditis (CLT) and thyroid cancer (DTC) have been repeatedly reported[32].

Conclusions

In patients with elevated serum TSH concentration the risk of developing thyroid malignancy is increased. This hormone should be used as an additional diagnostic tool in identifying patients who require further investigation and/or surgical treatment. The role of thyrotropin in a development of thyroid malignancy is not confirmed yet, further studies are needed for enlightening the

molecular mechanisms link of thyroid autoimmunity and thyroid cancer.

According to level of anti-thyroid antibodies and malignant cytopathological findings it can be assumed that the patients with chronic thyroiditis more often develop thyroid cancer. Understanding the relationship between thyroid autoimmunity and differentiated thyroid cancer provides implication of immunotherapy in treatment of thyroid carcinoma.

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Conflict of Interest

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