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Thyroid stimulating hormone levels and BRAFV600E mutation contribute to pathophysiology of papillary thyroid carcinoma: Relation to outcomes?

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ABSTRACT

Aims: The aim of this study was to evaluate the relation between the level of thyroid stimulating hormone (TSH) and progression of papillary thyroid carcinoma (PTC) with or without $BRAF^{V600E}$ mutation.
Methods: The medical records and laboratory data of 547 patients with PTC and 94 patients with follicular adenoma (FA) were collected. The relationship between hormones levels and such end-points as extrathyroid extension (ETE), lymphovascular invasion (LVI) and lymph node metastasis (LNM) was assessed. In addition, age, gender, $BRAF^{V600E}$ mutation status, histological type and Hashimoto's thyroiditis (HT) were considered.
Key findings: Most of the patients with PTC had hormones levels within the normal range, however, serum TSH concentration was significantly higher in PTC comparing with FA (P = 0.022). High levels of TSH in PTC were more frequent among women rather than men (P = 0.03) due to the gender differences in coexisting HT rate (P = 0.003). In contrast, LNM rate was higher in men (P = 0.0014). Coexisting HT significantly decreased the risk of ETE (OR = 0.67; 95%CI 0.44–1.00; P = 0.05) and LNM (OR = 0.59; 95%CI 0.37–0.94; P = 0.028) among males with PTC. However, there was no significant relationship between HT and PTC-related ETE and LNM in females. *$BRAF^{V600E}$* mutation was associated with presence of lymphocytic infiltration (P < 0.001) but not with HT (P = 0.08) and violation of thyroid function.
Conclusion: The present study showed the lack of significant relationship between TSH levels and PTC aggressiveness (LNM, TNM stage, *$BRAF^{V600E}$* mutation). Higher TSH levels were found in patients with coexisting HT that was associated with female sex and multifocality of PTC.

1. Introduction

Papillary thyroid carcinoma (PTC) is the most common cancer of the endocrine system [5,12,24]. Its incidence has been increasing rapidly for the last decades [5,12,17,24]. It is widely accepted that thyroid stimulating hormone (TSH) plays a role in nodular thyroid disease and risk of thyroid malignancies, including PTC [12]. This concept is based on TSH effects which include both activation of thyroid hormones production and follicular cells proliferation [22]. However, the detailed assessment of the TSH levels' significance in PTC prognosis is controversial. The number of studies reported an increased risk of thyroid cancer associated with elevated TSH levels [6,7,13,18]. Following the physiological effects, high serum TSH should lead to the increase of iodine uptake and thyroid hormones synthesis, however there is no confirmation of direct link between thyroid dysfunction and PTC. Moreover, such relationship conflicts with age-related trend of PTC development and thyroid functioning [28,36]. In contrast, the possibility of reverse causation or treatment effect due to measurement of TSH levels after surgery and histopathological confirmation of PTC diagnosis was suggested [14,34]. Several studies did not find any relations between TSH and PTC [14 hormoneslevels, 35]. In addition, there are some data demonstrating significantly reduced risk of thyroid cancer associated with elevated TSH levels [10,11,35]. An alternative factor associated with PTC progression is genetic alterations including the most frequent $BRAF^{V600E}$ mutation [2,8,20,26,30]. Some authors

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demonstrated the relationship between TSH-mediated and BRAF-signaling pathways in thyrocytes [21,23,40]. Nevertheless, it is still unclear if there are any relations or combined effects of *BRAF*^{V600E} mutation and TSH levels alteration in PTC progression. The aim of this study was to evaluate whether TSH and thyroid hormones levels are related to PTC clinicopathological features and *BRAF*^{V600E} mutation status.

2. Material and methods

It was a retrospective one-center cohort study that was conducted at Ukrainian Research and Practical Center for Endocrine Surgery. Enrolled cases met the following criteria: 1) histologically confirmed PTC in line with updated WHO classification (2017) [24]; 2) measurement of TSH and thyroid hormones levels was done prior to surgery; 3) patients' age was 21 years or older at the time of hospital admission. Exclusion criteria were the following: 1) patients who were diagnosed with any other malignancy prior to thyroidectomy; 2) patients who received hormonal replacement therapy prior to diagnostics and operation; 3) Graves' disease; 4) pregnancy. A total of 576 eligible cases of PTC were identified. The comparison group comprised 94 patients with follicular adenomas (FA) verified by histopathological examination. Enrolled patients with FA met the same inclusion criteria for PTC patients.

Information on demographic variables, clinical and laboratory data as well as tumor histology were collected. The following characteristics were considered during grossing and histopathological examination: unifocal/multifocal growth, coexisting Hashimoto's thyroiditis (HT), extrathyroid extention, intrathyroid invasion (lymphovascular or in surrounding tissues), lymph nodes metastasis (LNM). Histological type of PTC was defined according to the WHO classification (2017) [4,24]. Clinicopathological staging was performed in line with the American Joint Committee on Cancer TNM staging system (8th Edition; 2017) [1]. Diagnosis of HT was based on clinical and sonographic data, confirmed by serum autoantibodies to thyroglobulin and TPO and by histological evaluation of slides. The following criteria for HT diagnostics were used: diffuse lymphocyte infiltration, lymphoid follicles with germinal centers and Hürthle cell changes [25]. To measure the serum concentrations of TSH and thyroid hormones Roche Cobas e411 automatic analyzer, the manufacturer's reagents and calibrators were used. The normal ranges for serum concentrations of TSH, fT3 and fT4 were considered as 0.27–4.0 μIU/ml, 1.8–4.2 pg/ml and 0.8–2.3 ng/dl respectively.

The distributions of demographic characteristics between groups were compared by chi-square tests. To assess the association between TSH, thyroid hormones and PTC characteristics the odds ratio (OR) with 95% confidence interval (95% CI) were calculated. We divided all patients into subgroups according to serum concentrations of TSH by quartile within 90% confidence intervals of the reference limits in non-Gaussian distribution (5th percentile: 0.27 μIU/ml, 25th percentile: 0.95 μIU/ml, 50th percentile: 1.71 μIU/ml, 75th percentile: 2.47 μIU/ml, and 95th percentile: 3.56 μIU/ml). According to this there were 6 subgroups of patients with different levels of TSH: subgroup 1 – with TSH below the normal range (<027 μIU/ml), subgroup 2 – with normal low TSH (0.27–0.95 μIU/ml), subgroup 3 and subgroup 4 – having serum TSH in medium 2nd (0.95–1.71 μIU/ml) and 3^{d} quartile (1.71–2.47 μIU/ml), subgroup 5 – with high normal TSH (2.47–3.56 μIU/ml), and subgroup 6 with TSH levels above the normal range (>3.56 μIU/ml). Comparison between continuous variables was performed by *t*-test for unpaired data or ANOVA. Categorical variables were handled with the χ^2-test. To assess the association between TSH, thyroid hormones and PTC characteristics the odds ratio (OR) with 95% confidence interval (95% CI) were calculated. Data assessment was conducted using statistical software (MedCalc Software Inc, Belgium).

3. Results

3.1. Hormones levels in PTC patients of different sex and age

There were 468 females (81.3%) and 108 males (18.7%) among observed patients with PTC aged 47.5 ± 0.65 (95%CI 46.2–48.7) and 45.05 ± 1.39 (95%CI 42.3–47.8) years old. respectively. The group of patients with FA included 76 females (80.9%) and 18 males (19.1%) aged 45.2 ± 2.24 (40.7–49.7) and 46.3 ± 5.05 (34.7–57.9) years old respectively.

The distribution of TSH variables in patients with PTC demonstrated that more than 80% of observed patients had TSH level under 2.5 μIU/ml (Table 2). Serum fT3 and fT4 levels comprised respectively 3.82 ± 0.14 pg/mL and 1.26 ± 0.05 ng/dL among patients with PTC, that was comparable with thyroid hormones levels in FA (Table 1). However, serum TSH concentration was significantly higher in PTC comparing with FA (1.68±0.051 vs 1.28±0.14 μIU/ml respectively; P = 0.022). This difference was related to higher rate of coexisting HT in patients with PTC (38.6% vs 17.5% in PTC and FA respectively). The comparison of hormones levels in patients with FA and PTC with no HT did not find any significant differences in TSH as well as in fT3 and fT4 (Table 1).

Assessment of hormones levels in PTC patient of different sex did not revealed significant distinctions in TSH, fT3 and fT4 in men comparing to women (Table 1). Although there were no significant differences in ranges of TSH when compared to males and females, it was found that high-normal and high levels of TSH were more frequent among women rather than men (P = 0.03). Only 37 patients (6.14%) among observed cohort had high levels of TSH, majority of them (29 of 37; 78.38%) had coexisting Hashimoto's thyroiditis and about 93% (27 of 29) of these patients were females.

In addition, we found the significant differences in fT3 levels in PTC patients of different age groups. It was shown that PTC patients older than 55 years of age had lower levels of fT3 as compared to patients under 55 years old (3.47 ± 0.14 vs 4.07 ± 0.26 pg/ml respectively; P = 0.042), that could reflect age-related decline of thyroid functioning.

3.2. Relationship between hormones levels and histopathological features of PTC

Hormones levels were similar in patients with papillary microcarcinoma and PTC sized more than 10 mm among both men and women. However, comparison of tumor size in patients of different subgroups divided according to TSH levels percentile, demonstrated weak negative correlation (r=-0.182; p = 0.02): the lower levels of TSH were associated with larger size of tumors though these differences were not statistically significant due to diversity of variables. Nevertheless, we did not find significant size-related differences of serum TSH, fT3 and fT4 levels in patients with PTC. In contrast, patients of different sex demonstrated differences in PTC size (1.57±0.06 vs 1.89±0.13 cm in women and men respectively, P = 0.02) and rate of metastasis (17% vs 33% in females and males respectively; P = 0.0029).

Assessment of TSH levels in different histological types of PTC revealed that tall cell variant of PTC was associated with higher levels of TSH (2.27 ± 0.32 vs 1.65 ± 0.07 μIU/ml in patients with tall cell and conventional types of PTC; P = 0.008). In contrast, follicular type of PTC was associated with normal TSH with relatively higher levels of fT3 and fT4, however these differences were not statistically significant (Table 1).

Table 1
TSH, fT3 and fT4 levels in patients with PTC.

Parameters	TSH (μIU/ml)	fT3 (pg/mL)	fT4 ng/dl)
Type of tumors			
FA	1,28±0.14	4.09±0.15	1.09±0.06
PTC	1.68±0.051 P_{FA}=0.022	3.82±0.14 P=0.432	1.26±0.05 P=0.168
Sex			
Females	1.79±0.06	3.69±0.16	1.29±0.08
Males	1.56±0.09 P=0.076	4.10±0.37 P=0.269	1.31±0.05 P=0.902
Age			
Age <55	1.74±0.07	4.07±0.26	1.26±0.02
Age ≥55	1.75±0.07 P=0.904	3.47±0.14 P=0.042	1.33±0.13 P=0.599
Size			
≤1 cm	1.83±0.08	3.84±0.30	1.25±0.04
>1 cm	1.67±0.07 P=0.137	3.69±0.13 P=0.578	1.33±0.11 P=0.576
Histological variant*			
Microcarcinoma	1.77±0.08 P=0.264	3.91±0.37 P=0.796	1.24±0.12 P=0.953
Conventional	**1.65±0.07**	**3.79±0.29**	**1.23±0.13**
Tall cell	2.27±0.32 P=0.008	2.93±0.17 P=0.302	1.19±0.12 P=0.919
Follicular	1.57±0.12 P=0.538	3.46±0.19 P=0.426	1.58±0.37 P=0.171
Diffuse sclerosing	1.91±0.32	2.96±0.12	0.99±0.12
	P = 0.386	P = 0.278	P = 0.637
Encapsulated PTC	1.88±0.36 P = 0.306	3.56±0.13 P = 0.735	1.98±1.18 P = 0.167
Oncocytic	1.85±0.25 P = 0.498	4.23±0.18 P = 0.708	1.20±0.24 P = 0.947
Extrathyroid extension			
Present	1.63±0.08	3.94±0.28	1.26±0.03
Absent	1.79±0.06 P = 0.204	3.69±0.17 P = 0.490	1.31±0.09 P = 0.781
LN metastasis			
Present	1.69±0.09	3.79±0.27	1.52±0.28
Absent	1.76±0.06 P = 0.132	3.75±0.17 P = 0.923	1.23±0.02 P = 0.043
Concurred autoimmune thyroiditis			
Present	2.24±0.10	3.51±0.19	1.18±0.02
Absent	1.43±0.05 P < 0.001	3.84±0.18 P = 0.351	1.35±0.09 P = 0.318
Character of tumor growth			
Unifocal	1.67±0.08	3.90±0.32	1.25±0.05
Multifocal	1.92±0.1 P = 0.05	3.84±0.21 P = 0.881	1.49±0.22 P = 0.206
BRAF mutation status			
Positive	1.68±0.07	3.72±0.18	1.21±0.02
Negative	1.59±0.28 P = 0.643	4.03±0.30 P = 0.400	1.20±0.09 P = 0.986

Statistically significant differences are highlited in the table.
*all the data were compared with conventional variant of PTC.
P_{FA} – shows differences between PTC and FA.

The frequency of LNM was tightly associated with histological type of PTC (P < 0.0001). Among enrolled cases there were 114 patients (19.8%) with LNM. The highest rate of LNM were found in patients with diffuse-sclerotic and tall cell variant of PTC (53%). The conventional type of PTC comprised 39% LNM, follicular variant – about 11%, and the lowest incidence of LNM was found among patients with papillary microcarcimona (8%).

Assessing the relationship between PTC progression and hormones levels, we found that the LNM incidence was higher among patients of subgroup 1 with low TSH level (Table 2) whereas frequency of metastasis among patients with high normal and high TSH levels was the same to the rest of the patients. High TSH levels did not affect TNM stage of PTC (OR = 1.229; 95%CI 0.79–1.89; P = 0.35). In contrast, low TSH level was associated with higher stage (Table 2). In addition, the risk of LNM was closely related to sex and was much higher in men (P = 0.0014).

3.3. *Relationship between* BRAF^{V600E} *mutation, PTC characteristics and hormones levels*

The rate of *BRAF*^{V600E} mutation among observed patients was 43.2% and did not differ significantly in males and females (P = 0.985; Fig. 1a) and among patients of different age groups (P = 0.609, Fig. 1b). While assessing the association of *BRAF*-mutation with PTC characteristics we did not find any relations between *BRAF*^{V600E} mutation detection and tumor size in patietns of both sexes (Fig. 1c). In addition, there was no association between *BRAF*^{V600E} status and levels of TSH, fT3, fT4. As expected, there we did not detected BRAF mutation in patients with non-invasive incapsulated follicular variant of PTC. *BRAF*^{V600E} mutation was mostly found in the conventional type and tall cell variant of PTC (P = 0.007). Although the frequency of ETE was higher among PTC patients with *BRAF*^{V600E} mutation (18% comparing to 14% in PTC without mutation), these differences were not statistically significant (P = 0.155). The same relations were found between mutation status and lymph node metastasis incidence (23% vs 17% in patients with and without *BRAF*^{V600E} mutation; P = 0.084; Fig. 2). As far as presence of *BRAF*^{V600E} mutation was correlated with intratumor infiltration by lymphocytes (P < 0.001) we assessed whether coexisting HT is associated with *BRAF*^{V600E} mutation appearance. Surprisingly the rate of *BRAF*^{V600E} mutation detection in PTC with coexisting HT was slightly lower than in patients with PTC alone (40% vs 45% respectively; P = 0.326). Although this finding does not have statistical significance, this could mean that HT and BRAF mutation could be independent risk factors of PTC development.

3.4. *The impact of coexisting HT on hormones levels and PTC features*

HT was more frequent among women with PTC (41.6% vs 21% - in females and males, respectively; P = 0.003). Naturally, coexisting HT significantly affected TSH levels in patients with PTC (Table 2). TSH levels were higher in PTC with HT than in PTC alone (P < 0.001) though we did not find statistically important relationship between HT and fT3/fT4 levels in patients with PTC. Interestingly, coexisting HT was found significantly more often among patients with PTC (38.6%) comparing with FA (17.5%). In addition, PTC coexisting with HT was more likely to be bilateral and multifocal.

Surprisingly, presence of HT significantly decreased the risk of ETE (OR = 0.67; 95% CI 0.44–1.00; P = 0.05) and metastasis into LN (OR = 0.59; 95% CI 0.37–0.94; P = 0.028) among males with PTC. However, such effect was not found in females. We did not find significant relationship between coexisting HT and rate of both LNM (OR = 0.81 (95% CI 0.48–1.34); P = 0.407) and ETE (OR = 2.0 (95% CI 0.69–5.72; P = 0.19). This could be related to some gender-specific features of PTC progression and invasiveness and had to be clarified

Table 2
Characteristics of thyroid function and tumor's features in patients with PTC according to different TSH levels.

Parameters	Subgroup 1 TSH <0.27 μIU/ml	Subgroup 2 TSH 0.27-0.95 μIU/ml	Subgroup 3 TSH 0.95-1.71 μIU/ml	Subgroup 4 TSH 1.71-2.47 μIU/ml	Subgroup 5 TSH 2.47-3.56 μIU/ml	Subgroup 6 TSH >3.56 μIU/ml
Number of patients						
FA	0	28	36	22	6	2
		29.79%	38.30%	23.40%	6.38%	2.13%
PTC	6	139	196	126	72	37
	1.04%	24.13%	34.03%	21.88%	12.50%	6.42%
Thyroid hormones levels in patients with PTC						
fT3 (pg/ml)	4.01 ± 0.82	3.52 ± 0.10	3.68 ± 0.19	3.76 ± 0.38	3.78 ± 0.14	3.09 ± 0.23
fT4 (ng/dl)	1.79 ± 0.25	1.22 ± 0.03	1.40 ± 0.18	1.17 ± 0.03	1.19 ± 0.05	1.06 ± 0.07
PTC characteristics						
Size	1.7 ± 0.42	1.65 ± 0.12	1.65 ± 0.09	1.69 ± 0.12	1.51 ± 0.15	1.29 ± 0.16
LNM n = 114 (19.8%)	2	19	46	25	16	6
	1.7%	16.7%	40.4%	21.9%	14.0%	5.3%
HT	**0**	**0**	**0**	**57**	**38**	**29**
n = 124				**46.0%**	**30.6%**	**23.4%**
(2,15%)	**P < 0.0001**					
TNM stage						
Stage I n=474	3	113	162	103	62	31
	50%	81.2%	82.66%	81.7%	86.1%	83.8%
Stage II n=94	3	24	32	20	9	6
	50%	17.4%	16.32%	15.9%	12.5%	16.2
Stage III n=8	0	2	2	3	1	0
		1.4%	1.02%	2.4%	1.4%	

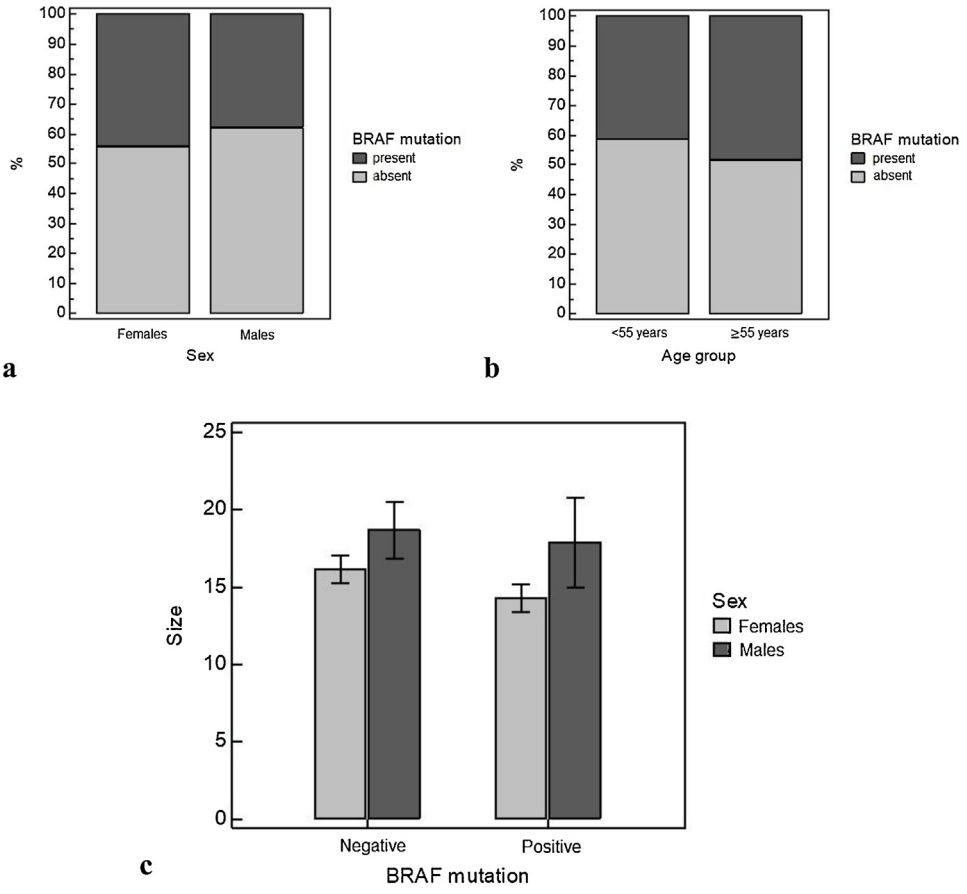

Fig. 1. Lack of relationship between BRAF mutation status and PTC patients age, sex, and tumor size.
There were no significant differences in BRAF mutation frequency among patients of different sex (Fig. 1a) and age groups (Fig. 1b). In addition, tumor size there was no relationship between BRAF mutation status and tumor size with regard to sex (Fig. 1c).

in future investigations. HT was not related to tumor size however it was associated with the increased rate of bilaterality and multifocality of PTC (OR = 1.65; 95% CI 1.15–2.34; P = 0.005) regardless of sex.

The comparison of fT3 and fT4 levels in patients with TSH of different ranges showed the inverse relationship between these hormones levels (r1=–0.370; r2=–0.501; p < 0.001). Patients with low TSH demonstrated higher levels of fT3 and fT4, and, in contrast,

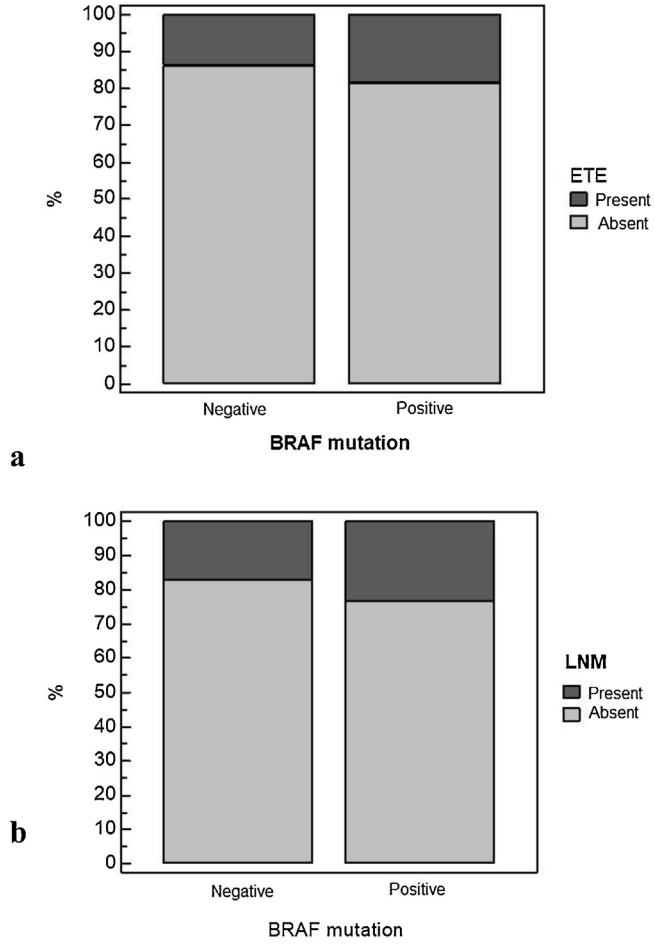

Fig. 2. Relationship between BRAF mutation and PTC progression.
ETE (Fig. 1a) and LNM rate (Fig. 1b) were higher in patients with detected BRAF mutation, however these differences were not statistically significant.

the lowest levels of thyroid hormones were found in patients with TSH above the normal range.

4. Discussion

In this study we did not find association between TSH, fT3 and fT4 levels and such PTC characteristics as size, intrathyroid invasiveness, extrathyroid expansion and lymph node metastasis. In fact, there were numerous studies discovering and discussing the role and informativeness of serum TSH concentration measurement in interpretation of the risk of PTC and its prognosis. For instance, several meta-analyses showed that higher TSH levels were associated with an increased thyroid cancer risk [13,29]. However, most of the studies selected for analysis were cross-sectional studies and measured TSH levels after treatment of thyroid cancer began and could be incorrect due to effects of treatment [14]. As we selected only the patients who were observed prior to therapy, this allowed us to exclude the impact of replacement therapy on TSH levels. As it was shown in this study, most patients with PTC had normal or low normal TSH levels. One more important issue to be mentioned is controls that were used for studies. Most of the studies control groups comprised patients with thyroid nodules or patients undergoing surgical treatment for a suspicious thyroid tumor [18,29]. In fact, some kinds of autonomous thyroid nodules were associated with alteration of thyroid hormones production that could lead to secondary changes of TSH production [14,35].

TSH level significantly differed in patients with benign tumors and PTC. Partly these differences were related to higher rate of coexisting HT among patients with PTC. There were no significant differences in TSH levels when compared between patients with FA and PTC with no HT. In fact, most of the patients with PTC demonstrated normal and low normal levels of PTC. And only about 6,42% of patients with PTC had TSH levels higher than the normal range. It is important to underline that most of these patients had coexisting HT that could lead to serum TSH elevation.

The concept postulating the role of TSH in PTC development is based on TSH impact in stimulation of follicular cells proliferation [22]. As it is widely accepted, TSH plays an important role in regulating thyroid function including stimulation of proliferation of follicular cells, their size and secretory activity. The main mechanisms of TSH actions are mainly mediated through TSHR that is associated with Gαs-protein activating adenylyl cyclase-cyclic adenosine monophosphate (cAMP) - protein kinase A-pathway [37]. This signaling pathway plays the crucial role in follicular cells differentiation and functional activities. There are some researches postulating that somatic TSHR mutations in thyroid epithelial cell can also activate the cAMP pathway, which facilitates the cell growth and clonal expansion, leading to the formation of an autonomously functioning thyroid adenoma [16,22]. Although activated cAMP pathway results in enhanced growth, it is not sufficient for malignant transformation of normal thyrocytes. It was shown that TSHR-mutations are associated with several diseases like familial gestational hyperthyroidism, autonomous toxic adenomas, hereditary or sporadic toxic thyroid hyperplasia, familial non-autoimmune hyperthyroidism, Graves' disease and autoimmune hypothyroidism but rarely occur in thyroid cancer [37].

In our study high TSH level was associated with HT. Coexistence of HT and PTC predominated among women. Although there are some debates about the role of HT in PTC development there is strong evidence demonstrating the role of autoimmune inflammation in papillary microcarcimona and PTC [32,38,37]. Inflammation causes oxidative cells damage and reactive oxygen species formation that may cause DNA damage, resulting in mutations that eventually can lead to malignant cell transformation and PTC development [15,25,33]. Dailey et al. was the first who proposed an association between HT and PTC many decades ago [31]. In fact, the incidence of PTC development in patients with such autoimmune thyroid lesions as HT and Grave's disease is 3–5 times higher than in patients without inflammatory lesions of thyroid gland [3,15,19,25].

Although the results of this study support the concept of relationship between HT and PTC, we did not find the evidence of HT role in PTC progression as the rate of metastasis in patients with combined PTC and HT lesions did not differ from patients without HT. In addition, some authors postulate that lymphocytic infiltrate of HT may be an immunological response with a cancer-retarding effect, contributing to a favorable outcome of PTC [32]. This concept could explain the negative correlation between PTC coexisting with HT and metastasis rate in females. Actually, the reciprocal association between HT and such features as extrathyroidal extension and lymph node metastasis was shown previously [33]. This allows to postulate a potentially protective role of HT in PTC. In addition, authors demonstrated the negative relationship between Hashimoto's thyroiditis and $BRAF^{V600E}$ mutation [9,20]. In our study we did not confirm the relation between HT and $BRAF^{V600E}$ mutation. In addition, we found a kind of protective effect of HT on PTC progression in males only that stimulates studying gender-related mechanisms of PTC development of progression.

In addition, we did not find any significant relationship between $BRAF^{V600E}$ mutation and thyroid functioning. This fact conflicts with data of Franco AT et al. [8], who showed that mice with induced $BRAF^{V600E}$ mutation become profoundly hypothyroid due

to down-regulation of the sodium-iodide-symporter and TSH-receptor (TSHR) expression [27]. Deregulation of genes involved in thyroid hormones synthesis was accompanied with further increase of TSH production. In our study, all the patients with *BRAF*^{V600E} mutation in PTC had a serum TSH, fT3 and fT4 within the normal ranges. Despite these controversies, the study of Franco underlined the secondary nature of TSH changes. This could mean that low levels of thyroid hormones (due to age-related or iodine deficiency medicated dysfunction of the thyroid gland) in patients with PTC could be confounding factors causing the induction of pituitary gland to overproduce TSH by negative feedback mechanisms. In part this concept is supported by the negative correlation between TSH and thyroid hormones, found in this and other studies [9,39].

4.1. Conclusion

The present study showed the lack of significant relationship between TSH levels and PTC aggressiveness (size, invasiveness, metastasis, TNM stage, *BRAF*^{V600E} mutation). Levels of TSH, fT3 and fT4 were within the reference range in most patients with PTC. Higher TSH levels were found in patients with coexisting HT that was associated with female sex and multifocality of PTC. Thus, TSH and thyroid hormones levels are not informative in PTC metastasis prediction.

4.2. Limitation of the study

Authors had no information on such important confounding factors, as history of benign thyroid disease and family history of thyroid cancer and ionizing radiation exposure.

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