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Comparison of ultrasound findings and fine needle aspiration results between anti-thyroid peroxidase positive and negative patients with nodular goiters

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Abstract

Introduction: Ultrasonography is an optimal approach for thyroid gland evaluation, nodule detection and cancer risk assessment in patients with thyroid nodules. Almost all patients with Hashimoto's thyroiditis have high levels anti-thyroid peroxidase (anti-TPO) antibodies.
Objectives: This study aimed to evaluate the relationship between ultrasonography and fine needle aspiration (FNA) results in anti-TPO antibodies positive and negative patients with nodular goiters.
Patients and Methods: This cross-sectional study included 128 patients with nodular goiters, referred to endocrinology clinic of imam Reza hospital, Tabriz, Iran. Anti-TPO levels above and below 16 IU/mL were considered as positive and negative, respectively. All patients underwent thyroid ultrasonography, and eligible nodules were subjected to FNA.
Results: Of 128 patients, 33.6% and 66.4% were anti-TPO positive and negative, respectively. FNA was conducted on 196 nodules. A significant relationship was observed between sonographic and FNA results in low and intermediate-suspicion nodules. Chronic lymphocytic thyroiditis (CLT) was more frequently reported in low-suspicion nodules of the anti-TPO positive group ($P \leq 0.0001$). In addition, in intermediate-suspicion nodules, CLT was reported in 33.3% of patients in the anti-TPO positive group ($P = 0.026$). No significant difference was observed between other nodules.
Conclusion: Based on our findings, when a nodule is classified in low or intermediate-suspicion categories, the possibility of CLT following FNA is significantly higher in the anti-TPO positive group, compared to the anti-TPO negative group.

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Introduction

Thyroid gland is one of the largest endocrine organs, with an approximate weight of 15-20 g and has a great growth potential. Thyroid gland enlargement can represent a nodular or diffuse goiter (1). Single or multiple thyroid nodules are common problems, detected in 3%-7% of adults during physical examinations and 50% of individuals in sonographic evaluations (2). Evidence shows that the prevalence of multinodular goiters, which increases with age, is higher in women than in men (1). The majority of thyroid nodules are benign and malignancies occur in 5%-15% of these patients (2,3). Therefore, to select eligible patients for surgery, it is crucial to divide nodules into benign and malignant types using appropriate methods.

Thyroid sonography, which was first conducted in 1966, has been frequently used for thyroid gland assessment in recent years. This modality is also suitable for the evaluation of thyroid parenchyma and nodule quality (2,4-7). Besides sonography, fine needle aspiration (FNA) has been applied for thyroid nodule and cancer risk evaluations. FNA is a precise, safe and valuable method for thyroid nodule evaluation, which can identify eligible patients with malignancies for surgery (2). However, this method is not affordable for all nodules during the physical examination or sonography of thyroid glands and performing FNA depends on sonographic findings (8-10). Thyroid autoimmune diseases, such as Graves' disease and Hashimoto's thyroiditis, typically occur due to genetic and environmental

Key point

Almost all patients with Hashimoto's thyroiditis have high levels of anti-thyroid peroxidase (anti-TPO) antibodies. This study aimed to evaluate the relationship between ultrasonography and fine needle aspiration (FNA) results in anti-TPO positive and negative patients with nodular goiters. This cross-sectional study included 128 patients with nodular goiters. Anti-TPO levels above and below 16 IU/mL were considered as positive and negative, respectively. A significant relationship was observed between sonographic and FNA results in low and intermediate-suspicion nodules. Chronic lymphocytic thyroiditis (CLT) was more frequently reported in low-suspicion nodules of the anti-TPO positive group ($P \leq 0.0001$).

factors. The activity of thyroid tissue autoantigens in patients with specific genetic backgrounds leads to the production of antibodies, causing thyroid gland stimulation or destruction. In the majority of patients with Hashimoto's thyroiditis, high levels of anti-thyroid peroxidase (ant-TPO) antibodies have been detected (11-13).

Objectives

The aim of this study was to evaluate the correlation between sonographic and FNA results and positive/negative anti-TPO antibodies in patients with nodular goiters.

Patients and Methods

Study design

In this descriptive cross-sectional study, individuals with nodular goiters, referred to the endocrinology clinic of Imam Reza hospital, Tabriz, Iran, were enrolled from June 1 to November 29, 2019. For sample size calculation, the two-proportion equation was used at an alpha level of 0.05 and power of 80% (14). Accordingly, 128 patients with nodular goiters were selected. The inclusion criteria were age above 18 years and having a nodular goiter. Moreover, patients with a history of thyroidectomy, hot nodules, sub-acute thyroiditis and history of iodine-131 treatment and also FNA over the last three months were excluded.

Initially, patients with nodular goiters were selected, based on their sonographic features and the American Thyroid Association (ATA) guidelines for FNA (15). To conduct FNA, the patients were placed in the supine position with the neck extended. After determining the nodule location by sonography (Affiniti 50, Philips), the region of interest was disinfected with 70% alcohol, and FNA was carried out using a 25G syringe. During sampling, the patients were asked not to swallow their saliva. From each nodule, two to four aspirates were obtained, fixed on a microscope slide using 96% alcohol and then, transferred to the cytopathology laboratory.

In addition, to measure the anti-TPO antibody, 2 mL of whole blood was collected from the patients. The levels of serum thyroid-stimulating hormone (TSH) and anti-TPO antibody were assessed by Liaison TSH (105201, Italy) and anti-TPO (311701, Italy) kits, using an electrochemiluminescence (ECL) instrument. Regarding the pathology reports, the patients were categorized into benign, suspicious for papillary thyroid carcinoma (SUSP PTC), PTC (papillary thyroid carcinoma), chronic lymphocytic thyroiditis (CLT), atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) and follicular neoplasm/suspicious follicular neoplasm (FN/SUSP FN).

Statistical analysis

SPSS version 22 was used for data analysis. Chi-square and Fisher's exact tests (if necessary) were used for comparing qualitative variables, and independent sample *t* test was applied for the analysis of quantitative data. *P* value less than 0.05 was considered statistically significant.

Results

Patients' general characteristics

In the present study, 128 patients (113 females and 15 males) were enrolled. The anti-TPO positive and negative groups included 43 (40 females and 3 males) and 85 (73 females and 12 males) patients, respectively. The average age and body mass index (BMI) of the anti-TPO (+) and anti-TPO (+) groups were 45.35 ± 13.84 years and 43.79 ± 12.08 years ($P = 0.513$) and 27.12 ± 2.84 kg/m^2 and 27.44 ± 4.21 kg/m^2 ($P = 0.674$), respectively. No significant difference was observed in serum TSH level between the groups (3.83 ± 3.82 versus 2.21 ± 2.48 IU/mL; $P = 0.05$). However, there was a significant difference in the anti-TPO level (402.92 ± 392.12 versus 5.76 ± 3.91 IU/mL; $P < 0.001$; Table 1).

Thyroid gland size and heterogeneity

Table 2 shows the thyroid gland size and heterogeneity in the studied groups. The thyroid volume was calculated in mL, according to the following formula:

Thyroid volume; Length (cm) × thickness (cm) × width (cm) × 0.479

The mean volume of the right and left lobules was calculated in the anti-TPO (+) and anti-TPO (+) negative groups [11.8 ± 12.7 and 10.3 ± 8.2 mL ($P > 0.05$) and 17.4 ± 19.3 mL and 13.6 ± 31.9 mL ($P > 0.05$), respectively]. Evaluation of the heterogeneity of the thyroid gland parenchyma also revealed a higher heterogeneity in the anti-TPO (+) patients ($P < 0.001$). In anti-TPO (+) patients, 17 (39.5%) and 26 (60.5%) patients were classified as homogenous and heterogeneous, respectively. On the contrary, in the anti-TPO (-) patients, 74 (87.1%) and 11 (12.9%) patients showed homogeneity and heterogeneity, respectively. Additionally, the average of the largest nodule diameter in anti-TPO (+) and anti-TPO (-) patients was 24.06 ± 14.78 mm and 23.64 ± 11.51 mm, respectively. Nonetheless, no significant difference was detected between the two groups regarding this parameter ($P = 0.82$).

Table 1. American Thyroid Association (ATA) sonographic pattern, estimated malignancy risk and FNA guidance for thyroid nodules

Sonographic pattern	US features	Estimated malignancy risk (%)	FNA size cutoff (largest dimension)
High suspicious	Solid hypoechoic nodule or solid hypoechoic component of a partially cyclic nodule with one or more of the following features: Irregular margins (infiltrative, microlobulated), microcalcification, taller than wide shape, rim calcification with small extrusive soft tissue component, evidence of extrathyroidal extension.	>70-90[a]	Recommend FNA at ≥ 1cm
Intermediate suspicious	Hypoechoic solid nodule with smooth margins without microcalcifications, extrathyroidal extension, or taller than wide shape.	10-20	Recommend FNA at ≥1cm
Low suspicious	Isoechoic or hyperechoic solid nodule or partially cystic nodule with eccentric solid areas, without microcalcification, irregular margin or extrathyroidal extension, or taller than wide shape.	5-10	Recommend FNA at ≥1.5 cm
Very low suspicious	Spongiform or partially cystic nodules without any of the sonographic features described in low, intermediate or high suspiciousicion patterns	<3	Consider FNA at ≥2 cm Observation without FNA is also a reasonable option
Benign	Purely cystic nodules (no solid component)	<1	No biopsy[b]

US-guided FNA is recommended for cervical lymph nodes that are sonographically suspiciousicious for thyroid cancer.
[a] The estimate is derived from high volume centers, the overall risk of malignancy may be lower given the interobserver variability in sonography. [b]Aspiration of cyst may be considered for symptomatic or cosmetic drainage.

Table 2. General characteristics of the patients

Parameters	Anti-TPO (+) (n=43)	Anti-TPO (-) (n=85)	*P* value
Age (y)	45.35 ± 13.84	43.79 ± 12.08	0.513
BMI (kg/m^2)	27.12 ± 2.84	27.44 ± 4.21	0.674
TSH (IU/mL)	3.83 ± 3.82	2.21 ± 2.48	0.05
Anti-TPO antibody (IU/mL)	402.92 ± 392.12	5.76 ± 3.91	<0.001

BMI, Body mass index; TSH, Thyroid stimulating hormone.
Data are presented as mean ± standard division (SD). $P<0.05$ was considered as statistically significant.

Pathology reports based on sonographic features in nodules under FNA

Table 3 shows the cytopathological findings of nodules under FNA, based on the sonographic features. In the anti-TPO negative group, 135 nodules were assessed. Two nodules with benign sonographic features (purely cystic nodules) were only cystic fluids in the pathology report. Of 29 very-low-suspicion nodules, 25 (86.2%), 3 (10.3%) and 1 (3.4%) were benign, CLT and PTC, respectively. Based on the pathology reports, among low-suspicion nodules (n = 63), 56 (88.9%), three (4.8%), three (4.8%) and one (1.6%) nodules were benign, AUS, SUSP PTC and PTC, respectively. Furthermore, of 26 intermediate-suspicion nodules, 14 (53.8%), 4 (15.4%), 2 (7.7%), 4 (15.4%) and 2 (7.7%) were diagnosed as benign, AUS, FN, SUSP PTC and PTC, based on the pathology reports, respectively. Finally, of 15 high-suspicion nodules, 7 (46.7%), 2 (13.3%) and 6 (40%) nodules were benign, SUSP PTC and PTC, respectively.

Meanwhile, 61 nodules with no benign sonographic features (purely cystic nodules) were assessed in the anti-TPO positive group. Of 10 very-low-suspicion nodules, 8 (80%), 1 (10%) and 1 (10%) were diagnosed as benign, AUS and FN, respectively. In addition, of all low-suspicion nodules (n = 35), 22 (62.9%), 11 (31.4%), 1 (2.9%) and 1 (2.9%) were reported as benign, CLT, AUS and PTC, respectively. Moreover, of 12 intermediate-suspicion nodules, 5 (41.7%), 4 (33.3%), 1 (8.3%) and two (16.7%) were benign, CLT, AUS and PTC, respectively. Finally, of high-suspicion nodules (n = 4), one (25%), one (25%) and two (50%) were reported as benign, SUSP PTC and PTC in the pathology reports, respectively.

Table 3. Volume and heterogeneity of thyroid gland in patients under study

Parameters	Anti-TPO (+) (n=43)		Anti-TPO (-) (n=85)		*P* value
Volume (mL)	**Right lobule**	**Left lobule**	**Right lobule**	**Left lobule**	>0.05
	11.8±12.7	10.3±8.2	17.4±19.3	13.6±31.9	
Heterogeneity	**Homogenous**	**Heterogeneous**	**Homogenous**	**Heterogeneous**	<0.001
	17	26	74	11	
Nodule size (mm)*	24.06±14.78		23.64±11.51		0.82

Data are presented as mean ± SD or number. $P < 0.05$ was considered as statistically significant. * Avarage of the largest diameter of nodules.

In low-suspicion nodules, significant differences were observed between the anti-TPO (+) and anti-TPO (-) patients regarding the FNA results. CLT in these nodules has more frequently been reported in anti-TPO (+) group, in comparison with their negative counterparts. In contrast, benign nodules (nodular goiter) were more frequent in the anti-TPO (-) patients, compared to the anti-TPO (+) group ($P \leq 0.0001$). In addition, of intermediate-suspicion nodules, the prevalence of CLT was 33.3% in the anti-TPO (+) patients; however, 0% was detected in the anti-TPO (-) patients ($P = 0.026$). Overall, there was a significant relationship between the FNA results and ultrasonography findings in both groups ($P \leq 0.0001$ and $P = 0.003$ for the anti-TPO negative and positive groups, respectively; Table 4). There were also two inadequate nodules (data not shown).

Discussion

Today, ultrasonography is frequently used as a diagnostic approach for thyroid tissue evaluation in patients with nodular goiters. Given the effective role of ultrasonography in evaluating thyroid nodules, FNA is also regarded as the gold standard approach for tissue analysis. The main limitation of FNA in the evaluation of thyroid nodules is when the pathology results are reported as AUS/FLUS or FN/SFN (16). The anti-TPO level is considered as a diagnostic marker for thyroid autoimmune disorders, especially at high titers. Moreover, this marker represents certain characteristics, including the presence of hypoechogenicity and heterogeneity, increased thyroid parenchyma vascularity and the presence of pseudonodules (17). In the current study, anti-TPO levels higher and lower than 16 IU/mL were considered as anti-TPO positive and anti-TPO negative, respectively.

In this regard, Willms et al (17), investigated the correlation between sonography results and anti-TPO antibody levels in 223 patients with Hashimoto's thyroiditis. They reported a significant correlation between the increased level of anti-TPO and hypoechogenicity, heterogeneity, and pseudonodule infiltration. However, no significant correlation was observed between the anti-TPO level and nodule volume/number. Similarly, in our study, evaluation of the thyroid gland parenchyma heterogeneity showed a higher heterogeneity in the anti-TPO (+) patients, compared to the anti-TPO (-) patients.

Moreover, Tugna et al (18), showed a significantly larger thyroid size in the anti-TPO positive group with diffuse goiter. Besides, thyroid parenchyma was heterogeneous with hypoechogenicity and high vascularity. Although the present study was conducted on patients with nodular goiters, similar results were found, as the anti-TPO positive group showed a significantly higher thyroid parenchyma heterogeneity in comparison with the opposite group. However, no significant difference was found in terms of thyroid gland volume.

Chehada et al (19) studied the prevalence of Hashimoto's thyroiditis in nodular goiter and investigated the association between cytological and serological results. The prevalence of CLT was estimated at 35% based on the cytological criteria versus 31% based on the serological results; these findings indicated the high prevalence of CLT in nodular goiters. Because there is a clear correlation between CLT and the increased level of anti-TPO, this marker can be used in patients with nodular goiters. In our study, of 61 nodules in anti-TPO (+) patients and 135 nodules in the anti-TPO (-) patients, 15 (24.6%) and three (2.2%) were classified as CLT, respectively, which indicates a significant difference ($P < 0.0001$).

In a study by Chehada et al (19), CLT was reported in 31.2% and 9.7% of patients in anti-TPO (+) and anti-TPO (-) patients, respectively. Comparison of the results of FNA between the anti-TPO (+) and anti-TPO (-) patients showed a significant difference in low and intermediate-suspicion nodules. Overall, 62.9% and 88.9% of low-suspicion nodules were benign in anti-TPO (+) and anti-TPO (-) groups based on the FNA reports, respectively. Likewise, 31.4% and 0% of these nodules were reported as CLT based on the cytopathological evaluations ($P < 0.0001$). In anti-TPO (-) patients, 89% and 0% of low-suspicion nodules were benign and CLT, respectively.

Table 4. Sonographic and pathological findings in studied groups

Pathology		Sonography											
		Benign % (No.)	*P* value	CLT % (No.)	*P* value	AUS % (No.)	*P* value	FN/SUSP FN % (No.)	*P* value	SUSP PTC % (No.)	*P* value	PTC % (No.)	*P* value
Benign	Anti-TPO(+)	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA
	Anti-TPO(-)	100 (2)		0		0		0		0		0	
Very low suspicious	Anti-TPO(+)	80 (8)	NA	0	NA	10 (1)	NA	10 (1)	NA	0	NA	0	NA
	Anti-TPO(-)	86.2 (25)		10.3 (3)		0		0		0		3.4 (1)	
Low suspicious	Anti-TPO(+)	62.9 (22)	0.002**	31.4 (11)	<0.001**	2.9 (1)	NA	0	NA	0	NA	2.9 (1)	NA
	Anti-TPO(-)	88.9 (56)		0		4.8 (3)		0		4.8 (3)		1.6 (1)	
Intermediate suspicious	Anti-TPO(+)	41.7 (5)	0.485	33.3 (4)	NA	8.3 (1)	NA	0	NA	0	NA	16.7 (2)	NA
	Anti-TPO(-)	53.8 (14)		0		15.4 (4)		7.7 (2)		15.4 (4)		7.7 (2)	
High suspicious	Anti-TPO(+)	25 (1)	NA	0	NA	0	NA	0	NA	25 (1)	NA	50 (2)	NA
	Anti-TPO(-)	46.7 (7)		0		0		0		13.3(2)		40(6)	

NA, Not acceptable; SUSP PTC, Suspicious for papillary thyroid carcinoma; CLT, Chronic lymphocytic thyroiditis; FN/SUSP FN, Follicular neoplasm/suspicious follicular neoplasm.
Data are presented as number or percent. $P < 0.05$ was considered as statistically significant.

In the anti-TPO (+) patients, 62% and 31% of low-suspicion nodules were reported as benign and CLT in the cytopathological evaluations, respectively. In addition, comparison of the FNA results in intermediate-suspicion nodules indicated CLT in 33.3% of patients in anti-TPO (+) patients; nevertheless, there was no CLT report in anti-TPO (-) patients ($P=0.026$). This finding can be justified because in anti-TPO positive patients, pseudonodules may be occasionally considered as intermediate-suspicion nodules in sonography.

Bera et al (20), also reported an association between PTC in patients with nodular goiters and high anti-TPO levels. In the present study, of 61 and 135 nodules in anti-TPO (+) and anti-TPO (-) patients, 6 and 19 nodules were reported as PTC, respectively; however, the difference was not statistically significant. Moreover, in patients with an uncertain diagnosis, such as AUS/FN, SUSP PTC or PTC, no significant difference was observed between the groups. Overall, only benign and CLT cytopathology results were significantly different between the anti-TPO positive and negative groups in low and intermediate-suspicion nodules. In the remaining pathology groups, that is, AUS, FN, SUSP PTC and PTC, no significant differences were observed.

Furthermore, comparison of sonographic features showed no significant differences in the benign, very low suspicion, low suspicion and intermediate suspicion nodules between the anti-TPO (+) and anti-TPO (-) patients. According to the ATA guidelines, the malignancy risk in very low suspicion, low suspicion, intermediate suspicion and high suspicion nodules is <3%, 5-10%, 10-20% and 70-90%, respectively (15). In the present study, the malignancy risk in these sonographic groups was 0% and 3.4%; 2.9% and 6.4%; 16.7% and 23.1%; and 75% and 53.3%, respectively in anti-TPO (+) and anti-TPO (-) groups, respectively.

The strength of the present study was the simultaneous comparison of sonographic and FNA results regarding the anti-TPO status in patients with nodular goiters. However, the limited number of nodules was the main limitation of this study, as it was not possible to directly compare the FNA and ultrasonographic results of nodules with other types of nodules.

Conclusion

Regardless of the strong correlation between thyroid parenchymal ultrasonographic findings and anti-TPO status, no significant relationship was observed between the nodules of anti-TPO (+) and anti-TPO (-) patients. Additionally, no significant differences were observed regarding the number, size, and characteristics of nodules between two groups; however, in anti-TPO (+) patients, when the nodule was categorized as low or intermediate suspicion, the possibility of CLT after FNA was higher, compared to the anti-TPO (-) patients.

Limitations of the study

Limitations of the study our sample size was relatively low as we excluded some patients because of missing hospital records.

Authors' contribution

Conceptualization: Jalil Houshyar, Mahsa Malekian.
Data curation: Mahsa Malekian.
Formal analysis: Mahsa Malekian.
Investigation: Jalil Houshyar.
Methodology: Nasser Aghamohammadzadeh.
Project administration: Jalil Houshyar.
Resources: Mahsa Malekian.
Supervision: Farshid Bozorgi.
Validation: Jalil Houshyar, Mahsa Malekian.
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Writing–review & editing: Reza Javad-Rashid, Javad Jalili, Monireh Halimi, Farshid Bozorgi, Jalil Houshyar.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (# IR.TBZMED.REC.1397.331). Written informed consent was taken from all participants before any intervention. This work was conducted as part of the sub-specialty (endocrinology fellowship) dissertation for Mahsa Malekian. Besides, ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission and redundancy) were completely observed by the authors.

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