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Association of Anti-Thyroglobulin and Anti-Thyroid Peroxidase Antibodies in Patients with Primary Hypothyroidism

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ABSTRACT

Background: Hypothyroidism is one of the most important thyroid disorders, and chronic autoimmune (Hashimoto's) thyroiditis is the most common cause in iodine-replete areas. Anti-thyroglobulin antibodies (anti-Tg Ab) and anti-thyroid peroxidase antibodies (anti-TPO Ab) are commonly associated with the development of chronic autoimmune thyroiditis. However, the precise relationship between them has not been defined yet.
Objectives: To study the relationship between anti-Tg Ab and anti-TPO Ab among patients with primary hypothyroidism.
Materials and methods: In a cross-sectional study of 169 patients with primary hypothyroidism, their anti-TPO Ab and anti-Tg Ab were measured, analyzed, and correlated together.
Results: Positive anti-TPO Ab and anti-Tg Ab were observed in 52.1% of the patients, while 26% and 8.9% of them were only positive for anti-TPO Ab or anti-Tg Ab, respectively, and the remaining 13% showed no positivity for both of them. Anti-Tg Ab titer increased steadily with advancing age in contrast to anti-TPO Ab (P-value = 0.009). The best and highest positive predictive value for having a positive anti-TPO Ab was 91%, and this was obtained at an anti-Tg titer of > 691 IU/mL with a sensitivity of 31% and specificity of 89% at an odds ratio of 3.72 (P-value = 0.014). The best negative predictive value was seen at an anti-Tg Ab titer < 11 IU/mL with a sensitivity of 99% and a specificity of 24% (P-value < 0.001), while a maximum Youden's value of 1.4 (i.e., maximum sensitivity at a maximum specificity) was obtained at a titer of 26 IU/mL with a positive predictive value of 86% and a negative predictive value of 59% with a sensitivity of 90% and a specificity of 51% (P-value < 0.001).
Conclusion: Both anti-Tg Ab and anti-TPO Ab had considerable information in patients with chronic autoimmune hypothyroidism. Anti-Tg Ab tends to be more informative in older people (> 50 years), where it is more prevalent than anti-TPO Ab. At an anti-Tg Ab titer > 691 IU/mL, we most likely will have positive anti-TPO Ab, while at an anti-Tg Ab titer < 11 IU/ml, we most likely have negative anti-TPO Ab.

Keywords: Hypothyroidism; Anti-Thyroglobulin antibodies; Anti-Thyroid peroxidase antibodies.

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INTRODUCTION

Hypothyroidism is a common condition that affects around 5% of the general population, and approximately 5% are undiagnosed [1]. There is a geographical variation in its occurrence; for example, the prevalence was 0.3-3.7% in the United States [2] and 0.2-5.3% in Europe [3]. A recent survey study from Iraq of 24568

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subjects reported that 4.48% of the studied population had hypothyroidism [4].

The causes of hypothyroidism are broad and can be classified into primary due to thyroid hormone deficiency, secondary due to thyroid stimulating hormone (TSH) deficiency, tertiary due to thyrotropin-releasing hormone deficiency, and finally peripheral hypothyroidism (extra-thyroidal) [5].

Primary hypothyroidism is diagnosed when TSH values are above the reference range (0.5 to 5.0 mIU/L) together with free thyroxine levels below the reference range of 0.7 to 1.9 ng/dL and this is dependent on the population being studied and assay method [6].

Autoimmune thyroid diseases (AITD) are the most common organ-specific autoimmune disorders of the body, with the resultant thyroid gland dysfunction (hypo- or hyperthyroidism) [7]. These thyroid antibodies are associated with a spectrum of disorders ranging from hypothyroidism (Hashimotos Thyroiditis) to hyperthyroidism (Graves Disease).

Historically speaking, the first mention of thyroid antibodies dates back to the year 1956, and since that time different types of thyroid antibodies have been recognized. Three sets of them have the most important clinical significance, and these are [8]: First, the Anti-Thyroid Stimulating Hormone Receptor antibodies (Anti-TSHR), which are classically the hallmark of Graves' disease and present in 90% of them, are pathogenic, and still, the usual assay methods are less sensitive in detecting a small amount of these antibodies [9].

Second; The Anti-Thyroid Peroxidase Antibodies (Anti-TPO Ab) formerly known as anti-microsomal antibodies, are very sensitive markers of autoimmune thyroid disease, and despite being traditionally associated with Hashimoto's thyroiditis, they can still be present in 5% of patients with Graves' disease. They are considered markers of thyroid dysfunction and can be indicative of future thyroid failure in patients with subclinical disease [10].

Third: The Anti-Thyroglobulin Antibodies (Anti-Tg Ab), represent early and dominant antigens in the course of AITD, however, their diagnostic value is not as useful as the Anti-TPO Ab [11].

Finally, other antibodies are less well studied and of no clinical or pathological significance, like Anti Na+/I- symporter, among others [12].

Anti-TPO Ab is traditionally associated with the presence of autoimmune hypothyroidism (Hashimoto Thyroiditis) rather than anti-Tg Ab [12]. A recent study showed that positive anti-TPO Ab and not anti-Tg Ab appeared before the onset of hypothyroidism in 73% of patients [13]. Anti-Tg Ab titers are usually declined on long-term therapy with levothyroxine and would rise again once TSH becomes elevated for prolonged periods, in contrast to anti-TPO Ab titers which are usually declined on long-term follow-up as the thyroid gland becomes more atrophied. Therefore, rising anti-Tg Ab titers, may reflect poor control of hypothyroidism in the long term [14].

Both anti-Tg Ab and anti-TPO Ab are commonly related to the development of chronic autoimmune thyroiditis. However, the precise association between them is not yet defined. Hence, the aim of conducting this study was to assess the relationship between anti-Tg Ab and anti-TPO Ab among patients with primary hypothyroidism in Basrah, Iraq.

MATERIALS AND METHODS

Study Design

The study was a cross-sectional study conducted at the Faiha Diabetes Endocrine and Metabolism Center (FDEMC), Basrah City, Iraq. The current study covered the period from January 2, 2022 to the 30th of September of the same year. The study included 169 patients with a diagnosis of primary hypothyroidism. This study was approved by the Ethical Approval Committee of the College of Medicine, University of Basrah. Informed consent was obtained from every participant.

Inclusion and exclusion criteria

The candidates involved in the study were regular attendants to FDEMC who have thyroid disease, namely primary hypothyroidism, seeking review of their thyroid status and further management and follow-up, or newly diagnosed patients with primary hypothyroidism. Subjects with central hypothyroidism (including patients with a known history of pituitary tumors, those with Sheehan's syndrome, post-head injury hypopituitarism, and all other patients with primary disease affecting the hypothalamus and pituitary gland), euthyroid sick syndrome, congenital hypothyroidism, transient hypothyroidism secondary to thyroiditis, hypothyroidism due to surgery involving the thyroid, whether total, sub-total, or partial thyroidectomy, patients on medicines and supplements that are known to interfere with thyroid assays (e.g. biotin), and those who declined to participate, were excluded from the study.

Clinical assessment

A detailed history and thorough physical examination were carried out for every participant to assess the physical status of the patients. Besides, the study excludes cases with a past medical history that is specifically directed towards the natural history of hypothyroidism, including conditions that cause hypothyroidism, detailed drug histories involving drugs that cause hypothyroidism, as well as other substances that interfere with the assay method that we should use for this study, and histories of thyroid surgery.

Radiological assessment

An ultrasound of the thyroid gland was conducted by the treating endocrinologist for selected patients when the diagnosis of primary hypothyroidism was still in doubt, looking for features of autoimmunity on thyroid ultrasound like pseudo nodularity, heterogeneous texture, increased vascularity, giraffe skin appearance, or white-knight lesions.

Biochemical assays

The eligible patients for the study were submitted to the FDEMC laboratory, where 10 ml of whole blood was taken from a peripheral vein into a gel tube labeled by patient name and number, which was kept on a tube rack for two hours to clot, then centrifuged at 3000xg for ten minutes and the serum was transferred the same day for measurement of anti-Tg and anti-TPO antibodies.

The equipments used in this study are:

1. Centrifuge: Nuve/Turkey
2. COBAS® e411 analyzer: Roche Diagnostics GmbH, Mannheim, Germany (a fully automated analyzer that uses

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a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis).

Two diagnostic kits used in the study are:

1. Elecsys Anti-TPO Ab 06368590 190 for cobas® e411 analyzer.
2. Elecsys Anti-Tg Ab 06368697 190 for cobas® e411 analyzer.

The diagnostic range of the Abs is:

1. Anti TPO Ab: 5-600 IU/mL, positive result > 34 IU/mL.
2. Anti Tg Ab: 10-4000 IU/mL, positive result > 114 IU/mL.

Serum anti-Tg Ab and anti-TPO Ab were measured according to the kit procedure. The Anti-TPO competitive principle is shown in Figure 1.

Statistical analysis

The data obtained from this study were entered and analyzed using SPSS (Statistical Package for Social Sciences) version 23.0 for Windows 10 (SPSS Inc., Chicago, IL, USA). The continuous variables were presented as mean value and standard deviation, while the categorical variables were presented in tables or figures as percentages and frequencies. The Chi-Square (X^2) test is used to compare the categorical variables. Independent Student *t*-test, ANOVA T-test, and Mann-Whitney test were used as appropriate. Univariate analysis of the general linear model was used to obtain odds ratio (OR) and 95% confidence intervals (CI) to show the interactions between different cofounders. We consider the two-tailed probability values with (P-value < 0.05) to be statistically significant. Receiver-operating characteristic (ROC) curve analysis was used to examine the value of anti-Tg Ab and also to compare the sensitivity and specificity according to the anti-TPO Ab value, correlation was done by Spearman rank. Positive predictive values (PPV) and negative predictive values (NPV) for the target markers were assessed by first applying a marker-specific cutoff value and consecutively calculating the corresponding values from a 2×2 table in the usual way that these quantities are defined. A P-value < 0.05 was considered a statistically significant difference.

RESULTS

The total number of patients recruited for this study was 169. The mean age was 44.3 ± 1.0 years (range 15–72 years). The majority of the cases were female (n = 141, 83.4%), with a male-to-female ratio of 1/5.04. The anti-TPO Ab (mean = 284.8 ± 17.8 IU/mL) was positive in 132 patients (78.1%), while, the anti-Tg Ab was positive (845.2 ± 103.3 IU/mL) in 103 (60.9%) of them, as shown in Table 1.

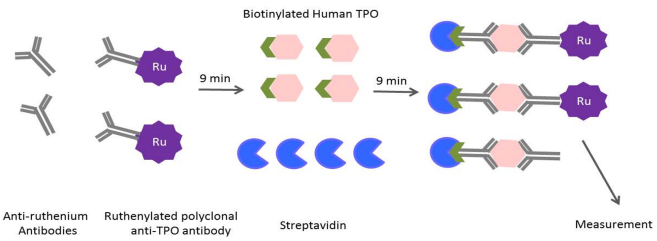

Figure 1. Anti-TPO competitive principle.

Table 1. General characteristics of the patients*.

Variables		N (%) or Mean ± SD
Age (year)	Mean	44.3 ± 1.0
	< 35	44 (26.0)
	35–44	42 (24.9)
	45–55	40 23.7)
	> 55	43 (25.4)
Gender	Males	28 (16.6)
	Females	141 (83.4)
Anti-TPO Ab	Mean	284.8 ± 17.8 IU/mL
	Positive	132 (78.1)
	Negative	37 (21.9)
Anti-Tg Ab	Mean	845.2 ± 103.3 IU/mL
	Positive	103 (60.9)
	Negative	66 (39.1)

* SD = Standard deviation, Anti-TPO Ab = Anti-thyroglobulin antibodies, Anti-Tg Ab = anti-thyroid peroxidase antibodies.

Regarding the anti-Tg Ab seropositivity status, there was no difference in the mean age of the total patients (P-value = 0.776) as well as among the different age groups designed for this study (P-value = 0.758). Males were more likely to be anti-Tg Ab positive (71.4%) in comparison to females (58.9%), but the results were statistically not significant (P-value = 0.213). When anti-TPO Ab correlated to anti-Tg Ab status, anti-TPO Ab titer was significantly higher in those with positive anti-Tg Ab (P-value = 0.007), and also for those who are anti-TPO Ab positive, anti-Tg Ab is more likely to be positive (66.7%) in comparison to those who are anti-TPO Ab negative where 59.5% also tested negative, for anti-Tg Ab (P=0.004) as shown in Table 2.

In comparison to anti-Tg Ab status, anti-TPO Ab seropositivity status shows also no difference in the mean age (P-value = 0.946) as well as among the different age groups of this study, apart from those between the ages of 45 and 55 years, where there are much more patients who are tested negative for anti-TPO Ab (40%) (P-value = 0.013). Primary hypothyroid female patients are more likely to be anti-TPO Ab positive (80.9%), in contrast to males, where only 64.3% are anti-TPO Ab positive (P-value = 0.053). Also, the mean

Table 2. General characteristics of the patients according to their anti-Tg Ab status.

Variables		Anti-Tg Ab		P-value
		+VE(n=103)	-VE(n=66)	
Age (year)	Mean ± SD	44.1±1.3	44.7±1.7	0.776
	<35	27(61.4)	17(38.6)	0.758
	35–44	28(66.7)	14(33.3)	
	45–55	22(55.0)	18(45.0)	
	> 55	26(60.5)	17(39.5)	
Gender	Males(n=28)	20(71.4)	8(28.6)	0.213
	Females(n=141)	83(58.9)	58(41.1)	
Anti-TPO Ab	Mean	323.2±22.0	224.9±28.7	0.007
	Anti-TPO Ab +VE	88(66.7)	44(33.3)	0.004
	Anti-TPO Ab -VE	15(40.5)	22(59.5)	

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Table 3. General characteristics of the patients according to their anti-TPO status +VE = Positive, and VE = Negative.

Variables		Anti-TPO Ab		P-value
		Positive (n = 132)	Negative (n = 37)	
Age (year)	Mean	44.3±1.2	44.5±2.1	0.946
	<35	35(79.5)	9(20.5)	0.013
	35–44	36(85.7)	6(14.3)	
	45–55	24(60.0)	16(40.0)	
	> 55	37(86.0)	6(14.0)	
Gender	Males(n=28)	18(64.3)	10(35.7)	0.053
	Females(n=141)	114(80.9)	27(19.1)	
Anti-Tg Ab	Mean	928.2±118.8	549.2±202.2	< 0.001
	Anti-Tg Ab +VE	88(85.4)	15(14.6)	0.004
	Anti-Tg Ab -VE	44(66.7)	22(33.3)	

anti-Tg Ab was higher among those who tested positive for anti-TPO Ab (P-value < 0.001). Finally, when we correlate the anti-Tg Ab positivity with the presence or absence of anti-TPO Ab, 85.5% of those who are anti-Tg Ab positive also tested positive for anti-TPO Ab, in contrast to only 66.7% of those who are anti-Tg Ab negative but tested positive for anti-TPO Ab (P-value = 0.004) as shown in Table 3.

Table 4 shows the effect of different variables on the mean anti-Tg Ab titer, where older age groups show a higher anti-Tg Ab titer than those who are younger (P-value = 0.19). Despite the fact that there is a numerical difference in the anti-Tg Ab level between males and females, that difference was not statistically significant (P-value = 0.286).

Table 5 shows the spatial relationship between anti-TPO Ab and anti-Tg Abs, where 88 patients (52.1% of the total) tested positive for both antibodies, while 22 patients both (13.0% of the total) tested negative for both antibodies, anti-TPO Ab only positive patients were 44 (26% of the total), while anti-Tg Ab alone was present in only 15 patients (8.9% of the total), which shows a significant correlation between the two antibodies (P-value = 0.004). For a positive anti-Tg Ab titer (> 115), the sensitivity for positive anti-TPO Ab would be 66.7%, while the specificity was 59.5%; as well as the PPV would be 85.4%, and the NPV would be 33.3%.

Table 4. Effect of age, gender, and anti-TPO Ab on the mean anti-Tg Ab titer.

Variables		Anti-Tg Titer	P-value
Age (year)	<35	628.8±172.6	0.019*
	35–44b	555.7±155.4	
	45–55	816.6±210.3	
	> 55 a, b	1376.1±255.6	
Gender	Males(n=28)	1369.8±312.3	0.286**
	Females(n=141)	741.1±105.5	
Anti-TPO Ab	Anti-TPO Ab+VE	928.2±118.8	0.001**
	Anti-TPO Ab-VE	549.2±202.2	

* Univariate analysis through a general linear model.
Post hoc: a: P-value = 0.043, b: P-value = 0.023.
** Mann Whitney test.

Table 5. Correlation between Anti-Tg Ab and Anti-TPO Ab status*.

Variables		Anti-TPO Ab		Total
		Positive (n = 132)	Negative (n = 37)	
Anti-Tg Ab	Positive(+VE)	88(85.4)	15(14.6)	103(100.0)
	Negative(-VE)	44(66.7)	22(33.3)	66(100.0)
Total		132(78.1)	37(21.9)	169(100.0)

* P-value = 0.004
For anti Tg Ab titer level at 115:
Sensitivity = 66.7%
Specificity = 59.5%
Positive predictive value = 85.4%
Negative predictive value = 33.3%
The risk estimates for having a TPO+VE in anti Tg +VE patients = 2.93 (1.39-6.21) at 95% CI

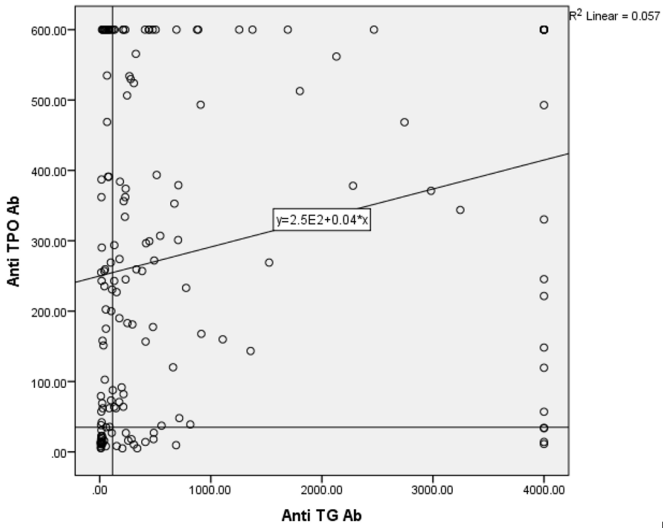

Figure 2. Blot for patients anti-Tg Ab and anti-TPO Ab levels.

The blot for their anti-Tg Ab and anti-TPO Ab titer is shown in Figure 2.

Figure 3 shows the sensitivity and specificity of anti-Tg Ab in contrast to the anti-TPO Ab level to predict the highest positive predictive value and negative predictive value of the anti-Tg Ab level for a positive anti-TPO Ab level.

The highest PPV (91%) for having a positive anti-TPO Ab was obtained at an anti-Tg Ab titer > 691 IU/mL with a sensitivity of 31% and a specificity of 89%, OR=3.72 (P-value = 0.014).

The highest NPV (90%) was obtained at an anti-Tg Ab titer < 11 IU/mL with a sensitivity of 99% and a specificity of 24% RRR = 0.579 (P-value = 0.001). A maximum Youden's value = 1.4 (maximum sensitivity at maximum specificity) was obtained at a titer of 26 with a PPV= 86% and an NPV = 59% with a sensitivity of 90% and specificity of 51% (P-value = 0.001).

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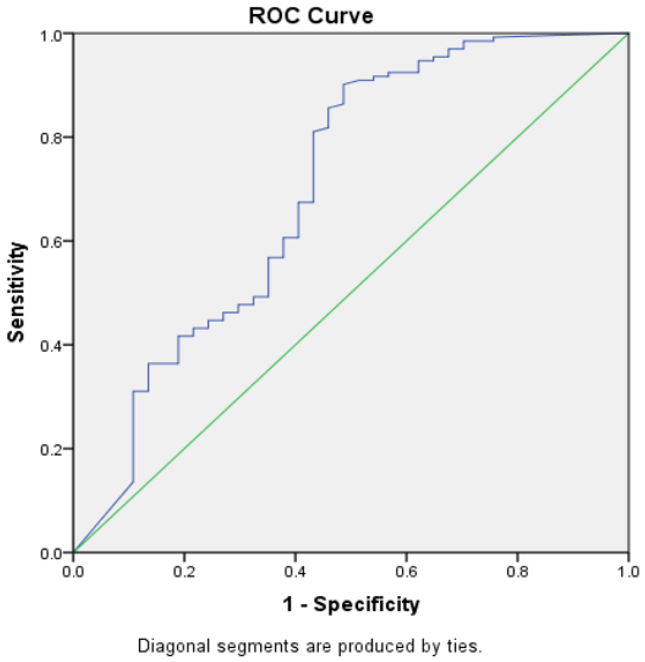

Figure 3. ROC curve of anti-Tg Ab concerning a positive anti-TPO Ab level.

DISCUSSION

Chronic autoimmune (Hashimoto's) thyroiditis is the most common cause of hypothyroidism in iodine-sufficient areas of the world, and it can be either goitrous or atrophic, which differ in the extent of fibrosis, follicular cell hyperplasia, and lymphocytic infiltration but not in their pathophysiology, which is autoimmune involving both humoral as well as cellular immunity [14]. Traditionally, autoimmune thyroiditis is associated with high serum levels of anti-Tg Ab and anti-TPO Ab, which vary in their prevalence from area to area [15].

It is wellknown that the prevalence of hypothyroidism in females is much higher than in males. In this study, the female to male ratio was around 5.04:1, which is much less than what was observed in the famous United States National Health and Nutrition Examination Survey (NHANES III) where the female -to -male ratio was 8:1 [16]. Recently, Koceak et al. illustrated that the family history of hypothyroidism elevated almost double the danger of anti-thyroid antibodies in Caucasian women [17].

Anti-TPO Ab and anti-Tg Ab were found in 78.1% and 60.9% of our patients with primary hypothyroidism, in comparison to 50% and 100%, respectively, in a previous study from Iran [18]. While, in the Western world, the prevalence of anti-TPO Ab and anti-Tg Ab was 90–100% and 80–90% ,respectively, in the famous Whickham survey for the United Kingdom and the Framingham Study for the United States [19, 20]. This can be explained by differences in ethnicity and iodine intake status. In a study from Bangladesh, the prevalence of anti-TPO Ab and anti-Tg Ab was 82.5% and 55.2% in patients with thyroid disease respectively, however, the abnormality of the thyroid's structure is associated with the risk of autoimmunity [21]. In Japan the prevalence was 81.4% and 98.6% for anti-TPO Ab and anti-Tg Ab, respectively [22]. This explains the heterogeneity in the prevalence of these thyroid antibodies among the different populations.

The study's result is consistent with a cohort study of 5 year follow-up by Li et al., which explained that TPO Ab titer and TG Ab are significantly related to an elevated risk of TSH levels [23]. While in the other two previous studies, anti-Tg Ab and anti-TPO Ab titers were 6974 and 373 IU/mL, respectively, which were higher than our records. This may be related to the difference in the duration of the disease as well as the degree of TSH control [24, 25].

Anti-Tg Ab titer increased steadily with advancing age among our patients in contrast to anti-TPO Ab (P-value = 0.009). This observation was similar to that of Arai et al., who recommended that anti-Tg Ab was more predictive of thyroiditis among elderly people than anti-TPO Ab [26]. Sulejmanovic's study in 2020 reported that both antibodiy levels (anti-TG and anti-TPO) were higher in Hashimotos Thyroiditis and Graves Disease patients (293.47 ± 429.50 IU/ml and 1715.58 ± 969.79 IU/ml, respecively) compared to the control healthy individuals (16.56 ± 7.4IU/ml; 25.31 ± 10.51 IU/ml) [27].

Kasagi et al. found that anti-Tg Ab was more closely associated with the histological diagnosis of Hashimotos thyroiditis than anti-TPO Ab (96.4% vs 73.5%, respectively). However, in our study, we lacked a parallel histopathological study [28]. Recently, Guldvog and his colleagues reported that higher levels of TPO Ab (greater than 1000 IU/mL) were found in Hashimotos Thyroiditis [29]. An increase in the levels thyroid antibodies, such as anti-TPO and anti-TG, is presented in cases of Hashimotos encephalopathy [30].

Finally, in this study, we tried to make a cut-off value where an anti-Tg Ab titer would predict positivity for anti-TPO Ab. Therefore, we can use either one of the antibodies for the evaluation of patients with hypothyroidism for autoimmunity. Unfortunately, we did not find comparable studies in the PubMed and Embase databases to compare our results with.

In this study, the risk of having positive anti-TPO Ab in anti-Tg Ab-positive patients would be 2.93 (1.39-6.21) at a 95% confidence interval. The best and highest PPV for having a positive anti-TPO Ab was 91%, and this was obtained at an anti-Tg titer of > 691 IU/mL with a sensitivity of 31% and specificity of 89% at an odds ratio of 3.72 (P-value = 0.014). The best NPV was seen at an anti-Tg Ab titer < 11 IU/mL with a sensitivity of 99% and a specificity of 24% (P-value < 0.001), while a maximum Youdens value of 1.4 (i.e., maximum sensitivity at a maximum specificity) was obtained at a titer of 26 IU/mL with a positive predictive value of 86% and a negative predictive value of 59% with a sensitivity of 90% and a specificity of 51% (P-value < 0.001).

There were three limitations to the current study. First, it is a single-center study. Second, the study is clinic-based. The lack of parallel histopathological examination or ultrasonographic study is considered the third shortcoming.

CONCLUSION

Anti-Tg Ab and anti-TPO Ab are both valuable information for patients with chronic autoimmune hypothyroidism. Anti-Tg Ab tends to be more informative in the age group of > 50 years where it is more prevalent than anti-TPO Ab. At an anti-Tg Ab titer > 691 IU/mL, we most likely will have positive anti-TPO Ab, while at an anti-Tg Ab titer < 11 IU/ml, we most likely have negative anti-TPO Ab. We recommend a future population-based study to define the cut-off

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values for positive anti-TPO Ab as well as anti-Tg Ab. Using anti-Tg Ab rather than anti-TPO Ab among elderly people. At extreme anti-Tg Ab levels, anti-TPO Ab will likely be positive or negative, respectively.

ETHICAL DECLARATIONS

Acknoweldgements

None.

Ethics Approval and Consent to Participate

The study was approved by the Ethical Approval Committee of the College of Medicine, University of Basrah, Basrah, Iraq. Informed consent was obtained from every participant.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented it to be published.

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