Journal of Advances in Medicine and Medical Research

24(3): 1-6, 2017; Article no.JAMMR.36949
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

The Role of Indirect Immunofluorescence Methods in the Detection of Autoimmune Antibodies in Thyroid Disease

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Authors' contributions

This work was carried out in collaboration between both authors. Author NK designed the study, performed the statistical analysis wrote the protocol and wrote the first draft of the manuscript. Author LK managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/36949
Editor(s):
(1) E. Umit Bagriacik, Department of Immunology, Gazi University, Turkey.
Reviewers:
(1) Emina Kasumagic-Halilovic, University Clinical Center Sarajevo, Bosnia and Herzegovina.
(2) Liudmila Ivanova, Kuban State Medical University, Russia.
Complete Peer review History: http://www.sciencedomain.org/review-history/21489

Original Research Article

Received 25th September 2017
Accepted 18th October 2017
Published 20th October 2017

ABSTRACT

Aims: In this study, we evaluated the prevalence of antithyroglobulin antibodies (anti-Tg Ab) and antipyroxidase antibodies (anti-TPO Ab) in thyroid disease and investigated the role of indirect immunofluorescence methods in its detection.
Study Design: The study was carried out on 87 patients with thyroid disorders.
Place and Duration of Study: The patients were recruited from the Department of Internal Medicine and the Department of Surgery at Aleppo University Hospital. A group of 25 matched volunteers were included as a control group for the investigation. Data analyzed in this study was collected over the period from December 2005 to February 2006.
Methodology: Immunofluorescence methods were used to study the prevalence of antithyroglobulin antibodies (anti Tg Ab) and anti pyroxidase antibodies (anti TPO Ab) in patients with different thyroid diseases.
Results: We found that anti-Tg Ab was positive in 33% of patients with goiters, 40% of patients with hyperthyroidism, 73% of patients with Hashimoto's thyroiditis, and 25% of patients with

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nodules. Anti-Tg Ab was positive in only 4% of the control group. Similarly, anti-TPO Ab was positive in 37% of patients with goiters, 73% of patients with hyperthyroidism, 88% of patients with Hashimoto's thyroiditis, and 50% of patients with nodules. Anti-TPO Ab was found positive in 8% of the control group.

Conclusion: Taken together, these results suggest anti-Tg Ab and anti-TPO Ab measurements are an important step in the evaluation and management of thyroid disorders. Anti-TPO Ab had the highest sensitivity in predicting thyroidal disorders. Immunofluorescence techniques, furthermore, were found to be a reliable method for the evaluation of these disorders.

Keywords: Thyroglobulin antibodies; autoimmune thyroid disorders; immunofluorescence; thyroid microsomal antibodies.

1. INTRODUCTION

Autoimmune thyroid disease (AITD) causes cellular damage and alters thyroid gland function by humoral and cell-mediated mechanisms. Cellular damage occurs when sensitized T-lymphocytes or autoantibodies bind to thyroid cell membranes, causing cell lysis and inflammatory reactions. Alterations in thyroid gland function result from the action of stimulating or blocking autoantibodies on cell membrane receptors [1,2]. Three principal thyroid autoantigens are involved in AITD, namely thyroperoxidase (TPO), thyroglobulin (Tg), and TSH receptor.

Traditional methods to measure thyroid autoantibodies include immunofluorescence techniques, using human or primate thyroid tissue, and passive (tanned) erythrocyte hemagglutination assays; newer techniques include (ELISAs) and radioimmunoassys. Thyroglobulin antibodies (Tg Abs) are circulating immunoglobulins directed against different epitopes of the thyroglobulin molecule [3].

Thyroid microsomal antibodies (TPO Abs) are circulating immunoglobulins directed against a component of the smooth endoplasmic reticulum of thyroid cells and are the earliest detectable abnormality in the course of developing hypothyroidism, secondary to Hashimoto's thyroiditis [2,3]. Progression to hypothyroidism is directly correlated to the antibody titer and initial TSH levels [4,5].

Thyroglobulin is polymerized and degraded during the process of thyroid hormone synthesis and release [6]. Its use is limited to cancer patients [5]. In most cases, the diagnosis of Hashimoto's disease is not difficult. However, patients with chronic renal disease may have symptoms that mimic those of hypothyroidism; in addition, free T4 tests are not always reliable. Therefore, Tg/TPO Abs testing helps to confirm the exact diagnosis. De Quervain's thyroiditis (granulomatous thyroiditis or subacute thyroiditis) is a rare disease. Both Tg Ab and TPO Abs are usually low or weakly positive in this condition and disappear with spontaneous healing of the gland [7].

2. MATERIALS AND METHODS

The current study was conducted on a group of 87 patients, with different thyroid disorders, from the Department of Internal Medicine and the Department of Surgery at Aleppo University Hospital. The data was collected over the period from December 2005 to February 2006. A group of 25 matched volunteers were included in the investigation as a control. Overweight patients (BMI > 33), patients with pituitary disease, patients on amiodarone or lithium therapy, and patients over 75 years old were excluded from the study.

We used an indirect immunofluoresence method (Euroimmun using Thyroid gland monkey with titer plane technique). Preparation of PBS Tween was carried out by dissolving 1 pack of PBS salt in 1 L of distilled water. 2 mL of Tween-20 was then added. 25 µm of diluted sample (10 µm of sample with 100 µm of PBS Tween) was applied to each reaction field of the reagent tray. Trays were incubated for 30 minutes at room temperature. Biochip slides were subsequently washed with PBS Tween solution for 5 min. 20 µl of fluorescein labeled anti-human globulin was added to each reaction field. Slides were incubated for 30 min (in a dark room). After incubation, the biochip slides were rinsed with PBS Tween for 5 minutes. Slides were covered with the prepared cover glasses. Fluorescence intensity was read by the fluorescence microscope at 495 nm. The same procedure was repeated with incremental dilutions (1/30-1/40).

All statistical analyses for this study were conducted using StataSE (version 8).

3. RESULTS

Demographic data of the studied patients are depicted in Table 1. We found that thyroid disease was concentrated among patients in their 30s. Patients included in this study were further subdivided into six main categories as shown in Table 2.

Among the female patients, thyroid goiter accounts for 31% of cases, hyperthyroidism 25%, hypothyroidism 26%, and nodule 4%. In male patients, thyroid goiter accounts for 30% of cases, hyperthyroidism 25%, hypothyroidism 15%, and nodule 5%. In our study, the goiter represented a third of thyroid disease cases; this can likely be attributed to iodine deficiency in the local population. It's worth noting, moreover, there is a notable prevalence of hyperthyroidism (Grave's diseases) and hypothyroidism (Hashimoto disease), as compared to thyroid nodules, among the patients studied. The most common cause of thyroid nodules is infection or adenoma.

In Table 3, we show the prevalence of anti-Tg Ab and anti-TPO Ab by type of thyroid disorder. Anti-TG Abs were found to be most prevalent in hypothyroidism; this is because patients with hypothyroidism have an increase in TSH concentration and this is commonly associated with an increase in antithyroglobulin antibodies. Similarly, anti-TPO Abs were found to be the most prevalent in hypothyroidism; this can be attributed to the observation that anti-TPO Ab are typically the *first* antibodies to arise in this disease.

In Table 4, we show the distribution of anti-Tg Abs and anti-TPO Abs, by patient sex, in different types of thyroid diseases. The high prevalence of anti-TPO and anti-Tg Ab (65% and 47%, respectively) indicate that AITD is common in our area, especially hyperthyroidism (Grave's diseases) and hypothyroidism (Hashimoto disease). When we compare these results with those of the control group, we found that the prevalence of antipyroxidase and antithyroglobulin antibodies in the general population is 8% and 4%, respectively. Moreover, as might be expected, both are more common in females than males because AITD in general is more common among female patients.

Patients with positive anti-TPO Ab and anti-Tg Ab were subdivided depending on the antibodies titer, and the TSH concentration (Table 5). After observation, it was found that the antibodies titer is related to the severity of the disease. When TSH is increased or decreased, beyond the normal range, the antibodies titer is increased which gives us a good evaluation of the case and this will help to control and treat the patient.

The correlation coefficient (r) between TSH and anti-TPO Ab was between 0.6 – 0.88, depending on the levels of TSH. The correlation coefficient between TSH and anti-Tg Ab was between 0.4 – 0.84, also depending on the levels of TSH. The diagnostic potential of antipyroxidase and antithyroglobulin antibodies in the detection of thyroid diseases is depicted in Table 6.

4. DISCUSSION

More than one mechanism is involved in the origin of thyroglobulin and thyroperoxidase antibodies, and this is consistent with antibody heterogeneity. Different groups have suggested that there could be an antigenic stimulation at the initial step of antibody production. It has been proposed that a viral infection such as subacute thyroiditis could induce occult antigenic changes within the thyroid. Other hypothesis referred to the abnormal antigenic stimulation in these patients, where thyroglobulin release in the bloodstream is accelerated, and iodinated forms, which are normally confined to the thyroid gland, are secreted. Another hypothesis suggests an immunoregulation disorder may play a role in the etiology of both Graves' and Hashimoto's disease [8]. TSH is also thought to stimulate TPO Ab production in thyroid cells through a complex pathway involving cyclic AMP [9,10].

In the current study, we found that 73.1% of Hashimoto's thyroiditis patients have positive anti-Tg Ab and 88.5% have positive anti-TPO Ab. Similarly, anti-Tg Ab and anti-TPO Ab were found in 40% and 73.3% of Graves' disease cases, respectively. Gardas A, et al. [11] studied Tg Ab and TPO Ab in a group of 109 patients with Hashimoto's thyroiditis. In these patients, 76% and 85% were positive for Tg Ab and anti-TPO Ab, respectively. In a group of 79 patients with Graves' disease he found that 47% and 78% of these patients were positive for anti-Tg and anti-TPO Ab, respectively. Dai WX, et al. [12] estimated the serum levels of anti-TPO Ab and anti-Tg Ab (using indirect immunofluorescence) in a group of 434 subjects; these included 51 patients with Hashimoto's thyroiditis, 58 with Graves' disease, 68 with nodular goiter, 56 with thyroid adenoma and carcinoma, and 56 with

Table 1. Distribution of the patient and control groups according to sex and age (SD, standard deviation)

	Patient group				Control group			
	No.	**%**	**Mean age**	**SD**	**No.**	**%**	**Mean age**	**SD**
Males	20	23	36.4	13.9	9	36	41.9	12.8
Females	67	77	38.7	16.4	16	64	39.3	9.8
Total	87	100			25	100		

subacute thyroiditis. He demonstrated that 86% of patients with hypothyroidism have positive anti-TPO Ab and 71% have positive anti-Tg Ab, while 72% of patients with hyperthyroidism have positive anti-TPO Ab and 44% have positive anti-Tg Ab. The similarity of results in these investigations can likely be attributed to the fact that all the studies, including ours, used indirect immunofluorescence techniques.

Table 2. Distribution of patients depending on clinical findings

Diagnosis	**Total number**	**Percentage (%)**
Hyperthyroidism without goiter	22	25.28
Hypothyroidism without goiter	21	24.13
Hyperthyroidism with goiter	8	9.19
Hypothyroidism with goiter	5	5.74
Euthyroid with goiter	27	31.03
Euthyroid with nodules	4	4.59
All patients	87	100

Savoie JC, et al. [13] screened 629 patients with thyroid disease and 100 controls for TPO Ab and anti-Tg Ab by hemagglutination. Thyroid antibodies were present in 4% of control patients. The overall prevalence of thyroid antibodies in autoimmune thyroiditis were: TPO Ab 93%, anti-Tg Ab 78%. In Graves' disease, anti-TPO Ab was 63%, while anti-Tg Ab was 33%. He found a slightly higher prevalence rate of anti-TPO Ab and anti-Tg Ab in hypothyroid patients (93% and 78%, respectively), compared to our study, but similar results in hyperthyroidism patients (63% and 33%, respectively). Mariotti S, et al. [14] investigated anti-TPO Ab in 596 patients with different thyroid disorders using monoclonal antibody-assisted RIA. Anti-TPO Abs was positive in 74% of patients with Graves' disease and 95% of patients with Hashimoto's. In contrast, anti-TPO Abs was detected in only 8.4% of the control group. Both *Savoie* and *Mariotti* used radioimmunoassay methods in their study to improve sensitivity. The correlation between the levels of TSH and the titer of anti-TPO Ab is consistent with Kontianen S, et al. [15], who demonstrated that 47% of patients with a titer of anti-TPO Ab more than 1/40 have abnormal TSH levels. Walsh JP, et al. [16] analyzed serum samples from 2115 adults for FT4, TSH, TPO Ab, and Tg Ab by immunochemiluminescent assays. He demonstrated that 63% of patients with high concentration of TSH (more than 4 micro u/ml) have high titer of anti-TPO Ab and 60% of them have high titer of anti-Tg Ab.

Table 3. The prevalence of anti-Tg Ab and anti-TPO Ab according to pathology

Diagnosis	**No. of patients**	**anti-Tg Ab**	**%**	**anti-TPO Ab**	**%**
Goiter	27	9	33.3	10	37
Hyperthyroidism	30	12	40	22	73.3
Hashimoto's thyroiditis	26	19	73.1	23	88.5
Nodules	4	1	25	2	50
All patients	**87**	41	47.1	57	65.5
Control group	**25**	1	4	2	**8**

Table 4. Sex distribution of anti-Tg Ab and anti-TPO Ab in the study and control groups

Diagnosis	anti-TPO Ab (%)		anti-Tg Ab (%)	
	Females	Males	Females	Males
Thyroid goiter	38.1	33.3	33.3	33.3
Hyperthyroidism	72.7	75	40.9	37.5
Hashimoto's thyroiditis	90.5	80	76.2	60
Nodules	33.3	100	33.3	0
All patients	65.7	65	49.3	40
Control group	12.5	8	6.25	0

Table 5. The prevalence of anti TPO and anti-Tg Ab depending on antibodies titer and their relation to TSH concentration

TSH concentration (micro unit /ml)	1/10		1/20 -1/30		1/40	
	anti-TPO Ab (%)	anti-Tg Ab (%)	anti-TPO Ab (%)	anti-Tg Ab (%)	anti TPO Ab (%)	anti-Tg Ab (%)
Less than 0.27	27.2	16.6	27.3	16.6	45.5	50
Between 0.27- 4.2	58.4	60	25	60	16.6	10
More than 4.2	21.7	21	30.5	21	47.8	42.2
All patient	31.5	29.3	28	34.1	40.5	36.6
Control group	50	100	50	0	0	0

Table 6. The sensitivity, specificity, positive and negative predictive values of anti TPO Ab and anti Tg Ab in the detection of thyroid diseases

Parameter	Anti-TPO Ab (%)	Anti-TG Ab (%)
Sensitivity	80	55
Specificity	61	67
Positive predictive value	78	75
Negative predictive value	63	45

5. CONCLUSION

In conclusion, anti-TPO Ab and anti-Tg Ab are important measurements in the evaluation and management of thyroid disorders. Immunofluorescence techniques are reliable methods for the evaluation of these disorders, bearing in mind the mainstay of diagnosis and management relays on clinical presentation and physical findings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21489