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Evaluation of the association of anti-thyroid peroxidase with antinuclear antibodies and different antinuclear antibodies patterns

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ABSTRACT

Aims: To investigate the relationship between anti-thyroid peroxidase (TPO), a marker for Hashimoto's thyroiditis, and antinuclear antibodies (ANA) and ANA patterns, biomarkers for systemic autoimmune diseases.

Methods: In this study, ANA and anti-TPO test results, obtained in our hospital laboratory between 2019 and 2022, were retrospectively evaluated. ANA was detected by the indirect immunofluorescence antibody method using commercial HEp-20-10 cell substrates and anti-TPO was determined by the sandwich immunoassay method using a commercial immunoassay analyzer.

Results: A total of 1750 patients' results were analyzed. ANA was positive in 28.7% of anti-TPO-positive patients and 19% of anti-TPO-negative patients. While 6.4% (112/1750) of patients were positive for both ANA and anti-TPO, both test results were negative in 62.85% of patients ($p<0.001$). When the ANA patterns' distribution in patients with ANA and anti-TPO positivity examined together, the homogeneous pattern was statistically significantly higher than the other patterns ($p=0.043$).

Conclusion: Individuals with autoimmune thyroid disease had a higher rate of autoantibodies not only to thyroid-specific antigens but also to non-thyroid-specific antigens. Further studies are needed on how epigenetic changes, such as histone modifications might cause other autoimmune diseases and increase their frequency.

Keywords: Autoimmunity, antinuclear antibodies, autoimmune disease, thyroid diseases, Hashimoto disease

INTRODUCTION

Autoimmune diseases affect the quality of life and increase health care costs. When there are multiple causes of death due to autoimmune diseases, mortality increases at least 1.5-fold.[1] Autoimmune diseases, estimated to have a prevalence of about 5% in developed countries, are more common in women.[2]

The main factors involved in the development of autoimmunity are infections, other environmental factors, and genetic predisposition. When autoreactive B and T cells are stimulated under the influence of these factors, autoimmune diseases develop due to formation of antibodies or cellular immune responses against autoantigens.[3]

Autoimmune diseases can be systemic or organ specific. The most commonly used parameter in screening for systemic autoimmune diseases is the detection of antinuclear antibodies (ANA).[4,5] In organ-specific autoimmune diseases, antibodies that directly target antigens in an organ or endocrine gland cause tissue damage, and the goal is to detect these antibodies in screening.[6] The best example of this group is anti-thyroid peroxidase (TPO) and anti-thyroglobulin (Tg) antibodies, which cause tissue damage in Hashimoto's thyroiditis.[7]

Because of the changes that can occur in autoimmune pathogenesis, other autoimmune disorders accompany the original disease in 25% of patients.[8] The incidence of multiple autoimmune diseases has been reported to be 33-45% for Systemic Lupus Erythematosus (SLE), 13-32% for Rheumatoid Arthritis, 26% for Systemic Sclerosis, 33-52% for Sjögren's syndrome, and the most common accompanying disease is autoimmune thyroid disease.[9] The overlap between Hashimoto's thyroiditis and some non-specific rheumatic symptoms has been reported to predict the development of some connective tissue diseases.[10]

Therefore, in this study, the association between anti-TPO, one of the markers for Hashimoto's thyroiditis, and ANA, a marker for systemic autoimmune disease, and different ANA patterns was investigated to reveal the presence of organ-specific autoimmune thyroid disease along with systemic autoimmune disease.

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METHODS

The study was carried out with the permission of University of Health Sciences İzmir Tepecik Training and Research Hospital Non-interventional Ethics Committee (Date: 15.04.2022, Decision No: 2022/04-23). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

ANA and anti-TPO test results of samples sent to the Medical-Microbiology and Medical-Biochemistry Laboratory of İzmir Tepecik Training and Research Hospital between 2019 and 2022 were retrospectively evaluated. The patients' data were obtained from the hospital medical records. Patients with both anti-TPO and ANA test results were included in the study. The first samples of patients were accepted. Other samples were excluded.

Indirect Immunofluorescence Antibody (IIF)- ANA Scanning

ANA was detected by the IIF method using commercial HEp-20-10 cell substrates (Euroimmun Luebeck, Germany). Testing and scoring were performed according to the manufacturer's instructions with an initial screening dilution of 1:100. After slide preparation, the test was scored according to the international consensus report on ANA samples.[11]

Anti-TPO Detection

Anti-TPO was determined by the sandwich immunoassay method using a commercial immunoassay analyzer (DxI 800, Beckman Coulter, Inc., CA, USA). The analytical range of the assay reported by the manufacturer is 0.25-1000 IU/mL, the analytical sensitivity is 0.25 IU/mL, and the reference range reported for the healthy adult population is 0-10 IU/mL.

Statistical Analysis

The SPSS (Statistical Packages for the Social Sciences) version 22.0 program (SPSS Inc., Chicago, USA) was used for the statistical analysis. The qualitative results of the tests (positive/negative) and the sex of the patients were recorded as categorical variables, and the age of the patients was recorded as a continuous variable. Numerical data were expressed as mean±standard deviation (SD), number (n), and percentage (%). The chi-square test was used to compare categorical variables such as anti-TPO positivity, ANA positivity, and sex. Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to analyze the normality assumption for groups such as sex and ANA patterns of continuous variables such as age. Analysis continued with parametric tests in the case of a normal distribution and nonparametric tests in the case of an abnormal distribution. Comparison of categorical variables by patient age was performed with Independent Sample T, One-Way Anova, and Mann-Whitney U tests. The Spearman correlation test was used to analyze the correlation between test dilutions of ANA, and anti-TPO levels. Post hoc tests were used for further pairwise comparisons. The statistical significance level (p value) was set at 0.05 for all analyzes.

RESULTS

In the study, 1750 patients (mean age±SD=40.0± 19.0) (76.5% female, 23.5% male) routinely screened for ANA, and anti-TPO tests from different clinics were included.

ANA and anti-TPO positivity were found in 21.2% (371/1750) and 22.3% (391/1750) of patients respectively. ANA was positive in 28.7% of anti-TPO-positive patients and 19% of anti-TPO-negative patients (p>0.001). Both ANA and anti-TPO positivity were detected in 6.4% of patients (112/1750), while both were negative in 62.85% (1100/1750) of patients. Anti-TPO positivity in patients with ANA positive test results and anti-TPO negativity in patients with ANA negative test results were statistically significant (p<0.001) (Table 1).

Table 1. Comparison of ANA and anti-TPO test results

	Anti-TPO positive	Anti-TPO negative	Total	p
ANA positive (n=371)	112	259	371	<0.001
ANA negative (n=1379)	279	1100	1379	
Total	391	1359	1750	

The most common IIF-ANA pattern detected in both ANA positive and anti-TPO positive patients (n:112) was homogeneous (n:43, 38.4%), followed by dense fine speckled (DFS)-70 (n:29, 25.9%), granular (n:26, 23.2%), nucleolar (n:6, 5.4%), nuclear membrane (n:5, 4.5%), nuclear dots (n:2, 1.8%), and centromere (n:1, 0.8%) (p=0.057, chi-squared) (Table 2).

Table 2. Relationship between positive ANA patterns and anti-TPO results (n=371)

	Anti-TPO positive, n=112	Anti-TPO negative, n=259	p*
Homogeneous (n=114)	43 (38%)	71 (27%)	0.035
DFS-70 (n=78)	29 (26%)	49 (19%)	0.130
Centromere (n=13)	1 (1%)	12(5%)	0.120
Speckled (n=110)	26 (23%)	84 (32%)	0.074
Nucleolar (n=29)	6 (5%)	23 (9%)	0.246
Nuclear envelope (n=16)	5 (5%)	11 (4%)	1.000
Nuclear dots (n=11)	2 (2%)	9(4%)	0.516

*Each group was tested against other patterns (chi-square)

When the ANA patterns' distribution in patients with ANA and anti-TPO positivity were examined together, the homogeneous pattern was found to be significantly higher than the other patterns (p=0.035, chi-square)

(Table 2). When the results were evaluated according to staining in the metaphase layer, a central element in the evaluation of ANA, ANA metaphase positivity was found to be statistically higher (n:73) in patients with anti-TPO positivity (p=0.001, chi-square) (Table 2). There was no statistically significant difference for DFS-70 (p=0.13), centromere (p=0.12), speckled (p=0.074), nucleolar (p=0.246), nuclear envelope (p=1), nuclear dots(p=0.516). No correlation was found between test dilutions of ANA, and anti-TPO levels (r=-0.01, p=0.80). There was no statistically significant difference between the age of patients who were simultaneously positive for ANA and TPO (p=0.361, Oneway Anova). The age and sex distribution of ANA positive patients showed in Table 3.

Table 3. The age and sex distribution of ANA positive patients (n=371)

	Age,mean±SD years	Women, n (%)
DFS-70 (n=78)	36.09± 17.59	64 (82%)
Homogeneous (n=114)	44.45±20.14	95 (83%)
Speckled (n=110)	36.83±19.63	83 (76%)
Nucleolar (n=29)	42.20±21.48	22 (76%)
Nuclear dots (n=11)	41.12±19.15	9 (82%)
Nuclear envelope (n=16)	54.73±16.47	14 (88%)
Centromere (n=13)	43.87±19.90	10 (77%)

DFS70: dense fine speckled 70; SD: standard deviation.

DISCUSSION

The most important elements in the development of autoimmune diseases (AD) are genetic predisposition and environmental factors.[12] Autoimmune diseases may develop as a result of disturbances in the regulation of the immune system due to these factors. Polyautoimmunity is defined as the presence of more than one autoimmune disease in a single patient, and in 13-52% of patients, other autoimmune diseases occur in addition to the original disease, with the most common concomitant disease being autoimmune thyroid disease.[9]

ANA positivity with low titers can also be observed in healthy individuals and in many different non-rheumatic diseases. An abnormal ANA should be a titer that is above the 95th percentile of a healthy control population.[13] In the current study, the ANA prevalence was 28.7% in anti-TPO positive patients with our ANA screening dilution (p <0.001). Prevalence studies of systemic autoantibodies in individuals with thyroid disease are limited. The prevalence of ANA in autoimmune thyroid disease patients was reported to be 35% by Tektonido et al.[14], 45% by Lazurova et al.[15], and 26% by Morita et al.[16]

In a study evaluating patterns with ANA in anti-TPO-positive individuals, a prevalence of 18% was found. The ANA patterns were found to be homogeneous in 50% and granular in 35% of anti-TPO-positive patients, but the DFS-70 pattern was not examined.[8] In the current study one of the nuclear patterns, DFS-70 was evaluated for the first time and our results suggest that DFS-70 and homogeneous patterns were significantly higher in anti-TPO positive patients.

The homogeneous pattern was the most frequent in the anti-TPO-positive patients. The antigens associated with the homogeneous pattern were dsDNA, histone, and nucleosome. In a study evaluating the extractable nuclear antigen (ENA) profile,[8] the prevalence of homogeneous patterns in anti-TPO positive patients was 50%, like our study (when the DFS-70 pattern was excluded, the prevalence of homogeneous pattern was 52%), and the prevalence of antibodies to histones was very high at 72%. The homogeneous pattern is more common in patients with SLE but can also be found in patients with mixed connective tissue disease and drug-induced lupus.[17] Therefore, follow-up and evaluation of patients with homogeneous patterns in ANA screening and histone positivity in ENA evaluation concerning anti-TPO may be useful for the early diagnosis of Hashimoto's thyroiditis. The overlap between Hashimoto's thyroiditis and some non-specific rheumatic symptoms has been reported to predict the development of some connective tissue diseases.[10]

In this study, the DFS-70 pattern, which has a high prevalence in the healthy population and correlates negatively with ANA-related autoimmune diseases, was the second most common (26%) pattern in anti-TPO-positive patients. In a study investigating the relationship between DFS-70 autoantibodies and other autoantibodies, the most common concomitant autoantibody was anti-TPO at 16%.[18] This rate was higher in this study and anti-TPO was positive in 37% (29/78) of DFS-70-positive patients. Therefore, it may be important to perform the anti-TPO test when the DFS-70 pattern is detected in healthy individuals, allergic diseases, non-systemic autoimmune diseases, ocular diseases, or cancers. Future studies are needed in these disease groups. No association between thyroid diseases and patterns other than homogeneous and DFS-70 has been described.

One of the limitations of our study is that it was a retrospective study, meaning that we could not determine which antibody appeared first and influenced the other or whether these autoantibodies were persistent or transient. We also do not know how these antibodies change with treatment. Second, we were unable to reveal whether cross-reactivity of the substrates used to detect these antibodies led to false positive results.

CONCLUSION

Individuals with autoimmune thyroid disease have a high rate of autoantibodies not only to thyroid-specific antigens but also nonspecific ones. Although there are many studies on epigenetics, the epigenetic changes in thyroid autoimmune diseases have not fully elucidated.[19-22] There is a need for further prospective studies on how epigenetic alterations, such as histone modifications may cause systemic autoimmune diseases and increase their frequency in autoimmune thyroid diseases. Those will provide new information that can adopted in the diagnosis, follow-up, and prognosis of thyroid disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences, Tepecik Training and Research Hospital, Non-interventional Clinical Researches Ethics Committee (Date: 15.04.2022, Decision No: 2022/04-23).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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REFERENCES

1. Hernández-Negrín H, Roque-Dapresa Y, Martínez-Morales O, Mederos-Portal A. Using multiple cause-of-death analysis to estimate systemic autoimmune disease mortality burden in low- and middle-income countries. *MEDICC Review.* 2021;23(2):69-74.
2. Frederick WM, Lars A, Karen HC, et al. Epidemiology of environmental exposures and human autoimmune diseases: Findings from a National Institute of Environmental Health Sciences Expert Panel Workshop. *J Autoimmun.* 2012;39(4):259-271
3. Tsokos GC. Systemic lupus erythematosus. *N Engl J Med.* 2011;365(22):2110-2121. doi:10.1056/NEJMra1100359
4. Andrade LEC, Damoiseaux J, Vergani D, Fritzler MJ. Antinuclear antibodies (ANA) as a criterion for classification and diagnosis of systemic autoimmune diseases. *J Transl Autoimmun.* 2022;5:100145. doi:10.1016/j.jtauto.2022.100145
5. Bilgin M, Keskin A, Aci R, Baklacioglu HS, Arslanbek Erdem M. Darkness hormone or daylight hormone in women with systemic lupus erythematosus? *Clin Rheumatol.* 2023;42(1):93-99.
6. Conrad K, Bachmann M. Autoantibodies and systemic autoimmune diseases. *Autoantibodies and Autoimmunity,* 2006;225-245.
7. Weetman A, DeGroot LJ. Autoimmunity to the Thyroid Gland. In: Feingold KR, Anawalt B, Blackman MR, et al (ed). Endotext (Internet). Jan 14. South Dartmouth (MA): MDText.com, Inc.; 2000-. PMID: 25905407.
8. Siriwardhane T, Krishna K, Ranganathan V, et al. Exploring Systemic Autoimmunity in Thyroid Disease Subjects. *J Immunol Res.* 2018;6895146.
9. Matusiewicz A, Stróżyńska-Byrska J, Olesińska M. Polyautoimmunity in rheumatological conditions. *Int J Rheum Dis.* 2019;22(3):386-391.
10. Giuffrida G, Bagnato G, Campennì A, et al. Non-specific rheumatic manifestations in patients with Hashimoto's thyroiditis: a pilot cross-sectional study. *J Endocrinolo Investigation.* 2020;43(1):87-94.
11. Damoiseaux J, Andrade LEC, Carballo OG, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the international consensus on ANA patterns (ICAP) perspective. *Ann Rheum Dis.* 2019;78(7):879-889.
12. Moroni L, Bianchi I, Lleo A. Geoepidemiology, gender and autoimmune disease. *Autoimmun Rev.* 2012;11(6-7):A386-A392.
13. Agmon-Levin N, Damoiseaux J, Kallenberg C, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis* 2014;73:17-23.
14. Tektonidou MG, Anapliotou M, Vlachoyiannopoulos P, Moutsopoulos HM. Presence of systemic autoimmune disorders in patients with autoimmune thyroid diseases. *Ann Rheum Dis.* 2004;63(9):1159-1161.
15. Lazúrová I, Benhatchi K, Rovenský J, et al. Autoimmune thyroid disease and autoimmune rheumatic disorders: A two-sided analysis. *Ann New York Acad Sci.* 2009;1173(1):211-216.
16. Morita S, Arima T, Matsuda M. Prevalence of nonthyroid specific autoantibodies in autoimmune thyroid diseases. *J Clin Endocrinol Metab.* 1995;80(4):1203-1206.
17. Solomon DH, Kavanaugh AJ, Schur PH. American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. *Arthritis Rheum.* 2002;47(4):434-444.
18. Carbone T, Pafundi V, Tramontano G, et al. Prevalence and serological profile of anti-DFS70 positive subjects from a routine ANA cohort. *Sci Rep*. 2019;18;9(1):2177.
19. Wang B, Shao X, Song R, Xu D, Zhang JA. The emerging role of epigenetics in autoimmune thyroid diseases. *Front Immunol.* 2017;7;8:396.
20. Yan N, Zhou JZ, Zhang JA, et al. Histone hypoacetylation and increased histone deacetylases in peripheral blood mononuclear cells from patients with Graves' disease. *Mol Cell Endocrinol.* 2015;15;414:143-147.
21. Angiolilli C, Kabala PA, Grabiec AM, et al. Histone deacetylase 3 regulates the inflammatory gene expression programme of rheumatoid arthritis fibroblast-like synoviocytes. *Ann Rheum Dis.* 2017;76(1):277-285.
22. Cheng F, Lienlaf M, Wang HW, et al. A Novel role for histone deacetylase 6 in the regulation of the tolerogenic STAT3/IL-10 pathway in APCs. *J Immunol.* 2014;15;193(6):2850-2862.