THE INJECTOR 2022;1(3):124-131

Original Article

Comparison of inflammatory markers in euthyroid patients with positive and negative thyroid autoantibodies

Lezan Keskin[1], Bülent Yaprak[2], Doğu Karahan[3], Fatih Eskin[4]

[1]Malatya Turgut Ozal University Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolic Diseases, Malatya, Turkey

[2]Malatya Training and Research Hospital, Department of Internal Medicine, Malatya, Turkey

[3]Malatya Turgut Özal University Faculty of Medicine, Department of Internal Medicine, Malatya, Turkey

[4]Hitit University Faculty of Medicine, Department of Internal Medicine, Çorum, Turkey

ORCID IDs of the authors

LK: https://orcid.org/0000-0001-8283-4516

BY: https://orcid.org/0000-0001-5592-9755

DK: https://orcid.org/0000-0002-5387-2000

FE: https://orcid.org/0000-0002-3636-4004

Correspondence:

Author: Doğu Karahan

Address: Malatya Turgut Özal University, Faculty of Medicine, Department of Internal Medicine, Malatya, Turkey

Phone: +90 422 341 06 60

e-mail: dogu.karahan@ozal.edu.tr

Received: 1 November 2022

Revised: 27 November 2022

Accepted: 2 December 2022

Abstract

Objective: Autoimmune thyroid diseases can cause certain levels of systemic inflammation in the body. In this research, it was aimed to evaluate the thyroid autoantibody levels and systemic inflammation markers of those applicants for our outpatient clinic who were found to be euthyroid.

Methods: In our study, 220 patients, aged between 18-75 years, who were found to be euthyroid after thyroid hormone evaluation and did not use any thyroid medication, were retrospectively included. Patients' age, height, weight, hemogram parameters, erythrocyte sedimantation rate (ESR), C-reactive protein (CRP), free T3 (fT3), free T4 (fT4), thyroid stimulating hormone (TSH), anti-thyroglobulin (anti-TG) and anti-thyroid peroxidase (anti-TPO) values were recorded retrospectively by the researchers by examining the patient files. The relationship between inflammatory parameters, thyroid autoantibody levels (anti-TG and anti-TPO), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) were subjected to our investigation.

Results: The individuals who were participated in the research had a mean age of 46.2±13.8 (min=18, max=75). When the participants were assessed with regard to thyroid autoantibody positivity and negativity, ESR, CRP, NLR, MLR, PLR, eosinophil percentage, eosinophil count, basophil count, basophil percentage, mean platelet volume (MPV), it was seen that there was no statistically significant difference between the values. While there was a weak positive correlation between TSH and anti-TPO and anti-TG, there was a weak and significant positive correlation between the proportions of eosinophils and both anti-TG and anti-TPO.

Conclusion: No significant difference has been found in hematological systemic inflammation parameters between euthyroid patients with positive and negative thyroid autoantibodies. In addition, a positive correlation was observed between the percentage and count of eosinophils and thyroid autoantibodies levels.

Keywords: Autoantibody, euthyroid, inflammatory markers.

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 DOI: https://doi.org/10.5281/zenodo.7389121

INTRODUCTION

Deciding with thyroid function tests (TFT) alone in the evaluation of thyroid diseases may cause thyroid disease (Hashimoto, Graves, etc.) that carries a risk for the future to be overlooked. Elevated thyroid autoantibodies may be the first indicator of thyroid problems in many cases. Because there may be high levels of thyroid autoantibodies starting about 5-10 years before an abnormality is detected in TSH measurements. Thyroid autoantibody elevation may mean that an active attack and destruction of thyroid tissue has begun. If the autoimmune process progresses, symptoms such as chronic fatigue, panic attacks, anxiety, depression, and hair loss can be seen even if TSH is normal in the person (1). It has been observed that some metabolic diseases and some kinds of cancer are associated with this developing inflammation (2-4). Parameters including CRP, tumor necrosis factor-alpha (TNF-alpha), total leukocyte count and ESR can be used to determine the level of systemic inflammation (5). In addition to all these, the NLR has been employed in recent years to detect the systemic inflammatory response (6). NLR is an inexpensive, simple method that can be obtained by routine complete blood count; numerous studies have demonstrated its utility in assessing inflammation in some chronic conditions (including rheumatoid arthritis, asthma, hypertension and diabetes) and determining the prognosis in malignancies (colorectal cancer, breast, lung, ovarian and prostate cancer) (7-8). There is also an increase in platelet count in response to systemic inflammation (9). The measure known as MPV depicts the size of platelets, and is studied during a complete blood count. In inflammatory conditions that stimulate the bone marrow, platelet production increases. Young platelets released into the circulation have a larger volume. According to reports, MPV increases during the acute stage of inflammation and decreases during the chronic process due to destruction in the inflammation area (10). In inflammatory events, the platelet count increases, the lymphocyte count decreases, and the PLR increase. As a prognostic factor in many chronic inflammatory and malignant diseases, PLR has been investigated, and it has also been found to be associated with mortality in patients with acute coronary syndrome and end-stage renal disease (11).

The purpose of this study was to assess the association between the existence of autoantibodies and NLR, MPV and PLR, which are indicators of systemic inflammation in euthyroid patient groups. In this way, it is planned to evaluate the inflammatory process in euthyroid and thyroid autoantibody positive cases.

MATERIALS AND METHODS

This retrospective study was performed in compliance with the Declaration of Helsinki and with permission from the Malatya Turgut Özal University Non-interventional Clinical Research Ethics Committee (2022/146). This study was conducted by retrospectively examining the data of patients who applied to endocrinology and internal medicine clinics. The files of patients between the ages of 18-75 who submitted an application to the outpatient clinic and was subsequently included in the research. The power analysis revealed, the effect size was 0.3, the power was 95%, and the Type 1 error was 5%, and the research's required sample size was evaluated as 220 individuals. Inclusion criteria for study were being age between 18-75, being euthyroid, high and normal anti-TG and anti-TPO values and not having a history of drug use for thyroid disease. Exclusion criteria for study were having hypothyroidism or hyperthyroidism, during pregnancy or lactation, being under the age of 18 or over the age of 75, having acute illness or infection just before enrolling, renal disease, using oral contraceptives, having hematological disease, having a known malignancy, using acetyl salicylic acid, chronic rheumatological disease, presence of other autoimmune diseases and having chemotherapy and/or radiotherapy. The age, height, weight, hemogram parameters, ESR, fT3, fT4, TSH, anti-TG and anti-TPO values of the participants were recorded by retrospectively examining the patient files by the researchers. Hemogram parameters were studied on Sysmex XE2100 (Japan). NLR and PLR were calculated by the division of the absolute neutrophil count by the lymphocyte count and the absolute platelet count by the lymphocyte count, respectively.

 DOI: https://doi.org/10.5281/zenodo.7389121

Statistical analysis

The study data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 21.0 software. Mean, standard deviation, median, minimum and maximum values were calculated for all parameters. Descriptive values are shown in tables as numbers and frequencies (%), mean, and standard deviation. Shapiro -Wilk test was utilized as the normal distribution test. Data are shown as arithmetic mean (AM), standard deviation (SD), and median. Mann Whitney U test and Spearman correlation analysis were used in the analysis. Statistical significance was defined as a p value of <0.05.

RESULTS

The individuals who participated in the research had a mean age of 46.2±13.8 (min=17, max=75). 82.3% of the patients were female (n=181) and 17.7% were male (n=39). A total of 220 people, 110 of whom were thyroid autoantibody positive and 110 thyroid autoantibody negative, were included in the study. When the inflammation parameters were compared according to the autoantibody positivity of the patients, it was seen that there was no statistically significant difference between the groups (Table 1).

Table 1. Comparison of inflammation parameter according to the presence of autoantibodies

Parameters	**Autoantibody (-) group (n=110)**	**Autoantibody (+) group (n=110)**	***P***
CRP (mg/dl)	0.27 (0.02-3.90)	0.25 (0.01-3.13)	0.958
ESR (mm/h)	23.62±13.03	23.55±13.70	0.900
Eosinophil (%)	2.18±1.42	2.62±1.83	0.105
Eosinophil count (10^3/µl)	0.16±0.1	0.19±0.14	0.183
Basophil count (10^3/µl)	0.023±0.02	0.02±0.01	0.602
Basophil (%)	0.30±0.26	0.31±0.25	0.649
MPV (fl)	10.26±0.98	10.25±0.86	0.646
NLR	1.98±1.31	1.95±0.82	0.549
PLR	121.12±50.73	126.12±42.71	0.218
MLR	0.23±0.09	0.24±0.09	0.208

***Abbreviations**; CRP: C reactive protein, ESR: Erythrocyte sedimentation rate, MPV: Mean platelet volume, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio MLR: Monocyte to lymphocyte ratio, p<0.05 is accepted significant.*

 DOI: https://doi.org/10.5281/zenodo.7389121

Considering the correlations between thyroid function tests, thyroid autoantibody levels and inflammation parameters, TSH, anti-TG and anti-TPO were found to have a weak positive correlation ($p<0.05$) (Table 2). It was found that there was a positive weak significant correlation between the percentage of eosinophils and both anti-TG and anti-TPO ($p<0.01$) (Figure 1).

Table 2. Correlations between thyroid function tests, thyroid autoantibody levels and inflammation parameters

	Anti-TG	**Anti-TPO**	**TSH**	**fT3**	**fT4**
Anti-TG	—				
Anti-TPO	0.686***	—			
TSH	0.150*	0.193**	—		
fT3	-0.000	-0.011	-0.107	—	
fT4	-0.055	-0.061	-0.190**	0.104	—
CRP	-0.067	0.035	0.105	-0.017	0.056
NLR	0.029	0.037	-0.032	0.129	-0.022
MLR	0.074	0.097	0.007	0.118	0.067
PLR	0.070	0.035	0.035	0.053	0.020
Eosinophil count	0.072	0.170*	0.141*	0.045	-0.120
Eosinophil %	0.127*	0.166*	0.155*	0.015	-0.067
Basophil count	0.070	0.041	0.078	0.137*	-0.026
Basophil %	0.075	0.012	0.080	0.122*	0.008

Note 1: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ Note 2: The values are the correlation coefficient

***Abbreviations**; Anti-TG: Anti-thyroglobulin, Anti-TPO: Anti-thyroid peroxidase, TSH: Thyroid stimulating hormone, fT3: Free tri-iodothyronine, fT4: Free tetra iodothyronine, CRP: C-reactive protein, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio.*

DISCUSSION

Parameters such as CRP, TNF-alpha, total leukocyte count and ESR can be used to determine the level of systemic inflammation (5). In recent years, the increase in NLR and PLR has been frequently evaluated, studied and used in determining the systemic inflammatory response (6,11). The relationship between these inflammation parameters and the presence of autoantibodies in euthyroid cases was analyzed in our research. It was found that there was no significant difference between inflammation parameters in thyroid autoantibody positive patients. A positive weak significant correlation was discovered between both anti-TG and anti-TPO and the percentage of eosinophils. Systemic inflammatory parameters and NLR, PLR have been studied in the literature in many different thyroid diseases. There are studies focused on the distinction between malignant and benign thyroid

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nodules (12-14). Bilge et al. made research, in which they examined the systemic inflammation markers in patients with Hashimoto's Thyroiditis (HT), 145 women with HT and 60 age-matched healthy controls were evaluated. There was no significant difference between the control and patient groups in terms of general leukocyte and neutrophil counts. Compared to the control group, the lymphocyte count was lower, the platelet count, PLR and NLR were higher in the patient sample (12).

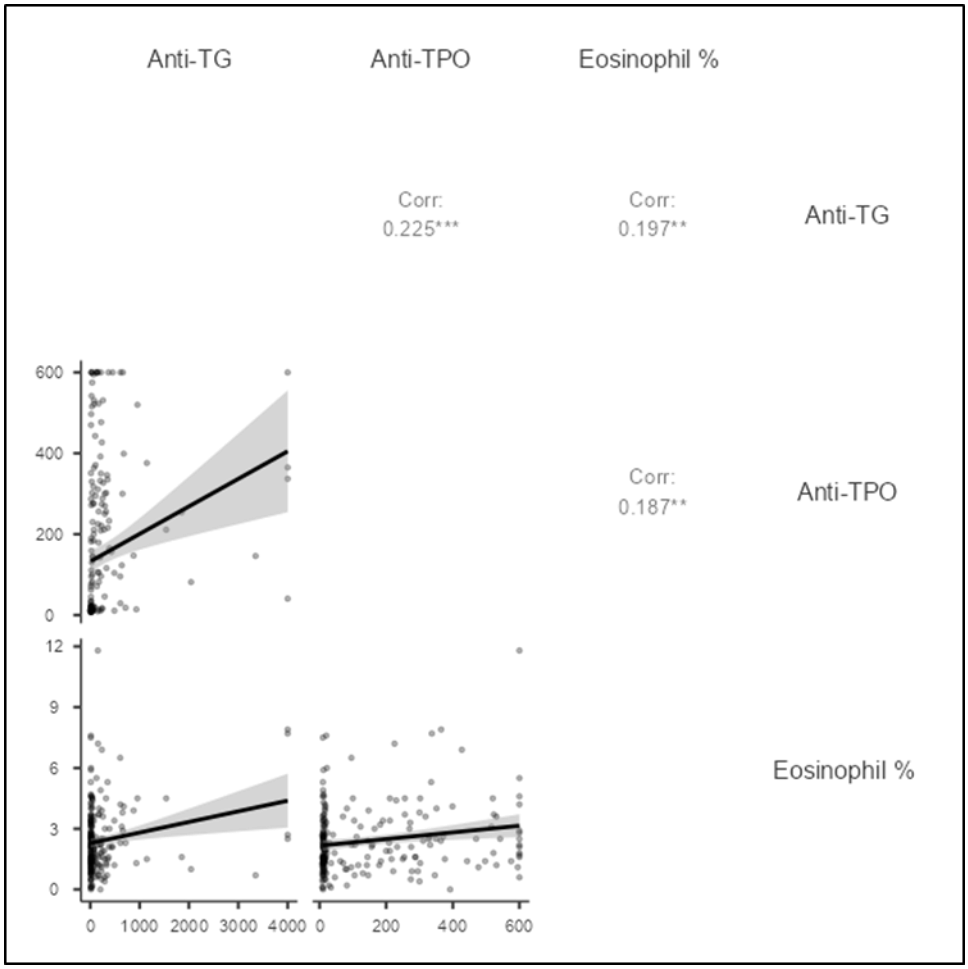

Figure 1. Correlation between % eosinophils and thyroid autoantibody levels

NLR and PLR are likely nonspecific indicators of immune disorder. In the study of Kutlutürk et al. when platelet count, NLR and PLR values were compared between euthyroid, hyperthyroid and subclinical hyperthyroid phases, they were not found to be statistically different (13). In another study by Martin et al. higher platelet count ($p<0.001$) and papillary thyroid cancer ($p=0.010$) were found in patients with papillary thyroid cancer under 55 years of age. MPV ($p=0.032$), NLR ($p=0.013$), ESR ($p=0.005$) and fibrinogen ($p=0.019$) levels were found to be higher in patients over 55 years of age (14). Yıldız et al. in their study, patients with papillary thyroid cancer and nodular hyperplasia were examined PLR, MPV and lymphocyte count were observed to be significantly higher in individuals with papillary thyroid cancer. Only the PLR can help in differentiating benign goiter from thyroid cancer. But MPV, lymphocyte count and platelet distribution width were found to be effective prognostic markers for papillary thyroid cancer (15). According to the study of Ergun et al. on patients with subacute thyroiditis, the lymphocyte count did not significantly differ between the subacute thyroiditis and control groups, but the ESR, leukocyte count, neutrophil count, NLR, fT4/fT3 ratio, and CRP were all significantly higher in the patient group compared to the control group. The patient group's MPV was shown to be considerably lower than that of the control group (16). In our study, however, no significant difference

 DOI: https://doi.org/10.5281/zenodo.7389121

was found between hematological systemic inflammation parameters and euthyroid patients with positive and negative thyroid autoantibodies. A positive correlation was observed only between the percentage and number of eosinophils and thyroid autoantibody levels. Koçer et al. in that study, level of NLR was observed to be significantly higher in papillary thyroid cancer cases compared to multinodular goiter patients, and it was emphasized that NLR value could be a potential marker in the differentiation of malign and benign thyroid disorders (17). In a meta-analysis of 3081 patients, it was stated that high NLR was not significantly associated with survival in thyroid diseases in general (18). Liu et al. in his study, it was found that there was no difference in NLR in patients with malignant and benign thyroid nodules, and high NLR values in cancer patients were associated with significantly larger tumor size (19). Sit et al. in his study, it was observed that NLR was higher in malignant thyroid diseases. (20). Ozmen et al. in his research, a significant correlation between NLR and CRP was found in thyroid cancer patients (21). The NLR, determined from peripheral blood, is accepted as a current and practical indicator of systemic inflammation. Kaya et al. in his study, it was found that while there was no difference for leukocytes or neutrophils in chronic autoimmune euthyroid patients, lymphocyte and NLR levels were higher. A positive correlation was found between NLR and CRP, anti-TPO, anti-TG, leukocyte and PLR (22). In a study conducted with Graves' patients, lymphocyte, monocytes, thrombocyte, fT3 and fT4 levels were found to be significantly higher in the graves group compared to the controls. TSH and NLR were found to be significantly lower in Graves compared to controls, and there were significant differences before and after treatment for monocytes, basophils, platelets and NLR (23). Onalan et al. in their study, it was found that PLR was statistically significantly lower and NLR was higher in patients diagnosed with hashimoto when compared to the healthy control group, and in addition to the existing findings, PLR and NLR were not significantly correlated with anti-TPO, TSH and fT4 (24). Arpaci et al. in their study, hashimoto patients and the control group were compared. CRP level was found to be higher in hashimoto patients than in the control group. There was no difference between the groups in terms of leukocytes, neutrophils, platelets or MPV. NLR and PLR were significantly different in HT patients (clinical, subclinical and euthyroid HT) compared to healthy subjects. A positive correlation was found between NLR, anti-TG and anti- TPO antibodies, and a negative correlation was found between PLR, TSH, anti-TPO and anti-TG (25). Ekambaram M et al. in a study evaluating whether eosinophilic infiltration of the thyroid gland is associated with HT, they found that the eosinophil-neutrophil ratio in smears with a diagnosis of HT was significantly higher than in smears with a diagnosis of colloid goiter. As a result of the study, eosinophilic infiltration of the thyroid gland was mostly evaluated as related to HT (26). Hidaka et al. the aim of this study was to evaluate the relationship between Graves' disease and allergic conditions. For this purpose, serum eosinophil-derived neurotoxin (EDN) levels were investigated in 30 Graves' patients, 50 HT and 39 normal healthy control groups. Compared to the serum level in normal subjects, EDN was increased in untreated patients with Graves' disease but normal in patients with HT. It was also found that there was a significant correlation between EDN level and serum activity of TSH receptor antibody (27). In our study, it is particularly striking that there is a positive correlation between the percentage and number of eosinophils and anti-TG and anti-TPO (r=0.127, r=0.166, r=0.177). Organized studies that will focus on these parameters and include more cases have the potential to highlight the importance of the relationship between eosinophils and anti-TG and anti-TPO.

Limitations There are some limitations in our study. These can be listed as the retrospective design of the study, its single-center design, and the relatively small number of cases. Although it was designed retrospectively, cases with complete clinical data and complete laboratory test results were included in the study.

CONCLUSION

According to the results of our study, no significant difference was found between the hematological systemic inflammation parameters in thyroid autoantibody-positive and negative euthyroid persons. A positive correlation was found between the

 DOI: https://doi.org/10.5281/zenodo.7389121

percentage and number of eosinophils and autoantibody levels. Based on the results of the study, even if the case group is euthyroid, it would be appropriate to follow up autoimmunity, which is reflected by thyroid autoantibody positivity, in terms of thyroid dysfunction in the future, especially in parallel with the increase in eosinophils. More studies are needed on thyroid autoantibody elevation and its contribution to inflammation in autoimmune thyroid disorders.

Conflicts of interest: The authors declare no conflict of interest.

Financial support and sponsorship: None.

Ethical approval: The study was performed in compliance with the Declaration of Helsinki and with permission from the Malatya Turgut Özal University Non-interventional Clinical Research Ethics Committee (2022/146).

Author contributions: Design of the study; LK - Supervision; LK, BY, DK, FE - Data collection &/or processing; LK - Performed data analysis; BY - Literature search; LK, DK, FE - Written by; LK, DK, FE - Critical review; LK, DK, FE.

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