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Evaluation of Thyroid Peroxidase Antibody Positivity and Cardiometabolic Markers in Healthy Individuals with Rheumatoid Factor Positivity

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Abstract

Background:

Rheumatoid factor (RF) may be positive in healthy individuals, yet its association with thyroid autoimmunity in this population remains unclear. This study evaluated anti-thyroid peroxidase antibodies (anti-TPO), thyroid function, lipid profile, inflammatory markers, and carotid intima–media thickness (CIMT) in asymptomatic RF-positive adults.

Material and Methods:

Fifty-nine clinically healthy adults without autoimmune disease were included and classified as RF-positive (n = 31) or RF-negative (n = 28). Thyroid function tests, anti-TPO levels, thyroid ultrasonography findings, lipid parameters, high-sensitivity C-reactive protein (CRP), and CIMT were compared between groups. Correlations between RF titers and metabolic or autoimmunity-related markers were analyzed.

Results:

Anti-TPO positivity was significantly higher in RF-positive individuals than in RF-negative controls (42.0% vs. 25.0%, $p < 0.001$), and mean anti-TPO levels were also higher ($p = 0.026$). Among thyroid function tests, only free thyroxine (fT4) levels were increased in the RF-positive group ($p = 0.034$), whereas free triiodothyronine (fT3) levels and the fT3/fT4 ratio were similar between groups. Thyroid ultrasonography findings, including heterogeneous parenchyma and nodule presence, did not differ significantly. RF-positive individuals exhibited higher low-density lipoprotein and total cholesterol levels ($p = 0.014$ and $p = 0.020$, respectively), while CRP, high-density lipoprotein, triglycerides, and CIMT values were comparable. Correlation analysis demonstrated a significant negative correlation between RF titers and anti-TPO levels ($r=–0.338$, $p = 0.009$).

Conclusion:

Asymptomatic RF-positive individuals showed increased anti-TPO positivity and less favorable lipid profiles compared with RF-negative controls, despite similar inflammatory markers and CIMT measurements. These findings suggest that RF positivity may coexist with subclinical thyroid autoimmunity independently of overt rheumatologic disease. Prospective studies are needed to clarify the clinical implications.

INTRODUCTION

Rheumatoid factor (RF) is a group of autoantibodies that develop against the Fc region of immunoglobulin G antibodies. RF is one of the oldest and most widely used serological markers in the

diagnosis of rheumatoid arthritis. [1] Rheumatoid arthritis (RA) is one of the best-studied examples of an autoimmune response. RF plays a central role in initiating and maintaining the autoimmune response. However, RF is not specific to RA; it can also be positive in healthy individuals, other autoimmune diseases, and infections. [2] RF can also be found as a normal part of the immune system and may play a role in defence against infections. [3] Anti-TPO antibodies are produced by the immune system against the TPO enzyme found in thyroid cells. These antibodies are found at high levels in autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease. Anti-TPO positivity is detected in almost all patients with Hashimoto's thyroiditis and in a significant proportion of patients with Graves' disease. [4, 5] Various studies have determined different frequencies of thyroid dysfunction, autoimmune thyroid diseases, and anti-thyroid peroxidase autoantibodies in RA patients in different geographical regions. Overall, 6% of rheumatoid arthritis patients have thyroid dysfunction, and 9% are positive for anti-thyroid peroxidase autoantibodies. [6]

Research indicates that anti-TPO antibodies may be positive not only in thyroid diseases but also in healthy individuals. Studies conducted in different populations show that anti-TPO positivity rates in healthy control groups vary between 6% and 25%. For example, studies conducted in China and Iran reported anti-TPO positivity rates of 9.2% and 10%, respectively, in healthy individuals. [7, 8] Considering the common pathways of anti-TPO and RF positivity and the possibility of these two autoantibodies being present in conditions other than their specific diseases, there is insufficient data in the literature on the frequency of anti-TPO positivity in patients who are rheumatoid factor positive but do not have a rheumatological diagnosis.

The aim of this study is to determine the status of anti thyroid peroxidase antibodies in individuals who tested positive for rheumatoid factor during a general health screening but who did not have a diagnosis of any autoimmune disease, and to evaluate the relationship between these positive results and metabolic markers and carotid intima-media thickness.

MATERIALS AND METHODS

This cross sectional study included 59 clinically healthy adults aged 18 to 70 years, who underwent routine health screening and were found to have no known autoimmune, rheumatologic, endocrine, infectious, or chronic systemic disease. Participants were classified into two groups based on their rheumatoid factor (RF) status: RF-positive (n = 31) and RF-negative (n = 28). Individuals with a history of thyroid disease, rheumatologic diagnosis, acute or chronic inflammatory conditions, cardiovascular disease, diabetes mellitus, hypertension, dyslipidemia requiring treatment, or use of any medication that could affect thyroid or metabolic parameters were excluded. All participants were asymptomatic at the time of evaluation. Fasting blood samples were collected from all individuals. Thyroid function tests (fT3, fT4, TSH) and anti-thyroid peroxidase antibody (Anti-TPO) levels were measured using standardized chemiluminescent immunoassay methods. Anti-thyroid peroxidase antibody (anti-TPO) positivity was defined according to the manufacturer’s recommended cut-off value (> 35 IU/mL). Serum lipid profile—including LDL cholesterol, HDL cholesterol, total cholesterol, and triglycerides—was analyzed

enzymatically. High-sensitivity C-reactive protein (CRP) levels were measured to assess systemic inflammation. Rheumatoid factor titers were determined using nephelometric or immunoturbidimetric methods, depending on laboratory standards. The study protocol was approved by the local institutional ethics committee, and written informed consent was obtained from all participants prior to enrollment.

Thyroid ultrasonography and carotid intima media thickness measurement

All participants underwent thyroid ultrasonography (USG) performed by a single experienced radiologist using high-resolution linear transducers. The thyroid gland was assessed for parenchymal echotexture (homogeneous vs. heterogeneous) and the presence of nodules (solitary or multiple). Nodule presence was recorded, but detailed nodule characteristics (such as size or risk classification) were not included in the primary analysis due to the asymptomatic nature of the study population.

Carotid intima–media thickness (CIMT) was measured by the same radiologist using B-mode ultrasonography with standardized protocols. Measurements were obtained from the far wall of the distal common carotid artery, approximately 1 cm proximal to the carotid bulb. Three measurements were averaged to calculate the final CIMT value. Subjects with carotid plaques or technically inadequate imaging were excluded from CIMT analysis. This approach minimized inter-observer variability; however, formal intra- or inter-observer reproducibility analysis was not performed. The study protocol was approved by the local institutional ethics committee (Decision No: 29/2024, Date: 26 June 2024). Written informed consent was obtained from all participants.

Stastistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test and visual methods. Normally distributed data are presented as mean ± standard deviation, whereas non-normally distributed data are expressed as median (interquartile range); categorical variables are reported as number and percentage.

Group comparisons were conducted using the independent samples t-test or the Mann–Whitney U test, as appropriate. Categorical variables were compared using the chi-square test or Fisher’s exact test. Associations between variables were evaluated using Pearson or Spearman correlation analyses. All tests were two-tailed, and $p < 0.05$ was considered statistically significant..

RESULTS

A total of 59 individuals were included in the study; 46 were women (78.0%) and 13 were men (22.0%). The frequency of heterogeneous thyroid parenchyma on thyroid ultrasonography was 41.9% in the RF-positive group and 25.0% in the RF-negative group ($p = 0.170$). The presence of nodules was 67.7% in RF-

positive individuals and 50.0% in RF-negative individuals (p = 0.166). Thyroid function tests showed no significant difference in fT3 levels (2.61 ± 0.52% vs. 2.78 ± 0.50%; p = 0.279) or fT3/fT4 ratio (2.39 ± 1.23 vs. 2.29 ± 0.80; p = 0.732) did not show significant differences, but the fT4 level was higher in the RF-positive group (2.05 ± 2.50 vs. 1.77 ± 2.31; p = 0.034). Anti-TPO positivity rate was higher in RF-positive individuals (42% vs.25%; p < 0.001). Among inflammatory markers, CRP was similar (3.89 ± 4.15 vs. 3.05 ± 2.83; p = 0.421). In the lipid profile, LDL (121.67 ± 33.88 vs. 102.86 ± 32.15; p = 0.014) and total cholesterol (198.94 ± 35.04 vs. 180.75 ± 35.81; p = 0.020) were significantly higher in RF-positive individuals, but no significant difference was found between HDL (50.72 ± 12.22 vs. 53.88 ± 12.73; p = 0.335) and triglyceride levels (139.54 ± 98.37 vs. 126.96 ± 61.67; p = 0.744). CIMT values were similar between the two groups (0.68 ± 0.33 mm vs. 0.62 ± 0.20 mm; p = 0.695). (Table 2) In the correlation analysis, a weak to moderate but statistically significant negative relationship was found between RF levels and anti-TPO (r = − 0.338, p = 0.009). In contrast, no significant relationship was observed between RF and thyroid function indicators such as the fT3/fT4 ratio (r = 0.085, p = 0.522) and the inflammatory marker CRP (r = 0.083, p = 0.532). (Table 3)

DISCUSSION

This study investigated thyroid autoimmunity and cardiometabolic parameters in asymptomatic but RF-positive individuals without a diagnosis of rheumatological disease. In our study, anti-TPO positivity was found in 42% of RF-positive individuals and 25% of RF-negative individuals. The prevalence of anti-TPO in healthy individuals in the general population may vary. European studies generally report anti-TPO positivity in women ranging from 5% to 16%. In some regions, such as Africa, this rate is reported to be in the range of 2.6% to 7%, while in some regions of Iran, this rate can reach high levels of 35.8% in women. [7, 9] The increased prevalence of anti-TPO positivity in RF-positive individuals is consistent with previous studies reporting a bidirectional association between rheumatoid arthritis and autoimmune thyroid diseases. Shared genetic susceptibility, including HLA-DRB1, STAT4, PTPN22, CTLA4, and VDR polymorphisms, may underlie this co-occurrence by contributing to impaired immune tolerance and enhanced autoreactive lymphocyte activation. [10–12] Importantly, our study extends these observations to RF-positive individuals without clinical autoimmune disease, suggesting that thyroid autoimmunity may emerge earlier or independently in genetically predisposed hosts. From another perspective, it may be worth considering that RF positivity alone does not necessarily indicate clinical disease and may coexist with other immunological markers. The absence of a significant relationship between RF and CRP, fT3/fT4 ratio, and CIMT in our study may make the notion that RF positivity is not directly related to systemic inflammation in asymptomatic individuals more plausible. Nevertheless, molecular, genetic analysis and studies involving larger, more homogeneous patient populations may provide guidance in resolving this dilemma.

Although anti-TPO positivity was more frequent in RF-positive individuals, an inverse correlation was observed between RF titers and anti-TPO levels. This finding suggests that increasing RF concentrations may not directly parallel the degree of thyroid autoimmunity. Rather than indicating a contradictory result, this pattern may reflect heterogeneity in immune activation pathways and the non-linear behavior

of autoantibody production. RF positivity alone may represent a distinct immunological phenotype that does not necessarily correspond to proportional increases in other autoantibody titers [13].

In our study, while LDL and total cholesterol levels were elevated in RF-positive individuals, HDL and triglyceride levels did not show a significant difference between the groups. Although CIMT values were higher in the RF-positive group, this difference was not statistically significant. Research shows that small increases in LDL and total cholesterol levels can indicate early changes in metabolism even in otherwise healthy individuals. [14] However, isolated increases may not always lead to measurable changes in blood vessel structure, especially in the absence of concomitant inflammation. CIMT, a marker of subclinical atherosclerosis, is known to correlate more strongly with age, gender, hypertension, diabetes, and chronic dyslipidemia than with isolated lipid abnormalities or single autoantibody positivity. [15] These factors were excluded in our study. Furthermore, studies emphasize that single CIMT assessments show limited sensitivity in predicting cardiovascular outcomes, whereas longitudinal variations have greater prognostic significance than static measurements. Collectively, these findings suggest that RF-positive individuals may exhibit minor lipid abnormalities, but these changes may not be of sufficient magnitude or duration to produce detectable differences in CIMT in a healthy, asymptomatic population.

Our study has certain limitations. Firstly, our sample size is limited; studies conducted on larger populations would increase the generalizability of the findings. Due to the cross-sectional design of the study, causal relationships between rheumatoid factor positivity and thyroid autoimmunity cannot be established. As all individuals included in the study were asymptomatic and clinically healthy, no long-term follow-up was conducted; therefore, no information can be provided on whether clinical disease will develop in the future. Furthermore, potential confounding variables such as genetic factors, dietary habits, and environmental factors that may be associated with thyroid autoimmunity and dyslipidemia were not evaluated.

Asymptomatic healthy RF-positive individuals showed higher anti-TPO positivity rates compared to RF-negative individuals, while no differences were observed in CRP, HDL, triglycerides, or CIMT. RF positivity may be observed in healthy individuals alongside the presence of anti-TPO antibodies. These findings were observed in clinically healthy, asymptomatic individuals without diagnosed autoimmune, metabolic, or cardiovascular disease, highlighting the potential coexistence of subclinical immune alterations in an otherwise healthy population. However, more comprehensive, prospective, and genetically based studies are needed to elucidate the biological significance of this relationship and its potential long-term consequences.

Declarations

Author Contribution

All authors contributed to the design of the study, data collection, analysis, and writing of the manuscript.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1
Comparison of Demographic Characteristics, Thyroid Ultrasonography Findings, and Thyroid Function Tests between RF-Positive and RF-Negative Groups

Variable		**RF-positive**	**RF-negative**	***p*-value**
Age (years, mean ± SD)		47 ± 14	46 ± 11	0.858
Sex	Male, n (%)	5 (16.1)	8 (28.6)	0.515
	Female, n (%)	26 (83.9)	20 (71.4)	
Heterogeneous echotexture on thyroid USG, n (%)		13 (41.9)	7 (25.0)	0.170
Presence of nodules, n (%)		21 (67.7)	14 (50.0)	0.166
fT3 (pg/mL, mean ± SD)		2.61 ± 0.52	2.78 ± 0.50	0.279
fT4 (ng/dL, mean ± SD)		2.05 ± 2.50	1.77 ± 2.31	**0.034**
fT3/fT4 ratio (mean ± SD)		2.39 ± 1.23	2.29 ± 0.80	0.732
Anti-TPO (IU/mL, mean ± SD)		22.30 ± 49.91	19.96 ± 28.40	**0.026**
Anti-TPO positivity, n (%)		13 (42.0)	7 (25.0)	**< 0.001**
RF, rheumatoid factor;				
Anti-TPO, anti-thyroid peroxidase antibody;				
fT3, free triiodothyronine;				
fT4, free thyroxine;				
USG, thyroid ultrasonography;				
SD, standard deviation;				
n, number of subjects;				
p < 0.05 was considered statistically significant				

Table 2
Comparison of Inflammatory and Lipid Profile Parameters between RF-Positive and RF-Negative Groups

Variable	RF-positive	RF-negative	p-value
CRP (mg/L, mean ± SD)	3.89 ± 4.15	3.05 ± 2.83	0.421
LDL cholesterol (mg/dL, mean ± SD)	121.67 ± 33.88	102.86 ± 32.15	**0.014**
HDL cholesterol (mg/dL, mean ± SD)	50.72 ± 12.22	53.88 ± 12.73	0.335
Total cholesterol (mg/dL, mean ± SD)	198.94 ± 35.04	180.75 ± 35.81	**0.020**
Triglycerides (mg/dL, mean ± SD)	139.54 ± 98.37	126.96 ± 61.67	0.744
CIMT (mm, mean ± SD)	0.68 ± 0.33	0.62 ± 0.20	0.695
RF, rheumatoid factor;			
CRP, C-reactive protein;			
LDL, low-density lipoprotein;			
HDL, high-density lipoprotein;			
CIMT, carotid intima–media thickness;			
SD, standard deviation;			
n, number of subjects;			
$p < 0.05$ was considered statistically significant			

Table 3
Correlation Analysis between Rheumatoid Factor Levels and Thyroid Functions, Anti TPO levels, Lipid Profile, and CIMT

Variable	**r**	**p-value**
fT3/fT4 ratio	0.085	0.522
Anti-TPO	-0.338	0.009
CRP	0.083	0.532
LDL cholesterol	0.251	0.055
HDL cholesterol	-0.157	0.236
Total cholesterol	0.252	0.054
Triglycerides	0.089	0.504
CIMT	0.052	0.697
RF, rheumatoid factor;		
Anti-TPO, anti-thyroid peroxidase antibody;		
fT3, free triiodothyronine;		
fT4, free thyroxine;		
CRP, C-reactive protein;		
LDL, low-density lipoprotein;		
HDL, high-density lipoprotein;		
CIMT, carotid intima–media thickness; r, correlation coefficient;		
SD, standard deviation;		
$p < 0.05$ was considered statistically significant.		