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Research Article

The relationship between homocysteine and autoimmune subclinical hypothyroidism

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Abstract

Objectives: Homocysteine, an atherosclerotic marker, is thought to be associated with chronic inflammation and autoimmunity. The aim of this study was to investigate the association between the homocysteine level, lipid parameters, high-sensitivity C-reactive protein (hs-CRP), and autoantibodies in subclinical hypothyroid patients.

Methods: A total of 100 cases, 50 patients who were older than 18 years of age and diagnosed with subclinical hypothyroidism due to Hashimoto's thyroiditis and 50 controls with no known disease, were included in the study.

Results: Homocysteine (9.6 µmol/L vs 5.5 µmol/L; p<0.001), hs-CRP (2.4 mg/L vs 0.9 mg/L; p<0.001), and low-density lipoprotein-cholesterol levels (115.9±32.7 mg/dL vs 100.3±23.9 mg/dL) (p=0.008) were determined to be higher in the subclinical hypothyroidism group, and the high-density lipoprotein (HDL) cholesterol level (51.2±12.7 mg/dL vs 58.8±15.2 mg/dL; p=0.008) was lower in the subclinical hypothyroidism group compared with the healthy control group. A positive correlation was determined between the homocysteine level and the hs-CRP (r=0.312; p=0.027), anti-thyroid peroxidase (r=0.505; p<0.001), and anti-thyroglobulin (r=0.318; p=0.031) levels in the subclinical hypothyroidism group.

Conclusion: In the regression analysis performed, HDL cholesterol, hs-CRP, and homocysteine levels were each found to be an independent risk factor for subclinical hypothyroidism. Our results indicated that homocysteine was associated with subclinical hypothyroidism.

Keywords: Atherosclerosis, autoimmunity, Hashimoto's disease, homocysteine

In Hashimoto's thyroiditis, an intensive inflammatory response occurs via cellular immunity in thyroid tissue due to various factors (genetic, hormones, excessive iodine, radiation, etc.). Proinflammatory cytokines (interleukin-6, interferon gamma, tumor necrosis factor alpha, interleukin-1b, etc.) synthesized principally from T cells play a role in this immune response [1]. CD4+ T helper 1 cells induce CD8+ cytotoxic T helper 2 cells in the thyroid gland via these proinflammatory cytokines as a result of the infiltration of T and B cells due to the reactivity of autoantibodies and loss of immune tolerance, and trigger an apoptotic process, leading to the destruction of thyroid cells [2-3].

It has been demonstrated in many studies that atherosclerosis in the cardiovascular system may occur as a result of increasing chronic inflammation and fluctuations of thyroid hormone levels during the subclinical hypothyroidism phase in Hashimoto's thyroiditis [4-7]. However, to our knowledge, there has been no study investigating the association between homocysteine level, autoimmunity, and chronic inflammation in subclinical hypothyroidism.

Homocysteine is an intermediate metabolite that emerges as a result of the metabolism of the amino acid methionine. The plasma concentration may vary due to genetic, pathological, or nutritional factors. Homocysteine causes endothelial dys-

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function through damage to the elastin lamina and accumulation of lipoprotein-proteoglycan complexes in the vessel wall as a result of a chemical injury in the endothelial tissue [8, 9]. Homocysteinemia, which presents with high levels of homocysteine, is considered to be a risk factor for coronary artery disease [10, 11]. Subclinical hypothyroidism is a clinical condition associated with increased chronic inflammation, oxidative stress, and lipid peroxidation. Therefore, homocysteine, an atherosclerotic marker, may be hypothesized to be high in cases of subclinical hypothyroidism. This study was designed to investigate the association between homocysteine level, lipid parameters, high-sensitivity C-reactive protein (hs-CRP) level, and autoantibodies in patients who had previously been diagnosed with subclinical hypothyroidism.

Materials and Methods

Study population

This research was a dissertation study conducted with a retrospective design at Ankara Numune Education and Research Hospital between February and March, 2018. The Clinical Research Ethics Board of Ankara Numune Education and Research Hospital unanimously granted scientific and ethical approval for the study on January 17, 2018 with decision number 1745/2018.

A total of 100 cases, 50 patients older than 18 years of age and diagnosed with subclinical hypothyroidism due to Hashimoto's thyroiditis, and 50 volunteers with no known disease, were included in the study.

Those with known cardiovascular or cerebrovascular diseases, venous or arterial acute or chronic thrombus in any site of the body, acute-chronic kidney or liver diseases, serious surgical intervention, hereditary homocysteinemia, B12 or folic acid deficiency, individuals using any medication that may lead to high levels of homocysteine and those who smoked or consumed alcohol were excluded from the study.

The relevant laboratory parameters of patients who had been diagnosed with subclinical hypothyroidism due to Hashimoto's thyroiditis in the clinic and were in follow-up, and whose homocysteine level had been tested in addition to assessment of thyroid function, thyroid autoantibodies, lipid parameters, thyroid ultrasonography, and hs-CRP were recorded. In addition, the patients' height, weight, body mass index (BMI), and gender were recorded. The hospital real-time health data analysis system was used to collect the parameters.

The diagnosis of Hashimoto's thyroiditis was made based on positive findings of one or both of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) among the laboratory parameters and/or an ultrasonographic result consistent with thyroiditis (heterogeneous parenchymal echogenicity of thyroid) [12]. Following a diagnosis of Hashimoto's thyroiditis, patients with a thyroid-stimulating hormone (TSH) level above the normal range but with a free thyroxin (fT4) level within the normal range were considered to have subclinical hypothyroidism.

The individuals selected as the control group had TSH, fT4, anti-Tg, and anti-TPO measurements within the normal range, and a diagnosis of Hashimoto's thyroiditis was discarded during ultrasonographic examination.

Parameters investigated

Blood samples were collected between 8:00 and 10:00 in the morning following 12 hours of fasting. All of the samples were assayed in the same laboratory with the same kit within a maximum of time interval of 1-2 hours. TSH (normal range: 0.5-4 µIU/mL), fT4 (normal range: 0.9-1.2 ng/dL), anti-TPO (normal range: 0-9 IU/mL), and anti-Tg (normal range: 0-4 IU/mL) levels were measured using a Cobas e 601 analyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland) using an electrochemiluminescence immunoassay method. Homocysteine levels were also measured with the Cobas e 601 analyzer using an electrochemiluminescence method.

Ultrasonographic examination of thyroid

Thyroid ultrasonography was performed by a single individual using a 7.5-mHz probe and a Logic 7 ultrasonography device (GE Healthcare, Inc. Chicago, IL, USA). The examination included evaluation of the size, echogenicity, and vascularization of the thyroid gland, the presence of a nodule, and assessment of peripheral lymph nodes.

Statistical analysis

Statistical evaluation was performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Numerical variables exhibiting a normal distribution were represented as mean±SD, and those that did not demonstrate a normal distribution were represented as median (min-max). Categorical variables were defined as count and percentage. For numerical variables, the Student's t-test and the Mann-Whitney U test were used for comparisons of subclinical hypothyroidism due to Hashimoto's thyroiditis and the control group. A chi-square and Fisher's exact test were used for the comparison of categorical variables. Pearson's and Spearman's correlation analysis were used to determine the association between numerical variables. Step-wise multivariate logistic regression analysis was used to determine potential independent predictors for the presence of subclinical hypothyroidism due to Hashimoto's thyroiditis. Receiver operating characteristic curve analysis was used to assess the diagnostic performance of independent predictors determined by regression analysis. The cut-off value was determined using the Youden index. A p value of <0.05 was considered to be significant.

Results

The study population comprised 50 controls (17 males, 33 females) and 50 patients with subclinical hypothyroidism due to Hashimoto's thyroiditis (10 males, 40 females). The distribu-

tion of demographic and clinical findings of the study population is summarized in Table 1. The mean BMI of the subclinical hypothyroidism patients was greater than that of the control group (28.6±4.7 kg/m^2 vs 25.0±3.8 kg/m^2; p<0.001). The mean systolic and diastolic blood pressures of the subclinical hypothyroidism patients did not differ significantly from those of the control group (respectively, 124.0±8.1 mm Hg vs 120.7±10.8 mm Hg; p=0.088 and 65.3±6.2 mm Hg vs 63.5±6.3 mm Hg; p=0.152).

The distribution of the laboratory findings of the study population is presented in Table 2. The median TSH level was higher in the subclinical hypothyroidism group compared with the control group (5.4 µIU/mL vs 1.7 µIU/mL; p<0.001), whereas the median fT4 level did not differ significantly (1.1 ng/dL vs 1.2 ng/dL; p=0.396). The median anti-Tg level (219.5 IU/mL vs 11.1 IU/mL; p<0.001) and the median anti-TPO level (163.6 IU/mL vs 7.1 IU/mL; p<0.001) were higher in the subclinical hypothyroidism group compared with the control group. In the lipid profile, the mean total cholesterol and median triglyceride levels did not differ significantly. In the subclinical hypothyroidism group, the median low-density lipoprotein (LDL) level and the mean non-high-density lipoprotein (HDL) level were determined to be higher than in the control group (respectively, 115.9±32.7 mg/dL vs 100.3±23.9 mg/dL; p=0.008 and 143.7±42.6 mg/dL vs 121.4±30.7 mg/dL; p=0.003) and the mean HDL level was found to be lower (51.2±12.7 mg/dL vs 58.8±15.2 mg/dL; p=0.008). In the subclinical hypothyroidism group, the median homocysteine level (9.6 µmol/L vs 5.5 µmol/L; p<0.001) and the median hs-CRP level (2.4 mg/L vs 0.9 mg/L; p<0.001) were higher than those of the control group.

Factors associated with the homocysteine level are presented in Table 3. In the subclinical hypothyroidism group, a positive correlation was determined between homocysteine level and hs-CRP (r=0.312; p<0.027), TSH (r=0.292; p<0.040), anti-TPO (r=0.505; p<0.001) (Fig. 1), and anti-Tg (r=0.318; p<0.031) (Fig. 2) levels.

The retrospective multivariate logistic regression model performed to identify predictors of subclinical hypothyroidism revealed that a low HDL level (odds ratio [OR]: 1.364; p=0.023), increased hs-CRP (OR: 1.364; p=0.019) and in-

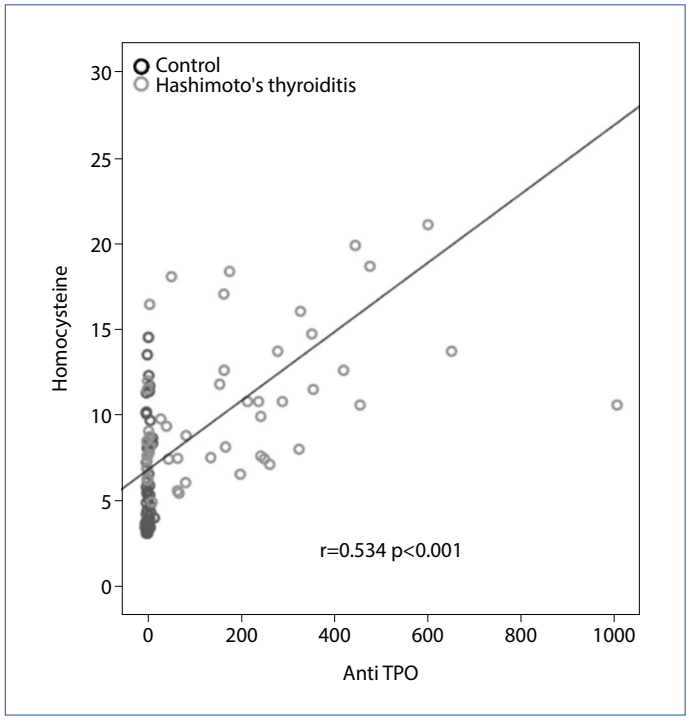

Figure 1. The relationship between homocysteine and anti-thyroid peroxidase levels.
Anti-TPO: Anti-thyroid peroxidase.

Table 1. Distribution of demographic and clinical findings in the study population

Variables	Subclinical hypothyroidism n=50	Control n=50	p
Gender, n (%)			
Female	40 (80.0)	33 (66.0)	0.176
Male	10 (20.)	17 (34.0)	
Age (years)	37.7±12.3	36.3±11.9	0.564
BMI (kg/m^2)	28.6±4.7	25.0±3.8	<0.001*
Smoking n (%)			
Non smoker	50 (100.0)	50 (100.0)	-
Smoker	0 (.0)	0 (.0)	
Chronic disease n (%)			
(-)	50 (100.0)	50 (100.0)	-
(+)	0 (.0)	0 (.0)	
SBP (mm Hg)	124±8.1	120.7±10.8	0.088
DBP (mm Hg)	65.3±6.2	63.5±6.3	0.152

*P<0.05 is statistically significant. Categorical variables were expressed as number (%), numerical variables were expressed as mean±SD. BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure.

Table 2. Distribution of laboratory findings in the study population

Variables	Subclinical hypothyroidism n=50	Control n=50	p
Ultrasonography			
Homogeneous	1 (2.0)	50 (100.0)	<0.001*
Heterogeneous	49 (98.0)	-	
TSH (µIU/mL)	5.4 (1.1-14.7)	1.7 (0.5-3.6)	<0.001*
FT4 (ng/dL)	1.1 (0.9-1.5)	1.2 (0.8-15.4)	0.396
Anti-Tg (IU/mL)	219.5 (10-4000)	11.1 (4-95.2)	<0.001*
Anti-TPO (IU/mL)	163.6 (5-1000)	7.1 (2-20.6)	<0.001*
Total cholesterol (mg/dL)	194.8±42.2	180.2±32.2	0.056
Triglycerides (mg/dL)	105.5 (36-771)	88.5 (39-280)	0.090
LDL cholesterol (mg/dL)	115.9±32.7	100.3±23.9	0.008*
HDL cholesterol (mg/dL)	51.2±12.7	58.8±15.2	0.008*
Non-HDL (mg/dL)	143.7±42.6	121.4±30.7	0.003*
Homocysteine (µmol/L)	9.6 (5-50)	5.5 (3.2-14.5)	<0.001*
Hs-CRP (mg/L)	2.4 (0.4-28.7)	0.9 (0.2-6)	<0.001*

*P<0.05 is statistically significant. Categorical variables were expressed as number (%), numerical variables were expressed as mean±SD. Anti-Tg: Anti-thyroglobulin; Anti-TPO: Anti-thyroid peroxidase; FT4: Free thyroxine; HDL: High-density lipoprotein; Hs-CRP: High-sensitivity C-reactive protein; LDL: Low-density lipoprotein; TSH: Thyroid-stimulating hormone.

Table 3. Clinical and demographic findings associated with homocysteine

Variables	Subclinical hypothyroidism r	p
Age	0.164	0.256
Systolic blood pressure	0.137	0.342
Diastolic blood pressure	0.197	0.145
BMI	0.083	0.568
TSH	0.292	0.040*
FT4	-0.252	0.077
Anti-Tg	0.318	0.031*
Anti-TPO	0.505	<0.001*
Total cholesterol	-0.180	0.211
Triglycerides	-0.110	0.449
LDL-cholesterol	-0.142	0.326
HDL-cholesterol	-0.083	0.568
Non-HDL	-0.175	0.225
Hs-CRP	0.312	0.027*

*P<0.05 is statistically significant. Anti-Tg: Anti-thyroglobulin; Anti-TPO: Anti-thyroid peroxidase; BMI: Body mass index; FT4: Free thyroxine; HDL: High-density lipoprotein; Hs-CRP: High-sensitivity C-reactive protein; LDL: Low-density lipoprotein; TSH: Thyroid-stimulating hormone.

creased homocysteine level (OR: 1.401; p<0.001) were independent factors (Table 4).

There was a significant difference in diagnostic performance between homocysteine and hs-CRP in predicting the presence of subclinical hypothyroidism (Δ area under the curve [AUC]: 0.041; p=0.473), and homocysteine was determined to have a higher diagnostic performance compared with HDL level (Δ AUC: 0.193; p=0.005). A homocysteine level of >6.19 µmol/L was found to predict the presence of subclinical hypothyroidism with a sensitivity of 92% and specificity of 60%. (+PPV: 97.8%, -NPV: 28.3%). An hs-CRP level of >1.65 mg/L was determined to predict subclinical hypothyroidism with a sensitivity of 66% and a specificity of 76%. (+PPV: 98.1%, -NPV: 10.5%) (Fig. 3).

Table 4. Independent predictors of subclinical hypothyroidism

Variables	Odds ratio	95% CI Lower	95% CI Upper	p
HDL-cholesterol	0.954	0.915	0.993	0.023*
hs-CRP	1.364	1.053	1.768	0.019*
Homocysteine	1.401	1.162	1.689	<0.001*
	Nagelkerke R^2=0.512, p<0.001			

*P<0.05 is statistically significant. HDL: High-density lipoprotein; Hs-CRP: High sensitivity C-reactive protein.

Discussion

In this study, the homocysteine, hs-CRP, and LDL cholesterol levels were observed to be higher and the HDL cholesterol level was lower in the autoimmune subclinical hypothyroidism group compared with the healthy control group. In the subclinical hypothyroidism group, a positive correlation was determined between homocysteine level and hs-CRP, anti-TPO, and anti-Tg levels. In the regression analysis performed, the HDL cholesterol, hs-CRP, and homocysteine levels were each found to be an independent risk factor for subclinical hypothyroidism.

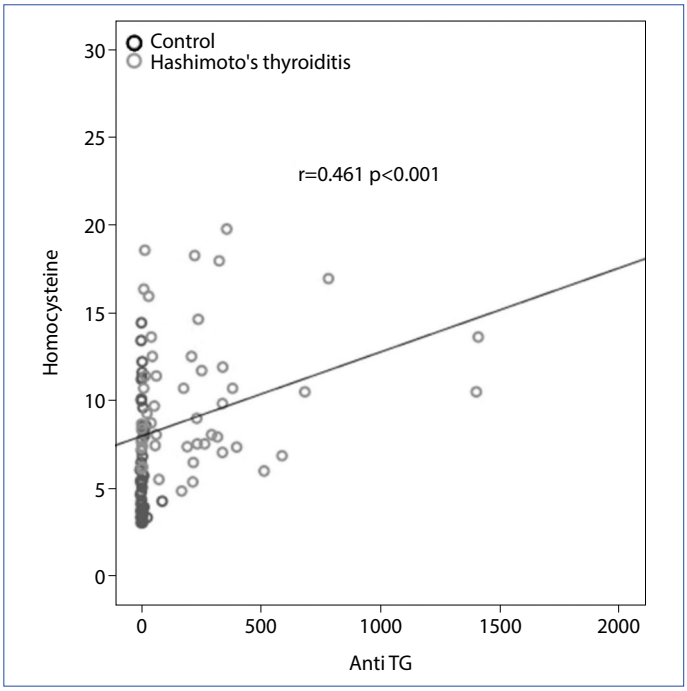

Figure 2. The relationship between homocysteine and anti-thyroglobulin levels.
Anti-Tg: Anti-thyroglobulin.

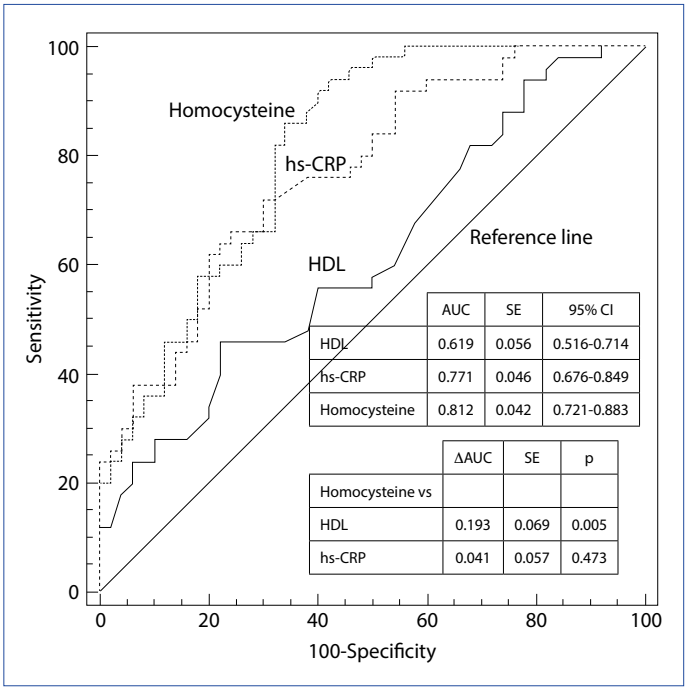

Figure 3. Diagnostic performance evaluation of independent predictors predicting the presence of subclinical hypothyroidism.
HDL: High-density lipoprotein; Hs-CRP: High-sensitivity C-reactive protein.

Homocysteine has primary atherogenic and prothrombotic properties. Histopathological characteristics of homocysteine-induced vascular damage include intimal thickening, destruction of elastic lamina, smooth muscle hypertrophy, marked thrombocyte accumulation, and the formation of a thrombocyte-rich occlusive thrombus [13, 14]. Homocysteine promotes monocyte chemoattractant protein-1 and interleukin-8 expression and secretion, facilitating leukocyte influx [15]. The thiolactone metabolite of homocysteine combines with LDL cholesterol, producing aggregates accumulated by vascular macrophages in the arterial intima, and these foam cells may then release the lipid into atherosclerotic plaques [16]. Homocysteine increases smooth muscle cell proliferation and collagen synthesis [17]. Prothrombotic effects of homocysteine previously demonstrated in patients with acute coronary syndrome were determined to be associated with weakening of plasminogen activator binding sites in endothelial cells, activation of factors VII and V, inhibition of protein C and heparin sulphate, an increase in fibrinopeptide A, a reduction of endothelial antithrombotic activity due to prothrombin fragments 1 and 2, increased blood viscosity, and alterations in the function of thrombomodulin [18]. The oxidative stress caused by free radicals formed during the oxidation of reduced homocysteine may directly damage endothelial cells [19]. Marked thrombocyte accumulation may be directly secondary to the proaggregant effects of homocysteine or may be secondary to endothelium-derived platelet inhibition [20].

Some studies in the literature have investigated the clinical implications of these pathophysiological mechanisms and the association between homocysteine and atherosclerosis and thrombosis. In a study of 830 cases of type 2 diabetes mellitus, Soinio et al., [21] found that homocysteine was an independent risk factor for coronary artery disease. Similarly, Pancharuniti et al., [22] observed a marked risk reduction in coronary artery disease with a lower plasma homocysteine level. Hoogeveen et al. [23] reported that in patients with type 2 diabetes mellitus, hyperhomocysteinemia significantly decreased 5-year mortality independently of other cardiovascular risk factors. In a study conducted by Schnyder et al., [24] however, homocysteine was shown to be closely associated with restenosis and major cardiac events in later periods in coronary angioplasty patients.

Considering the aforementioned pathophysiological mechanisms and previous clinical studies, it seems apparent that homocysteine is a parameter that is closely associated with atherosclerotic cardiovascular diseases.

It has been demonstrated in many preclinical and clinical studies that autoimmunity-mediated subclinical hypothyroidism contributes to the development of atherosclerosis via various mechanisms. In a study conducted by Tseng et al. [25] of 115.746 participants in Taiwan with a history of a thyroid disease, subclinical hypothyroidism was shown to be closely associated with all-cause deaths and deaths due to cardiovascular diseases. Carbotta et al. [26] found a significant increase in the incidence of coronary artery disease in chronic autoimmunity-mediated subclinical hypothyroidism. Moreover, Işguven et al. [27] studied 66 autoimmune euthyroid patients and it was demonstrated that a significant increase had occurred

in carotid intima media thickness, which is an early atherosclerotic indicator. Vaya et al. [28] reported a significant increase in the risk factors of plasma viscosity, fibrinogen, red blood cell distribution width, and homocysteine, all of which are closely associated with atherosclerosis, in autoimmune subclinical hypothyroidism patients. In the study conducted by Franca et al. [29], a significant increase was determined in the carotid intima thickness in the autoimmune subclinical hypothyroidism group and this was shown to be closely associated with metabolic syndrome.

Based on these studies, it can be said that autoimmune subclinical hypothyroidism is closely associated with the development of atherosclerosis as a result of chronic inflammation; increased immune reactions secondary to autoimmunity, hyperlipidemia and consequent lipid peroxidation due to thyroid hormone insufficiency; increased oxidative stress; increased plasma viscosity; and the development of endothelial dysfunction due to increased damage in vascular intimal tissues due to fibrinogen, fibrin degradation products, and homocysteine.

In our study, the homocysteine level was determined to be higher in the group with subclinical hypothyroidism due to Hashimoto's thyroiditis compared with the healthy control group. Deicher et al., [30] Turhan et al., [31] and Sengul et al. [32] also reported a homocysteine level higher in a subclinical hypothyroidism group. While some studies only reported on the homocysteine level in the subclinical hypothyroidism group, other studies noted that increased homocysteine levels may be associated with hyperlipidemia. In our study, in addition to the homocysteine level, the hs-CRP level was also determined to be significantly higher in this group. Furthermore, a positive correlation was observed between the levels of homocysteine and hs-CRP. This indicates that an elevated homocysteine level may be associated with chronic inflammation. Additionally, a positive correlation was also determined between the TSH level and the homocysteine level. This suggests that the homocysteine level is closely associated with thyroid functions. The determination of a significant reduction in the homocysteine level after treatment with levothyroxin given to euthyroid patients with Hashimoto's thyroiditis by Owecki et al. [33] suggests that increased homocysteine levels may also be influenced by thyroid function impairments that do not increase to clinical significance.

Additionally, our findings revealed a positive correlation between the homocysteine level and anti-TPO and anti-Tg levels. This correlation suggests that an increased homocysteine level may be closely associated with autoimmunity in the autoimmune subclinical hypothyroidism group. No significant correlation was determined between the homocysteine level and lipid parameters in the subclinical hypothyroidism group. However, a significant increase in the LDL cholesterol level and a significant reduction in HDL cholesterol were determined in the subclinical hypothyroidism group compared with the healthy control group. There may be an association between homocysteine level and lipid parameters that did not reach the level of significance.

In the regression analysis we performed, a low HDL cholesterol level, as well as hs-CRP and homocysteine levels, were determined to be independent risk factors for subclinical hypothyroidism. This analysis indicates that low HDL cholesterol levels and high hs-CRP and homocysteine levels are strong risk factors for atherosclerosis and indirect indicators of atherosclerosis as a result of the mechanisms described. These factors also appear to be risk factors for subclinical hypothyroidism and thereby indirect indicators of subclinical hypothyroidism, but this is speculation independent of clinical status. In that case, it may be concluded that autoimmune subclinical hypothyroidism may be closely associated with atherosclerosis.

The retrospective nature of our study, the small number of cases, and the fact that parameters such as carotid intima media thickness and left ventricular mass index, as indicators of atherosclerosis, were not studied and no association was revealed between these parameters and homocysteine level are among the major limitations of our study. However, the fact that hs-CRP and lipid parameters, which are equally valuable indicators for subclinical atherosclerosis demonstrates the value of our research.

In our study, the homocysteine level was higher in the subclinical hypothyroidism group. As homocysteine is an atherosclerotic risk factor, lowering the homocysteine level with medical therapies in patients with subclinical hypothyroidism will be a protective approach. Further studies are needed to determine the level of homocysteine that should be corrected by medical treatment in subclinical hypothyroidism. Subclinical hypothyroidism may be closely associated with atherosclerotic diseases.

Conflict of interest: None declared.

Ethics Committee Approval: Ankara Numune Education and Research Hospital 17.01.2018 with decision number 1745/2018.

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Authorship contributions: Concept – M.B., I.A., B.F.D., M.A., T.T., N.Y.; Design – M.B., I.A., B.F.D., M.A., T.T., N.Y.; Supervision – M.B., I.A., B.F.D., M.A., T.T., N.Y.; Data collection &/or processing – M.B., T.T., B.F.D.; Analysis and/or interpretation – T.T., M.A., N.Y.; Literature search – B.F.D., M.B., T.T., I.A.; Writing – I.A., M.B., N.Y., M.A.; Critical review – M.A., N.Y., T.T.

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