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Original Scientific Paper

EVALUATION OF SERUM HOMOCYSTEINE AND ASYMMETRIC DIMETHYLARGININE LEVELS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

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SUMMARY – Autoimmune thyroid diseases (AITD) may lead to increased cardiovascular risk; however, the underlying mechanism for this is unclear. The aim of our study was to investigate serum asymmetric dimethylarginine (ADMA), vitamin B12, folic acid and homocysteine (HCY) levels in patients with and without AITD. A total of 180 individuals were enrolled in this study, encompassing 45 patients with Hashimoto's Thyroiditis (HT), 45 patients with Graves' disease (GD), 45 individuals with multinodular goiter (MNG) and 45 healthy controls who were admitted to Selçuk University Medical Faculty's Department of Endocrinology. All statistical analyses were conducted using the R Statistical Software (version 4.2.1). We found increased levels of HCY and ADMA, and decreased levels of folic acid in AITD compared to the control group ($P=0.021$, $P=0.04$ and $P=0.017$, respectively). Vitamin B12 levels were similar between groups. HCY was negatively correlated with vitamin B12 and folic acid ($r=-0.028$, $P=0.002$ and $r=-0.368$, $P<0.001$, respectively). Our study revealed that a rise in ADMA and HCY levels in patients with AITD can provide insight into how AITD may contribute to endothelial dysfunction and cardiovascular risks.

Keywords: *Hashimoto disease; Graves' disease; Asymmetric dimethylarginine; Homocysteine*

Introduction

Autoimmune thyroid disease (AITD) represents a group of disorders characterized by the immune system attacking its own thyroid gland, with Graves' disease (GD) and Hashimoto's thyroiditis (HT) being

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the most prevalent variants[1]. The causation of AITD involves a combination of genetic and environmental factors. The primary characteristic of AITD is the generation of antibodies targeting thyroid peroxidase (TPO), thyroglobulin (TG) and the thyroid stimulating hormone receptor (TSH-R)[2].

Graves' disease is an autoimmune disorder presenting with exophthalmos, thyrotoxicosis findings and diffuse goiter. The classical triad is not observed in all patients and patients may present with only hyperthyroidism[3]. GD occurs as a result of the generation of IgG antibodies that attach to and trigger the TSH-R situated on the exterior of thyroid follicular cells. HT, alternatively recognized as chronic lymphocytic thyroiditis, stands as the prevalent contributor to hypothyroidism and represents an enduring inflammation of the thyroid gland[4,5]. It is diagnosed with clinical (diffuse enlargement of the thyroid gland, hypothyroidism findings) and laboratory findings (anti-TP, anti-TPO positivity)[4].

Asymmetric dimethylarginine (ADMA) is a methyl arginine derivative that inhibits nitric oxide synthase. Methylated arginines emerge through post-translational modification, arising from the methylation of arginine residues in proteins. Elevated concentrations of ADMA hinder nitric oxide (NO) synthesis, consequently compromising endothelial function and increasing the risk of cardiovascular disease[6].

Vitamin B12 is used as a cofactor for enzymes involved in the synthesis of DNA, fatty acids and myelin. Additionally, it acts as a coenzyme for methionine synthase, an enzyme responsible for transforming homocysteine (HCY) into methionine. Moreover, Vitamin B12 serves as a coenzyme for methylmalonyl-CoA mutase, facilitating the conversion of methylmalonyl-CoA to succinyl-CoA. In the absence of sufficient Vitamin B12, there is an elevation in methylmalonic acid (MMA) levels[7].

Folic acid is an essential water-soluble vitamin synthesized by plants and microorganisms. The significance of folic acid and sufficient folic acid consumption lies predominantly in its role as a precursor to 5-methyltetrahydrofolate, serving as a provider of methyl groups for the remethylation of HCY to methionine. Folic acid deficiency, therefore, may indirectly lead to high plasma HCY concentrations[8].

HCY, an intermediate in the biosynthesis of methionine and cysteine amino acids, is a sulfhydryl-containing amino acid and plays an important role in the methylation cycle. High HCY levels have been acknowledged as an independent risk determinant for cardiovascular disorders[9].

Thyroid diseases are risk factors for cardiovascular diseases[10]. However, there are considerable gaps in our understanding of the particular molecular and biochemical pathways regulating these effects. The aim of our study was to investigate ADMA, vitamin B12, folic acid and HCY levels in patients with GD, HT and nontoxic multinodular goiter (MNG), and to explore the relationship between them. It also aimed to shed light on studies on the biochemical mechanism of cardiovascular risk in AITD.

Methods

Study subjects

The study protocol was conducted according to the principles of the Declaration of Helsinki and approved by the Selçuk University Faculty of Medicine Ethics Committee (Number: 2017/210, Date: 21.06.2017).

The study comprised 45 Hashimoto's patients, 45 Graves' patients, 45 nontoxic multinodular goiter patients who underwent follow-up at the Endocrinology Outpatient Clinic of the Selçuk University Faculty of Medicine and 45 healthy individuals without any thyroid disorders. The patients were informed about the study and informed consent was obtained from the patients who agreed to participate. Our study did not include patients who were pregnant, had an active infection, or a chronic systemic disease other than thyroid disease. Peripheral venous blood samples were collected from all patients following a 12-hour fasting period. Then, the samples were centrifuged and serum samples were placed in Eppendorf tubes and stored at -80 ˚C until analysis.

Assays for biochemical parameters

Vitamin B12, folic acid, thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), anti-thyroglobulin antibody (anti-TG) and anti-thyroglobulin antibody (anti-TPO) analyses were performed on the Roche Cobas e-601 automated analyzer using the electrochemiluminescence immunoassay (ECLIA) method.

Serum HCY levels were determined by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) using the following protocol: 50 μL of the samples were mixed with 50 μL of internal standard (10 μM d8 homocysteine isotope DLM-3619-1) and 50 μL of reducing reagent (300 mmol/L 1,4-Dithiothreitol, Cat No: Merck 111474) and left at room temperature for 15 minutes. Then, 300 μL of a precipitation reagent (15% trichloroacetic acid, Cat No: Merck 100810) were added to precipitate the proteins, mixed for 10 seconds and centrifuged for 3 minutes at 13,000 rpm. Also, 10 μL of the supernatant was injected into the LC-MS/MS system for analysis.

Serum ADMA was analyzed with the Shimadzu HPLC system (Kyoto, Japan) and a Phenomenex Luna C18 column coupled with a API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) equipped with an electrospray ion source (ESI) operating in positive mode. Serum ADMA concentrations were measured using a modification of the previously published method[11,12]. Briefly, 100 μL of internal Standard (d7-ADMA) in methanol were added to 200 μL of serum and centrifuged at 13,000 rpm for 10 minutes to remove precipitated proteins. The supernatant was collected and evaporated under nitrogen gas at 60 °C. The derivatization step was carried out by dissolving the dried extract in 200 μL of a freshly prepared butanol solution containing 5% (v/v) acetyl chloride and incubated for 30 min at 60 °C. The solvent was removed by evaporation under nitrogen gas at 60 °C. The residues were dissolved in 200 μL of a water-methanol (90:10, v/v) mixture including 0.1% (v/v) formic acid, and 40 μL were injected into the LC-MS/MS system. Intra and inter-assay coefficient of variation (CV) values were lower than 8%.

Statistical Analysis

All statistical analyses were conducted using the *R* Statistical language (version 4.2.1; The *R* Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org). To check the normality of the data, Shapiro-Wilk's normality test and Q-Q plots were used. Numerical variables were expressed as a median with interquartile range (25th percentile to 75th percentile) and were compared between the four groups using the Kruskal-Wallis test followed by Dunn's post-hoc test with a Bonferroni correction applied. Considering the possible confounding effect of age, a Generalized Linear Models (GLM) analysis was performed by adjusting for age. We employed the GLM with a Poisson distribution for exploring the relationship between FT3 levels, TSH levels and study groups adjusting for age. The incidence rate ratio (IRR) was calculated with 95% confidence intervals (CIs). Generalized linear models with a Gamma distribution were performed to evaluate the association of study groups with FT4, Vitamin B12, Folic acid and ADMA levels adjusting for age. Generalized linear models with a Gaussian distribution were performed to evaluate the association of study groups with HCY levels adjusting for age. The odds ratio (OR) was calculated with 95% CIs. Additionally, a Fisher-Freeman-Halton test was run to determine whether there was significant association between study groups and sex. A value of *P* below 0.05 was considered statistically significant.

Results

The median age in patients with MNG (49 years [IQR, 43-60]) was significantly higher than in healthy controls (37 years [IQR, 30-51]) and patients with Graves (35 years [IQR, 27-48]). Also, the median age of patients with HT (44 years [IQR, 38-57]) was greater than that of patients with GD. The median BMI of patients with HT and MNG was significantly higher than that of healthy controls and patients with GD. The demographical characteristics and laboratory findings of the study population are given in Table 1.

In the laboratory findings, we observed a statistically significant difference in FT3 ($\chi^2 = 35.86$, $P < 0.001$), FT4 ($\chi^2 = 9.29$, $P = 0.026$), TSH ($\chi^2 = 49.74$, $P < 0.001$), Anti-TPO ($\chi^2 = 89.11$, $P < 0.001$), Anti-TG ($\chi^2 = 82.04$, $P < 0.001$), HCY ($\chi^2 = 8.82$, $P = 0.032$), Folic acid ($\chi^2 = 13.17$, $P = 0.004$) and ADMA ($\chi^2 = 9.84$, $P = 0.020$) levels among the study groups (Table 1). The multiple comparison results obtained by Dunn's post-hoc test with a Bonferroni correction showed that the median [IQR] HCY level was significantly higher in patients with Hashimoto's disease compared to healthy controls (12.5 [IQR, 10.9-16.1] vs. 10.9 [IQR, 7.8-13.4], adj. *P*-value = 0.022). Besides, the median ADMA [IQR] level of patients with GD was significantly

Table 1. Comparison of demographical characteristics and laboratory findings of the study groups.

	Control (*n*=45)	**Hashimoto (*n*=45)**	**MNG (*n*=45)**	**Graves (*n*=45)**	***P*-value**	**Adj. *P*-value**
Demographical characteristics						
Age (years)	37 (30-51)[ac]	44 (38-57)[ab]	49 (43-60)[b]	35 (27-48)[c]	<0.001[1]	
Sex (Female/Male)	34 (75.6)/ 11 (24.4)	39 (86.7)/ 6 (13.3)	41 (91.1)/4 (8.9)	29 (64.4)/ 16 (35.6)	0.008[2]	
BMI (kg/m^2)	26.3 (20.2-35.38)[a]	28.72 (17.92-39.54)[b]	28.04 (20.31-37.11)[b]	24.68 (19.47-35.96)[a]	<0.001[1]	
Laboratory findings						
FT3 (ng/L)	3.27 (2.91-3.68)[a]	2.91 (2.67-3.20)[b]	3.21 (2.91-3.43)[a]	4.07 (3.07-6.94)[c]	<0.001	<0.001[3]
FT4 (ng/dL)	1.28 (1.17-1.40)[a]	1.31 (1.13-1.42)	1.28 (1.17-1.42)	1.51 (1.16-2.18)[b]	0.026	<0.001[4]
TSH (mIU/L)	1.86 (1.47-2.57)[ac]	2.53 (1.41-4.30)[a]	1.27 (0.84-1.87)[c]	0.007 (0.005-1.23)[b]	<0.001[1]	<0.001[3]
Anti-TPO (>35 IU/mL)	1 (2.2)	36 (80)	3 (6.7)	36 (80)	<0.001[1]	
Anti-TG (>115 IU/mL)	0 (0)	21 (46.7)	1 (2.2)	20 (44.4)	<0.001[1]	
HCY (µmol/L)	10.9 (7.8-13.4)[a]	12.5 (10.9-16.1)[b]	11.8 (9.82-15.1)	12 (10.2-15.6)	0.032[1]	0.049[5]
Vitamin B12 (ng/L)	365 (290-417)	319 (250.4-417)	302 (271-449)	282.7 (245-427)	0.197[1]	0.314
Folic acid (mcg/L)	8.19 (5.73-10.49)[a]	6.87 (4.97-9.50)	7.46 (6.22-9.22)[a]	5.56 (4.05-7.77)[b]	0.004[1]	0.040[4]
ADMA (µmol/L)	0.43 (0.33-0.65)[a]	0.49 (0.39-0.64)	0.47 (0.30-0.64)	0.69 (0.42-0.95)[b]	0.020[1]	0.010[4]

Data were presented as median with interquartile range (25th percentile – 75th percentile) or count (n) percentage (%).
Difference small superscript letter in each row shows a statistically significant difference in each parameter between study groups after multiple comparison tests
[1] Kruskal Wallis test; [2] Fisher-Freeman-Halton test; [3] GLM with a Poisson distribution; [4] GLM with a Gamma distribution; [5] GLM with a Gaussian distribution
BMI = body mass index; FT3 = free triiodothyronine; FT4 = free thyroxine; TSH = thyroid stimulating hormone; Anti-TPO = anti-thyroid peroxidase antibody; Anti-TG = anti-thyroglobulin antibody; HCY = homocysteine; ADMA = asymmetric dimethylarginine.

higher than in healthy controls (0.69 [IQR, 0.42-0.95] vs. 0.43 [IQR, 0.33-0.65], adj. *P*-value = 0.029). However, no significant difference was found among other multiple comparisons in terms of HCY and ADMA levels (Figure 1-A and Figure 1-B). According to the results obtained by adjusting for age, HCY levels were about 8% (adj. OR = 1.08, 95% CI = 1.02-1.14, *P* = 0.010) and about 9% (adj. OR = 1.09, 95% CI = 1.03-1.15, *P* = 0.005) higher in patients with HT and GD, while ADMA levels were higher in patients with GD compared to healthy controls (adj. OR = 1.32, 95% CI = 1.11-1.58, *P* = 0.002).

The highest FT3 level was observed in patients with GD (4.07 [IQR, 3.07-6.94]), and the lowest FT3 level in patients with Hashimoto's disease (2.91 [IQR, 2.67-3.20]), while FT3 levels in patients with MNG (3.21 [IQR, 2.91-3.43]) and healthy controls (3.27 [IQR, 2.91-3.68]) were similar. After adjusting for age, FT3 levels were significantly higher than average, more specifically 1.82 times higher (adj. IRR = 1.82, 95% CI = 1.49-2.23, *P* < 0.001), in patients with GD than in healthy controls.

FT4 levels were significantly higher in patients with GD compared to healthy controls (1.51 [IQR,

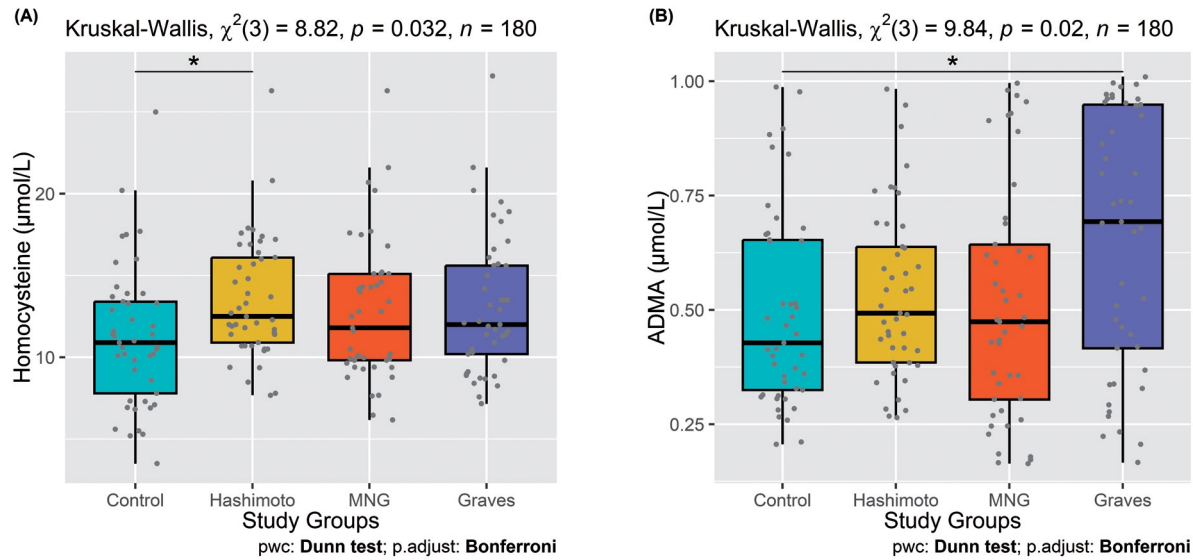

*Figure 1. The box-plots of (A) homocysteine and (B) ADMA levels in study groups. *P < 0.05 shows a statistically significant difference between groups. ADMA = asymmetric dimethylarginine; MNG = multinodular goiter.*

1.16-2.18] vs. 1.28 [IQR, 1.17-1.40], adj. *P*-value = 0.049), and after adjusting for age, they were still about 1.43 times higher (adj. OR = 1.43, 95% CI = 1.25-1.63, *P* < 0.001) than in healthy controls.

As shown in Table 1, the lowest TSH levels were observed in patients with GD. After adjusting for age, TSH levels in patients with Hashimoto were roughly 1.90 times higher (adj. IRR = 1.90, 95% CI = 1.46-2.50, *P* < 0.001) than in healthy controls, but 51% lower (adj. IRR = 0.49, 95% CI = 0.34-0.70, *P* < 0.001) in patients with GD compared to healthy controls.

Folic acid levels in patients with GD (5.56 [IQR, 4.05-7.77]) were significantly lower than in patients with MNG disease (7.46 [IQR, 6.22-9.22]) and healthy controls (8.19 [IQR, 5.73-10.49]), but Folic acid levels in patients with HT (adj. OR = 0.81, 95% CI = 0.67-0.99, *P* = 0.043) and GD (adj. OR = 0.78, 95% CI = 0.65-0.95, *P* = 0.015) were lower than in healthy controls after adjusting for age. No significant difference was found in terms of Vitamin B12 levels among the groups ($\chi^2 = 4.68$, *P* = 0.197).

When GD and HT are evaluated as a group as AITD, patients with AITD had significantly higher HCY levels compared to controls, even after adjusting for age (OR = 6.04, 95% CI = 1.46-24.95, *P* = 0.014). Vitamin B12 levels were similar between the groups. While folic acid levels were significantly lower in patients with AITD than in the control group and MNG patients, they were only lower than the control group after adjusting for age (OR = 0.79, 95% CI = 0.67-0.94, *P* = 0.009). In addition, while ADMA levels were significantly higher in patients with AITD compared to controls, it was observed that this elevation continued after adjusting for age (OR = 1.21, 95% CI = 1.03-1.42, *P* = 0.017). Demographic characteristics and laboratory findings for AITD, MNG and the control group are shown in Table 2 and Figure 2.

As shown in Table 3, FT3 levels positively correlated with FT4 levels and negatively correlated with TSH. There was a negative and significant relationship between TSH and FT4. While there was a negative and significant relationship between HCY and vitamin B12 and folic acid, there was a positive relationship between vitamin B12 and folic acid.

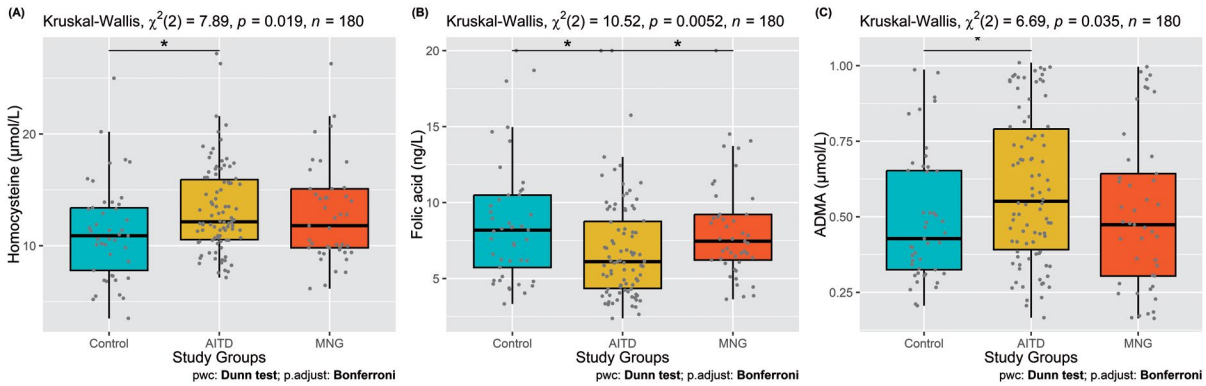

*Figure 2. The box-plots of (A) homocysteine, (B) Folic acid and (C) ADMA levels in study groups. *P < 0.05 shows a statistically significant difference between groups. AITD = Autoimmune thyroid diseases; ADMA = asymmetric dimethylarginine; MNG = multinodular goiter.*

Table 2. Comparison of demographical characteristics and laboratory findings of the study groups.

	Control (*n*=45)	**AITD (*n*=90)**	**MNG (*n*=45)**	***P*-value**	**Adj. *P*-value**
Demographical characteristics					
Age (years), median (IQR)	37 (30-51)[a]	40 (31-53)[a]	49 (43-60)[b]	<0.001[1]	
Sex (Female/Male), *n* (%)	34 (75.6)/ 11 (24.4)	68 (75.6)/ 22 (24.4)	41 (91.1)/4 (8.9)	0.080[2]	
BMI (kg/m^2), median (range)	26.3 (20.2-35.38)[a]	26.75 (17.92-39.54)[b]	28.04 (20.31-37.11)[c]	<0.001[1]	
Laboratory findings					
FT3 (ng/L), median (IQR)	3.27 (2.91-3.68)	3.19 (2.84-4.06)	3.21 (2.91-3.43)	0.386[1]	<0.001[3]
FT4 (ng/dL), median (IQR)	1.28 (1.17-1.40)	1.35 (1.13-1.73)	1.28 (1.17-1.42)	0.104[1]	0.005[4]
TSH (mIU/L), median (IQR)	1.86 (1.47-2.57)[a]	1.23 (0.01-2.80)[b]	1.27 (0.84-1.87)	0.033[1]	0.146[3]
Anti-TPO (>35 IU/mL)	1 (2.2)	72 (80)	3 (6.7)	<0.001	
Anti-TG (>115 IU/mL)	0 (0)	41 (45.6)	1 (2.2)	<0.001	
HCY (µmol/L), median (IQR)	10.9 (7.8-13.4)[a]	12.15 (10.55-15.93)[b]	11.8 (9.82-15.1)	0.019[1]	0.021[5]
Vitamin B12 (ng/L), median (IQR)	365 (290-417)	305.5 (246.25-425.5)	302 (271-449)	0.164[1]	0.314
Folic acid (mcg/L), median (IQR)	8.19 (5.73-10.49)[a]	6.11 (4.35-8.76)[b]	7.46 (6.22-9.22)[a]	0.005[1]	0.017[4]
ADMA (µmol/L), median (IQR)	0.43 (0.33-0.65)[a]	0.55 (0.39-0.79)[b]	0.47 (0.30-0.64)	0.035[1]	0.040[4]

Data were presented as a median with interquartile range (25th percentile – 75th percentile) or count (n) percentage (%).
Different small superscript letteres in each row show a statistically significant difference in each parameter between study groups after multiple comparison tests
[1] Kruskal Wallis test; [2] Fisher-Freeman-Halton test; [3] GLM with a Poisson distribution; [4] GLM with a Gamma distribution; [5] GLM with a Gaussian distribution
BMI = body mass index; FT3 = free triiodothyronine; FT4 = free thyroxine; TSH = thyroid stimulating hormone; Anti-TPO = anti-thyroid peroxidase antibody; Anti-TG = anti-thyroglobulin antibody; HCY = homocysteine; ADMA = asymmetric dimethylarginine; IQR = interquartile range.

Table 3. Correlations of laboratory findings.

	Spearman's Rho	**FT3**	**FT4**	**TSH**	**HCY**	**B12**	**FA**	**ADMA**
FT3	***r***	1	0.522*	-0.407*	-0.119	-0.069	-0.059	0.060
	P	-	<0.001	<0.001	0.112	0.358	0.433	0.425
FT4	***r***		1	-0.427*	-0.066	-0.053	-0.045	-0.026
	P		-	<0.001	0.380	0.480	0.551	0.727
TSH	***r***			1	0.091	0.064	0.056	-0.048
	P			-	0.225	0.395	0.456	0.525
HCY	***r***				1	-0.028*	-0.368*	0.118
	P				-	0.002	<0.001	0.116
B12	***r***					1	0.316*	0.111
	P					-	<0.001	0.138
FA	***r***						1	-0.131
	P						-	0.079
ADMA	***r***							1
	P							-

FT3 = free triiodothyronine; FT4 = free thyroxine; TSH = thyroid stimulating hormone; HCY = homocysteine; B12 = vitamin B12; FA = folic acid; ADMA = asymmetric dimethylarginine.

Discussion

Thyroid hormones are crucial for carrying out regular metabolic processes. Thyroid function issues cause metabolic activities to be disrupted, which in turn cause metabolic and cardiovascular illnesses[13]. The majority of thyroid disorders appear to be associated with subclinical imbalances in thyroid functioning. These irregularities in thyroid function are typically identified through analyzing the serum levels of relevant thyroid parameters like FT4, FT3, TSH and associated parameters, such as thyroid antibodies, lipid profiles, and cardiac functioning[14].

In general, AITD tends to be related to the existence of other coexisting autoimmune disorders. Given that autoimmune disorders such as atrophic gastritis and/or pernicious anemia impede vitamin B12 absorption, there is probably a connection between AITD and vitamin B12 insufficiency[15]. In some studies, B12 deficiency was found to be more common in patients with AITD[16, 17]. Wang et al. reported that B12 deficiency was more prevalent in those with thyroid-specific autoantibodies than in the control group, even though the concentration of vitamin B12 between the two groups was similar[18]. In the same study, thyroid autoantibody-positive patients exhibited elevated HCY levels; however, no difference in folic acid concentrations was observed between the two groups[18]. Lippi et al. reported that in patients with subclinical thyroid dysfunction, the prevalence of vitamin B12 and folic acid deficiency was similar to a healthy population, while there was a relationship between TSH and folic acid levels[19]. In a study conducted with 175 hypothyroid patients, serum folic acid and B12 levels were found to be lower compared to the euthyroid patient group[20]. In our study, although the control group had a higher vitamin B12 concentration than the patients with AITD, no statistically significant difference was noted. However, folic acid levels were observed to be reduced in patients with AITD regardless of whether they had GD or HT.

While HCY levels, a known cardiovascular risk factor, have generally been found to be increased in

hypothyroidism[21], conflicting results have been reported between hyperthyroidism and HCY levels. Berker et al. showed that HCY levels were increased in newly diagnosed Graves' patients, even though Vitamin B12 and folic acid levels were comparable[22]. Similarly, Colleran et al. reported that HCY and MMA levels were strongly correlated with free thyroxine levels in newly diagnosed and untreated Graves' patients, although vitamin B12 and folic acid concentrations were similar compared to the control group. These results may suggest that B12 enzyme activity may be impaired[23]. Bičíková et al. demonstrated that HCY levels in hypothyroidism decreased after achieving euthyroid status[21]. A recent study shows that anti-TG and anti-TPO, as markers of AITD, may be associated with increased HCY, and people who are positive for anti-TG or anti-TPO have higher HCY levels[24]. In our study, we found increased HCY in HT and GD compared to the control group, when adjusted for age. Additionally, HCY was negatively correlated with vitamin B12 and folic acid. The precise mechanism of the rise in HCY concentrations in individuals with AITD is still unclear; however, evidence suggests a potential link to decreased renal excretion of HCY or a decline in the activity or level of enzymes responsible for HCY metabolism[22].

ADMA, an endogenous inhibitor of nitric oxide synthase, has been associated with many diseases, particularly increased cardiovascular risk[6]. Hermenegildo et al. reported that ADMA levels were increased in hyperthyroidism compared to the control group and showed a positive correlation with FT4 ($r = 0.60$; $P < 0.001$), while an inverse relationship was found with NO ($r = -0.31$; $P < 0.05$)[25]. This may be because hyperthyroidism would reduce enzyme activity of the enzyme responsible for the degradation of ADMA through a raised production of oxygen free radicals and raised lipid peroxidation[25]. In another study conducted in patients with GD, ADMA levels were shown to be higher than in the control group and to be linked with FT4 and FT3 levels[26]. In research by Arıkan et al., a significant increase in ADMA concentrations was observed in both hypothyroidism and hyperthyroidism compared to the control group[27]. In our study, ADMA levels were found to be high in GD and no relationship with thyroid hormones was discovered.

Biochemically, HCY and ADMA are interconnected through several pathways. HCY suppresses the activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme responsible for the breakdown of ADMA[28]. HCY may promote protein degradation by causing structural instability and increasing oxidative stress, which then leads to the release of ADMA[29]. Additionally, two methyl groups from methionine are used for the posttranscriptional methylation of arginine, resulting in the formation of HCY and ADMA. Considering their biochemical relationship, a close correlation between ADMA and HCY would be expected, but interestingly, many studies in patients with increased cardiovascular risk have not found a relationship between ADMA and HCY[29-31]. Similarly, in our study, HCY and ADMA levels were found to be increased in AITD patients, yet no correlation was detected between the two parameters.

Thyroid dysfunction that is not sufficiently managed has recently been identified as one of the primary underlying causes of cardiovascular disorders. In a retrospective study conducted among 115,746 participants in Taiwan without a pre-existing thyroid condition, subclinical hypothyroidism was observed to be closely associated with all-cause mortality and deaths due to cardiovascular diseases[32].

Thyroid diseases have been linked to increased cardiovascular risk and possible underlying endothelial dysfunction; however, the precise cause of endothelial dysfunction in thyroid disease remains unclear. The results we found in our study may shed light on future studies to explain the increased cardiovascular risk in AITD through biochemical mechanisms.

This study had several limitations. Firstly, this was not a prospective study and it did not have a definitive endpoint for cardiovascular events. Secondly, the dynamic nature of the studied parameters with treatment over time remains unknown. Furthermore, since the thyroid disorders in the study group can occur at different ages, there was an age difference between the groups, but statistical age adjustment was applied to overcome it. Further studies in larger study groups with other molecules that are involved in this metabolic pathway (such as DDAH, NO) are required.

Conclusions

In conclusion, the current study revealed that a rise in ADMA and HCY levels in patients with AITD can provide insight into how AITD may contribute to some cardiovascular risks.

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Sažetak

PROCJENA RAZINE HOMOCISTEINA I ASIMETRIČNOG DIMETILARGININA U KRVI PACIJENATA S AUTOIMUNIM BOLESTIMA ŠTITNJAČE

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Autoimune bolesti štitnjače (AITD) mogu dovesti do povećanog kardiovaskularnog rizika, međutim temeljni mehanizam odgovoran za to nije jasan. Cilj naše studije bio je istražiti razine asimetričnog dimetilarginina (ADMA), vitamina B12, folne kiseline i homocisteina (HCY) u serumu pacijenata sa i bez AITD. U studiju je uključeno ukupno 180 pojedinaca: točnije, 45 pacijenata s Hashimotovim tireoiditisom (HT), 45 pacijenata s Gravesovom bolešću (GD), 45 pojedinaca s multinodularnom strumom (MNG) i 45 zdravih kontrolnih osoba koje su primljene na endokrinološki odjel Medicinskog fakulteta Sveučilišta Selçuk. Sve statističke analize provedene su softverom R Statistical Software (verzija 4.2.1). Pronašli smo povećane razine HCY i ADMA te smanjene razine folne kiseline u AITD u usporedbi s kontrolnom skupinom ($P = 0,021$, $P = 0,04$ i $P = 0,017$, redom). Razine vitamina B12 bile su slične u svim skupinama. HCY je bio u negativnoj korelaciji s vitaminom B12 i folnom kiselinom ($r = -0,028$, $P = 0,002$ odnosno r:-0,368, $P < 0,001$). Naša je studija otkrila da porast razine ADMA i HCY u bolesnika s AITD-om može pružiti uvid u to kako AITD može doprinijeti endotelnoj disfunkciji i kardiovaskularnim rizicima.

Ključne riječi: *Hashimotov tireoiditis; Gravesova bolest; asimetrični dimetilarginin; homocistein*