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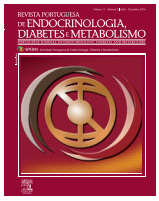

Artigo Original

Cardiovascular Risk Factors in Patients with Autoimmune Thyroiditis

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A B S T R A C T

Introduction: Thyroid dysfunction has been related to an increased cardiovascular risk resulting from alterations in lipid profile, insulin resistance, homocysteine levels and low grade systemic inflammation. The impact of normal TSH levels and subclinical hypothyroidism and autoimmunity in the increased cardiovascular risk remains controversial.

Our objective was to evaluate the interrelations between thyroid function, thyroid autoimmunity and cardiovascular risk factors, in patients with autoimmune thyroiditis.

Methods: 353 subjects with autoimmune thyroiditis were evaluated. We defined three groups based on TSH levels: TSH 0.5-2.5 µUI/mL, TSH 2.5-5.0 µUI/mL and TSH 5.0-10.0 µUI/mL. We recorded thyroid function tests, thyroid autoimmunity, insulin resistance markers including HOMA-IR (Homeostasis Model Assessment for Insulin Resistance), lipid profile, homocysteine, high-sensitivity C-reactive protein (hs-CRP) and vitamin B12 levels. Statistical analysis was performed using Kruskal-Wallis test and Spearman correlations.

Results: Our sample comprised 94% females with a mean age of 47.0 ± 16.3 years. The group TSH 5.0-10.0 µUI/mL presented higher levels of HOMA-IR when compared to the other two groups [2.96 (1.76-4.59) in TSH 5.0-10.0 µUI/mL vs 1.86 (0.97-2.56) in TSH 2.5-5.0 µUI/mL and 1.58 (1.06-2.46) in TSH 0.5-2.5 µUI/mL, $p = 0.002$]. In the total group we observed positive correlations between free T3 and both HDL ($r = 0.118$, $p = 0.028$) and apolipoprotein A-I (Apo A-I) ($r = 0.129$, $p = 0.024$); TSH was positively correlated with HOMA-IR ($r = 0.146$, $p = 0.018$) while free T4 was negatively correlated with homocysteine ($r = -0.119$, $p = 0.041$). In the group TSH 0.5-2.5 µUI/mL, positive correlations were found between TSH and both HDL ($r = 0.136$, $p = 0.031$) and homocysteine ($r = 0.147$, $p = 0.028$), between free T4 and CRP ($r = 0.136$, $p = 0.037$) and between anti-thyroglobulin antibodies and apolipoprotein B ($r = 0.165$, $p = 0.013$); anti-thyroglobulin antibodies were negatively correlated with homocysteine ($r = -0.186$, $p = 0.006$). Negative correlations between anti-thyroglobulin antibodies, total cholesterol ($r = 0.371$, $p = 0.004$), LDL ($r = -0.325$, $p = 0.011$), apolipoprotein B ($r = -0.342$, $p = 0.022$) and lipoprotein(a) ($r = -0.470$, $p = 0.001$) were revealed in the group TSH 2.5-5.0 µUI/mL. Regarding the group TSH 5.0-10.0 µUI/mL, we found positive correlations between free T3 and HDL ($r = 0.358$, $p = 0.030$), vitamin B12 ($r = 0.398$, $p = 0.024$) and HOMA-IR ($r = 0.424$, $p = 0.031$), and between anti-thyroglobulin and homocysteine ($r = 0.383$, $p = 0.033$).

Conclusion: We observed significant correlations between thyroid function, thyroid autoimmunity, insulin resistance, lipid profile and homocysteine that may contribute to an increased cardiovascular risk in patients with autoimmune thyroiditis.

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Fatores de Risco Cardiovascular em Doentes com Tiroidite Autoimune

Palavras-chave:
Complicações da Diabetes
Doenças Cardiovasculares/etiologia
Fatores de Risco
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R E S U M O

Introdução: O hipotiroidismo está associado a um risco cardiovascular aumentado. No entanto, o impacto de níveis normais de TSH bem como do hipotiroidismo subclínico e da autoimunidade permanece controverso. O nosso objectivo foi avaliar as relações entre função tiroideia, autoimunidade tiroideia e fatores de risco cardiovasculares, em doentes com tiroidite autoimune.
Métodos: Foram avaliados 353 doentes com tiroidite autoimune, tendo sido divididos em três grupos com base nos níveis de TSH: TSH 0,5-2,5 µUI/mL, TSH 2,5-5,0 µUI/mL e TSH 5,0-10,0 µUI/mL. Avaliou-se a função tiroideia, autoimunidade, marcadores de insulinorresistência, incluindo o HOMA-IR (*homeostasis model assessment for insulin resistance*), perfil lipídico e níveis de homocisteína, proteína-C-reativa (PCR) de alta sensibilidade e vitamina B12. Realizou-se análise estatística recorrendo ao teste Kruskal-Wallis e correlação de Spearman.
Resultados: A amostra era constituída por 94% de mulheres (média de idade: 47,0 ± 16,3anos). O grupo TSH 5,0-10,0 µUI/mL revelou níveis mais elevados de HOMA-IR em comparação com os restantes [2,96 (1,76-4,59) no TSH 5,0-10,0 µUI/mL *vs* 1,86 (0,97-2,56) no TSH 2,5-5,0 µUI/ml e 1,58 (1,06-2,46) no TSH 0,5-2,5 µUI/mL, $p = 0,002$]. No grupo total, observaram-se correlações positivas entre os níveis de T3 livre e HDL ($r = 0,118$; $p = 0,028$) e os níveis de T3 livre e apolipoproteína A1 ($r = 0,129$; $p = 0,024$) e entre TSH e HOMA-IR ($r = 0,146$; $p = 0,018$); a T4 livre correlacionou-se negativamente com a homocisteína ($r = -0,119$; $p = 0,041$). No grupo TSH 0,5-2,5 µUI/mL, encontraram-se correlações positivas entre o TSH e HDL ($r = 0,136$; $p = 0,031$), homocisteína ($r = 0,147$; $p = 0,028$), T4L e PCR ($r = 0,136$; $p = 0,037$) e também entre anticorpos antitiroglobulina e apolipoproteína B ($r = 0,165$; $p = 0,013$); os anticorpos antitiroglobulina correlacionaram-se negativamente com homocisteína ($r = -0,186$; $p = 0,006$). Observaram-se correlações negativas entre anticorpos antitiroglobulina, colesterol total ($r = 0,371$; $p = 0,004$), LDL ($r = -0,325$; $p = 0,011$), apolipoproteína B ($r = -0,342$; $p = 0,022$) e lipoproteína(a) ($r = -0,470$; $p = 0,001$) no grupo TSH 2,5-5,0 µUI/mL. Relativamente ao grupo TSH 5,0-10,0 µUI/mL, verificaram-se correlações positivas entre os níveis de T3 livre e HDL ($r=0,358$; $p=0,030$), vitamina B12 ($r = 0,398$; $p = 0,024$) e HOMA-IR ($r = 0,424$; $p = 0,031$) e entre anticorpos antitiroglobulina e homocisteína ($r = 0,383$; $p = 0,033$).
Conclusão: Observaram-se correlações significativas entre função tiroideia, autoimunidade, insulinorresistência, perfil lipídico e homocisteína que poderão contribuir para um risco cardiovascular aumentado em doentes com tiroidite autoimune.

Introduction

The term thyroiditis comprises many relatively common disorders.[1] Hashimoto thyroiditis (HT), also known as chronic autoimmune thyroiditis (CAT), is considered to be the most common organ-specific autoimmune disease and also the most common cause of hypothyroidism. Thyroid gland in patients with autoimmune thyroiditis (AIT) is characterized by diffuse lymphocytic infiltration and HT patients frequently present with clinical evidence of goiter or atrophic gland and thyroid dysfunction.[2,3] HT is characterized by elevated circulating antibodies to thyroid antigens, the anti-thyroid peroxidase antibodies (anti-TPO) being the most specific and sensitive for the diagnosis of the disease – positive in nearly 95% of patients; and anti-thyroglobulin antibodies (anti-Tg), positive in 60% - 70% of patients. Anti-Tg is also present in a higher proportion of healthy people.[2,4] Hypothyroidism may be the result of inadequate production of thyroid hormones or, less commonly, impaired activity at tissue level and it is classified as overt or subclinical. Overt hypothyroidism (OH) is characterized by the combination of elevated serum thyroid-stimulating hormone (TSH) concentrations and low thyroxine (T4) concentrations. Serum total and free triiodothyronine (T3) concentrations may only fall once the disease is far advanced. Subclinical hypothyroidism (SCH) is defined as the elevation in TSH levels in the presence of normal serum T4 and T3 concentrations.[1,5] SCH is estimated to occur in 4-20% of the adult population.[1] It was proven that the prevalence of thyroid autoimmunity is greater in women and increases with age.[6] It is clear that thyroid hormones play an important role in the modulation of the cardiovascular system and there is evidence of an association between hypothyroidism and atherosclerosis.[7] Several studies have established a positive correlation between OH and insulin resistance and dyslipidemia, both strong cardiovascular risk factors.[8-10] The cardiovascular risk associated with hypothyroidism is also due to hemodynamic, hormonal and metabolic changes and alterations in factors such as CRP (C-reactive protein) and homocysteine. Elevated levels of these factors in patients with OH were observed in several studies.[11,12] However, the associations between SCH and lipid profile, insulin resistance and homocysteine levels remain controversial, since many studies presented with controversial results.[7,8,13-15] Furthermore, the clinical significance of SCH may be difficult to determine due to controversies concerning the correct upper limit of the reference range for TSH levels.[7]

Also, there are controversies regarding whether there is a probable benefit to patients on initiating therapy when they are at a subclinical state of the disease, since there are conflicting results considering the effects of therapy on symptoms and cardiovascular risk in these patients.[16]

Since thyroid dysfunction has a major prevalence in the general population, it is essential to understand and establish the association between different levels of TSH, antibodies to thyroid antigens and an increased cardiovascular risk. Therefore, our main goal with this study is to determine the interrelations between

thyroid function, thyroid autoimmunity and the presence of cardiovascular risk factors in patients with AIT.

Material and Methods

Study population

The present study was conducted at the Endocrinology Service of São João Hospital, Porto, Portugal, between 2012 and 2016. This is a retrospective study involving a total of 353 subjects with AIT. Autoimmunity was defined based on positive levels of anti-Tg and anti-TPO, defined as above the normal laboratory levels. Patients were selected based on the diagnosis of AIT and we excluded those who were hypertensive, had cardiovascular disease, diabetes *mellitus* (DM) or at risk for DM; were taking thyroid-related medication or any medication that could interfere with our results (medications used for dyslipidemia, antidiabetic agents, supplements of folic acid or vitamin B12 or any kind of hormonal therapy). After the selection, the resulting age range was between 15 and 83 years. The study was approved by the institutional committee before its initiation.

We defined three groups based on TSH levels: a first group with TSH levels between 0.5 and less than 2.5 µUI/mL, a second group with TSH levels ranging from 2.5 to 5.0 µUI/mL and the third group included subjects with TSH levels between 5.0 and 10.0 µUI/mL.

We analyzed the levels and variations of thyroid function (TSH, FT4, FT3), lipid profile including total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL), apolipoprotein A-I (Apo A-I), apolipoprotein B (ApoB), Lipoprotein (a) [Lp(a)], homocysteine, anti-Tg, anti-TPO, high-sensitivity C-reactive protein (hs-CRP), insulin resistance markers comprising the HOMA-IR (homeostatic assessment insulin resistance index), QUICKI (quantitative insulin sensitivity check index), HISI (hepatic insulin sensitivity index), WBISI (whole-body insulin sensitivity index), IGI (insulinogenic index), folic acid and vitamin B12 levels.

Statistical analysis

The statistical analysis was performed in collaboration with the Department of Biostatistics and Medical Informatics of the Faculty of Medicine of University of Porto. Categorical variables were described through absolute and relative frequencies and continuous variables using mean and standard deviation or median and percentiles, depending on the distribution. Since the distribution of the continuous variables of each group was not normally distributed, we compared the different groups by using a non-parametric method, the Kruskal-Wallis test. To assess the strength of the associations between the analyzed variables, Spearman correlation coefficients were calculated. *P* values < 0.05 were considered as statistically significant. The analysis was performed using the statistical data analysis program SPSS (Statistical Package for Social Sciences) v.18.0.

Table 1. General characteristics of the population.

Parameters	Total Group	TSH < 2.5	TSH 2.5-5.0	TSH > 5.0	*p*-value
Age (years)	48 (34-58)	49 (35-58.5)	41 (31-58)	45 (27-58)	0.077
Weight (kg)	68 (59-78)	69 (59.5-79)	63 (58-73)	70 (61-80)	0.145
Height (m)	1.59 (1.55-1.65)	1.60 (1.55-1.64)	1.58 (1.55-1.65)	1.59 (1.56-1.63)	0.854
BMI (kg/m^2)	26.53 (23.38-30.25)	26.71 (23.59-30.45)	25.39 (23.05-29.09)	26.35 (22.76-32.46)	0.221
TSH (µUI/L)	1.66 (0.84-2.53)	1.28 (0.59-1.84)	3.15 (2.72-3.68)	7.47 (5.87-9.34)	<0.001*
FT3 (ng/mL)	2.78 (2.54-3.05)	2.76 (2.54-3.05)	2.86 (2.55-3.08)	2.78 (2.46-3.01)	0.534
FT4 (µg/mL)	1.05 (0.94-1.20)	1.06 (0.96-1.21)	1.00 (0.91-1.13)	0.96 (0.75-1.12)	0.026*
Anti-Tg (IU/mL)	77.2 (30.01-162.7)	75.6 (30.6-156.5)	75.3 (38.1-224)	92.9 (29.6-222.6)	0.636
Anti-TPO (IU/mL)	330.55 (64.6-1300)	303.6 (72.3-1300)	266.85 (45.1-1300)	833.9 (73.2-1300)	0.462
TC (mg/dL)	196 (174-220)	195 (175-218)	197 (172-231)	201 (173-254)	0.558
HDL (mg/dL)	57 (47-66)	57 (47-66)	56 (46.5-69)	53.5 (46-65)	0.864
LDL (mg/dL)	121 (104-141)	122 (105-140)	118 (95.5-140.5)	122.5 (105-152)	0.494
Apo A-I (mg/dL)	140 (129-154)	140 (128-153)	140 (129-159)	139 (129-163)	0.751
ApoB (mg/dL)	95 (82-111)	95 (82-110)	90 (78-113)	96 (77-128)	0.976
Lp(a) (mg/dL)	14.9 (6-42.9)	17 (6-42.9)	11.75 (5.5-43.45)	12.6 (5.5-68.5)	0.795
HOMA-IR	1.70 (1.07-2.70)	1.58 (1.06-2.46)	1.86 (0.97-2.56)	2.96 (1.76-4.59)	**0.002***
HOMA-β	119.06 (78.50-183.15)	113.84 (74.27-173.52)	129.75 (82.93-274.08)	161.56 (119.61-353.98)	**0.005***
QUICKI	0.57 (0.45-0.71)	0.58 (0.48-0.72)	0.55 (0.49-0.77)	0.45 (0.39-0.56)	**0.004***
HISI	58.87 (37.07-93.75)	63.29 (40.65-94.54)	53.62 (39.13-103.58)	33.84 (21.79-56.77)	**0.002***
WBISI	5.20 (3.32-7.42)	5.38 (3.71-8.02)	4.82 (3.20-6.92)	2.79 (1.99-5.64)	**0.011***
IGI	0.57 (0.37-0.97)	0.55 (0.37-0.96)	0.61 (0.44-0.94)	0.96 (0.49-1.33)	0.105
Homocysteine (µmmo/L)	7.9 (6.8-9.8)	8 (6.9-9.9)	7.9 (6.3-9.8)	7.75 (6.2-8.9)	0.719
CRP (mg/dL)	0.22 (0.10-0.46)	0.2 (0.09-0.43)	0.33 (0.13-0.57)	0.36 (0.13-0.64)	0.073
Vitamin B12 (pg/mL)	401 (305-553)	402.5 (305-565)	405.5 (323-528.5)	389 (285-552)	0.875

* Represents statistically significant differences between the group TSH 5.0-10.0 µUI/mL and the other two groups (TSH 0.5-2.5 µUI/mL and TSH 2.5-5.0 µUI/mL). BMI: body mass index; Apo A-I: apolipoprotein A-I; ApoB: apolipoprotein B; Lp(a): lipoprotein (a); HOMA-IR: homeostatic assessment insulin resistance index; QUICKI: quantitative insulin sensitivity check index; HISI: hepatic insulin sensitivity index; WBISI: whole-body insulin sensitivity index; IGI: insulinogenic index; CRP: C-reactive protein.

Results

Within our sample of 353 subjects with AIT, 94% of the population (331 subjects) were female while only 6% (22 subjects) were male. The mean age was 47.0 ± 16.3 years. Two hundred and fifty-three individuals (74% of the total population) belonged to the first group – TSH between 0.5 and 2.5 µUI/mL [mean TSH level of 1.28 (0.59-1.84)] - while the second group (TSH between 2.5 and 5.0 µUI/mL) comprised 62 individuals (18%), with a mean TSH level of 3.15 (2.72-3.68) and the third group (TSH between 5.0 and 10.0 µUI/mL) with 27 individuals (8%), having a mean TSH level of 7.47 (5.87-9.34) µUI/mL**.** There were no significant differences regarding the mean age or the mean anthropometric measurements (weight, height and BMI) between groups. The mean BMI of the total population was 26.53 (23.38-30.25) kg/m^2, meaning that more than 50% of the individuals were overweight (BMI > 25 kg/m^2).

The group TSH 5.0-10.0 µUI/mL presented higher levels of HOMA-IR when compared to the other two groups [2.96 (1.76-4.59) in TSH 5.0-10.0 µUI/mL *vs* 1.86 (0.97-2.56) in TSH 2.5-5.0 µUI/mL and 1.58 (1.06-2.46) in TSH 0.5-2.5 µUI/mL, p = 0.002]. The characteristics of the population are summarized in Table 1.

Regarding the total group, we found significant negative correlations between TSH and age (r = -0.114, p = 0.035). Positive correlations were found between FT3 and both HDL (r = 0.118, p = 0.028) and Apo A-I (r = 0.129, p = 0.024) and also between FT4 and IGI (r = 0.112, p = 0.045). TSH was positively correlated with HOMA-IR (r = 0.146, p = 0.018) (Fig. 1) negatively correlated with QUICKI (r = -0.122, p = 0.050), HISI (r = -0.146, p = 0.018) and WBISI (r = -0.146, p = 0.018). Negative correlations were observed between FT4 and both homocysteine (r = -0.119, p = 0.041) (Fig. 2) and WBISI (r = -0.124, p = 0.043) and also between anti-Tg and HDL (r = -0.122, p = 0.023) (Fig. 3).

Concerning the group with lower TSH levels (0.5-2.5 µUI/mL), positive correlations were observed between TSH and both HDL (r = 0.136, p = 0.031) and homocysteine (r = 0.147, p = 0.028) and between FT4 and both CRP (r = 0.136, p = 0.037) and IGI (r = 0.174, p = 0.014). Anti-TPO antibodies were positively correlated with total cholesterol (r = 0.141, p = 0.026) while anti-Tg positively correlated with ApoB (r = 0.165, p = 0.013) and showed negative correlations with HDL (r= -0.139, p = 0.028) and with homocysteine (r = -0.186, p = 0.006).

We found negative correlation in the group TSH 2.5-5.0 µUI/mL between anti-Tg and total cholesterol (r = 0.371, p = 0.004), LDL (r = -0.325, p = 0.011), ApoB (r = -0.342, p = 0.022), Lp(a) (r = -0.470, p = 0.001) and HOMABETA (r = -0.408, p = 0.015). The group with higher levels of TSH (TSH 5.0-10.0 µUI/mL) demonstrated positive correlations between FT3 and HDL (r = 0.358, p = 0.030), vitamin B12 (r = 0.398, p = 0.024), HOMA-IR (r = 0.424, p = 0.031) and IGI (r = 0.392, p = 0.023). FT3 was negatively correlated with HISI (r = -0.424, p = 0.031) and WBISI (r = -0.446, p = 0.022). Another positive correlation was observed between anti-Tg and homocysteine (r = 0.383, p = 0.033). The correlations between thyroid function, thyroid antibodies and cardiovascular risk factors are summarized in Table 2.

Discussion

Currently, several studies have questioned the need to lower the upper limit of normal for the serum TSH level.[17] Wartofsky proposed that the TSH levels should be lowered from 5.0 to 3.0 or even 2.5 µUI/mL, since there is a higher risk of progression

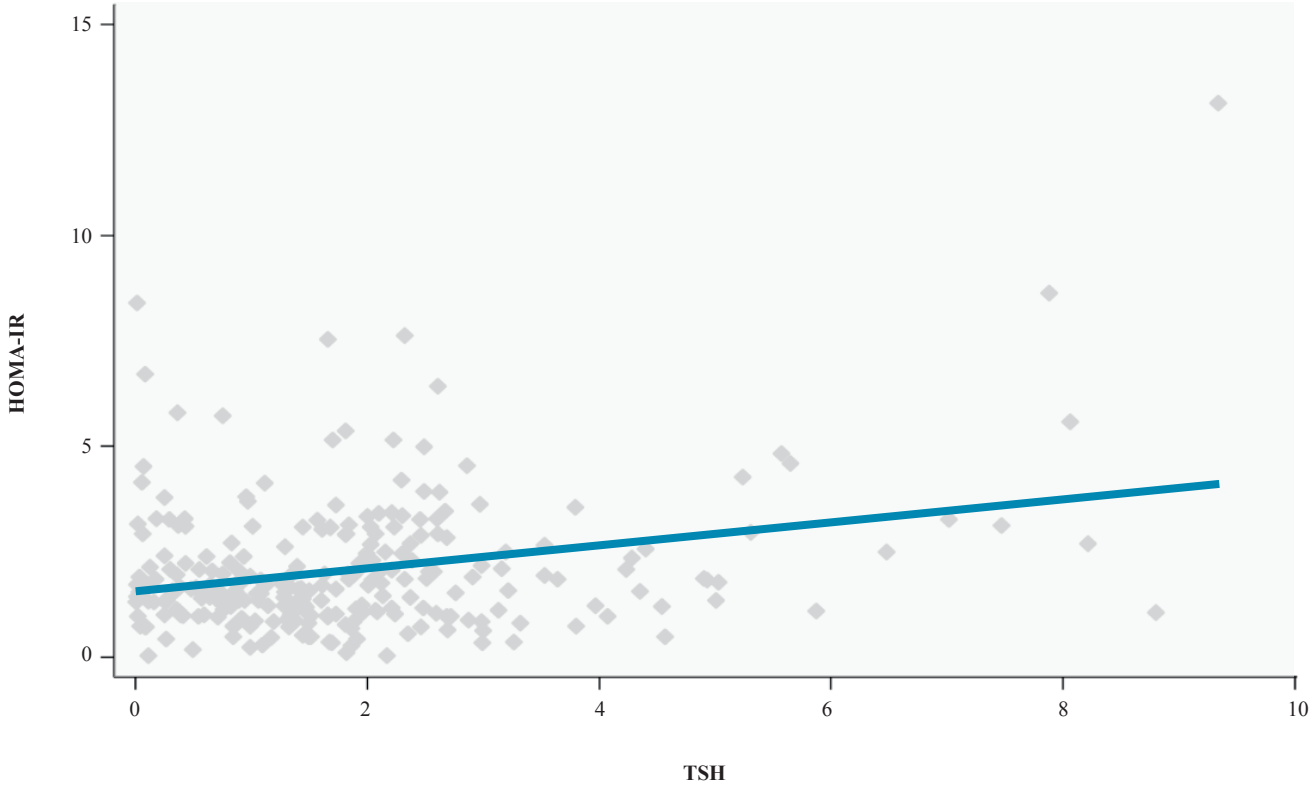

Figure 1. Scatterplot of correlations between TSH and HOMA-IR on the total group (r = 0.146, p = 0.018).
HOMA-IR: homeostatic assessment insulin resistance index

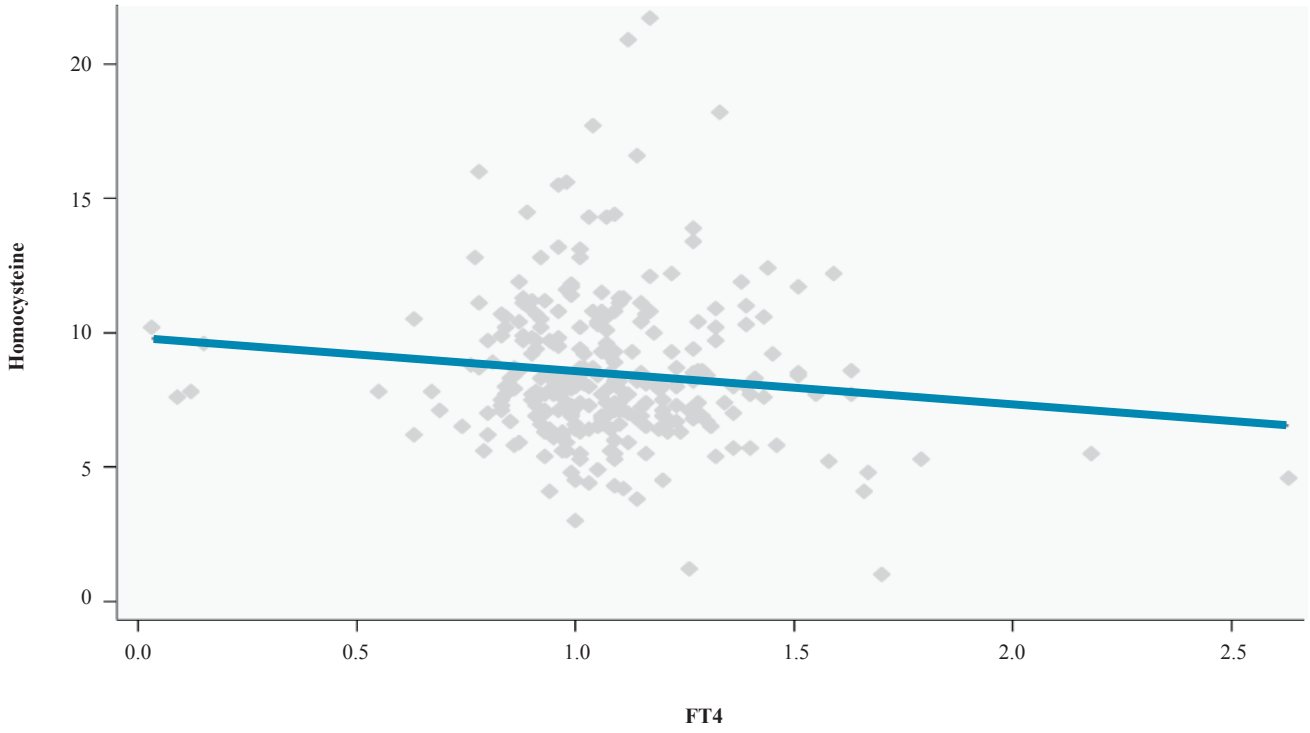

Figure 2. Scatterplot of correlations between FT4 and homocysteine on the total group (r = -0.119, *p* = 0.041).

to clinical thyroid disease with values between 3.0 and 5.0 µUI/mL.[18] Although the majority of studies regarding SCH consider a range of serum TSH levels between 4.5 to 10.0 µUI/mL, in this study we considered important to evaluate lower levels of TSH (TSH between 0.5 and 2.5, TSH between 2.5 and 5.0 and TSH ranging from 5.0 to 10.0 µUI/mL) in patients with the diagnosis of AIT, in order to understand whether levels of TSH lower than 10 µUI/mL may represent an increased cardiovascular risk.

Lipid profile

Our study revealed a positive correlation between FT3 levels and both HDL and Apo A-I levels in the total group. This association has been also reported in previous studies and may contribute to cardiovascular protection of patients with higher FT3 levels.[19-21] Also, the thyroid hormone T3 appears to increase the serum concentration of HDL by inducing the activity of Apo A-I.[22] Therefore, our data suggests that as the levels of FT3 decrease (accompanying the higher levels of TSH), there is also a decrease in HDL and Apo A-I levels, increasing the atherosclerotic risk.

There was found a negative correlation between anti-Tg levels and HDL, suggesting that autoimmunity and specifically higher levels of anti-Tg are associated with lower levels of HDL. Topaloglu *et al* also found negative correlations between these two variables.[23]

Regarding the group with TSH lower levels (0.5-2.5 µUI/mL), we found a positive correlation between TSH levels and HDL. Nevertheless, another study that evaluated TSH levels within the reference range of 0.50–3.5 µUI/mL found a persistent reduction in HDL cholesterol with increases in TSH levels.[24] In our study, levels of anti-TPO antibodies were found to be associated with higher levels of total cholesterol, in the same group**.** Topaloglu *et al* and Kang *et al* both studied euthyroid patients and found positive correlations between anti-TPO and total cholesterol.[23,25]

Anti-Tg levels correlated negatively with HDL and positively with ApoB levels. ApoB is a major component of LDL particles. These observations demonstrate that higher levels of circulating thyroid autoantibodies contribute to an atherogenic lipid profile. Pallas et al. also demonstrated a strong correlation between autoimmune thyroiditis and dyslipidemia.[26]

The second group (TSH levels between 2.5 and 5.0 µUI/mL) only revealed significant negative correlations. Interestingly, higher levels of anti-Tg were associated with lower levels of total cholesterol, LDL, ApoB and Lp(a). These findings are not consistent with observations by Tamer *et al*[27] where anti-Tg was positively associated with non-HDL cholesterol.

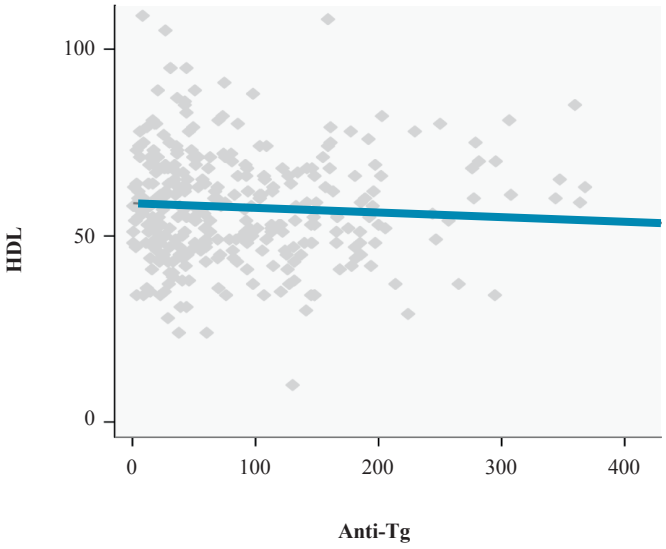

Figure 3. Scatterplot of correlations between anti-Tg and HDL on the total group (r = -0.122, *p* = 0.023).

Table 2. Correlations between thyroid function, autoimmunity, lipid profile, CRP, homocysteine, vitamin B12 and insulin resistance.

TSH		Total Group				TSH < 2.5 Group			
		FT3	FT4	anti-Tg	TSH	FT3	FT4	anti-Tg	
HDL (mg/dL)	r	0.073	**0.118**	0.004	**-0.122**	**0.136**	0.094	0.021	**-0.139**
	p	0.184	**0.028***	0.939	**0.023***	**0.031***	0.139	0.738	**0.028***
LDL (mg/dL)	r	0.025	-0.016	-0.029	0.008	0.049	-0.008	-0.002	0.111
	p	0.655	0.770	0.598	0.888	0.444	0.904	0.972	0.081
Apo A-I (mg/dL)	r	0.081	**0.129**	-0.005	-0.052	0.073	0.107	0.032	-0.039
	p	0.164	**0.024***	0.927	0.364	0.270	0.109	0.631	0.558
ApoB (mg/dL)	r	-0.003	-0.033	-0.034	0.067	-0.009	-0.035	-0.073	**0.165**
	p	0.957	0.563	0.555	0.246	0.898	0.598	0.275	**0.013***
Lp(a) (mg/dL)	r	-0.066	-0.038	0.051	-0.106	-0.075	-0.034	0.084	-0.044
	p	0.261	0.518	0.383	0.069	0.265	0.619	0.210	0.511
CRP (mg/dL)	r	0.086	0.008	0.078	0.027	-0.014	-0.043	**0.136**	0.008
	p	0.127	0.892	0.160	0.633	0.827	0.516	**0.037***	0.908
Homocysteine (µmmo/L)	r	0.061	0.035	**-0.119**	-0.060	**0.147**	0.080	-0.103	**-0.186**
	p	0.304	0.546	**0.041***	0.305	**0.028***	0.240	0.127	**0.006***
Vitamin B12 (pg/mL)	r	-0.033	-0.062	-0.011	-0.022	-0.063	-0.097	0.026	0.013
	p	0.577	0.286	0.854	0.708	0.350	0.147	0.697	0.846
HOMA-IR	r	**0.146**	0.115	0.045	-0.004	0.073	0.060	0.065	0.055
	p	**0.018***	0.060	0.468	0.948	0.308	0.402	0.364	0.438
TSH		**TSH 2.5 - 5.0 Group**				**TSH > 5.0 Group**			
		FT3	FT4	Anti-Tg	TSH	FT3	FT4	Anti-Tg	
HDL (mg/dL)	r	0.151	0.083	0.070	-0.046	-0.262	**0.358**	-0.129	-0.102
	p	0.250	0.527	0.595	0.727	0.195	**0.030***	0.445	0.549
LDL (mg/dL)	r	0.026	-0.192	-0.160	**-0.325**	0.035	0.247	-0.000	-0.037
	p	0.846	0.142	0.221	**0.011***	0.866	0.140	0.999	0.826
Apo A-I (mg/dL)	r	0.253	0.148	0.006	-0.108	0.012	0.290	-0.156	-0.145
	p	0.093	0.333	0.968	0.479	0.955	0.107	0.395	0.429
ApoB (mg/dL)	r	0.120	-0.015	-0.045	**-0.342**	-0.007	-0.038	0.175	0.045
	p	0.433	0.923	0.768	**0.022***	0.973	0.836	0.339	0.806
Lp(a) (mg/dL)	r	0.151	0.089	-0.050	**-0.470**	0.380	-0.302	-0.048	-0.033
	p	0.328	0.567	0.747	**0.001***	0.082	0.099	0.797	0.862
CRP mg/dL)	r	0.017	0.042	0.064	0.020	-0.149	0.328	-0.044	0.081
	p	0.905	0.764	0.648	0.890	0.479	0.055	0.803	0.644
Homocysteine (µmmo/L)	r	0.166	-0.110	-0.127	0.256	0.341	-0.089	-0.201	**0.383**
	p	0.288	0.481	0.417	0.098	0.121	0.633	0.278	**0.033***
Vitamin B12 (pg/mL)	r	-0.084	-0.222	-0.124	-0.296	-0.324	**0.398**	-0.025	-0.033
	p	0.588	0.149	0.423	0.051	0.131	**0.024***	0.893	0.856
HOMA-IR	r	-0.242	0.227	0.141	-0.075	-0.018	**0.424**	-0.077	-0.339
	p	0.118	0.143	0.369	0.667	0.943	**0.031***	0.710	0.093

* Represents statistically significant correlations between the analyzed variables. Apo A-I: apolipoprotein A-I; ApoB: apolipoprotein B; Lp(a): Lipoprotein (a); HOMA-IR: homeostatic assessment insulin resistance index; CRP: C-reactive protein.

Our findings show an inconsistent role of thyroid autoantibodies in cholesterol homeostasis. There are differences between the group with TSH levels between 0.5 and 2.5 µUI/mL and the one with TSH between 2.5 and 5.0 µUI/mL. Kang *et al*[25] also found an interesting result regarding anti-TPO levels, as they observed that higher anti-TPO levels were associated with higher HDL levels, results that were inconsistent with several previous studies.[23,27-29] Our results suggest that thyroid autoimmunity may affect lipid profile independent of thyroid function. This suggestion was also made by other authors.[27,30] The independent role of autoimmunity on lipid profile may be associated with higher levels of IFN-γ (interferon-gamma). It was observed that euthyroid patients with positive anti-TPO had higher INF-γ levels.[31] Also, Th1 immune response seems to be dominant in HT

patients when compared to Th2 response and Th1 cells mainly produce IFN-γ.[32] IFN-γ is responsible for stimulating foam cells production, cholesterol absorption and diminished cholesterol efflux, resulting on imbalance of lipid homeostasis.[33,34]

Our findings revealed that lower levels of FT3 are associated with lower levels of HDL in the group with TSH between 5.0 and 10.0 µUI/mL. Ness *et al* studied the effects of T3 in thyroidectomised rats and came with the conclusion that T3 increases levels of HDL that are probably due to positive effects on hepatic Apo A-I.[22] These findings suggest that T3 may have a beneficial effect on the synthesis of protective apolipoproteins.

Insulin resistance

Regarding the studied markers of insulin resistance, our results demonstrated that higher levels of TSH are associated with higher levels of HOMA-IR in the total group. This is in accordance with several previous studies.[8,35,36] We also found a positive association between higher levels of TSH and lower levels of WBISI, HISI and QUICKI. These observations allow us to perceive that as TSH levels increase the insulin sensitivity decreases.

In the total group, FT4 levels were positively correlated with IGI, a marker of beta cell function.[37] FT4 was negatively correlated with WBISI. Both these results show that lower levels of thyroid hormones are associated with lower sensitivity to insulin, as concluded by Maratou *et al*.[8]

In the group with TSH levels between 0.5 and 2.5 µUI/mL, FT4 was negatively correlated with TSH ($r = -0.271$, $p < 0.001$). As in the total group, we verified that lower levels of FT4 are associated with lower insulin sensitivity and that occurs even at normal levels of TSH.

In the group with higher levels of TSH (TSH 5.0-10.0 µUI/mL), FT3 was negatively correlated with TSH ($r = -0.474$, $p = 0.013$), showing the expected decrease in thyroid hormones with an increase in TSH levels and confirming that T3 begins to decrease more with an advanced stage of disease.[1] Our results demonstrated that as FT3 lowers, there is a decrease in HOMA-IR and IGI and an increase in HISI and WBISI, again supporting the known relation between lower thyroid hormone levels and less insulin sensitivity.[15]

Our results are in accordance with Duntas *et al*[38] as we also observed that dyslipidemia (manifested in our study as higher levels of LDL and total cholesterol and lower levels of HDL) occurs in SCH patients and, as we observed, starts to manifest at normal levels of TSH in patients with AIT. Duntas *et al* attributed the increased cardiovascular risk seen on these patients to a coexistence of other manifestations including overweight and insulin resistance and that the prevalence is higher in middle-aged women.[38]

Homocysteine

Several studies showed that overt hypothyroidism and SCH were associated with increased levels of homocysteine.[8,9,15]

In our total group, FT4 levels were negatively related to those of homocysteine. Our results demonstrate that lower levels of FT4 are related to higher levels of homocysteine.

Also, in the group with TSH between 5.0 and 2.5 µUI/mL, our results show that increases in TSH levels are associated with higher levels of homocysteine. A negative correlation was observed between levels of anti-Tg and levels of homocysteine in the same group. As previously referred regarding lipid profile, thyroid autoimmunity may also have different effects on homocysteine independent of thyroid function.

In group with higher TSH levels (5.0-10.0) µUI/mL, the correlation was opposite, as higher levels of anti-Tg were associated with increased levels of homocysteine. Carbotta *et al* found a strong relation between levels of anti-TPO and homocysteine, proving that autoimmunity contributes to hyperhomocysteinemia.[3]

Other findings

C-reactive protein (CRP), a low grade inflammation marker, has been considered as a cardiovascular risk factor and associated with atherosclerosis, an inflammatory process. Christ-Crain *et al* found that CRP levels increase with the progression of thyroid dysfunction even though there were no significant correlations between thyroid hormones and CRP levels.[39] The only significant correlation found in our study was between FT4 and CRP in the group with lower levels of TSH (TSH 0.5-2.5 µUI/mL). We demonstrated that in this particular group, lower FT4 levels were associated with lower hs-CRP levels.

Our findings revealed that FT3 in the third group (TSH 5.0-10.0 µUI/mL) were positively correlated with vitamin B12. Since FT3 tends to decrease with higher levels of TSH, as FT3 decreases, there is also a decrease in vitamin B12. Orzechowska-Pawilojc *et al* demonstrated that serum vitamin B12 was lower in patients with OH.[40]

One limitation of our study was the difference between the number of individuals that represented each group, mainly the two groups with higher levels of TSH.

The authors consider that one strength of this study was the fact that none of the individuals included were taking medications that could represent a confounding factor in the interpretation of the results. Also, the results found in the euthyroid group add an important contribution to understanding the characteristics of the population of patients with AIT and normal levels of TSH.

Conclusion

This study corroborates an association between thyroid function, thyroid autoimmunity and cardiovascular risk factors, including dyslipidemia, insulin resistance, levels of homocysteine, hs-CRP and vitamin B12 in patients with AIT. The correlations were observed not only in the subclinical range of TSH levels but also in patients classified as euthyroid. These results should raise awareness for the potential benefit of screening and treatment of patients with AIT. Further studies are required to understand whether autoimmunity contributes directly to the increased cardiovascular risk observed in patients with AIT.

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Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of patient data.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Responsabilidades Éticas

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