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ORIGINAL ARTICLE

Hashimoto thyroiditis is an independent cardiovascular risk factor in clinically hypothyroid patients

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KEYWORDS

Hypothyroidism;
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Nitric oxide

Abstract Hypothyroidism is a common disorder that confers an increased cardiovascular risk. The most common cause is Hashimoto thyroiditis (HT) but it can also be caused by thyroidectomy and radioiodine therapy. The aim of the study is to examine whether there is a relation between the cause of hypothyroidism and cardiovascular risk.

Subjects and methods: The study included 20 patients with Hashimoto thyroiditis and hypothyroidism, 20 patients with post-thyroidectomy hypothyroidism, 20 patients with post-radioiodine hypothyroidism, and 20 age and sex matched controls. In all the studied subjects we determined thyroid function tests; TSH and F.T4, thyroid auto-antibodies; anti-TPO and anti-TG antibodies, carotid intima media thickness (CIMT), flow mediated dilation (FMD) and serum nitric oxide.

Results: CIMT showed a trend to be higher in HT group (0.93 ± 0.08 mm) compared to other causes of hypothyroidism ($P = 0.090$). Multivariate analysis showed that HT is an independent predictor of CIMT ($P = 0.015$). FMD was significantly lower in HT group (5.74 ± 1.33%) compared to post-thyroidectomy (7.16 ± 1.05%) ($P = 0.001$), and post-radioiodine therapy (7.34 ± 1.34%) ($P = 0.000$). Multivariate analysis showed that HT is an independent predictor of FMD ($P = 0.000$). NO was significantly higher in hypothyroid patients (125.98 ± 5.03 μM/

Abbreviations: CIMT, carotid intima media thickness; FMD, flow mediated dilation; HT, Hashimoto thyroiditis; NO, nitric oxide

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ml) compared to controls (39.44 ± 3.63 μM/ml) ($P = 0.001$), both univariate and multivariate analyses showed that NO is an independent predictor of both CIMT and FMD ($P = 0.000$).
Conclusion: To our knowledge, this is the first study to show that Hashimoto thyroiditis is an independent cardiovascular risk factor in clinically hypothyroid patients.

1. Introduction

Hypothyroidism is the most common pathological hormonal deficiency and is more common in females and the elderly.[1] Overt hypothyroidism in the general population occurs in about 0.3% reaching 1.7% in elderly individuals ⩾65 years and up to 2% in women older than 60 years of age.[2,3]

Prevalence of subclinical hypothyroidism differs according to the studied population, from 4.3% to 8.5% in patients without history of thyroid disease [2,4] reaching 20% in females more than 65 years of age and up to 16% of males and 21% of females older than 74 years.[4]

Autoimmune thyroiditis (AIT) is an inflammatory condition of the thyroid gland characterized by intrathyroidal lymphocytic infiltration, ultrasonographic signs of inflammation and antibodies to thyroglobulin, thyroperoxidase (TPO) or both. AIT includes Hashimoto's thyroiditis, painless postpartum thyroiditis and painless sporadic thyroiditis.[5] A diagnosis of Hashimoto's thyroiditis is made when there is a diffuse swelling of the thyroid without any other cause in addition to any of the following: positive for anti-thyroid peroxidase antibodies, positive for anti-thyroglobulin antibodies or the presence of lymphocytic infiltration of the thyroid gland.[6]

Predictors of hypothyroidism in HT include: female sex, high normal TSH > 2.5 is the strongest predictor of hypothyroidism with the prevalence of hypothyroidism being 12% with baseline TSH of 2.5 and 87.5% with a baseline TSH of 4 and lastly positive anti-thyroid antibodies.[7]

Thyroidectomy operation may be total thyroidectomy (TT) in which the entire thyroid gland is removed or partial thyroidectomy which is further subdivided into: (1) near total thyroidectomy (NT), where less than 3 g of the gland is left behind; (2) subtotal thyroidectomy (ST), where no more than 25% of a single lobe is left and (3) hemithyroidectomy (HT) or lobectomy, where only one lobe is excised.[8]

Total thyroidectomy, by definition, causes hypothyroidism; near total thyroidectomy causes hypothyroidism in 85–100%.[9] Occurrence of hypothyroidism following subtotal thyroidectomy depends on a number of factors: age (there is a positive relation between age and thyroid function),[10] gender (there is a negative relation between male gender and thyroid function),[10] thyroid remnant volume/weight; the only modifiable factor (there is a strong negative correlation between remnant volume and TSH)[11] and ultrasound aspect of the thyroid remnant (hypoechoec aspect on ultrasound which may be due to inflammatory alterations) strongly predicted hypothyroidism. When ultrasound aspect and weight of the remnant are combined minimal thyroid remnant volume required to prevent hypothyroidism in isoechoec aspect is 4.2 ml).[11]

Occurrence of hypothyroidism following hemithyroidectomy depends on a number of factors: marked lymphocytic infiltration (grade 3 or 4),[12–14] preoperative TSH > 2–3,[15] detectable TPO Ab and Tg Ab,[16–18] Hashimoto's thyroiditis and MNG,[19,20] remnant lobe volume ⩽ 4 ml,[21] long standing disease before operation,[16] low iodine intake status,[22] low initial free T4[23] and right hemithyroidectomy.[24]

Radioiodine has been used for the treatment of hyperthyroidism since 1940; it is used for the treatment of hyperthyroidism due to Grave's disease or toxic multinodular goiter.[25,26] It is considered the 1st line treatment for Grave's disease and the treatment of choice for relapsed Grave's and toxic MNG.[27]

The optimum outcome is to achieve euthyroidism without relapse or hypothyroidism. However, it is impossible to titrate the dose of radioiodine to accurately guarantee euthyroidism.[28] Predictors of hypothyroidism following radioiodine for hyperthyroidism include: small thyroid volume, mild hyperthyroidism (mildly elevated initial free T4),[29] high radioiodine activity,[29–31] Grave's disease rather than toxic MNG,[31,32] higher TSH especially in toxic MNG,[32,33] use of recombinant human TSH (rhTSH),[34,35] female gender and older age.[29,36]

Hypothyroidism is an independent risk factor for myocardial infarction (MI), aortic atherosclerosis and CHD events and mortality through altering traditional and nontraditional risk factors for cardiovascular disease; including: dyslipidemia (high total cholesterol, LDL cholesterol, sd-LDL, [Lp(a)], chyomicron remnants and a low HDL cholesterol),[37,38] hypertension (three times more prevalent in hypothyroidism than in euthyroid subjects),[37] homocysteine (increased in hypothyroidism due to decreased clearance),[37] oxidative stress[39] and endothelial dysfunction.[40]

Nitric oxide (NO) is one of the most abundant free-radicals in the body and excess NO production causes mitochondrial respiratory enzyme inhibition. Although the formed O_2 is scavenged by super oxide dismutase (SOD), NO is the only known biological molecule that reacts faster with superoxide and is produced in high concentrations enough to outcompete endogenous levels of superoxide dismutase. If NO production is increased, short-lived NO rapidly reacts with O_2 to form a potent and powerful long-lived oxidant free-radical, peroxynitrite ($ONOO^-$) that decomposes into the hydroxyl radical OH^-, which is one of the most active ROS that oxidizes LDL.[41]

Increased peroxynitrite production, resulted in increased apoptotic cell death in atheromatous plaques of human coronary arteries.[42] Thus when oxidant defenses become depleted or endogenous tissue rates of oxidant production are accelerated, NO gives rise to secondary oxidizing species that increase membrane and lipoprotein lipid oxidation as well as foam cell formation in the vasculature.[43]

Endothelial cells respond to physical stimuli (e.g., shear stress), hormones, cytokines, drugs, and substances released by sensory and autonomic nerves or platelets by producing vasoactive relaxing substances (e.g. NO) and contracting substances (e.g. endothelin-1) that regulate vascular tone and permeability, hemostasis, angiogenesis, and inflammation. The vascular endothelium sustains the balance between

prevention and stimulation of platelet aggregation, thrombogenesis and fibrinolysis, promotion and inhibition of the smooth muscle cell proliferation and migration, and also between vasoconstriction and vasodilation. The disruption of this tightly controlled balance leads to the development of endothelial dysfunction which represents a predominant early feature of atherosclerosis.[44]

Endothelial dysfunction represents a key early step in the development of atherosclerosis and is also involved in plaque progression and the occurrence of atherosclerotic complications. Endothelial dysfunction significantly increased the risk of vascular events in asymptomatic patients with early stages of atherosclerosis.[45]

Evidence supports the view that the endothelial dysfunction associated with atherosclerosis is related to the local formation of reactive oxygen and nitrogen species in the vicinity of the vascular endothelium.[44]

Impaired brachial artery FMD; a marker of endothelial dysfunction; is an independent predictor of long-term in-stent restenosis after percutaneous intervention PCI,[46] incident cardiovascular events (MI, stroke and death) in an unselected, multi-ethnic population without a history of myocardial infarction or stroke[47] and postoperative cardiovascular events in patients undergoing nonemergent vascular surgery.[48] It also can identify subjects with asymptomatic coronary artery disease.[49]

CIMT (carotid intima media thickness) is a validated tool for noninvasive measurement of early asymptomatic (subclinical) atherosclerotic disease. Carotid IMT independently predicts future vascular events (MI, stroke, and cardiovascular death). Its predictive value is at least as high in younger subjects as in older subjects.[50] Increase in CIMT is not only associated with the presence of coronary artery disease CAD but also with the extent of CAD.[51]

Our aim is to study the relation between the cause of hypothyroidism, Hashimoto thyroiditis, surgery and radioactive iodine and cardiovascular risk in clinically hypothyroid patients.

2. Subjects

The study was carried out on 80 subjects attending the Internal Medicine and the Endocrinology Outpatient Clinics in Alexandria Main University Hospital, who were categorized into four groups: Group I: included 20 patients with chronic autoimmune thyroiditis (diagnosed by anti-thyroid antibodies and thyroid ultrasound) and hypothyroidism defined as TSH > 7 μIU/ml and F.T4 < 9 pmol/l, Group II: included 20 patients with post-thyroidectomy hypothyroidism, Group III: included 20 patients with post-irradiation hypothyroidism, Group IV: included 20 healthy subjects of matched age and sex as a control group.

Exclusion criteria were patients with a history of diabetes mellitus, hypertension, familial dyslipedemia, renal failures, any degree of glucose intolerance and known or symptomatic cardiovascular disease.

3. Methods

3.1. History and examination

Thorough history taking and complete physical examination for signs and symptoms of hypothyroidism, cardiovascular disease, diabetes and hypertension was performed. Height, weight and waist circumference were measured and BMI was calculated by the formula wt in kilograms/$(ht)^2$ in meters.

3.2. Assessment of thyroid function and autoimmunity

Thyroid function was assessed by the determination of thyroid-stimulating hormone (TSH) ELISA, LDN-Germany; reference range 0.4–7 μIU/ml[52] and free T4 (Free L-thyroxine ELISA, LDN-Germany); reference range 9.0–22.2 pmol/l.[53] Thyroid autoimmunity was assessed by the determination of thyroperoxidase antibodies (SERION ELISA classic, Institut Virion/serion GmbH-Germany); a normal range of less 50 IU/ml and thyroglobulin antibodies (anti-thyroglobulin ELISA, LDN-Germany); a normal range of less 100 IU/ml.[54]

3.3. Assessment of asymptomatic (subclinical) atherosclerotic; carotid intima media thickness (CIMT)

We assessed the CIMT in compliance with the recommendation of the American society of echocardiography consensus statement in 2008 for the use of ultrasonography for the measurement of CIMT.[55] Longitudinal images from three imaging planes: anterior, lateral, and posterior with clear images of distal CCA (distal 1 cm of each CCA) in a perfectly horizontal plane with double lines on near and far walls, indicating true perpendicular scanning plane with a linear-array transducer operating at a fundamental frequency of at least 7 MHz (B-mode imaging). Mean CIMT values from the far walls of the right and left CCAs (mean–mean) was reported; *Mean–mean* (average of segmental mean CIMT values) values are more reproducible because multiple points along the traced segment are averaged. CIMT values *greater than or equal to 75th percentile* are considered high and indicative of increased CVD risk.[56]

3.4. Assessment of endothelial function; flow mediated dilation (FMD)

The subjects were investigated by high-resolution color Doppler ultrasound imaging of the brachial artery in the dominant arm in a darkened, temperature controlled room. Furthermore, allowing the participants to rest for 10 min before the study permits hemodynamic stabilization and thus guarantees more accurate baseline readings.

Subjects were instructed to refrain from alcohol and strenuous exercise for 24 h prior to the study, abstain from smoking, vasoactive medicine, caffeine and high-fat food for at least 2 h, as these can all affect FMD.

The study was performed with subjects resting in the supine position. Study participants fasted for 8 h prior to the exam. The subjects' dominant arm was comfortably immobilized in the extended position to allow consistent access to the brachial artery. For each subject, optimal brachial artery images were obtained approximately 5 cm above the antecubital fossa. Arm pressure was caused by inflating a pneumatic arm cuff up to 50 mm Hg higher than the subject's systolic arterial pressure for 5 min. Doppler ultrasound measurements were performed before and 60 s after reactive hyperemia. To avoid interobserver variability, all measurements were performed by the same examiner, who was blind to the subjects' clinical status.

The internal diameter of the brachial artery (at the intima–lumen interface) was assessed at the end of diastole. The

baseline diameter is measured as an average of three frames and the peak as the mean of three consecutive images measured at the time of the maximal increase in diameter after cuff release and the values were recorded.

FMD was calculated according to the formula: FMD = (post-occlusion diameter − baseline diameter) × 100/ baseline diameter.[57]

3.5. Assessment of oxidative stress; serum nitric oxide (NO)

Nitric oxide levels were determined with a colorimetric method based on the Griess reaction. Sodium nitrite was used as a standard, and the results were expressed as μM/ml.[58]

4. Results

All studied groups were matched for age, sex, BMI and waist circumference. (Tables 1 and 2).

None of our subjects had renal impairment nor impaired glucose tolerance although there were statistically significant higher levels of FPG, urea and creatinine in hypothyroid patients compared to control, these parameters also showed a significant negative correlation with F.T4 and a positive correlation with TSH (Tables 1, 2 and 4).

Total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides (TG) showed statistically significant higher levels in hypothyroid subjects than in control group ($P = 0.001$) (Tables 1 and 2).

Statistical analysis shows that there was no significant difference in the mean high density lipoprotein (HDL) cholesterol between all studied groups ($P = 0.960$) (Tables 1 and 2).

Anti-TPO antibodies were significantly higher in hypothyroidism than in the control group ($P = 0.001$), also significantly higher in HT than in other studied groups (Tables 1–3).

Anti-TG antibodies were significantly higher in hypothyroidism than control group ($P = 0.007$), also significantly higher in HT than in other studied groups (Tables 1–3).

Thyroid ultrasound: Group I demonstrated 70% diffuse hypoechogenicity, 10% showed atrophic gland, 25% diffuse goiter and 10% multinodular goiter.

CIMT was significantly higher in hypothyroidism than control group ($P = 0.001$). CIMT was non-significantly (*a trend to increase*) higher in HT compared to post-thyroidectomy and to post-RAI ($P = 0.090$) (Tables 1–3).

FMD was significantly lower in hypothyroidism than control group ($P = 0.001$), also FMD was significantly lower in HT compared to post-thyroidectomy ($P = 0.000$) and to post-RAI ($P = 0.001$) (Tables 1–3).

Serum nitric oxide was significantly higher in hypothyroidism than control group ($P = 0.001$) (Tables 1 and 2).

Table 2 Clinical and laboratory characteristics of hypothyroid patients compared to control.

	Hypothyroid	Control	*P*
Age (Y)	41.85 ± 9.50	41.40 ± 9.75	0.936
BMI	25.91 ± 1.46	25.70 ± 1.34	0.766
WC (cm)	89.15 ± 7.23	86.30 ± 6.47	0.286
Urea (mg/dl)	35.65 ± 3.41	19.65 ± 2.14	0.001
Creatinine (mg/dl)	0.96 ± 0.04	0.76 ± 0.06	0.001
FPG (mg/dl)	90.82 ± 5.60	79.70 ± 5.27	0.001
Hb	11.96 ± 1.39	13.20 ± 0.66	0.001
TC (mg/dl)	262.05 ± 30.66	193.35 ± 14.58	0.001
TG (mg/dl)	140.37 ± 39.19	107.40 ± 18.03	0.001
HDL (mg/dl)	53.18 ± 10.30	54.80 ± 6.93	0.747
LDL (mg/dl)	173.00 ± 22.49	114.50 ± 14.64	0.001
TSH (μIU/ml)	34.06 ± 8.67	2.69 ± 1.81	0.001
Free T4 (pmol/l)	7.21 ± 1.21	14.47 ± 2.48	0.001
Anti-TPO (IU/ml)	74.07 ± 38.82	28.15 ± 9.44	0.001
Anti-TG (IU/ml)	195.12 ± 427.04	8.43 ± 7.26	0.112
NO (μM/ml)	125.98 ± 5.03	39.44 ± 3.63	0.001
CIMT (mm)	0.90 ± 0.08	0.67 ± 0.06	0.001
FMD (%)	6.75 ± 1.42	14.28 ± 1.65	0.001

Table 1 Clinical and laboratory characteristics of the studied groups and control.

	Group I	Group II	Group III	Group IV	*P*
Age (Y)	42.05 ± 9.54	41.30 ± 9.62	42.20 ± 9.82	41.40 ± 9.75	0.994
BMI	25.91 ± 1.44	25.98 ± 1.51	25.84 ± 1.49	25.70 ± 1.34	0.96
WC (cm)	87.80 ± 6.76	90.15 ± 7.59	89.50 ± 7.49	86.30 ± 6.47	0.448
Urea (mg/dl)	35.20 ± 3.59	37.23 ± 3.61	34.52 ± 2.47	19.65 ± 2.14	0.001
Creatinine (mg/dl)	0.95 ± 0.04	0.98 ± 0.05	0.94 ± 0.03	0.76 ± 0.06	0.001
FPG (mg/dl)	91.25 ± 5.73	90.90 ± 5.34	90.30 ± 5.96	79.70 ± 5.27	0.001
Hb	11.75 ± 1.40	12.00 ± 1.48	12.13 ± 1.35	13.20 ± 0.66	0.001
TC (mg/dl)	278.50 ± 20.28	265.40 ± 24.50	242.25 ± 34.61	193.35 ± 14.58	0.001
TG (mg/dl)	143.30 ± 40.62	136.90 ± 39.07	140.90 ± 39.63	107.40 ± 18.03	0.001
HDL (mg/dl)	53.55 ± 11.00	52.90 ± 10.10	53.10 ± 10.32	54.80 ± 6.93	0.960
LDL (mg/dl)	187.90 ± 9.43	177.70 ± 15.81	153.40 ± 23.89	114.50 ± 14.64	0.001
TSH (μIU/ml)	39.58 ± 2.01	37.29 ± 3.76	25.33 ± 9.59	2.69 ± 1.81	0.001
Free T4 (pmol/l)	7.35 ± 1.23	6.65 ± 1.40	7.65 ± 0.74	14.47 ± 2.48	0.001
Anti-TPO (IU/ml)	111.05 ± 15.07	51.00 ± 26.90	60.15 ± 39.05	28.15 ± 9.44	0.001
Anti-TG (IU/ml)	461.55 ± 662.00	70.09 ± 88.05	172.48 ± 428.37	8.43 ± 7.26	0.007
NO (μM/ml)	125.23 ± 5.16	126.72 ± 4.78	125.99 ± 5.27	39.44 ± 3.63	0.001
CIMT (mm)	0.93 ± 0.08	0.89 ± 0.07	0.88 ± 0.07	0.67 ± 0.06	0.001
FMD (%)	5.74 ± 1.33	7.16 ± 1.05	7.34 ± 1.34	14.28 ± 1.65	0.001

Group I: Hashimoto thyroiditis, Group II: post-thyroidectomy hypothyroidism, Group III: post-RAI hypothyroidism, Group IV: control.

Table 3 Important (significant) differences between HT group and post-thyroidectomy hypothyroidism and post-RAI hypothyroidism groups.

Dependent variable	(I) Diagnosis	(J) Diagnosis	Mean difference (I–J)	Sig.
TC	HT	RAI	36.2500(*)	0.000
		SUR	13.1000	0.132
LDL	HT	RAI	34.5000(*)	0.000
		SUR	10.2000	0.069
TSH	HT	RAI	14.2550(*)	0.000
		SUR	2.2950	0.236
Anti-TG	HT	RAI	289.0665	0.068
		SUR	391.4555(*)	0.014
Anti-TPO	HT	RAI	50.9000(*)	0.000
		SUR	60.0500(*)	0.000
FMD	HT	RAI	−1.5975(*)	0.000
		SUR	−1.4240(*)	0.001

HT: Hashimoto thyroiditis, SUR: post-thyroidectomy hypothyroidism, RAI: post-RAI hypothyroidism.
*The mean difference is significant at the .05 level.

There was a positive correlation between serum nitric oxide and anti-TPO antibodies ($r = 0.474$) which was significant ($P = 0.000$) (Table 4).

There was a statistically significant positive correlation between serum nitric oxide and CIMT ($r = 0.766$) ($P = 0.000$), as well as a statistically significant negative correlation between serum nitric oxide and FMD ($r = -0.883$) ($P = 0.000$) (Table 4).

There was a statistically significant positive correlation between CIMT and TSH ($r = 0.725$) ($P = 0.000$), age ($r = 0.566$) ($P = 0.000$), waist circumference ($r = 0.253$) ($P = 0.011$), Total cholesterol ($r = 0.630$) ($P = 0.000$), LDL cholesterol ($r = 0.689$) ($P = 0.000$), anti-TPO antibodies ($r = 0.566$) ($P = 0.000$) and serum nitric oxide ($r = 0.766$) ($P = 0.000$), while there was statistically significant negative correlation between CIMT and F.T4 ($r = -0.749$) ($P = 0.000$) (Table 4).

There was statistically significant negative correlation between FMD and TSH ($r = -0.910$) ($P = 0.000$), anti-TPO antibodies ($r = -0.529$) ($P = 0.000$), total cholesterol ($r = -0.826$) ($P = 0.000$), LDL cholesterol ($r = -0.911$) ($P = 0.000$) and TG ($r = -0.288$) ($P = 0.004$), and serum nitric oxide ($r = -0.883$) ($P = 0.000$), while there was a statistically significant positive correlation between FMD and F.T4 ($r = 0.799$) ($P = 0.000$) (Table 4).

All the correlations were made for all subjects in all studied groups.

4.1. Multivariate analysis of different factors which affect CIMT

Multivariate analysis showed that the diagnosis of Hashimoto thyroiditis is independent of thyroid function, age, WC and dyslipedemia affected or "predicted" CIMT ($P = 0.015$). There are other factors affecting CIMT in the same model which are age ($P = 0.000$), waist circumference ($P = 0.000$), F.T4 ($P = 0.027$) and interestingly serum nitric oxide ($P = 0.000$) (Table 5).

Table 4 Important correlations between studied parameters and NO, FMD and CIMT in patients with hypothyroidism.

	NO	CIMT	FMD
WC			
r	0.191	0.253(*)	−0.125
P	0.057	0.011	0.214
FPG			
r	0.443(**)	0.470(**)	−0.497(**)
P	0.000	0.000	0.000
TG			
r	0.299(**)	0.224(*)	−0.288(**)
P	0.003	0.025	0.004
TC			
r	0.642(**)	0.630(**)	−0.826(**)
P	0.000	0.000	0.000
LDL			
r	0.704(**)	0.689(**)	−0.911(**)
P	0.000	0.000	0.000
Age			
r	0.034	0.566(**)	80.003
P	0.734	0.000	0.977
F.T4			
r	−0.725(**)	−0.749(**)	0.799(**)
P	0.000	0.000	0.000
TSH			
r	0.822(**)	0.725(**)	−0.910(**)
P	0.000	0.000	0.000
Anti-TG			
r	0.240(*)	0.435(**)	−0.301(**)
P	0.024	0.000	0.004
Anti-TPO			
r	0.474(**)	0.566(**)	−0.529(**)
P	0.000	0.000	0.000
NO			
r	1	0.766(**)	−0.883(**)
P		0.000	0.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 5 Multivariate analysis of different factors which affect CIMT in patients with hypothyroidism.

Model	Unstandardized coefficients		Standardized coefficients	*t*	Sig.
	B	Std. error	Beta		
1					
Constant	0.264	0.069		3.845	0.000
Diagnosis	−0.009	0.004	−0.115	−2.468	0.015
WC	0.002	0.000	0.115	4.034	0.000
TC	5.800E−07	0.000	0.000	0.003	0.998
LDL	0.000	0.000	0.110	1.111	0.270
Age	0.006	0.000	0.497	17.441	0.000
F.T4	−0.004	0.002	−0.122	−2.242	0.027
TSH	0.001	0.001	0.098	0.995	0.322
Anti-TPO	0.000	0.000	0.081	2.401	0.018
NO	0.001	0.000	0.362	5.779	0.000

4.2. Multivariate analysis of different factors which affect FMD

Multivariate analysis showed that the diagnosis of Hashimoto thyroiditis is independent of thyroid function, age and dyslipedemia affected or predicted FMD ($P = 0.000$), other factors affecting FMD in the same model are LDL cholesterol ($P = 0.000$), F.T4 ($P = 0.046$) and interestingly serum nitric oxide ($P = 0.000$) (Table 6).

5. Discussion

5.1. Markers of subclinical atherosclerosis in hypothyroidism

Our results show that CIMT is significantly higher in overt hypothyroidism compared to control and correlated positively to TSH and negatively to F.T4. Univariate analysis showed that CIMT was also correlated to age, WC, total cholesterol, LDL cholesterol and serum nitric oxide.

Regarding the effect of the cause of hypothyroidism on CIMT, we observed a trend toward higher values of CIMT in HT compared to post-thyroidectomy and post-RAI that was not statistically significant; however, multivariate analysis showed that HT; independent of age, WC, F.T4 and NO; predicted CIMT with statistical significance.

In accordance with our work, Nagasaki et al. and Kim et al. showed that both overt and subclinical hypothyroidism increased CIMT. They explained this by an atherogenic lipid profile as both increased CIMT and dyslipidemia were reversible after achieving euthyroidism. They also suggested other explanations like low grade inflammation and activation of the immune system by Hashimoto thyroiditis.[59,60]

Takamura et al. showed that TSH, as well as fT4, within normal reference range, was significantly correlated with CIMT after adjustment for known cardiovascular risk factors.[61]

On the other hand, Cabral et al. showed that there is no significant difference observed with respect to CIMT between the SCH and control groups. The explanation was that they chose subjects who were matched with respect to age, BMI, smoking, menopausal status, and lipid profile.[62]

5.2. Markers of endothelial dysfunction

Our results show that FMD is significantly lower in overt and hypothyroidism compared to the control and correlated negatively to TSH and positively to F.T4, univariate analysis showed that FMD was also correlated to age, anti-TPO antibodies, total cholesterol, LDL cholesterol and serum nitric oxide.

Regarding the effect of the cause of hypothyroidism on FMD, we observed that FMD was significantly lower in HT compared to post-thyroidectomy and post-RAI; also, multivariate analysis showed that HT, independent of LDL-C, F.T4 and NO, predicted FMD with statistical significance.

In accordance with our work, Clausen et al. and Taddei et al. showed that both overt and subclinical hypothyroidism were associated with endothelial dysfunction and impaired FMD. They explained this partly by mild dyslipidemia as endothelial dysfunction persisted even after achieving euthyroidism and normolipidemia. They attributed this to the fact that most of their patients were HT and that autoimmune process may still be active in these patients despite levothyroxine substitution and this process could maintain the endothelial dysfunction despite euthyroidism.[63,64]

Völzke et al. showed that serum TSH levels within the upper reference range are associated with impaired endothelial function. They recommended that upper TSH reference limit should be redefined.[65]

On the other hand, Cabral et al. showed that there is no significant difference observed with respect to FMD between the SCH and control groups. The explanation was that they selected subjects who were matched with respect to age, BMI, smoking, menopausal status, and lipid profile.[62]

5.3. Markers of oxidative stress

Reactive oxygen species (ROS) consist of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), and increased free-radical production leads to oxidative stress with the formation of self-propagating lipid peroxidation.

Primary defense against oxidative stress includes the antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT). These ROS interact with nucleic acids, proteins and lipids causing cellular dysfunction and even death.[66]

Nitric oxide (NO) is produced by the enzyme NO synthase (NOS). NOS occurs in three forms: endothelial (e) (NOS3), neuronal (n) (NOS1) and inducible (i) (NOS2). The first two are constitutively expressed c-NOS. In general, n- and e-NOS release NO in the nM range whereas i-NOS, following

Table 6 Multivariate analysis of different factors which affect FMD in patients with hypothyroidism.

Model	Unstandardized coefficients		Standardized coefficients	*t*	Sig.
	B	Std. Error	Beta		
1					
Constant	21.819	1.310		16.650	0.000
Diagnosis	0.458	0.084	0.201	5.440	0.000
TG	0.002	0.003	0.024	0.700	0.486
TC	0.001	0.006	0.011	0.147	0.884
LDL	−0.066	0.009	−0.617	−7.126	0.000
F.T4	−0.079	0.039	−0.084	−2.028	0.046
TSH	0.006	0.018	0.026	0.324	0.747
Anti-TPO	−0.002	0.002	−0.023	−0.926	0.357
NO	−0.036	0.005	−0.394	−7.744	0.000

an induction/latency period, can release NO in the mM range for extended periods of time (often called a high-output source of NO).[44]

NO derived from c-NOS may occur in two functional forms: the first is always present at low 'tonal' or 'basal' levels which serve to dampen or control the threshold for activation of vascular and immune cells in response to nonspecific stimuli, i.e. homeostatic, and an enhanced release in response to certain signals, e.g., acetylcholine (ACH). This brief and enhanced release of c-NOS-derived NO can have profound physiological actions, e.g. endothelium-dependent vasodilation, whereas i-NOS is induced by various signal molecules, e.g. proinflammatory cytokines.[67].

NO, derived from c-NOS, may tonically inhibit i-NOS activity through NF-kB under non-stimulated conditions. However, if the proinflammatory event was extremely strong, i-NOS induction probably cannot be diminished.[67]

NO has a role as a neurotransmitter and a regulator of blood pressure, it has vasodilator and antiplatelet, tumoricidal, and microbicidal activities.[68] NO is converted under aerobic conditions to nitrite, and low concentrations of nitrite inhibit myeloperoxidase-mediated oxidation of LDL. NO also acts as an antioxidant by scavenging alkoxyl and peroxyl radicals. When NO is in excess of the surrounding oxidants, lipid oxidation and monocyte margination into the vascular wall are attenuated, producing anti-atherogenic effects.[43]

ROS can reduce NO bioavailability in endothelium by three mechanisms: (1) in the presence of $O^{\cdot -}$ by the formation of peroxynitrite, (2) inhibition of e-NOS, and (3) uncoupling of e-NOS with decreased NO and increased $O^{\cdot -}$ production, this is followed by endothelial dysfunction and atherogenesis.[69]

Every time NO and superoxide collide, they form peroxynitrite non-enzymatically and very rapidly. Under proinflammatory conditions, simultaneous production of superoxide and NO can be strongly activated to increase production 1000-fold, which will increase the formation of peroxynitrite by a 1000,000-fold.[62]

The peroxynitrite anion ($ONOO_2$) is an effective means of producing hydroxyl radical OH^-, which oxidizes LDL.[43] Peroxynitrite may contribute to endothelial dysfunction by various mechanisms including: (1) triggering apoptosis and/or necrosis in endothelial and vascular smooth muscle cells, (2) inducing upregulation of adhesion molecules in endothelial cells, (3) enhancing neutrophils adhesion and other mechanisms.[69]

Furthermore, clinically relevant concentrations of 3-nitrotyrosine, a marker of peroxynitrite load, result in concentration-dependent impairment of acetylcholine-induced, endothelium-dependent vascular relaxation. Numerous studies have demonstrated increased 3-nitrotyrosine and i-NOS expression in human atherosclerotic tissue, which correlated with plaque instability in patients.[62]

i-NOS-derived peroxynitrite has a central component of inflammation. Expression of inducible NOS, which is associated with increased peroxynitrite production, resulted in increased apoptotic cell death in atheromatous plaques of human coronary arteries.[43,44]

Our results show that serum NO is significantly higher in overt hypothyroidism compared to the control and correlated positively to TSH and negatively to F.T4, univariate analysis showed that NO was also correlated to anti-TPO antibodies, total cholesterol, LDL cholesterol.

Regarding the effect of the cause of hypothyroidism on serum NO, we observed that NO was *not* significantly different in the three studied groups of overt hypothyroidism; also, both univariate and multivariate analyses of different factors which affect CIMT and FMD showed that NO was an independent predictor of both FMD and CIMT.

Coria et al. showed somewhat similar results to ours, they proved that NO concentration significantly increased in overt hypothyroidism compared to the euthyroidism. Also NO in SCH tends to increase, though this was not significant compared to euthyroidism.[70]

Moustafa et al. demonstrated that serum total T3 showed negative correlation with serum NO, in a similar way we demonstrated a negative relation between F.T4 and NO. They explained their finding by the antioxidant function of thyroid hormones in two ways: by increasing ATP cycling, which enhances oxidative phosphorylation and decreases the formation of ROS and by increasing the supply of NADPH necessary for the regeneration of reduced glutathione (GSH), thus facilitating the scavenging of ROS.[71]

Kumari et al., unlike our results, showed that serum nitric oxide exhibits almost no change in hypothyroidism when compared to the normal levels.[72] Ozcan et al. on the other hand demonstrated that the NO levels in serum of the hypothyroid were lower than in the euthyroid controls.[73]

The apparent discrepancy in our results between impaired FMD (denoting impaired e-NOS derived NO availability) and increased serum NO can be resolved by the following arguments.

Virdis et al. demonstrated a significant i-NOS induction, together with an increased superoxide production, and impaired

endothelium-dependent relaxation in hypothyroidism. All these changes were reversible by i-NOS blockade and antioxidants.

These findings suggest opposing effects of hypothyroidism on i-NOS and e-NOS where there is an induction of i-NOS with increased NO and O^- (hence peroxynitrite formation), and down regulation of e-NOS with loss of tonal and stimulated c-NOS derived NO leading to non-dampening of vascular and immune cells activation and loss of endothelium-dependent vasodilation.[74]

A second argument to resolve discrepancy is that endothelial-dependent vasodilation EDV may not be NO dependent after all; supporting this hypothesis raised by Tschakovsky and Pyke are the following observations.

With an increase in shear stress at least three vasodilators are known to increase (NO, prostacyclin, endothelium-derived hypopolarizing factor). The FMD response to more sustained stimuli was mediated by NO-independent mechanism(s). Accordingly, FMD in response to sustained shear stress may constitute a critical component of endothelial function for which NO may not be obligatory. Shear stimulus also evokes a potent vasoconstrictor, endothelin. Thus alterations in endothelin release by the vascular endothelium would be expected to influence FMD.

Taken together, the common assumption that FMD represents NO-mediated endothelial function may be misleading in understanding endothelial function.[75]

To our knowledge, this is the first study to show that HT is an independent cardiovascular risk factor in clinically hypothyroid patients, Bilir et al. found no statistically significant difference regarding CIMT between 59 hypothyroid patients classified according to the cause of hypothyroidism; HT, post-surgical and post-radioiodine.

Possible mechanisms explaining the increased cardiovascular risk in Hashimoto thyroiditis

1. Low grade chronic inflammation leading to endothelial dysfunction: the association between low grade inflammation and endothelial function was demonstrated by Taddei et al., they showed that COX-2 dependent low grade inflammation as evidenced by high hs-CRP and IL-6 in patients with SCH due to Hashimoto thyroiditis causes impairment of endothelium-dependent vasodilation.[76]Taddei et al. proposed two possible mechanisms for systemic inflammation: high TSH itself may induce inflammation as evidenced by its ability to induce TNF-a production, but the persistence of impaired EDV even after L-thyroxine replacement and achieving euthyroidism precludes this explanation. The second and more accepted mechanism is the chronic stimulation of the immune system by Hashimoto thyroiditis, the persistence of only elevated ESR and thyroid Abs after achieving euthyroidism proves a role for Hashimoto thyroiditis in the residual endothelial dysfunction.[64]
2. Increased oxidative stress: in the aforementioned study by Taddei et al., they demonstrated that vitamin C (an antioxidant compound capable of scavenging superoxide anions at high concentrations) significantly improved EDV. They suggested that oxidative stress may be the pathogenetic mechanism linking inflammation to endothelial dysfunction.[76]Gerenova et al. demonstrated lower activities of Catalase and Glutathione peroxidase in euthyroid Hashimoto thyroiditis, suggesting an increased oxidative stress.[77]In addition, our work showed that nitric oxide as a marker of oxidative stress was an independent predictor of both CIMT and FMD in clinically hypothyroid patients.

Evidence from clinical trials linking euthyroid Hashimoto thyroiditis to increased cardiovascular risk and impaired health status demonstrated the following observations: (1) increased CIMT in overweight and obese women probably due to decreased immune tolerance in obesity, (2) increased IL-6, a key cytokine in atherosclerosis, (3) lower endothelial-dependent arterial dilation linked to autoimmunity and elevated LP(a), (4) impaired right and left ventricular function and cardiac autonomic dysfunction, (5) increased arterial stiffness in women and finally (6) decreased quality of life and early pregnancy loss.[78–83]

Staii et al. recently reported that euthyroid cytology proven HT has a similar prevalence to that of type 2 diabetes.[84]

In conclusion, to our knowledge, this is the first study to show that HT is an independent cardiovascular risk factor. Given its high prevalence, HT may have an impact on cardiovascular health that is more or less similar to that of type 2 diabetes.

References

1. Roberts CGP, Ladenson PW. Hypothyroidism. *Lancet* 2004;**363**:793–803.
2. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T4, and thyroid antibodies in the United States Population (1988–1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 2002;**87**:489–99.
3. Parle JV, Franklyn JA, Cross KW, Jones SC, Sheppard MC. Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. *Clin Endocrinol* 1991;**34**:77–83.
4. Canaris GJ, Manowitz NR, Mayor GM, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Intern Med* 2000;**160**:526–34.
5. Davies TF, Amino N. A new classification for human autoimmune thyroid disease. *Thyroid* 1993;**3**:331–3.
6. Li Y, Nishihara E, Hirokawa M, Taniguchi E. Distinct clinical, serological, and sonographic characteristics of Hashimoto's thyroiditis based with and without IgG4-positive plasma cells. *J Clin Endocrinol Metab* 2010;**95**:1309–17.
7. Walsh JP, Bremner AP, Feddema P, Leedman PJ, Brown SJ, O'Leary P. Thyrotropin and thyroid antibodies as predictors of hypothyroidism: a 13-year, longitudinal study of a community-based cohort using current immunoassay techniques. *J Clin Endocrinol Metab* 2010;**95**:1095–104.
8. Vaiman M, Nagibin A, Hagag P, Kessler A, Gavriel H. Hypothyroidism following partial thyroidectomy. *Otolaryngol Head Neck Surg* 2008;**138**:98–100.
9. Lankarani M, Mahmoodzadeh H, Poorpezeshk N, Soleimanpour V, Haghpanah V, Heshmat R. Hypothyroidism following thyroid surgery. *Acta Med Iranica* 2008;**46**(3):225–32.
10. Moreno P, Gómez JM, Gómez N. Subtotal thyroidectomy: a reliable method to achieve euthyroidism in graves disease. Prognostic factors. *World J Surg* 2006;**30**:1950–6.
11. Reynier J, Ngo P, Srania B. Technique de lexérèse dans la maladie de Basedow. *Lyon Chir* 1990;**86**:2.
12. Berglund J, Bondesom L, Christensen SB. The influence of different degrees of chronic lymphocytic thyroiditis on thyroid function after surgery for benign, non-toxic goiter. *Eur J Surg* 1991;**157**:257–60.

13. Wormald R, Sheahan P, Rowley S, Rizkalla H, Toner M, Timon C. Hemithyroidectomy for benign thyroid disease: who needs follow-up for hypothyroidism? *Clin Otolaryngol* 2008;**33**:587–91.
14. Bang U, Blichert-Toft M, Petersen PH. Thyroid function after resection for non-toxic goiter with special reference to thyroid lymphocytic aggregation and circulating thyroid autoantibodies. *Acta Endocrinol* 1985;**109**:214–9.
15. Moon H, Jung E, Park S, Jung TS, Jeong C, Ju Y, et al. Thyrotropin level and thyroid volume for prediction of hypothyroidism following hemithyroidectomy in an Asian patient cohort. *World J Surg* 2008;**32**:2503–8.
16. Koh YW, Lee SW, Choi EC, Lee JD, Mok JO, Kim HK, et al. Prediction of hypothyroidism after hemithyroidectomy: a biochemical and pathological analysis. *Eur Arch Otorhinolaryngol* 2008;**265**:453–7.
17. Su SY, Serpell J. Hypothyroidism following hemithyroidectomy. *ANZ J Surg* 2007;**77**(Suppl 1):A21–5.
18. Seiberling KA, Dutra JC, Bajaramovic S. Hypothyroidism following hemithyroidectomy for benign nontoxic thyroid disease. *Ear Nose Throat J* 2007;**86**(5):295–9.
19. Miller FR, Paulson D, Prihoda TJ, Otto RA. Risk factors for the development of hypothyroidism after hemithyroidectomy. *Arch Otolaryngol Head Neck Surg* 2006;**132**:36–8.
20. Buchanan MA, Lee D. Thyroid auto-antibodies, lymphocytic infiltration and the development of postoperative hypothyroidism following hemithyroidectomy for non-toxic nodular goiter. *J R Coll Surg Edin* 2001;**46**:86–90.
21. De Carlucci Jr D, Tavares MR, Obara MT. Thyroid function after unilateral total lobectomy risk factors for postoperative hypothyroidism. *Arch Otolaryngol Head Neck Surg* 2008;**134**(10):1076–9.
22. Thjodleifsson B, Hedley AJ, Donald D, Chesters MI, Kjeld M, Beck JS, et al. Outcome of sub-total thyroidectomy for thyrotoxicosis in Iceland and Northeast Scotland. *Clin Endocrinol (Oxf)* 1977;**7**(5):367–76.
23. Stoll SJ, Pitt SC, Liu J, Schaefer S, Sippel RS. Thyroid hormone replacement after thyroid lobectomy. *Surgery* 2009;**146**:554–60.
24. Ying M, Yung DMC. Asymmetry of thyroid lobe volume in normal Chinese subjects: association with handedness and position of esophagus. *Anat Rec* 2009;**292**:169–74.
25. Werner SC, Coelho B, Quimby EH. Ten year result results of I-131 therapy in hyperthyroidism. *Bull N Y Acad Med* 1957;**33**: 783–806.
26. Kabadi U, Cech R. Therapeutic 131I dose in hyperthyroidism: role of pretreatment with thionamide. *Thyroidology* 1994;**6**:87–92.
27. Franklyn JA. The management of hyperthyroidism. *N Engl J Med* 1994;**330**:1731–8.
28. Allahabadia A, Daykin J, Holder RL, Sheppard MC, Gough SC, Franklyn JA. Age and gender predict the outcome of treatment for Graves' hyperthyroidism. *J Clin Endocrinol Metab* 2000;**85**:1038–42.
29. Allahabadia A, Daykin J, Sheppard MC, Gough SCL, Franklyn JA. Radioiodine treatment of hyperthyroidism – prognostic factors for outcome. *J Clin Endocrinol Metab* 2001;**86**:3611–7.
30. Watson AB, Brownlie BE, Frampton CM, Turner JG, Rogers TG. Outcome following standardized 185 MBq dose 131 I therapy for Graves' disease. *Clin Endocrinol* 1988;**28**:487–96.
31. Metso S, Jaatinen P, Huhtala H, Luukkaala T, Oksala H, Salmi J. Long-term follow-up study of radioiodine treatment of hyperthyroidism. *Clin Endocrinol* 2004;**61**:641–8.
32. Adamali HI, Gibney J, O'Shea D, Casey M, McKenna TJ. The occurrence of hypothyroidism following radioactive iodine treatment of toxic nodular goiter is related to the TSH level. *Ir J Med Sci* 2007;**176**:199–203.
33. Pedersen-Bjergaard U, Kirkegaard C. Serum TSH and the response to radioiodine treatment of toxic multinodular goitre. *Eur J Endocrinol* 1997;**137**:365–9.
34. Albino CC, Mesa Jr CO, Olandoski M, Ueda CE, Woellner LC, Goedert CA, et al. Recombinant human thyrotropin as adjuvant in the treatment of multinodular goiters with radioiodine. *J Clin Endocrinol Metab* 2005;**90**:2775–80.
35. Nielsen VE, Bonnema S, Hegedus L, Grupe P, BoelJørgensen H. Recombinant human thyrotropin prior to radioiodine therapy improves the size reduction of non-toxic nodular goiter: a prospective randomized double-blinded trial. *Thyroid* 2005;**15**(Suppl 1):026.
36. Alexander EK, Larsen PR. High dose of (131)I therapy for the treatment of hyperthyroidism caused by Graves' disease. *J Clin Endocrinol Metab* 2002;**87**:1073–7.
37. Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. *J Clin Endocrinol Metab* 2003;**88**:2438–44.
38. Abbas JMK, Chakrborty J, Akanji AO. Hypothyroidism results in small dense LDL independent of IRS traits and hypertriglyceridemia. *Endocr J* 2008;**55**(2):381–9.
39. Nanda N, Bobby Z, Hamide A. Oxidative stress and protein glycation in primary hypothyroidism. Male/female difference. *Clin Exp Med* 2008;**8**:101–8.
40. Dagre AG, Lekakis JP, Protogerou AD. Abnormal endothelial function in female patients with hypothyroidism and borderline thyroid function. *Int J Cardiol* 2007;**114**:332–8.
41. Patel RP, Levonen A, Crawford JH, Darley-Usmar VM. Mechanisms of the pro- and anti-oxidant actions of nitric oxide in atherosclerosis. *Cardiovasc Res* 2000;**47**:465–74.
42. Esaki T, Hayashi T, Muto E, Kano H, Kumar TN, Asai Y, et al. Expression of inducible nitric oxide synthase and Fas/Fas ligand correlates with the incidence of apoptotic cell death in atheromatous plaques of human coronary arteries. *Nitric Oxide* 2000;**4**:561–71.
43. Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J* 2001;**15**:2073–84.
44. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007;**87**(1):315–424.
45. Corrado E, Rizzo M, Coppola G, Muratori I, Carella M, Novo S. Endothelial dysfunction and carotid lesions are strong predictors of clinical events in patients with early stages of atherosclerosis: a 24-month follow-up study. *Coron Artery Dis* 2008;**19**:139–44.
46. Akcakoyuna M, Kargina R, Tanalpa AC, Palaa S, Ozverena O, Akcay M, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events and restenosis in patients undergoing coronary stent implantation: a prospective study. *Coron Artery Dis* 2008;**19**:337–43.
47. Shimbo D, Grahame-Clarke C, Miyake Y, Rodriguez C, Sciacca M, Di Tullio M, et al. The association between endothelial dysfunction and cardiovascular outcomes in a population-based multi-ethnic cohort. *Atherosclerosis* 2007;**192**:197–203.
48. Gokce N, Keaney Jr JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 2002;**105**:1567–72.
49. Kaźmierski M, Michalewska-Wudarczyk A, Krzych LJ, Tendera M. Diagnostic value of flow mediated dilatation measurement for coronary artery lesions in men under 45 years of age. *Cardiol J* 2010;**17**(3):288–92.
50. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range prospective data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke* 2006;**37**:87–92.
51. Coskun U, Yildiz A, Esen OB, Baskurt M, Cakar MA, Kilickesmez KO, et al. Relationship between carotid intima-media thickness and coronary angiographic findings: a prospective study. *Cardiovasc Ultrasound* 2009;**7**:59–65.
52. Morimoto K, Inouye KA. Sensitive enzyme immunoassay of human thyroid-stimulating hormone (TSH) using bispecific F (ab') 2 fragments recognizing polymerized alkaline phosphatase and TSH. *J Immunol Methods* 1997;**205**(1):81–90.
53. Tiffany TO, Burts CA. Fluorometry, nephelometry and turbidimetry. In: Tietz NW, editor. *Fundamentals of clinical chemistry*. 3rd ed. Philadelphia: WB Saunders Company; 1987. p. 66–77.

54. Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. *Clin Chem* 1996;**42**:160–3.
55. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendal CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* 2008;**21**(2):93–111.
56. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke* 2006;**37**:87–92.
57. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;**39**:257–65.
58. Granger DL, Taintor RR, Boockvar KS, Hibbs Jr JB. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. *Methods Enzymol* 1996;**268**:142–51.
59. Nagasaki T, Inaba M, Henmi Y, et al. Decrease in carotid-intima media thickness in hypothyroid patients after normalization of thyroid function. *Clin Endocrinol* 2003;**59**:607–12.
60. Kim SK, Kim SH, Park KS, Park SW, Cho YW. Regression of the increased common carotid artery-intima media thickness in subclinical hypothyroidism after thyroid hormone replacement. *Endocr J* 2009;**56**(6):753–8.
61. Takamura N, Akilzhanova A, Hayashida N, Kadota K, Yamasaki T, Usa T, et al. Thyroid function is associated with carotid intima-media thickness in euthyroid subjects. *Atherosclerosis* 2009;**204**:e77–81.
62. Cabral MD, Teixeira PFS, Silva NAO, Morais FFC, Soares DV, Salles E, et al. Normal flow-mediated vasodilatation of the brachial artery and carotid artery intima-media thickness in subclinical hypothyroidism. *Braz J Med Biol Res* 2009;**42**:426–32.
63. Clausen P, Mersebach H, Nielsen B, Feldt-Rasmussen B, Feldt-Rasmussen U. Hypothyroidism is associated with signs of endothelial dysfunction despite 1-year replacement therapy with levothyroxine. *Clin Endocrinol* 2009;**70**:932–7.
64. Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, et al. Impaired endothelium-dependent vasodilatation in subclinical hypothyroidism: beneficial effect of levothyroxine therapy. *J Clin Endocrinol Metab* 2003;**88**:3731–7.
65. Völzke H, Robinson DM, Spielhagen T, Nauck M, Obst A, Ewert R, et al. Are serum thyrotropin levels within the reference range associated with endothelial function? *Eur Heart J* 2009;**30**:217–24.
66. Evereklioglu C, Hamdi Er, Doganay S, Cekmen M, Turkoz Y, Otlu B, et al. Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration. *Doc Ophthalmol* 2003;**106**:129–36.
67. Stefano GB, Goumon Y, Bilfinger TV, Welters ID, Cadet P. Basal nitric oxide limits immune, nervous and cardiovascular excitation: human endothelia express a mu opiate receptor. *Prog Neurobiol* 2000;**60**:513–30.
68. Rodríguez-Arnao MD, Rodríguez-Sánchez A, Rodríguez-Arnao E, Dulín-Íñiguez E, Cano JMB, Muñoz-Fernández MA. Undetectable levels of tumor necrosis factor-alpha, nitric oxide and inadequate expression of inducible nitric oxide synthase in congenital hypothyroidism. *Eur Cytokine Netw* 2003;**14**(1):65–8.
69. Siekmeier R, Steffen C, März W. Role of oxidants and antioxidants in atherosclerosis: results of in vitro and in vivo investigations. *J Cardiovasc Pharmacol Ther* 2007;**12**(4):265–82.
70. Coria MJ, Pastrán AI, Gimenez MS. Serum oxidative stress parameters of women with hypothyroidism. *Acta Biomed* 2009;**80**:135–9.
71. Moustafa AHA, Ali EMM, Mohamed TM, Abdou HI. Oxidative stress and thyroid hormones in patients with liver diseases. *Eur J Intern Med* 2009;**20**:703–8.
72. Kumari SN, Sandhya, Gowda KMD. Oxidative stress in hypo and hyperthyroidism. Al Ameen J Med Sci 2011;4(1):49–53.
73. Ozcan O, Cakir E, Yaman H, et al. The effects of thyroxine replacement on the levels of serum asymmetric dimethylarginine (ADMA) and other biochemical cardiovascular risk markers in patients with subclinical hypothyroidism. *Clin Endocrinol* 2005;**63**:203–6.
74. Virdis A, Colucci R, Fornai M, Polini A, Daghini E, Duranti E, et al. Inducible nitric oxide synthase is involved in endothelial dysfunction of mesenteric small arteries from hypothyroid rats. *Endocrinology* 2009;**150**:1033–42.
75. Tschakovsky ME, Pyke KE. Counterpoint: flow-mediated dilation does not reflect nitric oxide-mediated endothelial function. *J Appl Physiol* 2005;**99**:1235–7.
76. Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, et al. Low-grade systemic inflammation causes endothelial dysfunction in patients with Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 2006;**91**(12):5076–82.
77. Gerenova J, Gadjeva V. Oxidative stress and antioxidant enzyme activities in patients with Hashimoto's thyroiditis. *Comp Clin Pathol* 2007;**16**:259–64.
78. Ciccone MM, De Pergola G, Porcelli MT, Scicchitano P, Caldarola P, Iacoviello M, et al. Increased carotid IMT in overweight and obese women affected by Hashimoto's thyroiditis: an adiposity and autoimmune linkage? *BMC Cardiovasc Disord* 2010;**10**:22–9.
79. Siemińska L, Wojciechowska C, Kos-Kuda B, Marek B, Kajdaniuk D, Nowak M, et al. Serum concentrations of leptin, adiponectin, and interleukin-6 in postmenopausal women with Hashimoto's thyroiditis. *Pol J Endocrinol* 2010;**61**(1):112–6.
80. Xiang G, He Y, Zhao L, Hou J, Yue L, Xiang H. Impairment of endothelium-dependent arterial dilation in Hashimoto's thyroiditis patients with euthyroidism. *Clin Endocrinol (Oxf)* 2006;**64**:698–702.
81. Akgul E, Kutuk U, Ertek S, Cesur M, Cehreli S, Tore HF, et al. Cardiac autonomic function and global left ventricular performance in autoimmune euthyroid chronic thyroiditis: is treatment necessary at the euthyroid stage? *Echocardiography* 2011;**28**:15–21.
82. Stamatelopoulos KS, Kyrkou K, Chrysochoou E, Karga H, Chatzidou S, Georgiopoulos G, et al. Arterial stiffness but not intima-media thickness is increased in euthyroid patients with Hashimoto's thyroiditis: the effect of menopausal status. *Thyroid* 2009;**19**(8):857–62.
83. Ott J, Promberger R, Kober F, Neuhold N, Tea M, Huber JC, et al. Hashimoto's thyroiditis affects symptom load and quality of life unrelated to hypothyroidism: a prospective case-control study in women undergoing thyroidectomy for benign goiter. *Thyroid* 2011;**21**(2):161–7.
84. Staii A, Mirocha S, Todorova-Koteva K, Glinberg S, Jaume JC. Hashimoto thyroiditis is more frequent than expected when diagnosed by cytology which uncovers a preclinical state. *Thyroid Res* 2010;**3**:11–7.