

## RESEARCH

# Endothelial dysfunction and the risk of atherosclerosis in overt and subclinical hypothyroidism

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## Abstract

Hypothyroidism is associated with increased risk of atherosclerosis. We assessed carotid intima-media thickness (CIMT), as a marker of atherosclerosis, and endothelial function in patients with hypothyroidism. We included 70 female patients with hypothyroidism in the study, 40 patients with overt and 30 patients with subclinical hypothyroidism. Forty, age- and sex-matched, subjects with normal thyroid functions were also included as a control group. CIMT was measured using high-resolution color-coded Doppler ultrasonography. Endothelial function was assessed by measuring the percent of change in blood flow following heat-mediated vasodilation using laser Doppler flowmetry. CIMT was significantly higher in patients with overt and subclinical hypothyroidism as compared with the control group ( $0.7 \pm 0.2$  and  $0.6 \pm 0.2$  mm respectively vs  $0.45 \pm 0.07$  mm,  $P < 0.001$  for both). The percent of change in blood flow following heat-mediated vasodilation was significantly impaired in patients with overt and subclinical hypothyroidism as compared with the control group ( $328 \pm 17$  and  $545 \pm 406\%$  respectively vs  $898 \pm 195\%$ ,  $P < 0.001$  for both). The impairment was more significant in overt as compared with subclinical hypothyroidism ( $P = 0.014$ ). CIMT negatively correlated with percent of change in blood flow following heat-mediated vasodilation in patients with overt and subclinical hypothyroidism ( $P < 0.001$  for both). We concluded that CIMT is significantly higher in patients with overt and subclinical hypothyroidism compared with normal control subjects. Impairment of endothelial function is a contributing factor to the increased risk of atherosclerosis in both groups of patients.

## Key Words

- ▶ intima-media thickness
- ▶ endothelial function
- ▶ overt hypothyroidism
- ▶ subclinical hypothyroidism

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## Introduction

Hypothyroidism is associated with an increased risk for atherosclerotic cardiovascular disease owing to its metabolic and hemodynamic effects (1). Multiple mechanisms account for atherosclerosis in patients with hypothyroidism such as dyslipidemia, hypercoagulable state, increased arterial stiffness, obesity and endothelial dysfunction (1, 2, 3, 4). Current controversy centers on whether subclinical hypothyroidism (SCH) is associated

with atherosclerosis. Some studies showed that there is an association, but others did not confirm that (5, 6, 7). So, the benefit from thyroid replacement therapy in SCH is still debatable.

Endothelial dysfunction is believed to be an early step in the development and progression of atherosclerosis. Assessment of endothelial dysfunction, being an early biomarker, is helpful in predicting cardiovascular risk and

evaluating the outcome of treatment (8). Detecting and quantifying the existence of subclinical vascular disease, through vascular imaging, has been suggested as a means to enhance cardiovascular risk assessment (9). Carotid artery intima-media thickness (CIMT) measurement has increasingly been used as a marker of atherosclerosis in many studies (10). The aim of our work was to assess both CIMT and endothelial dysfunction in patients with hypothyroidism.

## Materials and methods

Our study included 110 Egyptian females, aged 18 to 55 years, recruited from the Endocrinology Clinic at Cairo University hospital. They were classified into three groups:

Group I: Forty patients with overt hypothyroidism (high serum thyroid-stimulating hormone (TSH) and reduced FT4).

Group II: Thirty patients with subclinical hypothyroidism (high serum TSH with normal FT4).

Group III: Forty age- and sex-matched subjects with normal thyroid functions as a control group.

Patients with diabetes mellitus, hypertension, known atherosclerotic cardiovascular disease (or ankle/brachial index < 0.9), liver or renal disease, primary hyperlipidemia and pregnant ladies were excluded from the study. The study was approved by Cairo University Ethical Committee and Review Board. All the patients and control subjects who participated in the study provided written informed consents.

Blood samples were drawn at 0800 h, after a 12-h fast. Total cholesterol, LDL-C, HDL-C, serum triglycerides, serum TSH and free T4 were measured. Normal reference ranges for TSH and FT4 were 0.3–4.8  $\mu$ IU/mL and 0.7–2 ng/dL, respectively.

Measurement of CIMT was performed using high-resolution color-coded Doppler ultrasonography (ALT HDI, Ultramark) using a 12-MHz linear array. Patients and control subjects were examined in the supine position, with the head turned 45° from the side during scanning. The reference point for the measurement of CIMT was the beginning of the dilatation of the carotid bulb, with loss of the parallel configuration of the near and far walls of the common carotid artery. The sonographer located the leading edges corresponding to the transition zones between lumen-intima and media-adventitia over a length of 1 cm proximal to the reference point at its thickest point, not including plaques. Plaque was identified as a localized thickened lesion ( $\geq 1.1$  mm).

All the results were the mean of the two sides. The mean IMT of four measurements determined by B-mode ultrasound using a linear transducer (7.5–10 MHz) was calculated in each patient. The Doppler examination for all patients and control subjects was performed by the same skilled sonographer.

Endothelial function has been assessed by measuring the percent of change in blood flow following heat-mediated vasodilation using laser Doppler flowmetry (Pen Flux System 5000, Perimed AB) in patients and controls. Skin microcirculation was assessed on dorsalis pedis artery at dorsum of the foot with a special laser Doppler probe for heating. We registered skin perfusion at baseline (24°C) and during local heating (40°C) for 1 min. After heating, we observed the perfusion till the end of hyperemic response. We evaluated both the resting and the peak flow.

Ankle/brachial index (ABI) was estimated as systolic pressure over the tibial arteries/systolic pressure over the brachial artery. Those with low ABI < 0.9 were excluded from the study.

Data were analyzed by the computerized SPSS, version 15. Quantitative variables from normal distribution were expressed as means  $\pm$  s.d. Student's *t*-test was used to compare continuous variables, and the chi-square test was used to compare differences among groups.  $P < 0.05$  was considered statistically significant. Pearson's correlation coefficient has been applied to correlate between two normally distributed quantitative variables.

## Results

In patients with overt hypothyroidism, there was a highly significant increase in CIMT in comparison with the control group ( $0.7 \pm 0.2$  mm vs  $0.45 \pm 0.07$  mm,  $P < 0.001$ ). Percent of change in blood flow after heat-mediated vasodilatation was significantly impaired in patients with overt hypothyroidism in comparison with the control group ( $328 \pm 17\%$  vs  $898 \pm 195\%$ ,  $P < 0.001$ ). CIMT was increased significantly in SCH patients as compared with the controls ( $0.6 \pm 0.2$  mm vs  $0.45 \pm 0.07$  mm,  $P < 0.001$ ). Percent change in blood flow following heat-mediated vasodilatation was impaired in SCH patients compared to control ( $545 \pm 406$  vs  $898 \pm 195\%$ ,  $P < 0.001$ ). Results are summarized in Tables 1 and 2.

The impairment of endothelial function was more significant in patients with overt hypothyroidism as compared with those with SCH ( $P < 0.014$ ). There was a significant negative correlation between CIMT and the

**Table 1** Clinical, laboratory and vascular data of the patients with overt hypothyroidism and healthy controls.

Characteristics	Hypothyroid	Control	P value
	n=40	n=40	
	Mean ± S.D.	Mean ± S.D.	
Age (year)	34 ± 8.3	36 ± 4.8	0.348
SBP (mmHg)	116 ± 7.6	115 ± 6.5	0.877
DBP (mmHg)	65 ± 6	67 ± 5.4	0.508
BMI (kg/m <sup>2</sup> )	24 ± 2.8	24 ± 2.3	0.97
FBG (mg/dL)	82 ± 8.9	81.5 ± 7.9	0.732
TSH (mU/L)	32.4 ± 30	3.2 ± 0.48	<0.001 <sup>a</sup>
FT4 (ng/dL)	0.92 ± 0.4	1.5 ± 0.2	<0.001 <sup>a</sup>
Cholesterol (mg/dL)	165 ± 34	141 ± 14	<0.001 <sup>a</sup>
LDL (mg/dL)	100 ± 30	62 ± 18	<0.001 <sup>a</sup>
HDL (mg/dL)	45 ± 10	61 ± 19	<0.001 <sup>a</sup>
TG (mg/dL)	92 ± 17	82 ± 34	0.102
CIMT (mm)	0.7 ± 0.2	0.45 ± 0.07	<0.001 <sup>a</sup>
% Change in blood flow due to heat mediated vasodilatation	328 ± 17	898 ± 195	<0.001 <sup>a</sup>

<sup>a</sup>Significant result.

BMI, body mass index; CIMT, carotid intima-media thickness; DBP, diastolic blood pressure; FBG, fasting blood glucose; FT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglycerides; TSH, thyroid-stimulating hormone.

percent of change in blood flow following heat-mediated vasodilation in patients with overt and subclinical hypothyroidism ( $r = -0.59$  and  $-0.71$  respectively,  $P < 0.001$  for both).

## Discussion

Hypothyroidism is a common progressive process ranging from mild subclinical to overt disease with a well-recognized effect on cardiovascular system. Dyslipidemia is considered one of the most important

cardiovascular risk factors in hypothyroidism (1). Our study showed a significant increase in the mean values of total cholesterol and LDL, in patients with overt and subclinical hypothyroidism as compared with the control group. These findings are in accordance with those of other studies, which documented the increased serum total and LDL cholesterol and serum triglycerides in both groups of patients (11). In our study, both groups of patients had decreased HDL cholesterol in comparison with the control group. This finding confirms previous reports but contradicts others (11, 12). Hypothyroidism is associated with accelerated atherosclerosis. A substantial

**Table 2** Clinical, laboratory and vascular data of the patients with subclinical hypothyroidism and healthy controls.

Characteristics	SCH	Control	P value
	n=30	n=40	
	Mean ± S.D.	Mean ± S.D.	
Age (year)	34 ± 8	36 ± 4.8	0.316
SBP (mmHg)	110 ± 7.4	115 ± 6.5	0.004 <sup>a</sup>
DBP (mmHg)	65 ± 5.4	67 ± 5.4	0.267
BMI (kg/m <sup>2</sup> )	26 ± 3.6	24 ± 2.3	0.24
FBG (mg/dL)	81 ± 9	81.5 ± 7.9	0.866
TSH (mU/L)	7.2 ± 2.2	3.2 ± 0.48	<0.001 <sup>a</sup>
FT4 (mU/L)	1 ± 0.4	1.5 ± 0.2	<0.001 <sup>a</sup>
Cholesterol (mg/dL)	150 ± 27.9	141 ± 14	0.06
LDL (mg/dL)	93 ± 26.6	62 ± 18	<0.001 <sup>a</sup>
HDL (mg/dL)	42 ± 9.9	61 ± 19	<0.001 <sup>a</sup>
TG (mg/dL)	94 ± 15.6	82 ± 34	0.084
CIMT (mm)	0.6 ± 0.2	0.45 ± 0.07	<0.001 <sup>a</sup>
% Change in blood flow due to heat-mediated vasodilatation	545 ± 406	898 ± 195	<0.001 <sup>a</sup>

<sup>a</sup>Significant result.

BMI, body mass index; CIMT, carotid intima-media thickness; DBP, diastolic blood pressure; FBG, fasting blood glucose; FT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; SCH, subclinical hypothyroid; TG, triglycerides; TSH, thyroid-stimulating hormone.

proportion of this can be explained by the association between hypothyroidism and traditional cardiovascular risk factors such as dyslipidemia. The early changes in lipid profile in patients with subclinical hypothyroidism could be a contributing factor for the risk of atherosclerosis. However, other factors may play a role in the increased atherosclerosis risk in those patients. This is further supported by studies that have shown an increased risk of all-cause mortality and cardiovascular events in hypothyroid patients, even after adjusting for conventional risk factors (13).

We assessed CIMT, as a marker of atherosclerosis, using high-resolution color-coded Doppler ultrasonography in patients with overt and subclinical hypothyroidism. Our patients had no risk for atherosclerosis other than dyslipidemia. In our study CIMT was significantly higher in patients with overt hypothyroidism as compared with the control group ( $P < 0.001$ ). Cakal *et al.* have similarly demonstrated higher CIMT in primary hypothyroid patients. They concluded that CIMT is an objective sign of accelerated atherosclerosis in patients with primary hypothyroidism (14). We also demonstrated that CIMT was significantly higher in patients with SCH as compared with the control group ( $P < 0.001$ ). Monzani and co-workers found early carotid wall alterations in subclinical hypothyroid patients that showed improvement after levothyroxine replacement (15). Our findings also confirmed those of Velkoska and his colleagues who reported that SCH is associated with increase in CIMT and presence of carotid plaques independent of traditional atherosclerosis risk factors (16). Onder *et al.* who showed lack of a significant difference in CIMT between overt and subclinical hypothyroid, indicating that the inflammation leading to increased CIMT starts in the subclinical phase (17). Our study confirmed that the increases in CIMT in patients with hypothyroidism start in the subclinical stage.

In our study, endothelial function was assessed by measuring the percent of change in blood flow following heat-mediated vasodilation using laser Doppler flowmetry. Local thermal hyperemia leads to a temperature-dependent increase in skin blood flow and reaches maximal vasodilatation between 42 and 44°C. This maximal thermal vasodilatation corresponds to the maximal vasodilator capacity of the vessels. Local heat-mediated vasodilatation is mediated by two independent mechanisms: the initial peak that occurs during the first 10 min and depends on local sensory nerves and is mediated by an axon reflex. The plateau phase that happens after 20–30 min of warming and mediated by

nitric oxide (NO). Heat-mediated vasodilation can be helpful in estimating local vascular NO bioavailability. Reduced NO in the setting of endothelial dysfunction results in reduction of its antimigratory, antiproliferative and anti-inflammatory functions (18). Kruger *et al.* demonstrated that thermal hyperemia parameters (first and second peak flow and area under the curve) were significantly associated with the calculated cardiovascular risk using Framingham and Cardio-risk scores. They found that cardiovascular mortality was significantly associated with the first thermal peak and the plateau (19).

In our study, the percent of change in blood flow following heat-mediated vasodilation was significantly impaired in patients with overt and subclinical hypothyroidism as compared with the control group ( $P < 0.001$  for both). The impairment was more significant in patients with overt hypothyroidism as compared with those with SCH ( $P = 0.014$ ). The significant impairment in patients with SCH confirmed the previous findings of Cabral *et al.* and proved that endothelial dysfunction in hypothyroidism starts early in the subclinical stage (20). Our study also showed a highly significant negative correlation between CIMT and the percent of change in blood flow following heat-mediated vasodilation in patients with overt and subclinical hypothyroidism ( $P < 0.001$  for both). This confirmed the results of Halcox *et al.* who demonstrated that systemic endothelial function was related to progression of preclinical carotid arterial disease and was more strongly related to CIMT changes than traditional risk factors (18). In a meta-analysis of 27 case-control studies, Yao *et al.* confirmed the significant association between SCH and cardiovascular risk with arterial wall thickening and stiffening and endothelial dysfunction. They suggested that these findings could help to establish cardiovascular prevention strategies for SCH patients (21). Our study results are consistent with that meta-analysis, and also proved the significant negative correlation between CIMT and endothelial function in both overt and subclinical hypothyroidism. This suggests that impairment of endothelial function is a contributing factor to the increased risk of atherosclerosis in patients with hypothyroidism even in the subclinical stage.

The absence of males in our study group is a major limitation that might affect the application of the results on all patients with hypothyroidism. A longitudinal study with higher number of patients would also help to increase the credibility of the results. To prevent SCH-related dyslipidemia from affecting the results, it would help to compare hyperlipidemic and non-hyperlipidemic SCH patients. The availability of a very good vascular

laboratory at Cairo University hospital with highly skilled experts in CIMT assessment facilitated its use as a credible marker of atherosclerosis in our study. But the absence of other parameters like pulse wave velocity, an index of arterial wall stiffness, is another limitation which we are going to overcome in our future research work in that field. Endothelial dysfunction has been assessed by heat-mediated vasodilation using laser Doppler flowmetry. But the use of more than one method to assess endothelial function could increase the credibility of the results.

## Conclusion

Our study revealed that CIMT, as a marker of atherosclerosis, is significantly higher in patients with overt and subclinical hypothyroidism compared with normal control subjects. Our results also highlighted the fact that impairment of endothelial function is a contributing factor to the increased risk of atherosclerosis in both groups of patients. Further studies are needed to assess the effect of levothyroxine therapy in endothelial function, especially in patients with SCH.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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