EVALUATION OF ENDOTHELIAL FUNCTION AND CAROTID ARTERY INTIMA-MEDIA THICKNESS IN PATIENTS WITH CLINICAL AND SUBCLINICAL HYPOTHYROIDISM

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Abstract

Background: The association between subclinical hypothyroidism (SCH) and cardiovascular risk, particularly with a TSH <10 µIU/ml, remains controversial.

Aim and objectives: The aim of this study was to evaluate the changes in endothelial function and carotid artery intima-media thickness in patients with clinical and subclinical hypothyroidism.

Subjects and methods: This study was a prospective study that was conducted on 150 Egyptian adults aged 18 to 50 years, recruited from endocrinology clinic at Kasr El-Ainy hospital during the period from March 2019 to April 2022. They were classified into the following three groups: Group (A): 50 patients with clinical hypothyroidism (CH group). Group (B): 50 patients with subclinical hypothyroidism (SCH group). Group (C): 50 euthyroid healthy subjects' age and sex matched as control.

Results: Regarding FMD, there is statistically significant difference in FMD among the three studied groups (P value < 0.001), CH and SCH groups had significant impairment in FMD compared to control group (P value < 0.001 & < 0.001 respectively), but there was no significant difference between CH and SCH groups (P value = 0.863). CIMT shows statistically significant difference among the three studied groups (P value < 0.001).

Conclusion: CIMT was significantly increased in studied hypothyroidism patients than controls and, it was significantly higher in overt hypothyroidism compared to subclinical hypothyroidism. FMD is significantly impaired in CH and SCH patients compared to controls.

Keywords: carotid intima-media thickness; Flow mediated diameter; subclinical hypothyroidism; clinical study; cardiovascular risk

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1. INTRODUCTION

Hypothyroidism is a common endocrine condition which caused by a thyroid hormone deficiency. It may be either overt, with an elevated serum thyroid-stimulating hormone (TSH) and decreased levels of free thyroxine (FT4) and free triiodothyronine (FT3), or subclinical, with normal levels of FT4 but an excessive serum TSH (1).

Subclinical hypothyroidism (SCH) affects a significant portion of patients who afterwards develop overt hypothyroidism. The likelihood of progression to overt hypothyroidism is correlated with the presence of Anti-thyroid peroxidase (Anti-TPO) and the initial serum TSH concentration (higher with TSH values >12 to 15 mU/L) (2).

Endothelial dysfunction (ED) is one of the earliest signs of atherosclerosis that can be noticed in clinical examinations prior to any overt CVD presentations (3).

Carotid intima media thickness (CIMT) is the area of tissue starting at the luminal edge of the artery and ending at the boundary between the media and the adventitia. It is measured using B-mode ultrasound as the thickness of the intima and media as a whole (4). More research are employing CIMT as an important parameter for analyzing heart disease and vascular atherosclerosis (5).

Because of its sensitivity and noninvasive nature, FMD is currently the most widely used method for evaluating ED (6).

The aim of this study was to evaluate the changes in endothelial function and carotid artery intima-media thickness in patients with clinical and subclinical hypothyroidism.

2. PATIENTS AND METHODS

This study was a prospective study that was conducted on 150 Egyptian adults aged 18 to 50 years, recruited from endocrinology clinic at Kasr El-

Ainy hospital during the period from March 2019 to April 2022. They were classified into the following three groups: **Group (A):** 50 patients with clinical hypothyroidism (CH group). **Group (B):** 50 patients with subclinical hypothyroidism (SCH group). **Group (C):** 50 euthyroid healthy subjects age and sex matched as control.

- **Inclusion criteria:**

For Patients with clinical and subclinical hypothyroidism: Age between 18 and 50, patients with BMI less than 35 and documented laboratory evidence of clinical or subclinical hypothyroidism. For control subjects: Sex and age matching with the patients, non-smokers, non-diabetic or prediabetic subjects, with fasting glucose < 100 mg/dl and an HbA1c < 5.7, no systemic hypertension (defined as systolic blood pressure > 130 mmHg, diastolic blood pressure > 80 mmHg)

- **Exclusion criteria:**

Individuals less than 18 years old or more than 50 years old, individuals with BMI higher than 35, smokers, patients with diabetes, hypertention, coronary artey disease, liver or renal diseases and ABI < 0.9

- **Ethical considerations:**

An informed consent was obtained from all participants or their families before getting them involved in the study and confidentiality of all data were ensured. Any of the patients and controls had the right to withdraw from the study any time without giving any reason.

- **Methodology in details:**

All patients and control group were subjected to the following:

History taking: All patients were subjected to complete history taking including: Personal history (name, age, sex, and residency). History of hypothyroidism: age of onset, duration, previous drug intake, treatment (medical or surgical), presenting symptoms (neck swelling cold intolerance, fatigue & tiredness, muscle cramps, bony pains, constipation & menstrual irregularities).

Complete clinical examination: All subjects had a complete clinical examination including anthropometric measurements

Anthropometric measurements of following indices: Weight, height and BMI.

Calculating and interpreting BMI: The BMI was calculated using the following standard formula: BMI=Weight (kg) / [height (m)]^2

Blood pressure measurement: The measurement was performed in a quiet room, systolic and diastolic BP was measured from the right brachial artery of the subjects in a supine position after 10 min of rest using pneumatic sphygmomanometer.

Detailed clinical examination of all body systems

Investigations: Laboratory investigations: Blood samples were obtained after 12 hours overnight fasting. Serum TSH and FT4 and lipid profile including: Serum cholesterol, low density lipoprotein (LDL) and serum triglycerides and fasting blood sugar and HbA1c. **Measurement of CIMT:** Measurement of carotid intima thickness was performed using a high-resolution color-coded Doppler ultrasonography (ALT HDI 5000 ultramark machine). **Measurement of flow mediated dilatation (FMD):** The technique measures the ability of the arteries to respond with endothelial NO release during reactive hyperemia (flow mediated) after a 5-minute occlusion of the brachial artery with a blood pressure cuff. During FMD test, vasodilation occurs after an increase in blood flow, induced via circulatory arrest in the arm (suprasystolic cuff occlusion) for a period of time. The subject's right arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. A satisfactory B-mode image was obtained that identified the endothelial layers with clear intima-intima walls of the artery. The image was frozen and the baseline diameter was measured.

After baseline measurements, a sphygmomanometer cuff, placed around the right upper arm proximal to the imaged artery segment, was inflated to 50 mm Hg above systolic pressure for 3 to 5 minutes to occlude arterial flow. After deflation of the cuff, the diameter was recorded every 1 minute for 5 minutes after deflation, and the maximum diameter was recorded.

Calculation of FMD: The calculation of FMD as a percentage change was calculated using the following equation:

$$\text{FMD}(\%) = \frac{\text{peak diameter} - \text{baseline diameter}}{\text{baseline diameter}}$$

and when multiplied by 100, FMD is expressed as a percentage of change in the vessel caliber.

Ankle /brachial index (ABI): ABI Will be estimated by ABI systolic pressure over the tibial arteries/systolic pressure over the brachial artery, those with ABI<0.9 will be excluded.

STATISTICAL ANALYSIS:

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 28. Numerical data were summarized using means and standard deviations or medians and/or ranges, as appropriate. Categorical data were summarized as numbers and percentages. Estimates of the frequency were done using the numbers and percentages. Numerical data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Chi square or Fisher's tests were used to compare between the independent groups with respect to categorical data, as appropriate.

3. RESULTS

A total of 150 adult subjects who met all the inclusion and exclusion criteria were included. All

patients had no significant risk factors for atherosclerosis except dyslipidemia and no evidence of clinical atherosclerosis.
They were classified into the following three groups: Group (A): 50 patients with clinical hypothyroidism (CH group). Group (B): 50 patients with subclinical hypothyroidism (SCH group). Group (C): 50 euthyroid healthy subjects' age and sex matched as control.

Table (1): Comparison of baseline demographic & clinical data among the studied groups

	CH (A)	**SCH (B)**	**Control (C)**	**P value**	**P value for pairwise**
	n=50 (%)	**n=50 (%)**	**n=50 (%)**		
Sex					
Female	48 (96)	48 (96)	24 (48)	<0.001	A vs C <0.001, B vs C <0.001
Male	2 (4)	2 (4)	26 (52)		A vs B = 1
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Age (years)	35±8	33±8	36±9	0.269	A vs C= 1 B vs C= 0.327 A vs B= 0.965
BMI (Kg/m^2)	29.9±4.1	29.6±4.1	28.5±4	0.178	A vs C= 0.255 B vs C= 0.432 A vs B= 1
Systolic BP (mmHg)	119.4±7	118.2±7	117±17	0.529	A vs C = 0.781 B vs C = 1 A vs B= 1
Diastolic BP (mmHg)	75±5	74.8±6	75±6	0.937	A vs C = 1 B vs C = 1 A vs B= 1
FBG (mg/dL)	91±6	91±5	88 ±7	0.010	A vs C = 0.016 C vs B = 0.045
					A vs B=1
Cholesterol (mg/dL)	198±38	182±27	155±29	<0.001	A vs C <0.001, B vs C <0.001
					A vs B = 0.054
LDL (mg/dL)	123±34	109±23	93±23	<0.001	A vs C <0.001, B vs C = 0.002, A vs B 0.049
TG (mg/dL)	130±34	109±21	97±21	<0.001	A vs C <0.001, B vs C = 0.015, A vs B <0.001

SD: Standard deviation, P value <0.05 is considered significant, BP: blood pressure, BMI: Body mass index, FBG: Fasting blood glucose, LDL: Low density lipoprotein, TG: Triglycerides

Demographic data showed that there is female sex preponderance in CH and SCH groups. There is no statistically significant difference in age, BMI, systolic BP and diastolic BP between the three groups.

Table (2): Comparison of thyroid functions and anti-TPO among the studied groups

	CH (A)	**SCH (B)**	**Control (C)**	**P value**	**P value for pairwise**
	Median (range)	**Median (range)**	**Median (range)**		
FT4 (mU/L)	0.6 (0.1-0.8)	1 (0.8-1.7)	1.3 (0.9-1.7)	<0.001	A vs C <0.001, B vs C <0.001,

					A vs B :0.001
TSH (mU/L)	39.5 (7.6-150)	11 (5.4 -92)	1.8 (0.8-3.2)	<0.001	A vs C <0.001, B vs C <0.001, A vs B :0.001
Anti-TPO (IU/ml)	107 (37-183)	56.5 (18-132)	19 (10-47)	<0.001	A vs C <0.001, B vs C <0.001, A vs B <0.001

P value <0.05 is considered significant, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody

Anti-TPO was statistically different among the three studied groups (P value < 0.001). Patients with CH had higher anti-TPO concentration compared to SCH and control groups (P value < 0.001 & < 0.001 respectively). Also, anti-TPO was significantly higher in subclinical hypothyroid than control (P value < 0.001).

Table (3): Comparison of Flow mediated diameter FMD and CIMT among the studied groups

	CH (A)	**SCH (B)**	**Control (C)**	**P value**	**P value for pairwise**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
FMD (%)	10 ± 2.8	11.5± 4.6	16.2±5.7	<0.001	A vs C <0.001, B vs C <0.001, A vs B = 0.863
CIMT (cm)	0.06±0.01	0.05 ±0.01	0.04 ±0.01	<0.001	A vs C <0.001, B vs C= 0.006, A vs B < 0.001

P value <0.05 is considered significant. FMD: Flow mediated dilatation, CIMT: carotid artery intima-media thickness

Regarding FMD, there is statistically significant difference in FMD among the three studied groups (P value < 0.001), CH and SCH groups had significant impairment in FMD compared to control group (P value < 0.001 & < 0.001 respectively), but there was no significant difference between CH and SCH groups (P value = 0.863). CIMT shows statistically significant difference among the three studied groups (P value < 0.001). Patients with clinical hypothyroidism had higher CIMT values compared to SCH and control groups (P value < 0.001& < 0.001 respectively). Also, CIMT was significantly higher in SCH group than control (P value < 0.006).

Table (4): Correlation between FMD with other variables in subclinical hypothyroidism group

	FMD		
	R	**P value**	**Degree of correlation**
CIMT	0.25	0.081	Non significant correlation
Age	-0.05	0.719	Non significant correlation
Height	0.26	0.074	Non significant correlation
BMI	-0.13	0.364	Non significant correlation
TSH	-0.25	0.080	Non significant correlation
Free T3	0.01	0.952	Non significant correlation
Free T4	0.21	0.135	Non significant correlation
Cholesterol	-0.08	0.597	Non significant correlation
LDL	-0.01	0.956	Non significant correlation
TG	-0.15	0.301	Non significant correlation
FBG	0.26	0.070	Non significant correlation
Anti TPO	-0.10	0.485	Non significant correlation
Systolic BP	0.02	0.920	Non significant correlation
Diastolic BP	0.24	0.099	Non significant correlation

r is the correlation coefficient & it ranges from -1 to +1, p value <0.05 is considered significant, BP: blood pressure, BMI: Body mass index, FBG: Fasting blood glucose, LDL: Low density lipoprotein, TG: Triglycerides, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, FMD: Flow mediated dilatation, CIMT: carotid artery intima-media thickness

There was no significant correlation between FMD and other studied parameters in SCH group.

Table (5): Correlation between FMD with other variables in clinical hypothyroidism group

	FMD		
	r	**P value**	**Degree of correlation**
CIMT	0.06	0.703	Non significant correlation
Age	-0.31	0.029	Significant fair negative correlation
Height	-0.24	0.089	Non significant correlation
BMI	-0.37	0.009	Significant fair negative correlation
TSH	-0.01	0.925	Non significant correlation
Free T3	0.04	0.776	Non significant correlation
Free T4	0.23	0.110	Non significant correlation
Cholesterol	0.06	0.689	Non significant correlation
LDL	0.06	0.657	Non significant correlation
TG	-0.09	0.548	Non significant correlation
FBG	-0.33	0.020	Significant fair negative correlation
Anti TPO	-0.21	0.151	Non significant correlation
Systolic BP	-0.35	0.014	Significant fair negative correlation
Diastolic BP	0.01	0.947	Non significant correlation

r is the correlation coefficient & it ranges from -1 to +1, p value <0.05 is considered significant, BP: blood pressure, BMI: Body mass index, FBG: Fasting blood glucose, LDL: Low density lipoprotein, TG: Triglycerides, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, FMD: Flow mediated dilatation, CIMT: carotid artery intima-media thickness.

In CH group, there was a significant fair negative correlation between FMD and age (r= -0.31, P= 0.029), BMI (r= -0.37, P= 0.009), FBG (r= -0.33, P= 0.020), and systolic BP (r = -0.35, P= 0.014). There was no significant correlation with other studied parameters.

Table (6): Correlation between CIMT with other variables in subclinical hypothyroidism group

	CIMT		
	R	**P value**	**Degree of correlation**
FMD	0.25	0.081	Non significant correlation
Age	0.20	0.159	Non significant correlation
Height	0.23	0.110	Non significant correlation
BMI	0.38	0.006	Significant fair positive correlation
TSH	0.01	0.956	Non significant correlation
Free T3	-0.29	0.043	Significant fair negative correlation
Free T4	0.15	0.135	Non significant correlation
Cholesterol	0.30	0.034	Significant fair positive correlation
LDL	0.38	0.007	Significant fair positive correlation
TG	0.16	0.275	Non significant correlation
FBG	0.02	0.917	Non significant correlation
Anti TPO	0.07	0.621	Non significant correlation
Systolic BP	0.56	<0.001	Significant moderate positive correlation
Diastolic BP	0.40	0.004	Significant fair positive correlation

r is the correlation coefficient & it ranges from -1 to +1, p value <0.05 is considered significant, BP: blood pressure, BMI: Body mass index, FBG: Fasting blood glucose, LDL: Low density lipoprotein, TG: Triglycerides, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, FMD: Flow mediated dilatation, CIMT: carotid artery intima-media thickness

In SCH group, there was a significant fair positive correlation between CIMT and BMI (r= 0.38, P= 0.006), cholesterol (r= 0.30, P= 0.034), LDL (r= 0.38, P= 0.007), and diastolic BP (r = 0.40, P= 0.004). Also, there was significant moderate positive correlation between CIMT and systolic BP (r= 0.56, P< 0.001) and significant fair negative correlation with FT3 (r= -0.29, P= 0.043). There was no significant correlation with other studied parameters.

Table (7): Correlation between CIMT with other variables in clinical hypothyroidism group

	CIMT		
	R	**P value**	**Degree of correlation**
FMD	0.06	0.703	Non significant correlation
Age	0.08	0.563	Non significant correlation
Height	0.15	0.298	Non significant correlation
BMI	0.11	0.429	Non significant correlation
TSH	-0.04	0.804	Non significant correlation
Free T3	0.14	0.324	Non significant correlation
Free T4	0.18	0.206	Non significant correlation
Cholesterol	0.32	0.026	Significant fair positive correlation
LDL	0.36	0.010	Significant fair positive correlation
TG	0.24	0.096	Non significant correlation
FBG	-0.23	0.111	Non significant correlation
Anti TPO	0.05	0.713	Non significant correlation
Systolic BP	0.23	0.102	Non significant correlation
Diastolic BP	0.07	0.633	Non significant correlation

r is the correlation coefficient & it ranges from -1 to +1, p value <0.05 is considered significant, BP: blood pressure, BMI: Body mass index, FBG: Fasting blood glucose, LDL: Low density lipoprotein, TG: Triglycerides, TSH: Thyroid stimulating hormone, Anti-TPO: Anti-thyroid peroxidase antibody, FMD: Flow mediated dilatation, CIMT: carotid artery intima-media thickness

In CH group, there was a significant fair positive correlation between CIMT and cholesterol (r= 0.32, P= 0.026), and LDL (r= 0.36, P= 0.010). There was no significant correlation with other studied parameters.

4. DISCUSSION

Atherosclerosis is the most important systemic aetiology leading to major adverse cardiovascular events (7). Therefore, the importance of detecting higher-risk individuals and early stages of sub-clinical atherosclerosis is paramount (8).

We prospectively enrolled 150 adult: 50 patients with overt hypothyroidism, 50 patients with subclinical hypothyroidism and 50 healthy controls. Cases were randomly recruited from endocrinology clinic at Kasr El-Ainy hospital during from March 2019 to April 2022.

Our results showed that there is a significant increase in the mean values of total cholesterol, LDL, and TG in patients with overt hypothyroidism and SCH in comparison to healthy controls (P value < 0.001 & < 0.001 respectively).

These findings are in accordance with Chen et al. who reported that patients with hypothyroidism presented with significantly higher serum levels of total cholesterol, TG and LDL than controls (9).

Another study done by **Haghi et al.,** (10) on 53 patients with SCH revealed that participants had significantly higher LDL levels than the control group regardless of age group and gender, but there was no difference in TG and total cholesterol levels.

This study also showed statistically significant difference between patients with CH and those with SCH concerning LDL and TG being higher in CH group. There was no significant difference between the 2 groups concerning total cholesterol in spite of its higher levels in CH group. In contrast to our study, a study done by **Kumari et al.,** (11) found that levels of TC and LDL were significantly higher in CH group than SCH, but levels of TG showed no significant difference between the 2 groups.

It is now a known fact that there is an increased risk of atherosclerotic diseases in hypothyroidism. Previously, it had been attributed to the associated risk factors like metabolic syndrome and hypertension, but now the studies have suggested the role of hypothyroidism as an independent risk factor for atherosclerosis even in subclinical hypothyroidism (11).

Our patient had no clinical evidence of overt atherosclerosis and has no risk factors for atherosclerosis except dyslipidemia and they were compared with healthy age-matched controls.

We have studied ED noninvasively using FMD of the brachial artery. We have also measured CIMT, as an early predictor of atherosclerosis in hypothyroid patients.

In our study there was a highly significant difference between patients with overt hypothyroidism and healthy controls regarding CIMT (P < 0.001) and There was a significant difference in mean CIMT between healthy control group and SCH groups (P value = 0.006).These results are in accordance with Saif et al., who found the same findings (12). **Beralkaret al.,** (13) also found the same results in their study. **Monzani et al.,** (14) were the first to show increase in CIMT in hypothyroidism.

Our findings also are in matching with who **Valentina et al.,** (15) reported that SCH is associated with increase in CIMT and presence of carotid plaques independent of traditional atherosclerosis risk factors.

In a systematic review and meta-analysis done by **Yao et al.,** (16) studies compared the CIMT between 494 SCH patients and 390 euthyoid subjects, revealed a significantly higher CIMT in SCH patients than in euthyoid subjects, with significant heterogeneity among studies.

In our study there was a significant fair positive correlation between CIMT and LDL and TC in patients with SCH (P= 0.007, P= 0.034 respectively) and CH (P=0.010, P=0.026 respectively).

So, increased CIMT can be due to the hypothyroid state or partially by the associated dyslipidemia. Wang et al., also found that CIMT and LDL levels were positively correlated (17).

Many researchers tried to highlight in their papers that the relation between hypothyroidism, endothelial dysfunction, and the alterations of FMD as independent risk factor for atherosclerosis. By analyzing the results of FMD, we found a significant statistical difference between SCH and CH patients and control group with the same P-value (< 0.001),

Furthermore, in their meta-analysis **Yao et al.,** (16) analyzed nine studies, evaluating a total of 230 SCH patients and 204 controls, indicated that SCH patients have a significantly lower FMD than control group, with significant heterogeneity among studies.

No relationship between CIMT or brachial FMD and free T4 and serum TSH was found in this study. This is supported by **Alibaz et al.** (18) who did not find significant correlation between serum TSH levels and FMD value in their patient group.

On the other hand, **Cakal et al.,** (19) have demonstrated positive correlation between CIMT, and TSH levels. They concluded that CIMT is an objective sign of accelerated atherosclerosis in patients with primary hypothyroidism.

Limitations of our study: The relatively small number of patients and female sex preponderance are major limitations that can affect the application of our results on all hypothyroid patients. Endothelial dysfunction was assessed only by FMD, but the use of other methods of endothelial dysfunction such as PWV and arterial wall stiffness can increase the credibility of the results.

5. CONCLUSION

CIMT was significantly increased in studied hypothyroidism patients than controls and, it was significantly higher in overt hypothyroidism compared to subclinical hypothyroidism. FMD is significantly impaired in CH and SCH patients compared to controls. There was a significant positive correlation between CIMT and LDL and TC in both overt and subclinical hypothyroidism patients.

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