Original Article

FMD and CIMT: Surrogate Markers of Atherosclerosis in Subclinical and Overt Hypothyroidism in Sub Himalyan Region

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Abstract

Introduction: Hypothyroidism increases the risk of atherosclerosis. Carotid intima-media thickness (CIMT) and flow-mediated dilation (FMD) have been used as a noninvasive method to detect atherosclerosis. But the literature is scarce on patients with subclinical hypothyroidism. Such a study was not done in our region, so we conducted this study at a tertiary care center to compare CIMT and FMD among subclinical and overt hypothyroid patients and to analyze the risk of atherosclerosis. **Methods:** We evaluated 68 patients aged 18–50 years, with newly diagnosed hypothyroidism. We divided them into overt and subclinical hypothyroidism groups and compared the findings. All analyses were performed by the computerized SPSS 17.0. The results were noted as means ± SD and percentage. Student's *t*-test was used to compare continuous variables, and the Chi-square test was used to compare differences. **Results:** The total number of patients with dyslipidemia in the subclinical hypothyroidism (SCH) group was 22 (45.83%) and in the overt hypothyroidism (OH) group was 26 (54.16%) with a *P*- value of 0.009.The mean FMD% in subclinical hypothyroidism patients was 6.9816 ± 3.4224 and in overt hypothyroidism patients was 5.3670 ± 2.7278 ($P = 0.03$). The mean CIMT was 0.5009 ± 0.0732, CIMT in the SCH group was 0.5082 ± 0.0672 and in the OH group was 0.5305 ± 0.0799 ($P = 0.2$). **Conclusion:** The outcome of this study specifies that hypothyroidism is associated with endothelial dysfunction as established by impaired FMD, and it may be the first marker of atherosclerosis appearing before any structural evidence like CIMT. We can speculate that there is a link between subclinical hypothyroidism and atherosclerosis, and thyroxine replacement in SCH may help to prevent the progression of atherosclerosis.

Keywords: Atherosclerosis, carotid intima thickness, endothelial dysfunction, flow-mediated dilation, overt hypothyroidism, subclinical hypothyroidism

Introduction

The prevalence of hypothyroidism in the developed world is 4%–5% and the prevalence of subclinical hypothyroidism (SCH) in the developed world is 4%–15%.[1,2] Cardiovascular system is rich in thyroid hormone receptors; therefore, it is relatively sensitive to changes in the levels of thyroid hormones.[3] It is well known that atherosclerosis is highly associated with overt hypothyroidism (OH) but there is still controversy about its association with SCH despite exclusive research.[4] Carotid intima-media thickness (CIMT) is a closer marker of early atherosclerotic changes and is a widely accepted surrogate end-point for cardiovascular events.[5] Rotterdam Study in 1149 women aged >55 years, concluded that SCH is a strong indicator of risk for atherosclerosis and myocardial infarction.[6] As limited studies have been done in India, and no study was done in the Himalayan region of India. Therefore, this study was done to contribute to the area by investigating the effects of SCH and OH on flow-mediated dilation (FMD) and CIMT.

Access this article online

Quick Response Code:

Website: www.ijem.in

DOI: 10.4103/ijem.IJEM_247_21

Material and Methods

Study design

The study was conducted for 1 year from July 2016 to June 2017 in the departments of Medicine and Cardiology in a tertiary care center, among indoor as well as outdoor patients.

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Submitted: 05-Jun-2021 **Published**: 26-Oct-2021
Accepted: 06-Aug-2021

How to cite this article: Kumari B, Kumar B, Gupta D, Ganju N. FMD and CIMT: Surrogate markers of atherosclerosis in subclinical and overt hypothyroidism in sub Himalyan Region. Indian J Endocr Metab 2021;25:220-5.

It was a hospital-based observational, cross-sectional study. Ethical clearance was taken from the institutional ethical committee. Patients presenting to outdoor clinics or admitted and having subclinical or overt hypothyroidism were included in the study. Patients with normal free thyroxine (FT4) and thyroid stimulating hormone (TSH) >5.5–10 m IU/mL were considered as SCH and TSH >10 m IU/mL with clinical symptoms were diagnosed as OH. TSH values were used for better delineation as the clinical symptoms may be diverse and subjective. Any cause leading to falsely elevated TSH such as thyroiditis, drugs, autoimmune disorders, and chemoradiation were ruled out on history and examination. A written informed consent was obtained from all the participants. A total of 38 patients in the SCH group and 30 in the OH group were included. The patients who were on treatment for hypothyroidism, had a history of cardiac disease, kidney disease, liver disease, malignancies, cerebrovascular disease, on statin therapy, hypertension, diabetes, obesity, or pregnant or lactating women were excluded from the study. A detailed personal and family history was taken regarding smoking, alcohol, diabetes mellitus, hypertension, and symptoms of coronary artery disease. Body mass index (BMI) was recorded.

Blood investigation

Levels of serum TSH and FT4 were measured by immunochemiluminescence (Cobas 6000, E601). Reference ranges for TSH and FT4 were 0.550–4.780 μ IU/mL and 0.93–1.7 ng/dL, respectively.

Blood pressure

Blood pressure was measured in both the arms in sitting in a chair with the armrest and backrest as per the Joint national committee (JNC) 7 guidelines.[7]

Echocardiography

Echocardiography was performed with the subject in the supine and left lateral position with the use of a Philips i33×-Matrix echocardiography machine, parameters given in the proforma were obtained in all the participants. 2D images were obtained in the standard parasternal and apical views. M-mode, 2D, and pulsed wave Doppler echocardiographic examination was performed in all these participants. The measurement of intima-media thickness of both the left and right common carotid artery was done by a single observer in the department of cardiology using a 11.3 MHz probe (Philips i33×-Matrix echocardiography machine).

CIMT

The subjects were examined in the supine position, with the head turned 45°from the side during the scanning procedure. Arterial wall segments were assessed longitudinally and perpendicular to the ultrasound beam with lateral probe position. CIMT was measured along a segment of the artery free of atherosclerotic plaque with clearly defined lumen-intima and media-adventitia interfaces at the end-diastole (R wave). The far wall of the common carotid artery was preferred. For optimal visualization of lumen-intima interface, the horizontal position of the artery was maintained in the image sector. The beginning of the dilatation of the carotid bulb was taken as the reference point for the measurement of CIMT, with loss of the parallel configuration of the near and far walls of the common carotid artery. The images were focused on the posterior far wall, and a linear portion of the artery was taken. Four images of the left and right common carotids were recorded at least 15 mm proximal to the reference point. CIMT was calculated as the mean of the eight measurements taken. The CIMT was measured as the distance between the leading edge of the first echogenic line to the leading edge of the second echogenic line as defined by Pignoli *et al*.[8]

FMD

On the vascular session day, the participants were instructed to report to the laboratory in the morning hours having fasted for at least 8 h, abstained from caffeine and tobacco products for 12 h and abstained from exercise for 12 h. Women were studied during the days 1–7 of their menstrual cycle to minimize the influence of cyclical changes in female hormones. All patients were advised to rest for at least 10 min before the first scan. Measurements were made while patients lying supine in a dark, climate-controlled quite room. All examinations were performed by the same investigator using a high-resolution ultrasound device with an 11 M-Hz linear array transducer. The baseline diameter of the brachial artery was measured after a 10-min rest by placing the probe at 5 cm above the anterior cubital cavity of the right arm. After a baseline rest image was acquired, arterial occlusion was created by a sphygmomanometer cuff inflation to at least 50 mm Hg above systolic pressure for 5 min. A second scan was obtained 60 s after cuff deflation. Vessel diameters after reactive hyperemia were compared to the diameters at rest and expressed as a percentage of the average lumen diameter at rest which was considered 100%. FMD (%) = [(VD reactive hyperemia – VD at rest) ×100]/VD rest.

Statistics

All analyses were performed by the computerized SPSS 17.0 package program (Statistical Package for Social Sciences, SPSS). The results were given as means ± SD. Student's *t*-test was used to compare continuous variables, and the Chi-square test was used to compare differences among groups. *P* value <0.05 was considered statistically significant.

Results

A total of 68 adult patients 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. Demographic parameters and BMI of SCH and OH groups were comparable, but the total- and low-density lipoprotein (LDL) cholesterol were significantly higher in the OH group [Table 1]. TSH had a positive correlation with total and LDL cholesterol [Table 2]. Echocardiography parameters were comparable [Table 3]. All

patients had a CIMT value less than 0.9 mm, which is a marker of clinical atherosclerosis. Out of the 68 patients, only 7 had CIMT >0.6. CIMT difference in the SCH and OH group was not statistically significant [Table 4]. FMD% less than 7% was seen in 22 patients of the SCH group and in 24 patients of the OH group. There was impaired FMD in both the groups demonstrating the statistically significant difference [Table 4].

Discussion

Hypothyroidism is quite prevalent in the Himalayan and sub-Himalayan regions, and it is more prevalent in females. Comparable to the literature, our study also had female preponderance, and females had more subclinical hypothyroidism as compared to overt hypothyroidism.[9] 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.[10] In our study, we found a higher BMI in both groups. SCH is also associated with higher BMI.[11] We found that the total cholesterol levels were high, and it was higher in the OH group as compared to the SCH group. A similar pattern of rising cholesterol levels was observed in other studies.[12-14] The level of cholesterol rises with age in all the groups. This demonstrates that with higher grades of hypothyroidism and increasing age there is an increase in total cholesterol levels.[14] We also found a significant difference in the mean LDL between both the groups. In our study, in SCH patients, the mean total triglyceride levels were lower as compared to the OH patients, but they were statistically insignificant. We observed that with increasing grades of hypothyroidism there is a decrease in serum HDL, but statistically insignificant. There was a positive correlation of TSH values with serum total cholesterol and LDL cholesterol in both groups, which was statistically

Table 1: Parameters of subclinical and overt hypothyroidism group of patients

Parameters	SCH (TSH 5.5-10 m IU/mL)	OH (TSH >10 m IU/mL)	*P*
Male	8	6	NS
Female	34	24	NS
Age	38.44±7.47	40.20±8.239	NS
Smokers	2	0	NS
BMI	24.38±2.0154	24.756±2.2129	NS
SBP	118.05±6.3499	119.066±8.1998	NS
DBP	77.89±5.7506	78.20±6.9153	NS
Total Cholesterol	160.50±42.0031	184.23±54.13	0.04
Triglycerides	149.97±57.037	159±57.037	NS
LDL	105.23±34.71	140.80±46.37	0.0005
HDL	48.71±10.22	46.0333±9.5321	NS

SCH: Subclinical hypothyroidism, OH: Overt hypothyroidism, TSH: Thyroid stimulating hormone, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LDL: Low density lipoprotein, HDL: High density lipoprotein

Table 2: Corelation of TSH with multiple variables

Parameters	r	*P*
TSH-TG	0.24	0.68
TSH-TC	0.00	0.04
TSH-LDL	0.44	0.0001
TSH-HDL	0.00	0.95

TSH-TG: Thyroid stimulating hormone-triglyceride, TSH-TC: Thyroid stimulating hormone-total cholesterol, TSH-LDL: Thyroid stimulating hormone-low density lipoprotein, TSH-HDL: Thyroid stimulating hormone-high density lipoprotein

Table 3: 2D Echo: Echocardiographic profile of the patients between subclinical and overt hypothyroidism was as follows

2D Echo parameters	TSH 5.5 – 10	TSH >10	*P*
Ao	24.4474±3.3987	25.90±3.1878	0.07673
LA	27.7368±4.0046	29.50±3.9282	0.07362
IVS Diastolic	9.1053±1.1807	9.6667±1.3218	0.06926
IVS Systolic	13.4737±2.0233	13.8000±1.8644	0.49675
LVPW Diastolic	9.1842±1.5744	9.6667±1.2954	0.18020
LVPW Systolic	12.8684±2.1203	13.6000±1.6103	0.12218
EF%	70.1053±5.7694	67.4333±7.1520	0.09276
LV Mass	121.7632±34.0782	134.3667±33.5410	0.13208
MV E/A	1.3184±0.4718	1.3633±0.3978	0.67793
EDT	140.00±140.0000	150.1667±34.1589	0.15944
TDI E′ (Med)	8.0197±2.3523	8.0437±1.4717	0.96133
E/E′ 2	8.2747±1.7190	9.0907±1.8671	0.06578

Ao: Aortic diameter, LA: Left atrium, IVS: interventricular septum, LVPW: Left ventricle posterior wall thickness, EF: Ejection fraction, LV: left ventricle, MV: Mitral valve, E: Early diastolic peak flow velocity, A: Late diastolic peak flow velocity, EDT: Deceleration time of E wave, E′: Early myocardial Dopler peak velocity

Table 4: CIMT AND FMD among patients of subclinical and overt hyppothyroidism

Parameter	TSH 5.5-10	TSH >10	*P*
CIMT			
<0.6	35 (92.1%)	26 (86.6%)	0.4
>0.6	3 (7.8%)	4 (13.3%)	0.4
MEAN CIMT	0.4903±0.0673	0.5143±0.0791	0.17
CIMT ABOVE AGE 35	0.5082±0.0672	0.5305±0.0799	0.2
FMD			
<7	22 (57.89%)	24 (80.00%)	0.05
>7	16 (42.11%)	6 (20%)	0.05
BASELINE VELOCITY	0.8782±0.0673	0.8830±0.0791	0.93
BASELINE DIAMETER	4.2431±0.2770	4.2308±0.2316	0.84603
DIAMETER AT 60 SECONDS	4.5429±0.2767	4.4591±0.2515	0.04
FMD%	6.9816±3.4224	5.3670±2.7278	0.03

CIMT: Carotid intima media thickness, FMD: Flow mediated dilation, TSH: Thyroid stimulating hormone

significant in our study, but the negative correlation for triglyceride and high-density lipoprotein (HDL) cholesterol was statistically insignificant. This was comparable to the Colorado thyroid disease prevalence study, which showed that total cholesterol and LDL-C in subclinical hypothyroidism were significantly higher, but triglyceride and HDL-C were not significantly different.[15] So, this points towards the higher risk for atherogenesis in both overt as well as subclinical hypothyroidism. Table 3 infers that the systolic functions were normal in both the groups except for a slight increase in posterior wall thickness of the left ventricle (LV) among the OH group patients. Diastolic dysfunction was observed in both the groups, which could be in the starting stage in both groups. Earlier studies on echocardiography in hypothyroidism have demonstrated diastolic dysfunction in both subclinical and overt hypothyroidism.[16,17] We observed that in our study, only 7 patients out of 68 had a CIMT of >0.6. The difference between the subclinical and overt hypothyroidism patients was statistically insignificant. So, our patients had no evidence of clinical or structural atherosclerosis because CIMT over 0.9 mm is considered a structural marker for subclinical atherosclerosis. Although normal, the CIMT values determined in our patients were higher in overt hypothyroidism than subclinical hypothyroidism patients. Similar findings were observed by Mariana Tudoran *et al*.[18] in their study. The assessment of FMD revealed that it was diminished in most of the hypothyroid patients, and the vasodilator response was depressed. By analyzing the results of FMD, we found a significant statistical difference between SCH and OH group patients. As the levels of total and LDL cholesterol were significantly higher in the OH group, but the FMD did not follow this trend and was impaired in both the groups irrespective of the levels of total and LDL cholesterol, so this may be independent of dyslipidemia. Many researchers tried to highlight in their papers that the relation between hypothyroidism, endothelial dysfunction, and the alterations of FMD is independent of other risk factors for atherosclerosis. The results of our study are comparable to the study by Cikim *et al*.[19] Similar results were described later, in other studies, all referring only to patients with subclinical hypothyroidism. Halcox *et al*.[20] and Kilic *et al*.[10] highlighted the abnormalities of FMD in patients with subclinical hypothyroidism having normal CIMT, as a marker for structural changes in the arterial wall, but there is little data about the fluctuations of the basal diameter of the brachial artery as a marker for increased basal arterial tone. A meta-analysis by Ochs *et al*.[21] screening 14,449 participants suggested that SCH may be associated with a modestly increased risk for coronary heart disease and mortality. In recent years, several meta-analysis have supported these conclusions from the perspective of evidence-based medicine. One significant meta-analysis based on the previous studies from 11 countries, including in America, Europe, and Asia showed that SCH is associated with the increased coronary artery disease incidence and mortality.[22] SCH may be related to endothelial dysfunction and atherogenesis in several ways. Thus, endothelial dysfunction, as one of the earliest signs of atherosclerosis, could be most frequently observed in clinical investigations before any overt manifestations of cardiovascular disease. Therefore, endothelial dysfunction would be a favorable initial factor to investigate the correlation between hypothyroidism and cardiovascular disease.[23] Tian L. *et al*.[24] used brachial artery responses to endothelium-dependent (FMD) and endothelium-independent stimuli (sublingual nitroglycerin) as indicators for endothelial dysfunction, and found that when confounding factors were excluded, patients with SCH have a statistically lower FMD and nitroglycerine (NTG) response compared to the controls, with FMD impairment correlating to serum TSH level. This study not only revealed the increased endothelial dysfunction incidence in patients with SCH, but also indicated a possible role of serum TSH in this phenomenon. In another study, it was concluded that SCH is associated with increased cardiovascular risk factors because of underlying atherosclerosis.[25] Some of the literature mentioned that subclinical hypothyroidism is also associated with increased risk of atherosclerosis as these subjects also share the same potential atherogenic factors such as higher total and LDL cholesterol, increased high sensitivity C-reactive protein (CRP), hyper homocysteinemia, altered coagulation profile, increased arterial stiffness, and endothelial dysfunction, which are present in overt hypothyroidism.[26-28] Similarly, some studies have indicated an association of hypertension in SCH, which further increases the risk of atherosclerosis.[29-31] Along with this, thyroid hormones have a substantial influence on the peripheral vasculature, and thyroid hormone receptors have also been identified in human vascular smooth muscle cells.[32] So, the role of these cells in the development of atherosclerosis arises the hypothesis that thyroid hormone deficiency and higher levels of TSH may be associated with atherosclerosis as an independent factor. Low-grade inflammation may also cause endothelial dysfunction and impaired nitric oxide availability. Patients with Hashimoto's thyroiditis, which is a leading cause of hypothyroidism, can have more of the underlying inflammation hastening the progression of atherosclerosis.[33] Subclinical hypothyroidism is usually diagnosed late as it is mostly asymptomatic, and the intervention from the physician side is also delayed, so it is not possible to know the duration of the disease but with the findings, one can speculate that impaired FMD is preceding change in the pathogenesis of atherosclerosis and CIMT. Yan RT *et al*.[34] in their study on CIMT and FMD among 1578 middle-aged healthy men speculated that CIMT and FMD may be independent surrogates that measure different aspects and stages of early atherosclerosis. As they observed a normal CIMT when the FMD was impaired in the brachial artery. However, not all studies showed an association between TSH and CIMT. In the study of Chiche *et al*.,[35] among a population of hyperlipidemic patients, investigators found that neither prevalence nor severity of carotid plaques or CIMT were significantly different between hypothyroid patients and controls. In SCH, the only noticeable change is the elevation of TSH. It is possible that the elevation of TSH can bind extra-TSH receptor (TSHR) to exert its function. The function

of the TSHR is further confirmed in a study carried out by Tian *et al.*,[36] which indicates that elevated TSH can promote endothelial dysfunction in human umbilical vein endothelial cells by attenuating endothelial nitric oxide synthase (eNOS) and prostacyclin (PGI2) expression in a dose- and time-dependent manner. CIMT is the measure of structural changes, whereas FMD is a dynamic measure that reflects the impact of both acute and chronic influences on endothelial function. Some inherent limitations of FMD have been described, and Thijssen DHJ *et al.*[37] have given some guidelines for evaluation of FMD in their review, which can improve the analysis of FMD in patients. As endothelial function is affected by a number of factors which are also risk factors for atherosclerosis, excluding patients with these risk factors may have led to exclusion of many patients with atherosclerosis at any level in all such studies as well as in our study. We conclude that exclusions resulted in small-sized groups, which in turn might have prevented statistical difference in CIMT.

Conclusion

We can conclude that subclinical hypothyroidism is highly prevalent in women. The present study has found that subclinical hypothyroidism is associated with higher BMI and atherogenic lipid profile, characterized by an increase in the concentration of total cholesterol, LDL, and triglycerides, whereas decrease in HDL levels. CIMT findings were normal in both groups, but FMD was impaired which signifies the endothelial dysfunction. So, impaired FMD is a preceding abnormality in the process of developing atherosclerosis and even subclinical hypothyroidism is an independent risk factor for atherosclerosis. So, the replacement of levothyroxine in subclinical hypothyroidism may be considered for decreasing the risk of atherosclerotic diseases. One of the limitations of our study was the small sample size, so we could not analyze the exact impact of the level of TSH on the FMD. Future research studies with a larger number of patients should be able to establish the fact. Another limitation of our study is that the FT4 and TSH were measured once in SCH and were not repeated after 8–12 weeks to give more strength to the diagnosis.

Ethical approval

The study was approved by the Institutional Ethics Committee.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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