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Study of association among various atherosclerotic risk factors in patients of subclinical hypothyroidism

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Abstract---Background & Objectives: Subclinical hypothyroidism is endocrine disorder where serum TSH levels are increased but FT4 levels are within the assay's reference interval. Atherosclerosis has been found to be associated with the increased level of Ox-LDL in SCH. The aim of the present study is to find out the association of Ox-LDL and lipid profile in SCH patients. Materials and methods: 120 SCH patients aged (18-70) years and 120 age & sex-matched healthy controls were included in the study. BMI & lipid profile were estimated in both SCH patients and controls. FT3, FT4, TSH and Ox-LDL) were estimated by enzyme-linked immunosorbent assay. Results: SCH patients and healthy controls had different BMIs ($p<0.001$). TC, Triglycerides, HDL-C, and LDL-C were significantly greater in the SCH group than in the control group, although HDL-C was decreased

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(p<0.001). SCH patients had a significantly higher blood TSH level than controls (p<0.001), although FT3 and FT4 levels were similar. SCH patients had higher Ox-LDL than controls (p<0.001). Conclusion: In our study, SCH patients had higher serum lipid profile and Ox-LDL levels than healthy persons, indicating susceptibility to atherosclerosis. Ox-LDL can be used to predict CVD risk in SCH patients.

Keywords---subclinical-hypothyroidism, atherosclerosis, cholesterol, Ox-LDL, CVD.

Introduction

Subclinical hypothyroidism (SCH) is a biochemical condition in which blood thyrotropin hormones (TSH) levels are higher than the upper limit of the assay's reference range, but free thyroxine (FT4) levels are within the assay's reference interval. SCH patients are either asymptomatic or have milder symptoms than individuals who have overt hypothyroidism.[1] Along with overt hypothyroidism, SCH has been linked to an increased risk of atherosclerosis. There is evidence that SCH influenced various key cardiovascular risk variables such as high blood pressure, cholesterol, and coagulability. However, until a recent population-based study revealed otherwise, it was unclear how important SCH is as an independent risk factor for atherosclerosis.[2]

Subclinical thyroid dysfunction has been linked to a variety of negative effects, including cardiovascular disease, osteoporosis, and cognitive impairment.[3] Hypothyroidism has been linked to lower thermogenesis and metabolic rate, as well as a higher body mass index (BMI) and obesity prevalence. Clinical data suggests that even minor thyroid malfunction, such as subclinical hypothyroidism, is associated with massive weight fluctuations and is a risk factor for obesity.[4] Patients with SCH has a disrupted lipid metabolism, according to several studies, with higher blood levels of total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C). Such alterations are widely recognized as atherosclerosis and coronary heart disease risk factors.[5]

Thyroid hormones exert big influence on lipid synthesis, mobilization, and metabolism. Overt hypothyroidism is linked to lipid metabolic problems, which may predispose to the development of cardiovascular disease. Patients with subclinical hypothyroidism have variety of lipid problems. Many studies have found that people with SCH have higher TC, LDL-C, and TG levels. HDL-C levels in SCH patients have been reported to be low as compared to control groups.[6]. Elevated serum cholesterol can be considered as an oxidation substrate in the presence of oxidant stress. Hypothyroidism may make the elevated LDL-C more susceptible to oxidation (Ox-LDL). Increased LDL-C can be oxidatively damaged by free radicals, which can change the receptor affinity of apolipoprotein B (apo B), increase LDL-C absorption by macrophages, and stimulate the formation of foam cells. Foam cells build-up underneath the endothelium plays a key function in the onset of atherosclerosis. Oxidation of LDL-C can produce oxidation products such as oxysterols, which have powerful physiologic effects in

atherosclerosis progression. Ox-LDL has the ability to change cell function and causes harm to endothelial cells. As a result, circulating Ox-LDL, as recently reported, might be a marker for atherosclerosis.[7]

The involvement of oxidized LDL (Ox-LDL) in the aetiology of atherosclerosis has been demonstrated. This study is inconsistency with other studies that have recently shown that increased levels of circulating Ox-LDL are linked to coronary heart disease (CHD). Receiver operating characteristic–curve analysis found that Ox-LDL had a greater sensitivity for coronary artery disease than LDL-cholesterol or total-to-HDL cholesterol ratio in middle-aged people. Furthermore, logistic regression results indicated that the predictive value of Ox-LDL was additive to that of the global risk evaluation score, which is according to Framingham risk factors such as age, total cholesterol, HDL cholesterol, systolic blood pressure, diabetes mellitus, and smoking for cardiovascular risk prediction.[8] In this study, mean serum TSH revealed a strong positive relationship with serum total cholesterol, triglyceride, low-density lipoprotein cholesterol, and very-low-density lipoprotein cholesterol. The association between BMI, TSH, total cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol and Ox-LDL is positively correlated and higher in patients with subclinical hypothyroidism than in healthy controls. Whereas, no significant correlation between TSH and HDL-C was found. [6, 9-12].

Material and Method

The present study was conducted in the Departments of Biochemistry at Shree Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, Uttarakhand and Adesh Medical College & Hospital, Shahbad, Haryana, India. A total of 120 patients with SCH in the age group of 18-70 years & an equal number of age and sex-matched healthy controls were included in the study as per the inclusion & exclusion criteria.

Inclusion criteria

Diagnosed cases of SCH with an elevated thyrotropin (TSH) (>6.5 mU/L), Normal free triiodothyronine (FT3) (2.0-4.0 pg/mL), Normal free thyroxine (FT4) levels (0.7–1.7 ng/dL).

Exclusion criteria

Obese people with BMI > 30 Kg/m^2, patients with diabetes mellitus, renal disorders, smokers & alcoholics, diagnosed cases of hypothyroidism, or those already on treatment, thyroid cancers, history of antipsychotic drugs, oestrogen therapy. All the subjects were asked to give written informed consent to participate in the study. The study was approved by the Institutional Ethics Committee (IEC) of the Shree Guru Ram Rai Institute of Medical and Health Science, Dehradun, Uttarakhand (SGRR/IEC/38/21) and Adesh Medical College & Hospital, Shahbad, Haryana (AMCH/BIO/2020/11/09) India.

After 12 hours of fasting, about 5 ml of venous blood sample were collected in plain tube from both the groups. Serum was separated and preserved at –80°C for the subsequent analyses. Serum free T3 (FT3), free T4 (FT4) and TSH and

cardiovascular risk factors; Interleukin-6 (IL-6) and assymetric dimethyl arginine (ADMA) were measured by enzyme linked immunosorbent assay (ELISA). Lipid parameters (triglycerides, total cholesterol and high density lipoprotein cholesterol) were analysed by fully autoanalyzer (EM-360). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated by Fredrickson Friedwald's formula. [13] BMI was calculated using the equation (body weight in kilograms divided by body height in meters squared) (kg/m^2). [13] The statistical analysis was done using SPSS (version 26.0). The descriptive analysis of BMI, lipid profile, thyroid profile, serum Ox-LDL was expressed in terms of the mean and standard deviation. The comparative study was done by Student t-test. The correlation analysis among the thyroid profile, lipid profile, BMI, & serum Ox-LDL was studied by Pearson's correlation coefficient. $p<0.05$ was considered statistically significant.

Results

A total of 120 patients (87 females and 33 males) with subclinical hypothyroidism were enrolled for the study and compared with 120 euthyroid controls (77 females and 43 males). There were no significant differences in age between groups. There was statistically significant difference in BMI among patients with subclinical hypothyroidism (23.35±2.21 kg/m^2) and the healthy control group (22.32±2.29kg/m^2) (Table-1). The level of TSH was significantly higher in the SCH group as compared to the control group (9.57±6.84 vs 2.97±0.91). Whereas, the levels of FT3 & FT4 were not significantly different in the SCH group (4.27±0.82 vs 4.51±1.2), (13.81±18.5 vs 13.55±2.52) as compared to the controls respectively. TC, Triglycerides, HDL-C, and LDL-C levels were significantly different in SCH patients compared to controls. Total cholesterol was significantly higher in the SCH group as compared to the control group (190.66±42.08 vs 164.67±7.4). Other parameters of lipid profile like triglycerides (247.49±115.89 vs 99.88±21.37), and LDL-C (194.77±59.84 vs 124.63±10.9) were also significantly higher in the SCH group. The serum concentration of HDL-C (45.38±11.96 vs 60.02±7.25) was significantly lower in the SCH group. While the level of Ox-LDL was significantly higher in the SCH group compared to the control group (3700 ±619.81 vs 905.75 ±428.68) (Table 2). Further, significant positive correlations between serum lipid profile and Ox-LDL were found except for the HDL (good cholesterol) which was negatively correlated with the cardiovascular marker i.e. Ox-LDL in our study (Table 3).

Discussion

In Western societies, 4–10% of people have subclinical hypothyroidism (SCH), which is characterized as an increased serum thyroid-stimulating hormone (TSH) level with serum thyroid hormone values within the reference range. [14] Although subclinical hypothyroidism (SCH) has been linked to an increased risk of atherosclerosis, the pathophysiology of the condition is yet unknown. The present study predicted that TSH could have a crucial role as potential risk factor of developing atherosclerosis in SCH patients. [15] Hypothyroidism affects the number of risk factors and is linked to a higher risk of cardiovascular disease morbidity. Disturbance in atherogenic lipid metabolism, which results in higher total cholesterol, low-density lipoprotein cholesterol (LDL-C), and reduced levels of

high-density lipoprotein cholesterol (HDL-C), has been proposed as a mechanism connecting hypothyroidism to cardiovascular disease. [16]

Endothelial cells, monocytes, macrophages, lymphocytes, and smooth muscle cells can all accelerate the oxidation of LDL- cholesterol. A few cell types create and deliver phospholipase A2 during aggregation. Myeloperoxidase, a heme protein delivered by dynamic phagocytes, changes over L-tyrosine to a tyrosyl radical, which act as a physiological impetus for lipid oxidation in LDL. The myeloperoxidase-catalyzed process isn't reliant upon free metal particles, dissimilar to other cell-interceded pathways for LDL-C oxidation. Lipid oxidation produces aldehydes, which replace lysine remnant in the apo B-100 moiety of LDL, Producing oxidized LDL. [17]

All these factors tend to increase the risk of atherosclerosis. Thus, the present study observed significantly increased BMI in patients with subclinical hypothyroidism as compared to healthy controls. These results are in accordance with the studies done by Geng H et al, Turhan C et al, and Harada PHN et al. [9, 12, 18] and in contraindication with the results reported by Zhu C et al, and Cerbon M et al. [19, 20]. In this study, we observed significantly increased total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C, and decrease HDL-C were observed in the case of SCH patients in comparison to healthy individuals. These results are in accordance to that reported by Laway BA et al, Jayasingh IA et al, and Abdel-Gayoum AA et al. [6, 11, 21] and in contrast to the finding of the study done by Zhu C et al, and Akini B et al. [19, 22]

The present study also evaluated Ox-LDL as a cardiovascular marker in patients with subclinical hypothyroidism and found significantly increased levels of Ox-LDL in SCH patients as compared to healthy individuals. The results of the present study are in accordance with Duntas LH et al, Cebeci E et al, and Holovoet P et al. [7, 17, 23] and contradictory to that of. Shoji T et al, Karvonen J et al. [24, 25] However, there is a limitation in our study that a large population has to be studied to elucidate the CVD risk among SCH patients.

Conclusion

In the present study, the outcome of SCH patients showed elevated levels of serum lipid profile along with serum Ox-LDL as compared to healthy individuals, which indicates a predisposition towards atherosclerosis. Hence, the estimation of serum biomarkers like Ox-LDL can be considered as a potential indicator of impending CVD risk in SCH patients.

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