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Original article

Significance of early biochemical markers of atherosclerosis in subclinical hypothyroidism patients with normal lipid profile.

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ABSTRACT

Aims & Objectives: The present study was conducted to determine the significance of early novel markers of atherosclerosis in subclinical hypothyroidism patients with normal lipid profile. Materials & Methods: This study was conducted on 50 subclinical hypothyroidism patients of either sex > 18 years, and 50 age and sex matched euthyroid controls. Lipid profile, T3, T4, TSH, hs-CRP and Nitric oxide levels were estimated among both the groups. Results: TSH and hs-CRP levels were significantly increased in subclinical hypothyroidism patients compared to controls and showed a significant positive correlation. Nitric oxide levels were significantly decreased in subclinical hypothyroidism patients compared to controls and showed a significant negative correlation between TSH and Nitric oxide levels. Conclusion: This study shows an association of increased hs-CRP levels and endothelial dysfunction with progression of subclinical hypothyroidism which might be helpful to plan for levothyroxine therapy and avoid the possible future cardiovascular disease.

1. Introduction

Subclinical Hypothyroidism (sHT) is characterized by normal serum free thyroxine concentrations with elevated serum thyroid-stimulating hormone concentrations. It has been associated with atherosclerotic cardiovascular disease even in the absence of hypercholesterolemia. 1 The endothelium which is the potential target of thyroid hormone, plays a major role in the maintenance of vascular function and integrity. The most important vasodilator substance produced by the endothelium is nitric oxide (NO). Endothelial dysfunction, a condition characterized by decreased NO availability, acts as a promoter of atherosclerosis and is associated with an increased risk of cardiovascular events, though the exact pathogenesis is unclear. 2 Circulating inflammatory markers are strongly associated with cardiovascular events, even in individuals with normal lipoprotein profiles, but little is known regarding the impact of sHT . 3 The decision to treat patients with sHT with Levothyroxine for restoring euthyroidism and improving cardiovascular risk should depend on the presence of risk factors, rather than on a TSH threshold . This study aims at determining the significance of early biochemical markers as effective tool for the detection of preclinical cardiovascular alterations in subclinical hypothyroidism patients with normal lipid profile.

2. Materials and methods

This cross-sectional study was conducted at the Central Research Laboratory and Department of Biochemistry, Sri Siddhartha Medical College (SSMC) Teaching Hospital and Research Centre, Tumkur. The study group were comprised of patients with subclinical hypothyroidism (normal tri-iodo-thyronine [T3], normal tetra-iodo-thyronine [T4] and thyroid stimulating hormone [TSH] levels between 5-25 µIU/ml) patients of either sex (Males-27, Females -23) with age >18 years [n=50]. The blood samples of the age and sex matched euthyroid patients were the control samples [n=50]. Cases were selected regardless of race, religion, occupation and socioeconomic status. This study was approved by the Ethical Committee of the Institute.

Obese (BMI _ 30 kg/m2) subjects, smokers, hypertension, diabetes mellitus, chronic renal failure which can affect endothelial function, antithyroid medications, chronic liver disease,

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atherosclerotic cardiovascular disease or signs and malignancies were excluded from the study. Women were premenopausal, with regular menses, and none were pregnant.

After overnight fasting, blood samples were collected under aseptic precautions from antecubital venipuncture into plain vacutainer tubes. Samples were then centrifuged at 1000 rpm for 10 minutes within one hour of collection and separated serum were analyzed for the biochemical parameters: Lipid profile, Serum nitric oxide end products, high sensitivity C-Reactive protein (hs-CRP) and thyroid profile.

Determination of serum nitric oxide end products (nitrates & nitrites) by modified Griess assay. 4 The principle of this assay is reduction of nitrate by vanadium(III) combined with detection by the acidic Griess reaction. In acid solution, nitrite is converted to nitrous acid (HNO2) which diazotizes sulfanilamide. This sulfanilamide-diazonium salt is then reacted with N-(1-Naphthyl)-ethylenediamine (NED) to produce a chromophore which is measured at 540 nm. The total nitrite determination is an indicator of nitric oxide production.

LIPID PROFILE: Cholesterol was estimated by the end point enzymatic method, serum triacylglycerol by glycerol phosphate oxidase method. HDL estimation was carried out by Phosphotungstate Precipitation method. LDL and VLDL were calculated from the estimated values of Cholesterol, Triglyceride and HDL-C, using the equation of Frieldwald et al. [5, 6]

Estimation of serum hs-CRP by turbidimetry latex-high sensitivity method. It was based on the principle that Serum C - reactive protein (CRP) causes agglutination of latex particles coated with anti-human C -reactive protein. The agglutination is proportional to the CRP concentration and can be measured by turbidimetry. [7]

T_3, T_4, TSH levels were measured by Chemiluminescent Immunoassay. [8]

Statistical comparisons were carried out using Student's t test at 5% level of significance. The Pearson's correlation coefficients were determined between

- NOx and TSH and

- CRP and TSH

at 5%level of significance.

3. Results

TABLE 1 – Measured parameters in euthyroid controls

PARAMETERS	CONTROLS (Mean ± S.D)	CASES (Mean ± S.D)
BMI (Kg / m^2)	20.3±1.9	21.1±1.3
Total Cholesterol (mg / dl)	170±19.4	177.7±18.3
Triglycerides (mg / dl)	147.4± 9.03	153.4±12.3
Low Density Lipoprotein (mg / dl)	99±18.3	108.9±17.2
High Density Lipoprotein (mg / dl)	41.4±2.1	41.5±3.9
hs-CRP(mg/l)	3.2±0.6	8.0±1.1
T3 (ng / dl)	110.3±8.1	141.6±31.9
T4 (µg / dl)	8.6±3.4	8.4±2.4
TSH (µIU / ml)	3.4±0.7	12.4±7.2
NOx (µmol / l)	28.4±3.5	21.7±3.0

From table 1, it is evident that both euthyroid and subclinical hypothyroid patients had normal lipid profiles, normal BMI values and normal T3, T4 values.

TABLE 2 – Comparison of biochemical parameters between euthyroid controls and subclinical hypothyroid cases

PARAMETERS	EUTHYROID CONTROLS	SUBCLINICAL HYPOTH-YROIDISM CASES	t value	p value
TSH	3.4±0.7	12.4±7.2	8.7974	<0.01 (highly significant)
hs – CRP	3.2±0.6	8.0±1.1	27.08	<0.01 (highly significant)
NOx	28.4±3.5	21.7±3.0	10.2773	<0.01 (highly significant)

From table 2, it is evident that TSH and hs-CRP values were significantly increased in subclinical hypothyroidism patients compared to euthyroid controls ($p<0.01$) and NOx levels were significantly decreased in subclinical hypothyroidism patients compared to euthyroid controls ($p<0.01$).

TABLE 3 – Correlations between the measured parameters in subclinical hypothyroid patients

PARAMETERS	r value	p value
TSH and hs-CRP	0.6655	<0.001 (highly significant)
TSH and NOx	-0.7659	<0.001 (highly significant)

As evident from table 3, hs-CRP shows significant positive correlation with TSH in subclinical hypothyroid patients and NOx shows significant negative correlation with TSH in subclinical hypothyroid patients.

4. Discussion

Subclinical hypothyroidism (sHT) is a disorder characterized by elevated serum TSH levels despite normal free thyroid hormone (FT3 and FT4) values. sHT has been associated with higher levels of some cardiovascular risk factors like hypercholesterolemia, high low-density lipoprotein cholesterol levels, increased C-reactive protein values etc which increases the risk for atherosclerosis. However, data on risk factors of coronary heart disease (CHD) in subjects with subclinical hypothyroidism are conflicting. 9

The cardiovascular system is an important target of thyroid hormone action and is sensitive to slight variations in circulating thyroid hormone levels. The myocytes and the smooth muscle cells of the aorta and the coronary arteries share the pituitary cell's ability to generate tri-iodothyronine from deiodination of thyroxine by type II 5'-monodeiodinase. Tri-iodothyronine modulates the expression of proteins (the a- and b-isoforms of the myosin heavy chains, the sarcoplasmic reticulum Ca2+-ATPase), and interferes in the expression of Na+/K+-ATPase, Na+/Ca2+ exchange, and some K+ channels like which play a crucial role in the physiologic activity of myocytes. sHT may act directly on the heart, impairing both systolic and diastolic functions or indirectly, sHT may increase cardiovascular risk by altering peripheral vascular resistance and serum lipid and coagulation profiles. 10

Several studies have investigated the relationship between sHT, lipoprotein profile, and the risk of cardiovascular disease, with controversial results. Few studies have demonstrated an increase in total cholesterol and low density lipoprotein-cholesterol (LDL-C). sHT does not significantly affect high-density lipoprotein-cholesterol (HDL-C). LDL-C levels are markedly elevated in overt hypothyroidism. The increase in total and LDL-C in hypothyroidism is due to a decrease in LDL receptor number. Furthermore, several steps involved in lipid metabolism are impaired in sHT, and a direct influence of thyroid hormones on both cholesterol ester transfer protein and hepatic lipase, leading to a reduction of LDL clearance, has been reported. 11 In the present study, we intended to study the nature of risk factors like CRP and endothelial dysfunction (NOx levels) without dyslipidemia.

Because endothelial dysfunction has been reported in association with aging, postmenopause, hypertension, and smoking status, 12 all our patients were young, nonobese, nonsmokers with normal blood pressure and rigorously matched to a control group of euthyroid subjects. Additionally, because sHT patients typically show moderately increased serum total and LDL-cholesterol levels, which per se could alter endothelial function, we selected sHT patients who had normal lipid profile, by purposive sampling method.

CRP release from the liver is promoted by IL-1, IL-6 and TNF-α as an acute-phase reactant in response to inflammation. CRP may directly promote atherosclerosis and endothelial inflammation by attenuating the release of NO, a key molecule in the endothelium that plays a pivotal role in the maintenance of vascular tone. The levels of serum CRP usually amount to several hundred milligrams/l. In contrast, a cut-off limit level >3 mg/l indicates low-grade inflammation, which in turn might be a prognostic marker for further cardiovascular events. CRP values increase with progressive thyroid failure and may count as an additional risk factor for the development of coronary heart disease in hypothyroid patients. 13 In contrast to overt disease, in sHT, CRP is increased yet without significant improvement after L-thyroxine therapy. In the present study, there was an increase in the hs-CRP levels which correlated positively with increasing TSH levels.

The endogenous production of nitric oxide (NO) by nitric oxide synthase (NOS) has been established as playing an important role in vascular homeostasis, neurotransmission, and immunological host defense mechanisms. NO is involved in many physiological processes and also becomes perturbed during disease. A methodology for NO detection is complex. Therefore its accurate detection and quantification is critical to understanding health and disease. Due to the extremely short physiological half life of this gaseous free radical, alternative strategies for the detection of the reaction products of NO biochemistry have been developed. Nitrite and nitrate in blood is widely used as an index of endothelial NO synthase activity as routine indirect measures of NO levels. Nitrite is now considered a central homeostatic molecule in NO biology and may serve as an important signaling molecule. Most recently nitrite has emerged as an endogenous signaling molecule and regulator of gene expression that can serve as a diagnostic marker and also as a potential therapy for cardiovascular disease. 14, 15 These data together with the recent discovery of nitrite as a signaling molecule have opened a new avenue for the diagnostic and therapeutic application of nitrite, especially in cardiovascular diseases, using nitrite as marker as well as an active agent. 16 In the present study, NOx levels significantly decreased in subclinical hypothyroidism patients when compared to euthyroid controls and there was a significant negative correlation of NOx levels with progressive increase in TSH levels.

5. Conclusion

Thus, present study shows an association of increased CRP levels and endothelial dysfunction with progression of sHT. This study is unique as it shows an association of cardiovascular risk factors before the onset of dyslipidemia in sHT and thus can possibly be considered early novel markers of future cardiac disease in sHT patients. This study might be helpful to plan for initiating, monitoring and optimizing levothyroxine therapy in sHT patients.

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