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Association between oxidative stress and coronary lipid risk factors in hypothyroid women is independent of body mass index

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Abstract

Hypothyroidism enhances the progression of atherogenesis. Furthermore, dyslipidemia, hypertension, and obesity are known risk factors for atherosclerosis. Oxidative stress is implicated in the pathogenesis of cardiovascular diseases. However, there are contradicting reports on the existence of oxidative stress in hypothyroidism. Thus, the aim of the study is to evaluate the presence of oxidative stress in hypothyroidism and, if so, its possible association with various coronary lipid risk factors. The present study was carried out in a group of 27 freshly diagnosed normotensive primary hypothyroid female patients in comparison with healthy subjects. Their body mass index (BMI), serum thyroid profile, lipid profile, glucose, protein carbonylation, thiobarbituric acid reactive substances (TBARS), and blood antioxidant enzyme levels were estimated. The TBARS and protein carbonylation were significantly higher in cases compared with those in controls. Reduced glutathione was lower and glutathione peroxidase was higher in the test group compared with those in controls. Various lipid risk factors for coronary artery disease were significantly higher among the hypothyroid women in comparison with those in controls. The level of TBARS correlated significantly with various lipid risk factors among the hypothyroid women even after correcting the effect of BMI. However, no significant associations were observed between BMI and these risk factors when the effect of TBARS was nullified. In hypothyroidism, the coronary lipid risk factors seem to be more associated with lipid peroxidation than BMI. In conclusion, the present study indicates the presence of oxidative stress in hypothyroid patients and its association with atherogenic dyslipidemia, which is independent of BMI.

1. Introduction

Oxidative stress results from either overproduction of free radicals or decreased efficiency of scavenger antioxidant system. This stress gets amplified and propagates because of the autocatalytic cycle of metabolic stress, tissue damages, and cell death that further intensifies the oxidative stress [1].

Oxidative stress has been implicated in a number of diseases such as cardiovascular diseases, neurologic diseases, malignancies, renal diseases, diabetes, inflammatory problems, skin diseases, aging, respiratory diseases, liver diseases, and different types of viral infections [2]. However, in hypothyroidism, the presence of oxidative stress is controversial [3-8]. There are reports stating a decrease in oxidative stress in experimental hypothyroid animal models [3]. Hypothyroidism prevented oxidative and nitrosative stress induced by ischemia and reperfusion in rats [4]. It protected against lipid peroxidation in a rabbit model [5]. On the contrary, an increase in oxidative stress has also been reported in other studies of experimental hypothyroidism. The plasma, red blood cell (RBC), liver, heart, and skeletal muscle levels of thiobarbituric acid reactive substances (TBARS) were increased in a propylthiouracil-administered group of hypothyroid rats in comparison with those in the control rats [6,7]; and this was ameliorated by the antioxidants taurine and vitamin E. These controversial findings can be attributed to the differences in experimental models adopted by the various investigators.

There is only scanty information in the literature on the existence of oxidative stress in human hypothyroidism. Increased low-density lipoprotein (LDL) oxidation has been reported in hypothyroidism [9]. Resch et al [10] have demonstrated the presence of oxidative stress by the increased titer of antioxidized LDL antibody in hypothyroid

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patients compared with that in controls. Hypothyroidism and atherosclerosis are found to be linked by a number of clinical case reports, epidemiological studies, and biochemical observations [11-13]. One factor that has been commonly implicated as an important atherosclerotic risk factor in various pathological states is body mass index (BMI). Apart from this, oxidative stress and dyslipidemia have also been considered as potential risk factors for atherosclerosis and coronary artery diseases [14,15]. There is no single study in the literature demonstrating the association of lipid risk factors with the degree of oxidative stress and the extent of BMI alteration in human hypothyroidism. Hypothyroidism is more common in women than in men. On the other hand, oxidative stress has been reported to be less pronounced in women during their reproductive phase in comparison with that in men, which has been attributed to the protective effects of estrogen on oxidative stress [16,17].

The present study was carried out in a group of hypothyroid women to assess the status of oxidative stress and its possible connection with coronary lipid risk factors along with its confounding factor, BMI.

2. Materials and methods

2.1. Subjects

Twenty-seven female patients who were clinically diagnosed as hypothyroid were recruited from the outpatient Department of Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research (Pondicherry, India), for the study. The patients were selected based on their thyroid profile (thyroid-stimulating hormone [TSH] >10 IU/mL because it is the level beyond which they are administered with thyroxine (T_4) by the clinician in our institute). Smoking, lifestyle, history of alcohol intake, and family history of thyroid dysfunctions, diabetes, and coronary artery diseases were recorded. Only the recently diagnosed hypothyroid patients who were not on any medication were included in this study. Patients diagnosed with hypothyroidism but already on treatment of the disease, patients taking lipid-lowering drugs or antioxidant vitamin supplements, smokers, patients with hypertension, alcoholics, patients with a sedentary lifestyle, pregnant women, women on hormone replacement therapy, and patients with diseases other than hypothyroidism were excluded from the study. Only 2 of 27 cases had a family history of thyroid dysfunction, and none were first-degree relatives of patients with diabetes or coronary artery disease. Of the 27 cases, 2 were postmenopausal women, 2 had postpartum hypothyroidism, 12 had complaint of painful neck with swelling for more than 2 months, 3 had simple goiter, 2 had cystic colloidal goiter, 5 had oligomenorrhea, 2 had multinodular goiter, and some had mixed clinical features mentioned above.

Twenty-six healthy, euthyroid female volunteer subjects of similar age were taken as controls. The control group consisted of clinically healthy subjects without any infectious diseases or chronic ailments. Women taking lipid-lowering drugs or antioxidant vitamin supplements, smokers, those with hypertension, alcoholics, pregnant women, and women on hormone replacement therapy were excluded from the control group. Among the participants in the control group, only one had a family history of diabetes mellitus; and none were first-degree relatives of patients with thyroid and coronary artery dysfunctions. The study was approved by the human ethics committee of our institute. Written consent was taken from all the participants of the study groups.

2.2. Blood collection

Blood samples were collected from patients before they were administered T_4 therapy. An overnight fasting sample was collected for the assay of reduced glutathione (GSH), glucose, lipid profile, thyroid profile, and antithyroperoxidase (anti-TPO) antibody titer. Red blood cells were separated and washed thrice with saline for the estimation of antioxidant enzymes. Serum samples were refrigerated at −50°C until the estimations of protein carbonyls and TBARS were carried out.

2.3. Methodology

2.3.1. Thyroid profile

Thyroid profile was assessed by radioimmunoassay assay for triiodothyronine (T_3) and T_4 with kits from the Bhaba Atomic Research Centre (Mumbai, India). The intra-and interassay coefficients of variation (CVs) were 3.3% and 7.3%, respectively, for estimation of T_3 at 150 ng/dL and 4.7% and 8.2%, respectively, for estimation of T_4 at 15 μg/dL. Thyroid-stimulating hormone was assayed by immunoradiometric assay method using kits from the Bhaba Atomic Research Centre. Intra- and interassay CVs were 3.6% and 7.8%, respectively, at 6.0 μIU/mL.

2.3.2. Lipid profile

Estimation of lipid profile was carried out by using kits from Biocon (Vöhl-Marienhagen, Germany) for total cholesterol by enzymatic method, Accurex (Mumbai, India) for triglyceride (TG) by enzymatic method, and high-density lipoprotein (HDL) cholesterol precipitating reagent and enzymes from Agappe diagnostics (Thane, India) using a clinical chemistry autoanalyzer (550 Express Plus, Ciba Corning, Oberlin, OH). Low-density lipoprotein cholesterol was calculated using the formula of Fridewald et al [18] because the total TG level was less than 400 mg/dL. Various lipid risk factors were calculated from the ratio of molar concentrations of the above parameters. A new index called *atherogenic index* was calculated by taking the logarithm of the ratio of molar concentrations of TG and HDL cholesterol [atherogenic index = log (TG/HDL cholesterol)] [19].

2.3.3. Glucose

Glucose was estimated by the glucose oxidase method in the autoanalyzer using glucose oxidase kits from Dr Reddy's laboratory (Hyderabad, India). The intra- and interassay CVs

for glucose estimation were 2.8% and 8.8%, respectively, at 6 mmol/L.

2.3.4. Anti-TPO antibody

Antithyroperoxidase antibody titer was estimated by Varelisa TPO antibody enzyme-linked immunosorbent assay kit from Pharmacia and Upjohn (Freiburg, Germany).

2.3.5. Reduced GSH

Whole-blood reduced GSH activity was estimated with 5,5′-bisdithionitrobenzoic acid [20] and expressed as micromoles per gram of hemoglobin. Hemoglobin was estimated with Drabkin reagent (Merck, Bombay, India) for the calculation of glutathione assay.

2.3.6. Glutathione peroxidase

Measurement of glutathione peroxidase (GPx) activity was based on Wendel's [21] method. Glutathione peroxidase catalyzed the oxidation of glutathione using hydrogen peroxide for the estimation of the peroxidase activity.

2.3.7. Glutathione S-transferase

Glutathione *S*-transferase was assayed by Habig's method [22] using 1-chloro-2,4-dinitrobenzene as the substrate.

2.3.8. Catalase

Catalase activity was measured by the method of Aebi [23].

2.3.9. Protein carbonyls

Protein carbonylation was estimated by the di-nitrophenyl hydrazine method [24].

2.3.10. Lipid peroxides

Thiobarbituric acid reactive substances, one of the by-products of lipid peroxidation, were estimated by the thiobarbituric acid method [25].

2.4. Statistical analyses

Data are expressed as mean ± SD. Significance of the differences between control and test groups was checked by independent-sample *t* test, and the association between the parameters was assessed by Pearson correlation analysis. Partial correlation analysis was carried out where other factors are known to influence oxidative stress. All statistical analyses were performed using the SPSS (Chicago, IL) program.

3. Results

Mean and SD of age, thyroid profile, lipid profile, other biochemical profiles, and oxidative stress parameters in controls and hypothyroid individuals and their correlations in hypothyroid individuals are compiled in Tables 1-6. The results show that fasting plasma glucose and total protein and albumin levels were not different among the groups. Lipid profile indicated hypertriglyceridemia (>1.69 mmol/L) in 19 (70%) and hypercholesterolemia (>6.21 mmol/L) in 18 (66.66%) of 27 cases. Although the mean anti-TPO antibody titer was higher in hypothyroid women compared with that in the control group, it was within the reference range (<100 IU/mL). Body weight and corresponding BMI were significantly higher in hypothyroid women in comparison with those in controls (Table 1). There was a significant increase in total cholesterol and TG levels among the hypothyroid patients. Low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol were significantly increased, whereas there was no significant change in the HDL cholesterol level (Table 2). The atherogenic lipid risk factors were found to be higher in the hypothyroid women (Table 3).

The TBARS, an index of lipid peroxidation, were increased significantly among the hypothyroid women in comparison with those in controls. In the study group, GPx, an antioxidant enzyme, was increased and reduced glutathione was decreased significantly when compared with those in the age-matched controls (Table 4). Protein carbonyl is an early indicator of oxidative stress. In the test group, there was a significant increase in its level and it correlated with the level of TBARS ($r = 0.67$, $P < .001$). Severity of the disease as assessed by the TSH level correlated with the increase in the level of TBARS ($r = 0.42$, $P = .028$) as well as with carbonylated proteins ($r = 0.49$, $P = .009$). However, in our study, the TSH levels had no correlation with any of the lipid risk factors.

The TBARS had a positive correlation with each of the lipid risk factors: non-HDL cholesterol ($r = 0.59$, $P = .001$), TG/HDL cholesterol ($r = 0.50$, $P = .007$), total cholesterol/HDL cholesterol ($r = 0.61$, $P = .001$), and LDL cholesterol/HDL cholesterol ($r = 0.61$, $P < .001$) (Table 5). *Atherogenic index* defined as the logarithm of the ratio between molar concentrations of TG and HDL cholesterol was also significantly higher among the hypothyroids. Because BMI is known to be associated with altered lipid profile and oxidative stress, we nullified the effect of BMI on the relationships between TBARS and various lipid risk factors for coronary artery disease through partial correlation analysis. The results are given in Table 5. We also checked for association of BMI with each of these lipid risk factors for coronary diseases and nullified the role of TBARS in the

Table 1
Mean ± SD of biochemical parameters of controls and hypothyroid women (cases)

	Control (n = 26)	Cases (n = 27)
Age (y)	35.53 ± 14.22	31.03 ± 12.03
Body weight (kg)	54.96 ± 9.94	65.85 ± 11.09 **
BMI (kg/m^2)	23.22 ± 4.06	29.11 ± 5.08 **
T_3 (ng/dL)	135.32 ± 30.01	74.92 ± 28.82 **
T_4 (μg/dL)	8.86 ± 1.65	4.62 ± 1.94 **
TSH (IU/mL)	2.24 ± 0.93	54.25 ± 36.22 **
Glucose (mmol/L)	4.19 ± 0.71	4.52 ± 0.78
Total serum protein (g/L)	70.19 ± 5.05	70.96 ± 3.45
Serum albumin (g/L)	37.87 ± 3.31	36.92 ± 4.48
Anti-TPO antibody (IU/mL)	12. 41 ± 11.81	49.99 ± 57.02 *

* $P < .05$.
** $P < .01$.

Table 2
Mean ± SD of serum lipid profile of controls and cases

	Control (n = 26)	Cases (n = 27)
Total cholesterol (mmol/L)	4.53 ± 0.82	6.92 ± 1.27 *
HDL cholesterol (mmol/L)	1.40 ± 0.34	1.29 ± 0.36
LDL cholesterol (mmol/L)	2.46 ± 0.95	4.56 ± 1.26 *
VLDL cholesterol (mmol/L)	0.27 ± 0.01	0.53 ± 0.30 *
TG (mmol/L)	1.36 ± 0.32	2.49 ± 0.94 *

VLDL indicates very low-density lipoprotein.
* $P < .01$.

association using partial correlation analyses. By this, we addressed the question of whether the relation between BMI and lipid risk factors is direct or is mediated through the enhancement of lipid peroxidation. It was found that BMI per se had no association with any of these risk factors when the positive influence of TBARS was nullified. The results of these partial correlation analyses are given in Table 6.

4. Discussion

Atherosclerosis is an associated complication of hypothyroidism [26]. Independent risk factors for the development of coronary artery disease include a family history of premature coronary artery disease, cigarette smoking, diabetes mellitus, hypertension, hyperlipidemia, a sedentary lifestyle, and obesity. In our study, we have taken a uniform group composed only of women with no history of coronary artery disease, hypertension, diabetes mellitus, smoking, and sedentary lifestyle diagnosed as hypothyroids for the first time before therapeutic intervention. Thus, we have brought forward the hidden link between oxidative stress, atherogenic lipid-related risk factors for atherosclerosis, and BMI in overt hypothyroidism.

Hyperlipidemia is a common feature in hypothyroidism and a risk factor for atherosclerosis [15]. The ratio of LDL cholesterol to HDL cholesterol [27] and the ratio of total cholesterol to HDL cholesterol [28] give an indication of cardiovascular disease. According to one study [29], the non-HDL cholesterol and the ratio of total cholesterol to HDL cholesterol are as good as or even better indicators than the apolipoprotein fractions for predicting the risk of cardiovascular diseases in women. In our study, the mean LDL cholesterol–HDL cholesterol ratio was 3.81; and the mean ratio of total cholesterol to HDL cholesterol was 5.79 among the hypothyroid women, indicating moderate risk for coronary artery disease. The atherogenic index, although studied in many ethnic populations, has not been studied in an Indian adult population [30].

Table 3
Mean ± SD of serum lipid risk factors for coronary artery disease and atherogenic index of controls and cases

	Control (n = 26)	Cases (n = 27)
TG/HDL cholesterol	1.05 ± 0.48	2.17 ± 1.25 *
Non–HDL cholesterol (mmol/L)	3.13 ± 0.92	5.62 ± 1.39 *
Total cholesterol/HDL cholesterol	3.46 ± 1.17	5.82 ± 2.28 *
LDL cholesterol/HDL cholesterol	2.05 ± 0.94	3.92 ± 1.92 *
Atherogenic index (SI units)	−0.01 ± 0.16	0.26 ± 0.25 *

Atherogenic index = log (TG/HDL cholesterol).
* $P < .01$.

Table 4
Mean ± SD of parameters of oxidative stress in controls and cases

	Control (n = 26)	Cases (n = 27)
Blood GSH (μmol/g Hb)	10.42 ± 3.18	8.39 ± 2.09 *
RBC catalase (K/mL)	23.00 ± 4.22	25.54 ± 6.86
RBC GPx (U/g Hb)	26.82 ± 6.54	36.74 ± 10.21 **
RBC GST (μmol/min/mg Hb)	4.45 ± 0.91	4.21 ± 0.73
Serum TBARS (μmol/L)	1.33 ± 0.39	2.21 ± 0.82 **
Serum protein carbonyls (nmol/mg protein)	1.14 ± 0.34	1.60 ± 1.03 *

Hb indicates hemoglobin; GST, glutathione *S*-transferase.
* $P < .05$.
** $P < .01$.

Most of the information relating oxidative stress with hypothyroidism have conflicting conclusions. In the human cases of hypothyroidism, some of the reports state an increase in TBARS [31-33]. Sarkar et al [8] demonstrated no reduced production of reactive oxygen species in the isolated mononuclear cells from hypothyroids in comparison with euthyroids by flow cytometry. In our study, we found an increase in plasma TBARS and protein carbonylation as well as alteration in various antioxidants that get altered in oxidative stress. This illustrates the existence of oxidative stress among the hypothyroid women of our study. The conflicting reports as to the presence or absence of oxidative stress in hypothyroidism could be attributed to the ethnic variation and the nutritional and environmental differences. Rise in the levels of both TBARS and protein carbonyls and their correlation in the hypothyroid women indicate simultaneous direct damage to lipid and protein structures of the body via free radical. Concurrent decrease in glutathione and an increase in GPx observed in the study point toward increased generation of free radicals and the induction of the enzyme as a mechanism for the enhanced sequestration of the same. The negative correlation of TBARS with GSH ($r = -0.54$, $P < .01$) and its positive

Table 5
Pearson correlations of lipid risk factors for coronary artery disease with TBARS in freshly diagnosed hypothyroid women (n = 27) and partial correlations after nullifying the effect of BMI

Risk factors	Correlation coefficient	*P*	Partial correlation coefficient	*P*
Non-HDL cholesterol	0.59	.001	0.53	.005
TG/HDL cholesterol	0.50	.007	0.41	.03
TC/HDL cholesterol	0.61	.001	0.53	.005
LDL cholesterol/HDL cholesterol	0.61	.001	0.53	.005
Atherogenic index	0.49	.009	0.40	.04

TC indicates total cholesterol.

Table 6
Pearson correlations of lipid risk factors for coronary artery disease with BMI in freshly diagnosed hypothyroid women (n = 27) and partial correlations after nullifying the effect of TBARS

Risk factors	Correlation coefficient	P	Partial correlation coefficient	P
Non-HDL cholesterol	0.36	.06	0.18	.35
TG/HDL cholesterol	0.39	.03	0.26	.19
TC/HDL cholesterol	0.46	.01	0.32	.10
LDL cholesterol/HDL cholesterol	0.45	.01	0.30	.13
Atherogenic index	0.40	.03	0.27	.17

correlation with GPx (r = 0.60, P < .01) show that the alteration in antioxidant activity is consistent with the rise in the lipid peroxidation process.

Oxidative stress is known to be associated with dyslipidemia [34,35] and obesity [36]. Increase in body weight in hypothyroidism is mostly due to fluid retention in the myxedematous tissues [37]. However, the presence of obesity before the genesis of hypothyroidism cannot be ruled out. The association between BMI and TBARS (r = 0.371, P = .057) among our study subjects was marginal. The higher BMI of the patients did not influence the relation between lipid peroxidation and coronary lipid risk factors (Table 5). Furthermore, the association between BMI and coronary lipid risk factors was lost when corrected for TBARS using partial correlation analyses (Table 6). Atherogenic index is a recently proposed plasma marker of atherogenicity because of its higher level in the coronary artery disease and its inverse relationship with LDL particle size [19]. Its association with TBARS in this study further strengthens the risk of atherogenesis in hypothyroidism in advent with oxidative stress. In hypothyroidism, the coronary lipid risk factors seem to be more associated with lipid peroxidation than BMI. These results emphasize the importance of the association of oxidative stress per se with coronary risk factors detected in hypothyroidism that is independent of BMI.

Hypothyroidism is often accompanied by diastolic hypertension that in conjunction with the dyslipidemia may promote vascular complications. We have selected only normotensive patients for the study to rule out the putative role of hypertension on oxidative stress parameters. Recently, we have published reports stating the presence of oxidative stress in prehypertensive stage [38]. It could be suggested that oxidative stress in overt hypothyroidism might even precede the development of diastolic hypertension. However, prospective studies in a larger population are required to substantiate this hypothesis.

Our study suffers from few drawbacks, such as a small sample size and the lack of data on the free T_3 and free T_4 levels. Nevertheless, the study group was a strictly uniform and homogenous one because of stringent adherence to exclusion and inclusion criteria. The free radical accumulation in hypothyroidism could be due to various reasons such as (1) decreased clearance of oxidants, (2) decreased antioxidant induction at the genetic level, (3) decreased effective activity of the body's antioxidant defense mechanism, and (4) atherogenic hyperlipidemia providing substrate for enhanced lipid peroxidation, among others. The interplay of various such mechanisms could finally result in oxidative stress.

In summary, the present study suggests a perturbation in the oxidant-antioxidant status in hypothyroidism despite its being a hypometabolic state. There was alteration in the body's antioxidant barrier and an increase in the process of oxidation of proteins and lipids. The increased lipid peroxidation was consistent with higher level of lipid risk factors for atherosclerosis, and this relationship was independent of other traditional risk factors for atherosclerosis such as BMI and hypertension in our study.

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