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Protein carbonylation in freshly diagnosed hypothyroidism is independent of thyrotropin levels

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Abstract

Background: Hypothyroidism is a common endocrine disorder with female preponderance. Protein carbonyls (cP) and malondialdehyde (MDA) are generated due to protein and lipid peroxidation, respectively. Oxidative stress (OS) in freshly diagnosed hypothyroidism met with conflicting data in the research. And, a clear relationship between OS and early hypothyroidism is very limited and obscure. Therefore, we aimed to investigate the association between levels of MDA vs. thyrotropin (TSH) and cP vs. TSH among freshly diagnosed hypothyroid subjects.
Methods: We collected blood samples of 80 hypothyroid subjects prior to initiation of thyroxine therapy to know the association between OS and freshly diagnosed hypothyroidism. Serum MDA, cP along with total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triacylglycerols (TG) were quantified in patients as well as 80 age- and sex-matched control subjects.
Results: Levels of MDA and cP were significantly elevated among hypothyroid subjects as compared to control. The rise in MDA levels positively correlated with TSH values among the patients. In addition, cP levels were substantially elevated as compared to MDA values; however, it does not correlate with TSH among hypothyroid subjects.
Conclusions: Our study found no relationship between cP and TSH in freshly diagnosed hypothyroidism. Though it may be due to differential degradation of protein peroxidation products, the mechanism needs further elucidation in future studies.

Keywords: hypothyroidism; malondialdehyde; oxidative stress; protein carbonylation; thyrotropin.

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Introduction

There are 31 enzymes including membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and 11 mitochondrial enzymes [1] that are cellular sources for the generation of reactive oxygen species (ROS) inside the cell; moreover, mitochondria have an edge in this respect especially in the basal level cellular metabolism [2]. Any electron that enters the electron transport chain (ETC) finally reduces molecular oxygen partially to ROS (superoxide anion [$O_2^{\cdot-}$] which may be further dismutated to hydrogen peroxide [H_2O_2]) or completely into water. Though ROS acts as an efficient signaling molecule inside the cell, its implication in the disease processes has been strengthening ever since its discovery. Increased production of ROS or decreased clearance by anti-oxidant defense systems in the body results in oxidative stress (OS) and damage to biomolecules of the cells, either lipids, protein or deoxyribonucleic acids, and consequently cell death.

Thyroid hormones (TH), namely triiodothyronine (T3) and tetraiodothyronine (T4), are involved in diverse functions in the body including regulation of growth, development, cellular metabolism [3] and peripheral homeostasis [4]. These functions have been categorized as genomic and nongenomic. They bind to five subcellular structures, plasma membrane, lipid droplets, nuclear envelope, nuclear matrix and mitochondria [5] to perform these functions. Genomic actions are mediated through the nuclear receptors involving transcription followed by translation and bring the required changes. On the other hand, the nongenomic actions are the result of TH interaction with receptors other than nuclear receptors. Mitochondria are

an important location for genomic as well as nongenomic actions of TH. Mitochondrial binding sites for TH include p43 (43 kDa; in the mitochondrial matrix) and p28 (28 kDa; inner mitochondrial membrane), which are truncated forms of thyroid receptor (TRα1) [6]. TH binding with p43 increases transcription of mitochondrial DNA (mtDNA; which encodes for 13 polypeptides, seven for complex I, one for complex III, three for complex IV and two for complex V) and protein synthesis inside the mitochondria [7]. Thus, it increases the pace of oxidative phosphorylation (oxPhos) as well as the efficiency of adenosine triphosphate (ATP) generation.

Hypothyroidism is a common endocrine disorder characterized by low T4 and high thyrotropin (TSH) and is often accompanied by dyslipidemia [8]. As THs act as important regulators of oxPhos, their low levels should result in a decrease in oxPhos and associated ROS generation through ETC. And, low OS should be reflected in hypothyroidism. Additionally, raised γ-glutamyl transpeptidase (GGT) levels are associated with hypothyroidism due to liver insult (non-alcoholic fatty liver disease; NAFLD) following disturbed ROS generation [9]. However, there are conflicting reports found in the literature regarding OS [10–12] as well as levels of GGT [13, 14] in hypothyroidism. Moreover, only a few studies on OS are available in freshly diagnosed overt hypothyroidism [15]. Therefore, the present study aimed to estimate markers of lipid and protein peroxidation, i.e. malondialdehyde (MDA) and protein carbonyls (cP), respectively, in freshly diagnosed hypothyroid patients in whom treatment is still not initiated. Moreover, association between markers of peroxidation and TSH as well as lipid moieties and GGT is ascertained.

Materials and methods

The study included 80 hypothyroid subjects (39.7 ± 12.5 years; 10 males) visiting for the first time to the hospital for their current illness and 80 euthyroid controls (39.9 ± 10.8 years; 12 males) from medical outpatient department (OPD) of Hakeem Abdul Hameed Centenary hospital, Jamia Hamdard, New Delhi. The study was approved by the Institutional Review Board of Jamia Hamdard, Delhi. The control group comprised healthy subjects without any evidence of thyroid disease or any chronic illness. The patients were clinically diagnosed with hypothyroidism followed by confirmation of TSH (>5.5 mIU/mL) and free T4 (fT4; <9 pmol/L) levels in their sera. The hypothyroid patients included in the study were not on any medication. Patients previously diagnosed with hypothyroidism and already on treatment for the disease or who discontinued thyroxine therapy due to any reason in the past were excluded. Patients as well as controls taking lipid-lowering drugs or antioxidant therapy, smokers, those with hypertension, alcoholics, pregnant women, females on hormone replacement therapy and any other chronic diseases including diabetes and cardiovascular disease were also excluded from this study. Any thyroid illness found or discovered among the first-degree relatives of patients and controls were also not included for the study.

Blood samples were drawn after overnight fasting into plain Vacutainer (VACU-ETTE®, Greiner Bio-One India Pvt. Ltd., Noida, UP, India) from hypothyroid subjects before the initiation of thyroxine therapy and from euthyroid subjects. Serum TC, HDL-C, TG, LDL-C and GGT were quantified using Siemens dimension Xpand plus biochemistry analyzer with kits and consumables as per the instruction of the supplier i.e. Siemens India Ltd., Worli, Mumbai, India. Free T3 (fT3), fT4 and TSH levels were analyzed in Cobas e411 using supplier's (Roche Products [India] Pvt. Ltd., Bandra, Mumbai, India) manual, kits and instruction. Euthyroid status is defined as TSH (0.5–5.5 mIU/mL), fT3 (3.5–5.5 pmol/L) and fT4 (9–22 pmol/L) with an inter-assay coefficient of variation (CV) (n = 15) of 1.96, 1.12 and 3.11, respectively. The levels of cP and MDA in patient and control sera were estimated using colorimetric method defined by Levine et al. [16] and Satoh [17] and standard protocol, respectively. The percent CV for MDA at 0.99 μmol/L and cP at 0.29 nmol/mg were 5.43 and 4.87, respectively.

Statistical analysis

All the experiments were performed in duplicate and data expressed as mean ± standard deviation (SD). Spearman's correlation was analyzed using Microsoft Excel 2016. Statistical analysis of data was done by employing two-tailed Student's t-test as described by Bennet and Franklin [18]. At 95% confidence interval, p-values less than 0.05 were considered significant.

Results

As seen in Table 1, a significant decrease of 37% and 46% was observed in fT3 and fT4 levels, respectively, in hypothyroid subjects when compared to the corresponding values from euthyroid subjects. In addition, a significant increase of 15% and decrease of 19% were observed in the levels of serum TG and HDL-C in hypothyroid subjects in

Table 1: Average serum levels of TC, LDL-C, HDL-C, TG, GGT, fT3, fT4, TSH, MDA and protein carbonyls (cP) from euthyroid and hypothyroid subjects expressed as mean ± SD.

Parameter	Euthyroid (n = 80)	Hypothyroid (n = 80)	[a]p-Value
fT3, pmol/L	4.52 ± 0.76	2.86 ± 0.82	<0.001
fT4, pmol/L	15.5 ± 2.4	4.4 ± 2.4	<0.001
TSH, mIU/mL	2.71 ± 1.3	10.9 ± 5.3	<0.001
cP, nmol/mg	0.25 ± 0.14	0.68 ± 0.21	<0.001
MDA, µmol/L	0.84 ± 0.25	1.2 ± 0.55	<0.001
GGT, mg/dL	36.8 ± 18.6	37.7 ± 18.3	0.758
TC, mg/dL	177.9 ± 29.1	200.3 ± 60.1	<0.001
TG, mg/dL	143.3 ± 12.2	164.5 ± 67.5	<0.001
HDL-C, mg/dL	43.5 ± 12.2	35.4 ± 9.4	<0.001
LDL-C, mg/dL	120.6 ± 37	107.9 ± 59.1	0.105

[a]p-Value <0.05 is significant.

comparison to euthyroid subjects. Similarly, markers of lipid peroxidation, MDA and protein carbonylation were significantly higher among hypothyroid subjects; however, no significance was seen in the levels of LDL-C and GGT.

In order to examine the variation in the levels of lipid peroxidation and protein carbonylation with the severity of hypothyroidism, the values of hypothyroid subjects were categorized into two groups, I (<10 mIU/mL) and II (>10 mIU/mL) on the basis of TSH values (Table 2). Protein carbonylation values were found to be substantially higher by 164% and 184% in groups I and II, respectively. Similarly, MDA was also increased in the sera of hypothyroid subjects by 33% and 86% in groups I and II, respectively, in comparison to values from euthyroid subjects (Table 2).

To recognize an association between hypothyroidism and OS, Spearman's correlation was performed between TSH vs. MDA and TSH vs. cP, in the respective groups. A significant correlation was observed between MDA and TSH levels in hypothyroid subjects in group II (r-value = 0.64, n = 50; $p < 0.05$). On the other hand, no significant correlation was seen between cP and TSH levels or with any of the lipid moieties among hypothyroid subjects irrespective of the group (Tables 3 and 4). Similarly, levels of fT4 were lacking any significant association with cP, MDA and any of the lipid parameters in both the groups of hypothyroid subjects (Table 5).

Table 2: Average serum levels of thyroid hormones, lipid parameters, MDA and cP from euthyroid and two groups of hypothyroid subjects.

Parameters	Euthyroid (80)	Hypothyroid	
		I (30)	II (50)
fT3, pmol/L	4.52 ± 0.76	2.88 ± 0.59[a]	2.60 ± 1.01[a]
fT4, pmol/L	15.5 ± 2.4	8.61 ± 1.9[a]	8.09 ± 1.9[a]
TSH, mIU/mL	2.71 ± 1.3	7.22 ± 0.98[a]	17.12 ± 3.32[a]
cP, nmol/mg	0.25 ± 0.14	0.66 ± 0.15[a]	0.71 ± 0.13[a]
MDA, µmol/L	0.84 ± 0.25	1.12 ± 0.20[a]	1.56 ± 0.59[a]
GGT, mg/dL	36.8 ± 18.6	32.73 ± 11.36[b]	37.86 ± 14.88[b]
TC, mg/dL	177.2 ± 29.1	185.3 ± 39.6[b]	223.1 ± 60.0[a]
TG, mg/dL	143.3 ± 12.2	148.3 ± 51.9[b]	162.86 ± 49.49[a]
HDL-C, mg/dL	43.5 ± 12.2	33.13 ± 6.13[a]	32.8 ± 6.22[a]
LDL-C, mg/dL	105.9 ± 34.1	103.2 ± 56.2[b]	109.2 ± 58.4[b]

Values are expressed as mean ± SD; significantly different from euthyroid subjects at [a]$p < 0.001$, [b]not significant.

Discussion

Hypothyroidism is known to have a dyslipidemic profile with increased vascular events. Our data of high TC and low HDL-C among hypothyroid subjects are in accordance with the previous reports [8]. TH decreases TC and LDL-C by multiple ways, first by repressing the activity of the rate limiting enzyme, hydroxymethylglutaryl-CoA (HMG-CoA) reductase, in cholesterol biosynthesis, second by enhancing excretion of cholesterol by accelerating conversion of cholesterol into bile and finally by decreasing absorption of dietary cholesterol from the intestine [19].

Table 3: Spearman's correlation of cP with thyroid and lipid parameters in hypothyroid subjects as a whole and in groups I and II.

Parameters	Hypothyroid	Group I	Group II
TSH	0.151	0.021	−0.070
GGT	0.103	0.001	0.164
TG	0.092	0.214	−0.175
TC	0.033	0.135	−0.191
HDL	0.089	0.171	−0.119
LDL	0.043	0.176	−0.165

Values are expressed as correlation coefficient "r".

Table 4: Spearman's correlation of MDA with cP, thyroid and lipid parameters in hypothyroid subjects as a whole and in groups I and II.

Parameters	Hypothyroid	Group I	Group II
TSH	0.616[a]	0.099	0.643[a]
GGT	0.038	−0.089	−0.270
TG	0.117	−0.046	−0.093
TC	−0.024	0.027	−0.308
HDL	0.088	0.041	0.190
LDL	−0.062	0.069	−0.281
cP	0.214	0.163	0.154

Values are expressed as correlation coefficient "r". [a]Statistically significant at $p < 0.001$.

Table 5: Spearman's correlation of fT4 with cP, MDA and lipid parameters among hypothyroid subjects in their respective groups.

Parameters	Group I	Group II
cP	0.123	0.34
MDA	0.174	0.125
TC	−0.018	0.003
TG	−0.011	−0.067
LDL	0.005	0.096
HDL	−0.170	0.160
GGT	0.123	0.038

Values are expressed as correlation coefficient "r".

Some researchers reported higher levels of GGT in hypothyroid subjects [13], whereas others found lower levels compared to the euthyroid population [14]. In the present study, we did not find significantly different levels of GGT in hypothyroid subjects compared with euthyroid subjects. Increased OS coupled with insulin resistance and dyslipidemic profile among hypothyroid subjects promotes NAFLD [9]. Despite identifying risk factors associated with hypothyroidism, GGT, a surrogate measure of NAFLD, quantification met with conflicting reports [20]. Moreover, our data lack any significant relationship between TSH and GGT levels among hypothyroid subjects. However, a lacking association between TSH and GGT or an insignificant difference between GGT levels among hypothyroid and euthyroid populations does not rule out ROS-induced liver insult in hypothyroidism. Therefore, GGT estimation coupled with imaging studies might quantify the damage to the liver in hypothyroidism.

The majority of studies measuring OS in hypothyroidism among humans are irrespective of the duration of disease and therapy status. OS in freshly diagnosed hypothyroidism, in subjects who are not on hormone replacement therapy, is infrequently reported [15]. In our study, it was evident that hypothyroidism is associated with high OS with an increase in MDA and cP due to lipid and protein peroxidation. It is reflected by an increase in the levels of peroxidation products in group I by 164, 33% and II by 184, 86% in cP and MDA, respectively, as compared to euthyroid subjects. Therefore, OS increases with the rise in TSH even in newly diagnosed subjects. There are several mechanisms contributing to the increased OS among hypothyroid subjects including a weak antioxidant defense system [15], and a decrease in uncoupling proteins and basal proton leak, despite low OxPhos, resulted in increased ROS generation in the mitochondria [21]. Our findings are in line with the previous reports of OS in overt hypothyroidism [10, 15]. However, in the present study, the rise in cP (172%) is more than four-fold as compared to MDA (43%) among hypothyroid subjects compared to controls. As THs regulate protein turnover, the degradation and clearance of damaged and/or modified proteins, i.e. cP, decreases in conditions of low thyroid levels [22]. Moreover, protein peroxidation occurs either by the direct action of ROS or by the adduct formation with MDA and/or intermediates of lipid peroxidation, i.e. reactive aldehydes, ketones and hydroperoxides [23]. Thus, the increased formation of cP as well as its prolonged persistence resulted in substantially increased levels of cP in the sera of hypothyroid subjects.

Similar to earlier studies [10, 15], we observed a significant positive association between TSH and lipid peroxidation in group II having TSH levels more than 10 mIU/L. However, our data do not show any correlation between lipid peroxidation and TSH, especially when TSH levels are low (<10; in group I). The variation of association in the two groups could be due to a number of reasons, e.g. there were no male patients in group I and the mean age of group II subjects (41.4 ± 13.5 years) was higher than that of group I subjects (38.6 ± 11.9 years), or a combination of them. It is further supported by previous studies that OS is more pronounced in male subjects and increases with advancing age [15]. Moreover, the relationship between fT4 and TSH is nonlinear and dependent on age as well as sex [24]. In contrast to lipid peroxidation, protein carbonylation does not correlate with TSH irrespective of its levels despite a substantial increase in cP levels among hypothyroid subjects. Although we were unable to confirm the nonconformity in the association of TSH and cP levels in our study, as found in previous studies, there may be various reasons for the same. The lack of association between protein peroxidation and TSH levels may reflect that following formation of cP, their degradation by the ubiquitin-proteasomal pathway is differentially expressed among hypothyroid subjects in the present study and pertains further exploration. Moreover, due to the nature of the study (cross-sectional), we were unable to determine how many patients progress from subclinical to overt hypothyroidism, and at what age, or in which time span. In addition, we were lacking data suggesting precisely when a patient was free from thyroid illnesses either subclinical or overt. Therefore, in extended future studies the causes of the substantial increase in cP and its relationship with TSH levels among freshly diagnosed hypothyroidism need to be determined.

In summary, high OS is found to be associated with hypothyroidism with the evidence of raised MDA and cP among patients. The increase in lipid peroxidation is proportionate with the severity of hypothyroidism. Both the increase in mitochondrial ROS generation and increased

lipid peroxidation contribute to protein peroxidation in a combined fashion. On the contrary, cP did not correlate with TSH levels among hypothyroid subjects. This could be due to differential metabolism of products of protein peroxidation. However, it needs further exploration to ascertain the substantial rise in cP as well as its association with TSH in freshly diagnosed hypothyroidism.

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