Original Article

Serum Prooxidant-Antioxidant Balance and hs-CRP in Patients with Clinical and Subclinical Hypothyroidism

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Abstract

Background: Oxidative stress (OS) is caused by an imbalance between prooxidant substance production and antioxidant defense. OS is involved in physiologic interactions in the body and the pathogenesis of various disorders. This study aimed to evaluate serum prooxidant-antioxidant balance (PAB) as a selective prooxidant, antioxidant defense, and acute phase reactant protein in patients with subclinical and clinical hypothyroidism. **Methods:** This case-control study was conducted in three groups including clinical hypothyroidism (32 patients), subclinical hypothyroidism, (42 cases), and healthy controls (32 individuals). This study was performed in the Endocrine Clinic of Arash Training and Research Hospital, Tehran, 2017. In the study groups, thyroid hormones including T4 and Thyroid Stimulating Hormone (TSH), fasting blood glucose (FBG), lipid profile, PAB, and hs-CRP as inflammatory markers were measured and compared between the groups. **Results:** Among 106 participants, 95.3% were females, the gender balance was similar across groups and mean age was 30.79 ± 7.65 years. FBG and lipid profile except for cholesterol level were not significantly different between the three study groups. However, cholesterol level in the clinical hypothyroid group was significantly higher than the other two groups. PAB was higher in subclinical hypothyroidism compared to healthy controls after adjustment for age and TSH levels (*P* value: 0.04) but there was no significant difference in the clinical hypothyroid group in comparison with healthy controls. In addition, there was no significant difference in high-sensitivity C-reactive protein (hs-CRP) between the three study groups. **Conclusions:** This study suggests that that subclinical hypothyroidism increases PAB in comparison to healthy control which could indicate OS response in patients with subclinical hypothyroidism, respectively.

Keywords: *Antioxidant, CRP, hypothyroidism, OS, prooxidant*

Introduction

Thyroid dysfunction is a common endocrine disorder which increases with age and is more common in women. The rate of thyroid dysfunction is different from 1 to 10% in different areas of the world.[1] The prevalence of hypothyroidism varies from 0.9 to 17.5% depending on the sensitivity of the thyroid function test methods and the population evaluated.[2] The rate of subclinical hypothyroidism (SCH) is higher than that of clinical hypothyroidism.[2]

SCH is characterized by elevated TSH levels with normal thyroxin (T4) levels. The prevalence of SCH in the general population is 3–8% and is higher in females and increases with age.[3,4] Clinical hypothyroidism is defined by elevated TSH levels with reduced thyroxin levels. Both SCH and clinical hypothyroidism have been associated with the same cardiovascular consequences.[5]

Oxidative stress (OS) occurs due to the imbalance between the production of prooxidant substances and antioxidant defense system. Reactive oxygen species (ROS) are important prooxidant substances. ROS are produced in different pathways and their harmful effects are neutralized by antioxidant defenses. OS is a routine event in the body. In normal conditions, the physiological levels of ROS are preserved at low levels in the cell by various antioxidant defenses.

OS is involved in the pathogenesis of various disorders such as inflammatory and immune-mediated disorders, cardiovascular disease,[6] chronic kidney disease,[7-9] diabetes and its complications,[9] cancer,[2] thyroid disorders,[7,10] and the aging process.[9]

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The production of ROS and other free radicals is ongoing in the thyroid. Many biochemical compounds can be damaged irreversibly or reversibly by free radicals and these prooxidant components contribute to the physiological and pathological processes in the thyroid gland. It has been shown that in a hypermetabolic condition in hyperthyroidism, the production of free radicals increases,[11] while their production reduces in a hypometabolic condition in hypothyroidism.[12]

The thyroid hormones have a significant effect on OS[13,14] due to their role in cellular metabolism and oxygen consumption in the mitochondria.[15]

OS and inflammation are closely related to each other and OS is an important underlying mechanism of inflammation. High-sensitivity C-reactive protein (hs-CRP) is a marker of subclinical inflammation and is an independent predictor of atherosclerosis and cardiovascular disease.

Studies regarding OS in SCH and clinical hypothyroidism are not enough and their results are contradictory and inconsistent.[12,14,16] Different methods have been developed to measure the total oxidants and antioxidants that are indirect, expensive, and time-consuming. PAB is an assay that simultaneously determines the serum prooxidant load and antioxidant capacity in a single test.[17] Although measuring OS in thyroid disorders is not new, the PAB method for measuring OS is new in this study. The main objective of this study was to examine whether subclinical and clinical hypothyroidism before starting therapy with levothyroxine are associated with an increase in OS and inflammatory markers.

Methods

Study design

This case-control study was conducted prospectively during the year 2017 in the Endocrinology Outpatient Clinic of Arash Training and Research Hospital, Tehran, Iran.

This study included three groups: Subclinical hypothyroidism group (42 patients), clinical hypothyroidism group (32 patients), and control group (32 individuals). Patients with TSH levels of equal to or greater than 5 but less than 10 mIU/L and with normal T4 levels were in the subclinical hypothyroidism group. Patients who had clinical hypothyroidism symptoms or signs and TSH levels of equal or greater than 10 mIU/L were in the clinical hypothyroidism group. Healthy volunteers with normal thyroid function (normal T4 and TSH levels less than 5 mIU/L) and anti-thyroid peroxidase (anti-TPO) less than 34 IU/mL were considered under the control group.

Patients aged between 17 and 42 years and confirmed in the subclinical or clinical hypothyroidism group before starting the therapy with levothyroxine and healthy volunteers with normal thyroid function were included in the study. Patients taking levothyroxine, lipid-lowering agents, antihypertensive drugs, and vitamin supplements effective on oxidative stress as well as those with a history of diabetes and cardiovascular, renal, and hepatic diseases, pregnancy, or planning for pregnancy, smoking, and alcohol use were excluded from the study. The body mass index (kg/m^2) was calculated by dividing weight by height square.

Laboratory evaluation

After 12-h of overnight fasting, the blood sample was collected and stored at −80°C until assay. The thyroid hormones as well as fasting blood sugar (FBS), total cholesterol, and fractions, triglycerides, hs-CRP as an inflammatory marker, and serum prooxidant-antioxidant balance (PAB) were measured.

The FBG and lipid profile were measured using the calorimetric method. Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula. Serum TSH and T4 were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method (Pars Azmoon kit, Iran).

High-sensitivity C-reactive protein measurement

This protein was measured by a (Polyethylene Glycol (PEG)-enhanced immuno-turbidometry method using commercially-obtained kits with an Alcyon® analyzer.

Serum prooxidant-antioxidant balance assay

The serum prooxidant-antioxidant balance was measured by the PAB assay. The chemical solutions were peroxidase enzyme (Applichem: 230 U/mg, A3791,0005, Darmstadt, Germany), Tetramethylbenzidine (TMB) powder (3,3′,5,5′-tetramethylbenzidine, Fluka), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), and hydrogen peroxide (30%) (Merck). The standard solutions were prepared by mixing various ratios (0–100%) of 250 mM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH). The TMB powder (6 mg) was dissolved in 1 mL Dimethyl Sulfoxide (DMSO). To prepare the TMB cation, 400 µL of the TMB/DMSO solution was added to 20 mL of acetate buffer (0.05 M buffer, pH 4.5), and then 70 µL of fresh chloramine T (100 mM) solution was added to this 20 mL. The solution was mixed thoroughly and incubated for 2 h at room temperature in a dark place. Then 25 U of peroxidase enzyme solution was added to 20 mL of TMB cation solution, dispensed in 1 mL aliquots, and stored at −20°C. To prepare the TMB solution, 200 µL of TMB/DMSO was added to 10 mL of acetate buffer (0.05 M buffer, pH 5.8) and the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution. This working solution was incubated for 2 min at room temperature in a dark place and was then used immediately. Ten microliters of each sample, standard or blank (distilled water) were mixed with 200 µL of working solution in each blank well of a 96 well-plate, which was

then incubated in a dark place at 37°C for 12 min. After the incubation time, 100 μL of 2 N HCl was added to each well, and the optical density (OD) was measured by an ELISA reader at 450 nm with a reference wavelength of 630 nm. A standard curve was provided from the values relative to the standard samples. The values of the PAB are expressed in an arbitrary (HK) unit, which is the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.[17]

The Ethics Committee at the Tehran University of Medical Sciences approved the study protocol (Approval ID: IR.TUMS.REC. 1395.29.7, Approval Date: 2017-1-30) and written informed consent was obtained from all the participants before enrollment. The study was conducted according to the principles of the revised version of the Helsinki Declaration.[18]

Statistical analysis

Numerical data are presented as mean and standard deviation and categorical data as number and percentage. For comparison of three groups, Analysis of variance (ANOVA) using the Tukey test was used for data with normal distribution and the Kruskal–Wallis test was used for data without normality. Simple linear logistic regression was used for comparison of hs-CRP and PAB between three study groups and the 95% confidence interval (95% CI) was calculated for the strength of association. The SPSS software version 20.00 for Windows was used for data analysis. A *P* value less than 0.05 was considered significant.

Results

In the three study groups, 106 participants including 101 women (95.3%) and 5 men (4.7%) with a mean age of 30.79 ± 7.65 years were included and the male/female ratio was comparable between the three study groups (*P* value: 0.66). The demographic characteristics, as well as thyroid hormones and lipid profile in the three study groups, have been shown in Table 1.

Age in the overt hypothyroid group was significantly higher than in the control group [Table 1]. There was no significant difference in the BMI between the three study groups [Table 1].

The fasting blood glucose (FBG) and lipid profile, except for cholesterol level, were not significantly different between the three groups [Table 1]. However, the cholesterol level in the clinical hypothyroid group was significantly higher than in the other two groups [Table 1].

A significant difference was observed in the anti-TPO and TSH levels between the three study groups and they were higher in the clinical hypothyroid group [Table 1].

Due to significant differences in age and TSH levels between the three study groups, the prooxidant-antioxidant balance (PAB) and hs-CRP were compared after an adjustment for age and TSH levels in the multiple linear regression model [Table 2]. PAB was significantly higher in subclinical hypothyroidism in comparison with the control group (*P* value: 0.04) but there was no significant difference in the clinical hypothyroid group. In addition, there was no significant difference in hs-CRP as a marker of inflammation between the groups [Table 3].

Discussion

In this study, we found a significantly increased PAB in patients with subclinical hypothyroidism in comparison with normal healthy controls and no difference in hs-CRP between clinical, subclinical hypothyroidism, and control groups.

The thyroid hormones determine the basal cell metabolic rate and regulate metabolism and oxygen consumption.[19] This role of thyroid hormones in energy metabolism is performed through the mitochondria that are the main intracellular target for calorigenic effects of the thyroid hormones. These hormones are associated with an oxidative and antioxidative status. The mitochondria also are the main intracellular target for tissue damage induced by OS due to the thyroid hormones. The changes in the thyroid hormone's levels could increase ROS generation by changes in the mitochondrial respiratory chain which is diluted by the antioxidants.[20]

The increased oxygen consumption by triiodothyronine due to the overproduction of ROS disrupts PAB and leads to OS which consequently damages cellular structures such as lipids, proteins, and Deoxyribonucleic acid (DNA).[21]

Numerous studies have shown that altered thyroid function causes an alteration in the antioxidant defense system in various tissues.[22-24] Some studies have demonstrated a reduction in the antioxidant status[25] while other studies reported an unaltered antioxidant system in SCH.[26]

The information about the status of OS in hypothyroidism is controversial, but a greater OS has been reported in patients with SCH and Clinical Hypothyroidism (CH) in comparison with euthyroid controls. We observed a significant difference in the PAB in SCH and control group in the linear multiple regression model but in CH there was no difference in the PAB in CH compared to the normal healthy group. However, these groups were older, which could affect the measurement results of OS. In some other studies, a significant increase of OS status in CH compared to SCH has been shown.[27-29] This finding could be related to different methods of oxidative antioxidative status measurement in these studies. In addition, it might be due to more advanced disease and destruction of the thyroid in CH that could reduce ROS production. On the other hand, significant differences in PAB were found between SCH and controls after the adjustment of age and TSH. This means that the variable TSH and age are not

Table 1: Demographic characteristics and biochemical parameters in patients with subclinical and clinical hypothyroidism and control groups

Variable	Control *n*=32	Subclinical Hypothyroidism *n*=42	Clinical Hypothyroidism *n*=32	*P*
Gender (M/F)	1/31	2/40	2/30	0.66
Age (year)	28.34±6.74	30.40±7.14	34.60±8.13	0.002*
BMI (kg/m^2)	26.60±6.17	25.32±3.97	28.53±5.43	0.28
FBG (mg/dL)	85.17±10.11	80.89±14.28	87.07±14.35	0.13
TG (mg/dL)	96.20±50.11	100.54±56.82	113.70±69.66	0.67
CHOL (mg/dL)	132.88±30.05	138.97±27.07	153±30.90	0.01*
HDL (mg/dL)	28.10±17.02	28.09±7.50	26.53±7.78	0.83
LDL (mg/dL)	69.28±19.39	72.29±17.05	78.20±26.59	0.21
TSH (mU/L)	2.77±1.11	6.60±1.19	21.04±17.19	<0.001*
T4 (µg/dL)	7.85±3.42	6.14±3.99	6.37±1.93	0.38
Anti-TPO (IU/mL)	17.10±20.64	141.40±251.34	276.75±214.66	<0.001*

The results are expressed as mean±standard deviation. BMI: Body mass index, Anti-TPO, anti-thyroid peroxidase, FBG: Fasting blood glucose, TG: Triglyceride, CHOL: Cholesterol, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol. *$P\leq0.05$ significant

Table 2: Prooxidant-antioxidant balance and acute phase reactant protein in the serum of patients with different levels of hypothyroidism

Variable	Euthyroid state	Subclinical Hypothyroidism	Overt Hypothyroidism	Range
hs-CRP (mg/L)	1.88±0.35	2.67±0.75	2.05±0.33	0.15-7.55
PAB (HK unit, arbitrary unit)	81.54±9.17	109.63±8.33	105.46±10.14	10.10-228.02

The results are expressed as adjusted mean±standard error after adjustment for age and TSH in multivariate analysis. PAB: Prooxidant-antioxidant balance

Table 3: Relationship between euthyroid state, subclinical, and clinical hypothyroidism with hs-CRP and PAB after adjustment for TSH and age

Variable		Regression coefficient	CI 95%	*P*
hs-CRP (mg/L)	Euthyroid state			
	Subclinical hypothyroidism	0.79	-0.85-2.44	0.34
	Clinical hypothyroidism	0.17	-2.21-2.41	0.93
PAB (HK unit, arbitrary unit)	Euthyroid state			
	Subclinical hypothyroidism	28.09	0.72-50.98	0.04*
	Clinical hypothyroidism	23.92	-45.6-25.46	0.051

PAB: Prooxidant- antioxidant balance. *$P\leq0.05$ statistical significance

responsible for the differences observed and it could have been due to various types of food such as oxidized fat and environmental pollutants. Also, some factors influence the antioxidant efficacy and levels such as dietary intake, physical activity, stress, and socioeconomic status.[30]

Both hyperthyroidism and hypothyroidism are associated with OS but the mechanisms of OS generation in these two conditions are different.[31] An increased generation of ROS and impaired antioxidant system have been shown in hyperthyroidism and hypothyroidism which indicates a strong impact of the thyroid hormones on OS and PAB.[32]

In the hyperthyroid state, ROS production increases, and the overall antioxidant capacity of the mitochondria reduces. This condition increases the susceptibility of the mitochondria to OS. Some studies demonstrated that in hypothyroidism, the antioxidants reduce and the depression of metabolism reduces oxidant production, and thus, protects the tissues against oxidant damage.[33] Despite a few studies reporting the presence of OS in hypothyroidism, its occurrence has been denied by others, therefore, OS in hypothyroidism is a relatively controversial topic.[32] This OS is the cause of some complications of hyperthyroidism and hypothyroidism in target tissues.[32] Furthermore, the thyroid hormones by themselves can act as oxidants and cause DNA damage.[10] In addition, the OS increases in the other thyroid disorders including Hashimoto's thyroiditis and autoimmune thyroiditis.[34-36]

The thyroid hormones which influence the metabolic rate also increase the synthesis and degradation of cholesterol and triglycerides.[32] In our study, the cholesterol level in the clinical hypothyroidism was significantly higher than the SCH and normal group. Dyslipidemia is common in thyroid disorders which occurs through different mechanisms and mainly affects the total and LDL cholesterol which is

consistent with our current study findings.[34-36] In addition, LDL and high-density lipoprotein (HDL) particle oxidation have been reported in this condition.[32] However, our study did not represent an increase in the OS or inflammatory status in CH. In this study, the clinical hypothyroid patients seem to have mild to moderate hypothyroidism, so they did not show other lipid profile abnormalities such as hypertriglyceridemia.

In our experience, this is the first study to evaluate OS and antioxidant defense with a single test (PAB) in a group of patients with clinical and subclinical hypothyroidism. It can be said with some certainty that PAB increases in subclinical hypothyroidism.

The main limitation of the current study was a relatively small sample size that may have caused the difference not to be significant in overt hypothyroidism, marginally.

Future studies with greater sample size and comparing the findings after the replacement therapy with levothyroxine are required to confirm our study findings.

Conclusions

This study suggests that subclinical hypothyroidism increases PAB in comparison to healthy control which could indicate OS response in patients with subclinical hypothyroidism, respectively. Further studies are required to show whether the OS is a cause or result of subclinical hypothyroidism.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/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.

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Conflicts of interest

There are no conflicts of interest.

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