International Immunopharmacology 14 (2012) 349–352

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Oxidative stress parameters in serum and low density lipoproteins of Hashimoto's thyroiditis patients with subclinical and overt hypothyroidism

Ümmühan Öztürk [a], Pervin Vural [a,*], Ayşenur Özderya [b], Berrin Karadağ [b], Semra Doğru-Abbasoğlu [a], Müjdat Uysal [a]

[a] Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Çapa, Istanbul, Turkey
[b] Department of Internal Medicine, Division of Endocrinology, Şişli Etfal Research and Training Hospital, Şişli, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 11 April 2012
Received in revised form 30 July 2012
Accepted 13 August 2012
Available online 28 August 2012

Keywords:
Hashimoto's thyroiditis
Subclinical hypothyroidism
Overt hypothyroidism
Oxidative stress
Serum
Low density lipoproteins

ABSTRACT

Background: Although prooxidant and antioxidant status were reported to be changed in clinical and experimental hypothyroidism, obtained results are conflicting. In addition, in subclinical hypothyroidism, scarced and controversial data are available about oxidative stress. Therefore, we aimed to investigate prooxidant–antioxidant status only in Hashimoto's thyroiditis (HT) patients with subclinical (sHT) and overt hypothyroidism (oHT).

Subjects and methods: Thirty sHT and 18 oHT patients and 30 healthy control subjects were included in the study. Endogenous and prooxidant 2,2′-azobis-(2-amidinopropane) hydrochloride (AAPH)-induced malondialdehyde (MDA), diene conjugate (DC), protein carbonyl (PC) and nitrotyrosine (NT) levels as well as ferric reducing antioxidant power (FRAP) were determined in serum. In addition, endogenous DC and copper-induced MDA levels were measured in low density lipoprotein (LDL) fraction.

Results: Although there were no significant difference in serum endogenous MDA and DC levels, AAPH-induced MDA levels were significantly increased in sHT patients. All these parameters increased in oHT patients. Serum PC levels were detected to be increased in both sHT and oHT patients. Serum FRAP values did not alter in sHT patients, but they lowered in oHT patients. Endogenous DC and copper-induced MDA levels in LDL fraction did not change in sHT patients. However, these parameters were detected to be increased significantly in oHT patients as compared to controls and sHT patients.

Conclusion: In conclusion, there were significant increases in oxidative stress parameters in serum and LDL-fraction in oHT patients. However, oxidative stress was detected to stimulate partly in serum, but not LDL fraction in sHT patients.

1. Introduction

Hashimoto's thyroiditis (HT) is the most common organ-specific autoimmune disorder affecting approximately 18% of population, characterized by diffuse lymphocytic infiltration of the thyroid gland, elevated levels of serum anti-thyroid antibodies, evidence of goitrous or atrophic gland, and frequent thyroid dysfunction in varying degrees [1]. There is an increased risk for atherosclerotic cardiovascular disease together with endocrinological problems in HT patients. The accelerated atherosclerosis in hypothyroid state has been ascribed to atherogenic lipid profile, presence of low-grade inflammation, and impaired endothelial function [1–6]. In addition, changes in oxidative status and antioxidant defense were also reported to be involved in experimental [7,8] and clinical hypothyroidism [9–14]. However, available results on oxidative stress in hypothyroidism are conflicting. Indices of oxidative stress were found to be increased [7,9–13], decreased [8] or unchanged [14] in hypothyroid state. Meanwhile, there is little and controversial knowledge about oxidative stress in subclinical hypothyroidism defined as the presence of high levels of thyroid stimulating hormone (TSH) and normal values of free triiodothyronine (fT_3) and free thyroxine (fT_4) [9,14–17].

Therefore, the aim of our study was to investigate the changes in lipid levels and the parameters related to lipid and protein oxidation

Abbreviations: AAPH, 2.2′-azobis-(2-amidino-propane) hydrochloride; anti-Tg, anti-thyroglobulin antibody; anti-TPO, anti-thyroid peroxidase antibody; BMI, body mass index; BP, blood pressure; DC, diene conjugate; ESR, erythrocyte sedimentation rate; FRAP, ferric reducing antioxidant power; fT_3, free triiodothyronine; fT_4, free thyroxine; hs-CRP, high sensitive C-reactive protein; HT, Hashimoto's thyroiditis; sHT, Hashimoto's thyroiditis with subclinical hypothyroidism; HDL-C, high density lipoprotein-cholesterol; HOMA, homeostasis model assessment; LDL-C, low density lipoprotein-cholesterol; LDL-DC, low density lipoprotein-diene conjugate; LDL-MDA, low density lipoprotein-malondialdehyde; MDA, malondialdehyde; NT, nitrotyrosine; oHT, Hashimoto's thyroiditis with overt hypothyroidism; PC, protein carbonyl; ROS, reactive oxygen species; TSH, thyroid-stimulating hormone.

* Corresponding author at: Istanbul Faculty of Medicine, Department of Biochemistry, Çapa 34093, Istanbul, Turkey. Tel.: +90 212 414 21 88; fax: +90 212 6215642.
E-mail address: pervinvural@yahoo.com (P. Vural).

1567-5769/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.intimp.2012.08.010

such as endogenous and prooxidant-induced malondialdehyde (MDA), diene conjugate (DC) and protein carbonyl (PC), nitrotyrosine (NT) levels, a nitrosative stress marker, and ferric reducing antioxidant power (FRAP) in serum of HT patients with subclinical (sHT) and overt hypothyroidism (oHT). In addition, endogenous DC and copper-induced MDA levels were determined in low density lipoprotein (LDL) fraction in patients with sHT and oHT.

2. Material and methods

2.1. Subjects

Thirty sHT and 18 oHT patients and 30 healthy control subjects were included in the study. The diagnosis of sHT was based on the finding of high TSH levels (>5 mIU/L) associated with normal fT_3 and fT_4 levels and high serum levels of autoantibodies (anti-thyroglobulin antibody = anti-Tg and anti-thyroid peroxidase antibody = anti-TPO), while the diagnosis of oHT was based on the findings of high serum TSH levels associated with low fT3 and fT4 levels and high serum levels of anti-TG and anti-TPO. Normal or enlarged size thyroid gland, loss of ecogenity in parenchyma, heterogeneous echotexture, fibrotic separations, pseudonodular images, micronodules with chronic thyroiditis were detected in Doppler ultrasonography in our patients. The patients having atrophic gland did not include in this study. Only the recently HT diagnosed patients who were not on any medication were included in the study. The control group consisted of 30 individuals matched for age and sex. None of the controls had personal or family history of thyroid disease and goiter on examination, they had normal thyroid functions and were negative for thyroid autoantibodies. Exclusion criteria for both groups were the existence of any comorbid cardiac, endocrine, autoimmune, infectious, musculoskeletal or malignant disease, a recent history of operation or trauma, as well as smoking, alcohol consumption and taking of any form of vitamin supplementation. The study was approved by the Institutional Review Board at Şişli Etfal Research and Training Hospital. Informed consent was obtained from each subject.

2.2. The determinations related to serum lipids and thyroid function

Blood samples were collected from patients before they were administered with thyroxine therapy. Overnight (12 h) fasting samples were taken in the morning in dry tubes. Serum triglyceride, cholesterol, HDL- and LDL-cholesterol and glucose measurements were performed on Cobas Integra 800 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum TSH, fT_3, fT_4, anti-Tg, anti-TPO and insulin were measured on Elecsys autoanalyzer (Roche Diagnostics, Mannheim, Germany). Homeostasis model assessment (HOMA) index was calculated as [fasting insulin concentration ($lU\ mL^{-1}$) × fasting glucose concentration ($mg\ dL^{-1}$)]/405. The reference ranges for thyroid status were as follows: TSH (0.35–4.94 mIU/L), fT_3 (2.6–5.7 pmol/L), fT_4 (9.0–18.0 pmol/L), anti-Tg up to 4.11 IU/ml, and anti-TPO up to 5.61 IU/ml. High sensitive C-reactive protein (hs-CRP) measurement was done by ELISA kit (Biomerica, CA, USA). Erythrocyte sedimentation rate (ESR) was measured by using Berkhun SDM-60 (Turkey).

2.3. Oxidative stress parameters in serum

The degree of endogenous lipid peroxidation in serum was assessed by two different methods: (a) MDA levels in the serum were evaluated by the spectrophotometric method based on the reaction between MDA and thiobarbituric acid [18]. The MDA concentration of samples was calculated using an extinction coefficient of $1.56\times10^5 M^{-1}\ cm^{-1}$. (b) Serum DC formation was determined spectrophotometrically at 234 nm [18]. For this reason, serum lipids were extracted with a chloroform/methanol (2:1) mixture. The extracted lipids were redissolved in cyclohexane and the approximate amounts of hydroperoxides were calculated using a molar extinction coefficient of $2.52\times10^{-4}\ M^{-1}\ cm^{-1}$.

For the evaluation of serum oxidizability, serum samples were incubated with or without 100 mM of the free radical generator 2,2′-azobis-(2-amidinopropane) hydrochloride (AAPH) for 2 h at 37 °C [19] and MDA levels were determined by using Buege–Aust procedure [18]. PC levels were measured by an enzyme immunoassay kit from Cell Biolabs (San Diego, CA, USA) according to the manufacturer's instructions. In this assay, the protein samples derivatized by making use of the reaction between 2,4-dinitrophenylhydrazine (DNPH) and PC to form a DNP hydrazone which is assayed using an anti-DNP antibody and a HRP-conjugated secondary antibody. A standard curve from the oxidized bovine serum albumin standards was run with each microplate. This kit assay is essentially a modification [20] of the method described by Buss et al. [21]. Serum NT levels, an indicator of peroxynitrite formation, were measured using an OxiSelect NT competitive ELISA kit (Cell Biolabs, San Diego, CA, USA). Antioxidant status was evaluated using a FRAP assay [22]. This assay uses antioxidants as reductants in a redox-linked colorimetric method. In this assay, at low pH, a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous form, which can be monitored by measuring the change in absorption at 593 nm.

2.4. Oxidative stress parameters in LDL fraction

Endogenous DC levels and the susceptibility of LDL to copper-induced lipid peroxidation were measured in LDL fraction. In brief, serum LDL was precipitated by using a precipitation buffer consisted of 0.064 M trisodium citrate adjusted to pH 5.05 and 50000 IU/L heparin and resuspended 0.1 M Na-phosphate-buffered, pH 7.4, containing 0.9% NaCl [23]. To determine endogenous DC levels, lipids were extracted from LDL samples by a mixture of chloroform and methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm [24]. Absorbance units were converted to molar units using the molar extinction coefficient $2.95\times10^{-4}\ M^{-1}\ cm^{-1}$.

To determine the susceptibility of LDL to copper-induced lipid peroxidation, LDL fraction (200 μg protein) was incubated with copper-sulfate (final copper concentration 50 μM) at 37 °C for 3 h. MDA produced during this period was estimated by taking the difference in levels from 0 h [25]. Values were expressed as nmol MDA/mg LDL protein.

2.5. Statistical analysis

Results were expressed as mean ± SD. Experimental groups were compared using Kruskal–Wallis variance analysis test. Where significant effects were found, post-hoc analysis using Mann–Whitney *U* test was performed, and $p<0.05$ was considered to be statistically significant. Correlation coefficients were determined by Spearman correlation test.

3. Results

Clinical characteristics and thyroid hormonal status of controls, sHT and oHT patients are shown in Table 1. Cholesterol, triglyceride, HDL-, LDL-cholesterol, glucose, insulin and hs-CRP levels and HOMA values were observed not to be changed in sHT and oHT patients. However, ESR values increased in all patients as compared to controls (Table 1).

Although there were no any significant difference in serum endogenous MDA and DC levels, AAPH-induced MDA levels were significantly increased in sHT patients. However, these parameters increased in oHT patients as compared to controls and sHT patients. Although there were no changes in serum NT levels, serum PC levels were detected to be increased in both sHT and oHT patients. Serum FRAP