Original article

Serum oxidative stress parameters of women with hypothyroidism

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Abstract. In order to study the effects of hypothyroidism on parameters of oxidative stress in woman, we determined the values of thiobarbituric acid reactive substances (TBARS), Nitric Oxide (NO) and Paraoxonase (PON-1) using phenylacetate as substrates in serum. Women in fertile age were separated into three groups: a- euthyroidism (ET) control group, b- overt hypothyroidism (OHT) and c- subclinical hypothyroidism (SHT). TBARS concentration and the PON-1 activity as aryl esterase activity did not show differences between OHT, SHT and ET woman. The concentration of NO increased in OHT compared to ET. The SHT and ET NO values were not significantly different, but the NO level was higher in the serum of OHT compared to SHT. The OHT selectively increased the NO levels but did not modify the parameters of oxidative stress in the serum of fertile-age women. (www.actabiomedica.it)

Key words: Hypothyroidism, women, oxidative stress, paraoxonase, nitric oxide

Introduction

Thyroid hormones are the most important factors involved in the regulation of the basal metabolic condition, as well as in the oxidative metabolism (1).

Thyroid hormones are associated to the oxidative and antioxidative status of the organism. Depression of metabolism due to hypothyroidism has been reported to decrease oxidant production and thus protects tissues against oxidant damage. Oxidative stress, characterized by an elevation in the steady-state concentration of reactive oxygen species (ROS), has been involved in a wide range of biological and pathological conditions (2). However, data on the oxidative status of hypothyroidism are limited and controversial (3, 4).

Hypothyroidism is a clinical entity resulting from the deficiency of thyroid hormones or, more rarely, from their impaired activity at the tissue level. The term subclinical thyroid disorders is applied to patients who show an abnormal serum thyroid-stimulating hormone (TSH) concentration but thyroxine and triiodothyronine levels within their reference ranges. Subclinical hypothyroidism occurs in 4% to 10% of the general population, and is especially prevalent in elderly women. Depending on the study, subclinical hypothyroidism has been found in 0.6-16% of the population (5).

Nitric oxide (NO), hydroxyl radical (OH*), superoxide anion (O_2^-) and hydrogen peroxide (H2O2) are free-radicals released in oxidative stress. Excess NO, formation of peroxinitrites, and/or defective antioxidants causes lipid peroxidation, and cellular dysfunction. Thiobarbituric acid-reactive substances (TBARS) are an index of lipid peroxidation and these, as well-known indexes of oxigen reactives species (ROS) activity, are associated to membrane lipid destruction. ROS production is inevitable in all aerobic organisms, including humans, who necessarily posses a complex system of antioxidant defenses (6). If the homeostasis is interrupted due to ROS, an oxidative stress situation is

created. An example of the damage hydrogen peroxide can cause is that it can stimulate the phosphorylation of the NF-*K*B-I*K*B complex, activating the NK-*K*B and facilitating nuclear translocation and upstream of the nitric oxide syntethase. The hydroxyl radical is able to trigger a classical chain reaction, known as lipid peroxidation, leading to the vasodilatation associated to nitric oxide production (7, 8).

Paraoxonase-1 (PON-1) is a high-density lipoprotein (HDL) associated - enzyme capable of hydrolyzing lipid peroxides with three activities, namely: paraoxonase, arylesterase and dyazoxonase, which have lipophilic antioxidant characteristics, and their activities are inversely related to cardiovascular diseases (9). Thus, PON-1 plays a preventing role in atherosclerosis by protecting against lipid peroxidation, modulates the susceptibility of LDL to atherogenic modifications, such as glycation and homocysteinylation, and even plays an antiinflammatory role. However, the physiological substrate of PON-1 still remains to be discovered. Neither PON's arylesterase/paraoxonase activities nor the protection against LDL oxidation involve the active site on the enzyme in exactly the same way, and PON's ability to protect LDL from oxidation requires the cysteine residue at position 283. PON-1 displays a high variability in human populations. The paraoxonase family consists of three members (PON-1, PON-2 and PON-3) that share structural properties and enzymatic activities, among which is the ability to hydrolyze oxidized lipids in LDL.

Considering that there are different results regarding the values of serum oxidative stress marker in women with hypothyroidism, the aim of this paper was to determine some of oxidative stress marker in a female population in order to analized if the effects of thyroid hormone on the oxidant-antioxidant system are specifics for this hormone, or if they are conditioned by other factors, such as environmental ones.

Materials and Methods

Subjects

A total of 81women between 20 and 45 years old were studied. The participants in the study were recruited from healthy volunteers and patients attending the San Luis Hospital for clinical controls. None of the women was included when presenting history or underlying symptoms of diabetes, cardiovascular disease, as well as if receiving hormonal treatment. In all cases the patients and controls were non smokers. The study protocol was approved by the Research Ethic Committee of San Luis Hospital. The women were separated into two groups. The ET group (28 women) presented normal serum-free T4 (FT4) and TSH values. The experimental group was separated into two subgroups: the first one, presenting FT4 and TSH values indicative for OHT (20 women), and the second one presenting values for SHT (33 women). The OHT and SHT patients presented the classic symptoms, which are often nonspecific and subtle: lethargy, mild weight gain, edema, cold intolerance, constipation, mental impairment, dry skin, depression, irregular menses, hoarseness, myalgias, hyperlipidemia.

Samples

After overnight fasting, blood samples were collected from a vein in the antecubital fosse without venous occlusion. All collections were made between 8:00 and 9:00 am. The samples were immediately centrifugated for serum separation and stored at -20 degrees centigrade: one aliquot was used for FT4 and TSH determination at the Clinical Analysis Laboratory of the San Luis Hospital and the other one was stored at -70°C and transported on dry ice to the National University of San Luis, where the assays were performed.

Thyroid profile

Thyroid profile was assessed by chemiluminescence assay. FT4 and TSH were determined using the commercial kit for the autoanalyzer ACS: 180 PLUS, Cina Corning, Bayer Health Care. For FT4 the intra assay coefficients of variation (CV) were 1.54% for estimation of T4 at 1.42 ng/dL. For TSH the intrassay CV were 3.01% at 18.53 μIU/mL

TBARS determination

The levels of malondialdehyde and malondialdehyde-like substances (TBARS) were spectrophotometrically determined to 535 nm with the thiobarbituric acid assay, with the addition of 0.02% (w/v) butylated hydroxytoluene;1,1,3,3 tetrametoxypropano (TMP) with HCL 0.01N was used as standard value. The TBARS level was expressed in μM/ml serum (10).

Assay of Paraoxonase-1 as serum arylesterase activity

Arylesterase activity (ARE) was spectrophotometrically measured using phenylacetate as substrate. The phenol formed after the addition of a 40-fold diluted serum sample was spectrophotometrically measured at 217 nm, following an established procedure. Blanks were included to correct the spontaneous hydrolysis of phenylacetate. The activity of ARE was expressed in U/ml serum. One unit was defined as the enzyme quantity that disintegrates 1 nmol phenylacetate per minute (11)

Nitric Oxide assay

Nitric Oxide levels were determined with a colorimetric method based on the Griess reaction (12). Sodium nitrite was used as a standard, and the results were expressed as μmoles/ml.

Reagents

The reagents used for the tests were provided by Sigma Chemical Co, except thiobarbituric acid (TBA), butylated hydroxytoluene (BHT) and 1,1,3,3 tetrametoxypropano(TMP) that were provided by Fluka, Switzerland

Statistical analysis

All data were evaluated with the Kolmogrov-Smirnov test for normality and were expressed as the mean ± standard deviation. Statistical analysis was performed using an ANOVA test and the Turkey´s multiple comparison test.

Results

The results obtained are shown in Table 1.

The FT4 and TSH values correspond to the ET, SHT and OHT: FT4: 1.11±0.04, 1.10±0.04 and 0.59±0.04 ng/dl and TSH: 2.29±0.17, 11. 46±1.9 and 65.96±17.88 μUI/ml respectively.

Table 1. Parametrs of oxidative stress in serum of women with different levels of hypohyroidism

	Euthyroidism	Subclinics Hypothyroidism	Overt hypothyroidism
TSH (μUI/ml)	2.29 ± 0.17[a] (28)	11. 46 ± 1.9[b] (33)	65.96 ± 17.88[c] (20)
FT4 (ng/dl)	1.11 ± 0.04[a] (28)	1.10 ± 0.04[a] (33)	0.59 ± 0.04[b] (20)
TBARS (μM/ml)	2.24±0.32 (28)	1.58±0.27 (33)	1.94±0.31 (20)
NO (μM/ml)	19.91±1.69[a] (28)	28.38±2.48[a] (33)	37.11±4.6[b] (20)
PON-1 (arylesterase) (U/ml)	537.12±130.57 (23)	506.05±126.46 (27)	513.24±95.53 (16)

The results are expressed as m ± SD. The numbers in brackets represent the number of women in each case. The numbers that have different letters as super index are significantly different P< 0.01

FT4: serum-free T4, TBARS: malondialdehyde and malondialdehyde-like substances, NO: Nitric Oxide, PON-1: Paraoxonase-1 measured as serum aryl esterase activity

We observed that the TBARS concentration were 1.94±0.31, 1.58±0.27, 2.24±0.32 μM/ml respectively. There were not modified by thyroid hormone deficiency in OHT and SHT compared to ET women. The concentration of NO, 37.11±4.6, 19.91±1.69, 28.38±2.48 μM/ml, respectively increased in OHT compared to ET and SHT. The PON-1 activity measured as ARE activity 537.12±130.57, 506.05±126.46, 513.24±95.53 U/ml respectively, did not show any differences between ET, SHT and OHT. We did not observe any correlation between all the parameters measured.

Discussion

No agreements regarding the effect of thyroid hormone on the marker levels of serum oxidative stress are observed. Thyroid hormones are the most important factors involved in the regulation of the basal metabolic condition, as well as in the oxidative metabolism (13). Thyroid dysfunctions bring about pathological changes in different organs of the body. The findings obtained from in vivo and in vitro studies show that thyroid hormones have a strong impact on oxidative stress. Data on the oxidative status of hypothyroidism are limited and controversial. Our results showed that the TBARS concentration, an index of lipid peroxidation, is not modified in OHT and SHT compared to ET. Our results are in agreement with some Authors, who observed that there is not significant alteration of plasma TBARS in SHT compared to ET (14). However, other Authors observed that TBARS increased in OHT compared to ET (15, 16). We found that the NO concentration significantly increased in OHT compared to the ET, according to some Authors (16, 17). However, other laboratory findings reveal that the NO levels in serum of OHT were lower than in the ET (18). On the other hand, we observed that the value of NO in SHT tends to increase, though this is not significant compared to ET. We did not observe changes in the activity of PON-1 between OH, SHT and ET, but another report showed that PON-1 activity decreased in patients with hypo and hyperthyroidism (19). The evaluation of the real effects of thyroid hormone on parameters of oxidative stress appears very difficult considering that thyroid hormone levels are influenced by diet, environmental factors, and reaction capacity to daily stress situations, in which cases, the parameters of oxidative stress are also modified (20-22). Moreover, the selection of the patients is very important, because some secondary disease, concomitant with the hypothyroid function can alter the results. For this reason, we believe that the effect of thyroid hormone on oxidative stress parameters in women is not clear and depends on different variables.

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