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The Effects of Hyperthyroidism on Lipid Peroxidation, Erythrocyte Glutathione and Glutathione Peroxidase

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Previous studies have suggested that hyperthyroidism has some effects on lipid peroxidation and antioxidant systems. This study was designed to determine if lipid peroxid ation, glutathione, and glutathione peroxidase can be modified by hyper-thyroidi sm. 23 subjects with hyperthyroidism (18 females / 5 males), and 19 euthyroid subjects (11 females / 8 males) participated in this study. Plasma and erythrocytes malondialdehyde, erythrocytes glutathione and glutathione peroxidase were measured . An increase in lipid peroxidation products was observed in the hyperthyroid patients ($p< 0.001$). This was accompanied by a decrease in glutathione and glutathione peroxidase in the same subjects ($p< 0.001$). The results suggested that hyper-thyroidism has some effects on lipid peroxidation and free radical scavengers .

Key words: Hyperthyroidism, hypothyroidism, lipid peroxidation, glutathione, glutathione peroxidase

Introduction

Oxygen free radicals have important effects on the pathogenesis of tissue damage of several pathologic conditions (1). Many biochemical compounds, namely nucleic acid, amino acid, protein, lipid, lipoprotein, carbohydrate, and macromolecules of collagen tissue, can be damaged irreversibly or reversibly by free radicals. Free radicals accumulate in tissues due to intracellular and extracellular processes. There are different antioxidant systems against free radicals. The glutathione (GSH) and glutathione peroxidase (GSH – PX) system, one of them, removes free radicals from the environment (2). Clinical and experimental studies showed an elevated free radical level in hyperthyroidism. The aim of this study is the investigation of the relatior between lipid peroxidation (Malondialdehyde, MDA) GSH and GSH - PX in hyperthyroidism.

Subjects and Methods

23 hyperthyroid patients (18 females / 5 males, mean age 37.00 ± 14.50 years, BMI 23.10 ± 3.36 kg/m^2), and 19 euthyroid subjects (11 females / 8 males, 34.55 ± 9.43 years, BMI, 24.12 ± 5.25 kg/m^2) participated in this study. None of them smoked cigarettes or were taking medications. T_3, T_4, TSH, plasma MDA, erythrocyte MDA, erythrocyte GSH and GSH - PX levels were measured for each of these groups. A blood sample was collected from each subject while fasting, and serum was frozen at -20°C until analysis for T_3, T_4, and TSH. LKT31, DPC, USA, 1997 was used for T_3 and its normal range is 1.25 -2.74 nmol/L; LKT41, DPC, USA, 1997 was used for T_4 and its normal range is 57.9 -160.8 nmol/L, LKTR1; DPC, USA, 1995 was used for TSH and its normal range is 0.4 - 4 μIU/ml. To measure GSH and GSH - PX levels, blood samples

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(10 mL) were obtained in heparinized tubes, and centrifuged at 2000 rpm for 10 minutes.

Plasma was separated and the buffer coat was discarded. Erythrocytes were washed with a cold sterile 9 g/L sodium chloride three times after 1/10 dilutions.

Lipid peroxidation was assayed by measurements of malondialdehyde generation. One volume of plasma or washed erythrocyte was mixed thoroughly with two volumes of stock solution of 15% trichloroacetic acid (w/v), 0.375% thiobarbituric acid (w/v), and 0.25 mol/L hydrochloric acid (w/v). The combination of the sample and the stock solution was heated for 30 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 3200 rpm at 15 min. The absorbance of the clear supernatant is determined at 535 nm and MDA concentrations were calculated using 1.50x 10^5 M^{-1} cm^{-1} as plasma coefficient (3).

Erythrocyte GSH levels were determined according to the method of Beutler et al (4) using metaphosphoric acid for protein precipitation and 5-5' dithio.2 nitrobenzoic acid for color development at 412 nm.

GSH - PX activity was determined by the method of Paglia and Valentine (5). Enzyme activity was determined from the oxidation of NADPH in the presence of H_2O_2 as substrate and monitored spectrophotometrically at 340 nm. One unit enzyme activity was defined as 1 μM NADPH oxidized per minute. Activity was expressed as U/gHb. Results were evaluated by independent T test and expressed as mean ± standard deviation.

Results

Plasma MDA, erythrocyte MDA, erythrocyte GSH, and GSH-PX levels in subjects with hyperthyroidism, and controls are shown in Table 1.

When the results of the two groups were compared, plasma and erythrocytes MDA levels were significantly higher in the patient group than in the control group ($p< 0.001$). Erythrocyte GSH and GSH-PX levels were also significantly lower in the patient group than the control group ($p<0.001$). T_3, T_4 and TSH levels were different in these groups.

Table 1. The results of hyperthyroid, and euthyroid subjects.

Parameters	Hyperthyroidism	Control Group	p
Age (year)	37.0 ± 14.5	34.5 ± 9.4	> 0.05
BMI (kg/m^2)	23.1 ± 3.3	24.1 ± 5.2	> 0.05
T_3 (nmol/L)	5.5 ± 2.8	2.0 ± 0.5	< 0.001
T_4 (nmol/L)	197.4 ± 77.0	104.7 ± 18.7	< 0.001
TSH (μIU/ml)	0.02 ± 0.0	1.0 ± 0.6	< 0.001
Cholesterol (nmol/L)	4.8 ± 0.5	4.9 ± 0.7	> 0.05
Triglyceride (nmol/L)	2.2 ± 0.5	2.3 ± 0.7	> 0.05
Hb %	12.3 ± 0.5	12.8 ± 0.4	> 0.05
Plasma MDA (nmol/ml)	4.0 ± 2.0	2.3 ± 1.1	< 0.001
Erythrocyte MDA (nmol/gHb)	235. ± 53.1	129.6 ± 54.7	< 0.001
Erythrocyte GSH (nmol/gHb)	6.6 ± 1.4	10.3 ± 1.2	< 0.001
GSH-PX (U/gHb)	8.9 ± 1.8	20.0 ± 1.0	< 0.001

Discussion

It was reported that there is a relation between hyperthyroidism and deteriorations of free radicals and antioxidant systems that increase lipid peroxidation (6). In aerobic cells, active oxygen species are generated as by products of oxidative metabolism in mitochondria. Hyperthyroidism leads to an enhancement of the metabolic rate and, more specifically, of the oxidative metabolism (7). In previous studies, different interpretations were given. It was demonstrated that thyroxin decreased the concentration of the products of lipid peroxidation in animal experiments (8, 9). However Fernandez et al. (10) showed that the products of lipid peroxidation were increased in rats that were given triiodothyronine. Dumitriu et al. (11) found high plasma MDA levels in hyperthyroidic patients as opposed to the control group. Costantini et al. (12) demonstrated that hyperthyroidism stimulated lipid peroxidation. Venditti et al. (13) investigated the effects of hyperthyroidism on lipid peroxidation in rats. They reported that hyperthyroidism increased the products of lipid peroxidation in several tissues. Iangalenko et al. (14) found that lipid peroxidation was increased in hyperthyroid patients. There was a correlation between lipid peroxidation and thyroid iodine uptake. Mano et al. (15) suggested that lipid peroxidation and GSH-PX were increased in the thyroid tissue in hyperthyroidism. Asayama et al. (7) observed that lipid peroxide was increased in the heart and

soleus muscles and GSH-PX was decreased in all tissues of hyperthyroid rats, and in contrast, no adverse reaction mediated by active oxygen species was found in hypothyroid rat tissues. Venditti et al. (16) showed that lipid peroxide was increased, but the antioxidant system was not affected in hyperthyroid rats. Asayama and Kato (17) showed that the damaging effect of lipid peroxidation was increased diminishing antioxidant enzymes in experimental hyperthyroidism.

We determined that the level of lipid peroxidation products was higher in the hyperthyroidism group than control levels, however GSH and GSH-PX were significantly lower than control levels. So, this study confirms some previous studies.

In conclusion, there is an important relation between hyperthyroidism and free radicals / antioxidant systems.

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