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Der Pharmacia Lettre, 2016, 8 (6):113-118  
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## Antioxidant defense system as a protector against oxidative stress induced by thyroid dysfunction

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### ABSTRACT

Thyroid is one of the principal glands in the body's endocrine system. It produces T3 [triiodothyronine] and T4 [thyroxine] hormones responsible for cellular differentiation, growth, and metabolism. In hyperthyroidism and hypothyroidism, disturbance of oxidant/antioxidant balance leads to reactive oxygen species (ROS) generation. This study is to assess the effect of thyroid dysfunction on the antioxidant roles of vitamins (A, E) and Paraoxonase-1. The study involved 55 individuals who were classified into three groups; 15 as control group, 20 as hyperthyroidism group and 20 as hypothyroidism group referred to Internal Medicine Department, 6 October University, Cairo, Egypt. Serum levels of vitamins (A, E) and Paraoxonase-1 as antioxidants, Malondialdehyde as an index of lipid peroxidation state, T3, T4 and Thyroid stimulating hormone (TSH) as to maintain the appropriate level of thyroid hormones in the body's cells were measured. The values of serum vitamins A, E and Paraoxonase-1 were significantly decreased in all patients with thyroid dysfunction as compared to the control group. The mean value of malondialdehyde level was significantly increased as compared to the control group. Correlation between Malondialdehyde levels with vitamin A, vitamin E and PON-1 showed a significant negative correlation in hyperthyroidism patients. It can be concluded that, the increased of the reactive oxygen species (ROS) in patients with hyperthyroidism and hypothyroidism has been accompanied with impairment of the antioxidant system.

**Keywords:** Thyroid gland dysfunction, antioxidant vitamins, paraoxonase-1, lipid peroxidation, oxidative stress.

### INTRODUCTION

Thyroid is one of the principal glands in the body's endocrine system. It produces T3 [triiodothyronine] and T4 [thyroxine] hormones responsible for cellular differentiation and growth. Thyrotropes in the anterior pituitary produce Thyrotropin (Thyroid stimulating hormone [TSH]) to maintain the appropriate level of thyroid hormones [T3 and T4]. Thyroid hormones (THs) act on gene transcription, synthesis and degradation of proteins, regulate the basal metabolic rate and mitochondrial oxidative metabolism, and induce changes in the antioxidant protective system [1,2].

In hyperthyroidism and hypothyroidism, disturbance of oxidant/antioxidant balance leads to the production of reactive oxygen species (ROS). These are highly reactive molecules that normally present in all cells and tissues at relatively low levels, but when present in excess, they cause, cellular damage, severe metabolic dysfunctions, and damage to biological macromolecules such as proteins, lipids and DNA [3].

Oxidative stress has been associated with a number of human diseases, such as cancer, coronary heart disease, arthritis, diabetes, cataract and degenerative disease, so The maintenance of adequate antioxidant levels is essential to prevent or even control a great number of disease conditions [4]. Malondialdehyde (MDA) is a natural product of

peroxidation of unsaturated fatty acids used as a marker for lipid peroxidation to maintain the state of oxidative stress. Vitamin A is an antioxidant, that is vital for vision, the immune system, sperm production, and maintaining tissues including skin, respiratory tract, gastrointestinal tract, genital and urinary systems. The interaction between vitamin A metabolism and the thyroid hormones has long been established [5]. Vitamin A is mobilized from liver stores and transported in plasma in the form of the lipid alcohol retinol, bound to a specific transport protein, retinol-binding protein (RBP). RBP interacts strongly with plasma prealbumin, and normally circulates in plasma as a 1:1 molar RBP-prealbumin complex. [6]. Vitamin E is involved in immune function, gene expression regulation and metabolic processes [7].

(PON-1) is an antioxidant enzyme present in the structure of high-density lipoprotein (HDL). It has a protective effect by preventing the LDL oxidation [8] and thereby decreasing the incidence of atherosclerosis [9]. PON-1 activity has been suggested to be inversely related to oxidative stress [8]. There are increasing data supporting the theory that thyroid disease is associated with increased cardiovascular risk [10].

The aim of this study was to investigate the role of oxidative stress and antioxidant status markers in both patients with hyperthyroidism and hypothyroidism by measuring the level of vitamins (A,E) and paraoxonase -1 and finding the correlation between them and ROS represent in measuring lipid peroxidation, given by MDA in the sera of the corresponding patients.

## MATERIALS AND METHODS

### Subjects

This study was applied on 55 individuals with mean age (45 $\pm$ 10 years) who were divided into three groups, 20 patients with hyperthyroidism, 20 patients with hypothyroidism and 15 healthy volunteers with normal TSH serving as control group. These patients were selected from Internal Medicine Department, 6 October University, Cairo, Egypt.

Subjects who were smokers, menopausal or had systemic diseases such as hypertension, diabetes or inflammatory diseases, those with abnormal liver or renal function and those taking medications including vitamin supplementation or with evidence of malabsorption were excluded from the study. All laboratory measurements were performed at the research laboratory of the national research center.

### Study design

Informed consent was obtained from patients and control prior to the study. After obtaining a complete history and physical examination, blood samples were collected for measurement of thyroid function (TSH, F T3 and F T4), Vitamins A, E and PON-1 activity were measured. MDA also was measured. All blood samples were collected in the morning after an overnight fasting, left to clot, and then centrifuged at 3000 rpm for 15 min to separate the serum. The serum was stored at -20°C until analysis. The study was approved by the ethics committee of the National Research Centre and all subjects gave their informed consent prior to entering this study.

### Methods:

#### Determination of FT3, FT4, and TSH :

Free form of T3 and T4 were preferred than total form as they are not affected by the level of their bounding proteins.

Serum FT3, FT4, and TSH concentrations were measured by luminescence - based immunoassay using Immulite 2000 (Diagnostic Products Corp., Los Angeles, California, USA) [11].

#### Analysis of vitamins A and E by high performance liquid chromatography(HPLC):

Sample extraction: one hundred  $\mu$ l of serum was mixed with ethanol. The micronutrients were extracted from the aqueous phase in hexane and dried under vacuum. The extract was re-dissolved in ethanol and acetonitrile and filtered to remove any insoluble materials [12].

#### HPLC condition for vitamin A

Twenty  $\mu$ l of the filtrate were injected onto a C18 reversed phase column (25cm $\times$ 10.00 mm, 5  $\mu$ m particle size) and isocratically eluted with a mobile phase consisting of ethanol/acetonitrile 50:50 with 0.1% triethylamine, and was delivered at a flow rate of 1 ml/min. UV detection was performed at 325 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas vs the corresponding concentrations. The concentrations in samples were obtained from the curve.

**HPLC condition for vitamin E**

Twenty  $\mu$ l of the filtrate were injected onto a C18 reversed phase column (15cm $\times$ 10.00 mm, 5  $\mu$ m particle size) and the thermostat was adjusted to 30°C with a mobile phase consisting of 100% methanol delivered at a flow rate of 1 ml/min. Fluorescence detector was used and performed at 295 and 330 (excitation and emission). Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas *vs* the corresponding concentrations. The concentrations in samples were obtained from the curve.

**Determination of paraoxonase activity**

Arylesterase activity of paraoxonase (PON1) was measured spectrophotometrically in supernatants using phenyl acetate as a substrate [13]. In this assay, arylesterase/paraoxonase catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol was measured by monitoring the increase in absorbance at 270 nm at 25°C. The working reagent consisted of 20 mM Tris-HCl buffer, pH 8.0, containing 1 mM calcium chloride and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer were added and the change in absorbance was recorded following a 20 sec lag time. Absorbance at 270 nm was taken every 15 sec for 120 sec using a UV-VI8 Recording Spectrophotometer (Shimadzu, Kyoto, Japan). One unit of arylesterase activity is equal to 1  $\mu$ M of phenol formed per minute. The activity was expressed in kU/L, based on the extinction coefficient of phenol of 1,310 M/cm at 270 nm, pH 8.0, and 25°C. Blank samples containing water were used to correct for the spontaneous hydrolysis of phenylacetate.

**Determination of lipid peroxidation**

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA). Malondialdehyde forms a 1:2 adduct with thiobarbituric acid measured by spectrophotometry. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea *et al.* [14], in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

**Statistical analysis**

All analysis was done using the statistical package for the social science (SPSS) software version 9 on a personal computer. All numeric variables were expressed as a mean  $\pm$  standard deviation (SD). The independent-sample T test was used to compare means. Pearson's correlation coefficient was obtained and a 'p' value <0.05 was considered as statistically significant. Step wise multiple regressions were applied to illustrate relationship between several independent or predictor variables and a dependent or criterion variable. The Beta (standardized regression coefficients) value is a measure of how strongly each predictor variable influences the criterion variable. P value <0.05 was considered as an entrance criterion, while p >0.05 was considered as removal criterion.

**RESULTS**

FreeT3 and FreeT4 were significantly higher in hyperthyroid patient group compared to the control group (table 1). While the hypothyroid patient group showed significant decrease of FT3 and FT4 compared to the control group (p <0.001 for each). On the other hand there was highly significant increase in TSH value of hypothyroidism patient, and a significant decrease in hyperthyroidism patients when compared to the control group (table 1).

Vitamin A shows highly significant decrease in both hyper- and hypothyroidism compared with the control group (table 2). A similar trend of significance was found in the serum level of vitamin E in diseased groups compared to control group. There was a highly significant increase of serum MDA level in both hyper- and hypothyroidism groups as compared to the control group. PON-1 activity was significantly lowered in both diseased groups compared to the control (table 2). Table 3 showed the results of correlation between oxidative stress index represented by MDA and levels of antioxidant vitamins A and E in addition to PON-1 in thyroid dysfunction. There was a significant negative correlation between MDA and vit. A, E and PON-1 in hyperthyroidism, also there was a positive correlation between MDA and FT3, FT4, while there was a negative correlation between MDA and TSH. In hypothyroidism there was a non significant negative correlation between MDA and vit. A, E, PON, FT3, FT4, and a non significant positive correlation with TSH.

Table 1: serum levels of FT3, FT4 and TSH in patients with hyperthyroidism, hypothyroidism and controls

Group description	Control	Hyperthyroidism	p	Hypothyroidism	p
N.	15	20	-	20	-
FT3 (pg/ml)	2.209 (±0.359)	6.995 (±1.352)	<0.001	1.267 (±0.208)	<0.005
FT4 (ng/ml)	1.223 (±0.202)	3.911 (±0.593)	<0.001	0.494 (±0.091)	<0.001
TSH (μIU/ml)	1.647 (±0.347)	0.126 (±0.059)	<0.001	8.55 (±0.545)	<0.001

values are expressed as Mean ± SD  
The main difference is significant at the <0.05 level

Table 2: serum levels of antioxidant vitamins (A &amp; E), PON-1 and MDA in different cases of thyroid dysfunction and controls

Group type (No. of studies)	Component	Mean	SD	P
Hyper(N=20)	Vitamin A (mg/ml)	12.614	2.835	<0.001
Hypo(N=20)		16.547	3.383	<0.005
Control(N=15)		19.219	3.0	
Hyper(N=20)	Vitamin E (mg/ml)	14.780	3.874	<0.001
Hypo(N=20)		26.546	5.15	<0.001
Control(N=15)		47.978	7.716	
Hyper(N=20)	PON-1 (U/ml)	132.46	5.793	<0.001
Hypo(N=20)		140.274	5.221	<0.001
Control(N=15)		161.8	6.538	
Hyper(N=20)	MDA (μmol/L)	13.84	0.592	<0.001
Hypo(N=20)		12.748	1.094	<0.001
Control(N=15)		9.788	0.574	

values are expressed as Mean ± SD  
The main difference is significant at the <0.05 level.

Table 3: Correlation coefficients between MDA level and serum levels of chemical components in patients of thyroid dysfunction

Component vs. MDA	Hyperthyroidism		Hypothyroidism	
	r	p	r	p
FT3	0.450	0.044*	-0.300	0.082
FT4	0.530	0.018*	-0.365	0.062
TSH	-0.436	0.055	0.396	0.071
Vit. A	-0.447	0.048*	-0.352	0.089
Vit. E	-0.663	0.001**	-0.262	0.078
PON-1	-0.523	0.020*	-0.371	0.074

P < 0.05 is significant.

## DISCUSSION

The present study expose the relationship between the thyroid dysfunction and oxidative stress. Oxidative stress results from an imbalance between formation and neutralization of reactive oxygen species (ROS). The antioxidants are one of the mechanisms that counteract this attack. [15, 16]. Our present study was conducted on hyperthyroidism and hypothyroidism as proved by their laboratory findings (table 1). These results were in agreement with [17, 18]. A low TSH in the presence of elevated thyroid hormones is logical because secretion of TSH from the anterior pituitary is regulated by negative feedback mechanism [19].

Results in table 2 showed a highly significant decrease ( $p < 0.001$ ) in Vitamins A, E and PON-1 concentrations, while the MDA increased significantly in both hyperthyroidism and hypothyroidism patients when compared with the control group. These results were in agreement with [18]. Vitamin A is a potent antioxidant, so it decreases with excess production of free radicals [20]. It decreases tissue responsiveness to thyroid hormones, as evidenced by down regulation of Na-K-ATPase activity in the liver along with the decrease in size of the thyroid gland. Also, it has been shown that vitamin A enhances the conversion of T4 to the active hormone T3 [6]. Our results were in agreement with [21]. Vitamin E was reported to be an important factor in breaking up free radicals and increasing the capability of the immune system [7]. It can be concluded from the lower vitamin E level in thyroid dysfunction (Table 2), is due to its use in preventing free radical damage [6]. Additional studies have explain that sufficient vitamin E levels may reduce the effect of active oxygen radicals which inhibit the activity of an enzyme responsible for the conversion of T4 to the active hormone T3 [17]. Zamora et al [22] stated that, vitamin E as an antioxidant might have an indirectly caused the destruction of  $H_2O_2$ , the required oxidizing agent for iodide oxidation, thus leading to a decrease in thyroid hormone biosynthesis. Our findings are consistent with the researches that

highlighted the importance of the effects of vitamin E in oxidative stress and as component of the antioxidant defense system [23].

Paraoxonase-1 (PON-1) is an HDL-associated enzyme capable of hydrolyzing paraoxon and preventing lipid oxidation *in vitro* [24, 25]. In hyperthyroidism, oxidative metabolism increases in mitochondria and also (LDL-C) oxidation is more pronounced [26, 27], leading to increased production of free radicals that would reduced PON-1 activity [28] (table 2).

In our study, the lipid peroxidation marker (MDA) was elevated in both hyper- and hypothyroidism (table 2). Dumitriu et al. [29] found high plasma MDA levels in hyperthyroidic patients as opposed to the control group. Costantini et al. [26] demonstrated that hyperthyroidism stimulated lipid peroxidation. Our study showed that there was increased oxidative stress in both hypothyroid and hyperthyroid patients. In hyperthyroidism increased concentrations of thyroid hormones T4 and T3 result in increased basal metabolic rate, increased oxygen consumption and in the production of large quantities of reactive oxygen species which enhance oxidative stress [30]. In hypothyroid the increase in the lipid peroxide is due to high concentration of TSH in plasma. When plasma TSH concentration is high the production of H<sub>2</sub>O<sub>2</sub> is increased. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is an important factor for thyroid hormone synthesis. It acts as an acceptor of electrons that are generated during oxidative reactions of hormone synthesis. The production of H<sub>2</sub>O<sub>2</sub> in thyroid gland is by NADPH oxidase system in the apical membrane of the thyroid cell. H<sub>2</sub>O<sub>2</sub> levels enhance free radicals which is present in hypothyroidism. Increased amount of free radicals and increased levels of antioxidants, excessive vascularization and H<sub>2</sub>O<sub>2</sub> and TSH levels contribute to developing thyroid diseases which lead to oxidative stress [30]. Increase in free radical increases the oxidation of LDL which causes injury to endothelial layer leading to atherosclerosis [31].

There was a positive significant correlation of MDA levels with FT3 and FT4 in hyperthyroidism patients, while there was a negative non-significant correlation in case of TSH (table 3). In hypothyroidism patients, there was a negative non-significant correlation between FT3 and MDA, a positive non-significant correlation between FT4 and MDA and non-significant change was observed between TSH and MDA in hypothyroidism patients (table 3).

A significant inverse correlation between MDA and Vitamin A was apparent in table 3 in diseased groups, suggesting that the decrease in Vitamin A level as an antioxidant is due to the excessive production of free radicals [32]. In the present study, a highly significant negative correlation between MDA and Vitamin E levels was observed in hyperthyroidism and hypothyroidism patients. Vitamin E converts the peroxy radical to the much less reactive hydroperoxides, thus inhibiting the propagative step in lipid peroxidation [33].

In hypothyroidism, a decrease in free radical production is expected because of the metabolic suppression brought about by the reduction in thyroid hormones levels [34]. On the other hand, hyperthyroidism is characterized by an increasing cellular metabolic rate, and thus an increased amount of free radicals [35], and an increase in peroxides levels [36].

## CONCLUSION

It can be concluded that the increased of the reactive oxygen species accompanied with impairment of the antioxidant system occurs in patients with hyperthyroidism and hypothyroidism. These results indicate that thyroid hormones have a strong impact on oxidative stress and the antioxidant system.

## Acknowledgement

The authors wish to appreciate the department of medical biochemistry National Research Centre for making use of their facilities to carry out this work.

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