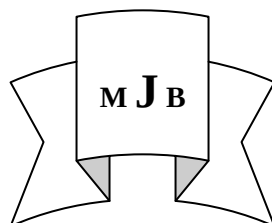


The Correlation between Oxidative Stress and Thyroid Hormones in Serum and Tissue Homogenized of Hypothyroidism Patients

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Abstract

Background: Hypothyroidism is a condition resulting from insufficient production or diminished action of either T3 and/or T4 thyroid hormones.

Objective: The present study is to look for a correlation between oxidative stress and thyroid function in hypothyroidism patients.

Patients and Methods: 30 patients with hypothyroidism patients and 30 healthy individuals as control subjects were included in the study. We measured their serum tri-iodothyronine, thyroxine and thyroid-stimulating hormone (T3, T4, TSH). Estimation of Malondialdehyde (MDA) and Glutathione (GSH) in serum and in homogenized thyroid tissue were carried out by standard methods.

Results: TSH was elevated in both serum and in homogenized tissue of patients compared to controls, while T4 and T3 were significantly lower in the patients than in controls. Malondialdehyde (MDA) as Lipid Peroxidation were significantly higher in both serum and in homogenized tissue of patients than in the controls, while (GSH) were significantly lower in both serum and in homogenized tissue of patients than in the control subjects.

Conclusions: According to the present study we could conclude that hypothyroidism cause tissues undergo several biochemical changes that predispose them to oxidative damage. Therefore we suggest that hypothyroidism patients may benefit from supplements of antioxidants.

Keywords: Glutathione; Oxidative stress, thyroid gland, Hypothyroidism.

العلاقة بين فرط الاكسدة وهرمونات الغدة الدرقية عند المصابين بقصور الغدة الدرقية

الخلاصة

المقدمة: قصور افراز الغدة الدرقية ينتج عن قلة افراز هرمون الثيروكسين وهرمون ثلاثي الايرونين او كلاهما ..

الهدف من الدراسة الحالية هو القاء الضوء على العلاقة ما بين فرط الاكسدة و قلة افراز هرمون الثيروكسين وهرمون ثلاثي الايرونين او كلاهما في المرضى المصابين بقصور الغدة الدرقية.

المرضى وطرائق العمل: اجريت الدراسة على ٣٠ من المرضى المصابين بقصور الغدة الدرقية و ٣٠ من الاشخاص الاصحاء كمجموعة سيطرة في مستشفى الحلة التعليمي، تم قياس هرمون الثيروكسين وهرمون ثلاثي الايرونين والهرمون المحفز للغدة الدرقية في مصل الدم. كما تم قياس الملانوداهايد والكلوتانيون في مصل الدم والانسجة.

النتائج: من خلال النتائج لوحظ ارتفاع معنوي في الهرمون المحفز للغدة الدرقية في المرضى المصابين بقصور الغدة الدرقية مقارنة مع الاشخاص الاصحاء ، كما لوحظ انخفاض معنوي في هرمون الثيروكسين وهرمون ثلاثي الايرونين في المرضى المصابين بقصور الغدة الدرقية مقارنة مع الاشخاص الاصحاء في مصل الدم والانسجة. فضلا عن ذلك لوحظ ارتفاع معنوي في مستوى الملانوداهايد في

المرضى المصابين بقصور الغدة الدرقية مقارنة مع الأشخاص الأصحاء، كما لوحظ انخفاض معنوي في مستوى الكلوتاثيون ا في المرضى المصابين بقصور الغدة الدرقية مقارنة مع الأشخاص الأصحاء في مصل الدم والأنسجة.

الاستنتاج: من خلال هذه الدراسة تم الاستنتاج ان المرضى المصابين بقصور الغدة الدرقية معرضين للإصابة بفرط الاكسدة الذي يؤدي الى اتلاف انسجة الجسم لذلك نقترح على المرضى المصابين بقصور الغدة الدرقية تناول مضادات الاكسدة.

Introduction

In basal conditions, thyroid epithelial cells produce moderate amounts of reactive oxygen species (ROS) that are physiologically required for thyroid hormone synthesis. They are not necessarily toxic because they are continuously detoxified either in the process of hormone synthesis or by endogenous antioxidant systems [1].

It is noted that oxidative stress arises when highly reactive free radicals produce oxidative damages to the macromolecular structures of the cell. Thyroid gland plays a central role in generating generalized oxidative stress in diseased condition [2].

The thyroid gland is located in the lower part of the neck near your Adam's Apple. It secretes two essential thyroid hormones: triiodothyronine (T3) and thyroxine (T4) which are responsible for regulating cell metabolism in every cell in your body[3]. They promote optimal growth, development, function and maintenance of all body tissues. They are also critical for nervous, skeletal and reproductive tissue as well as regulating body temperature, heart rate, body weight and cholesterol [3].

In a healthy people a normal thyroid gland secretes all of the circulating T4 (about 90 to 100mg daily) and about 20% of the total circulating T3 (about 30mg daily). The T4 made by the thyroid gland circulates throughout the body and is converted into roughly equal amounts of T3 and reverse T3[3]. All of the biological activity of thyroid

hormones is due to T3. Because 80% of serum T3 is derived from T4 in tissues such as the liver and kidney [4], T4 is considered a pro-hormone [6]. The synthesis and secretion of the two thyroid hormones is influenced by a hormone released by the pituitary gland called thyroid-stimulating hormone (TSH). The synthesis and release of TSH from the pituitary gland is influenced by thyroid hormone levels as well as a hormone released from the hypothalamus called thyrotropin-releasing hormone (TRH) [5]. The activity of the thyroid gland is regulated by a negative feedback loop, in which thyroid hormones interact with receptors in the pituitary gland to inhibit TSH and at the hypothalamus to inhibit TRH secretion [6].

Hypothyroidism is a condition resulting from insufficient production or diminished action of either T3 and/or T4 thyroid hormones. Hypothyroidism is characterized by a generalized reduction in metabolic function that most often manifests itself as slowing of physical and mental activity [7]. The most common signs and symptoms of hypothyroidism are: weight gain, fatigue, lethargy, sleepiness, cold hands and/or feet, low body temperature, depression/anxiety, constipation, headache, menstrual problems, reduced sex drive, hair loss, swollen eye lids and general fluid retention, poor memory and concentration and dry skin, hair and/or nails [7].

Patients and Methods

The present study was conducted in Al-Hilla Teaching Hospital in the period between 2010 to 2011.

30 patients with hypothyroidism patients and 30 healthy individuals as control subjects were included in the study.

Blood samples were collected from both hypothyroidism and the control healthy groups. From each patient 10 mls of venous blood was collected to use in this study. The blood was left to clot and then centrifuged at 3000 rpm for 10 minutes. The separated serum samples were stored at 20 °C until the biochemical analysis was performed. Hormonal analysis (T3, T4 ,TSH) was performed by radio-immunoassay technique (RIA) using immunotech-Kits[8] .

Serum glutathione is determined by a modified procedure utilizing Ellmans reagent[9].

In brief; an equal volume (150 ml) of serum and 4% sulfosalicylic acid were mixed, centrifuged (2000 rpm at 4 °C for 15 minutes to 150 of supernatant add 4.5 ml of 0.1 ml Ellman's reagent (5.5 dithiobis, 2- Nitrobenzoic acid DTN) in phosphate buffer of pH 8.0 (prepared by a mixer of 0.6 M KH₂PO₄ and 0.08 M. Na₂ HPO₄), then read at spectrophotometrically at 412 nm.

Malondialdehyde is an end product of lipid peroxidation. It reacts with thiobarbituric acid (TBA) to produce a colored complex. Malondialdehyde was measured according to the method of (Fong *et al .*) [9]

The byproduct of lipid peroxidation malondialdehyde (MDA) level was measured in (thyroid glands) tissues

homogenates depends on the formation of pink chromosphere because of the reaction between (MDA) and thiobarbituric acid TBA, which can be measured spectrophotometric ally according to the method of (Buege and Aust,)[10]

In this method 2 ml of TBA reagent [10.375 g (TBA), 15g trichloroacetic acid (TCA) dissolved in 100 ml of 0.25 N hydrochloric acid at 45 °C], was added to 1 ml of the homogenate.

The mixture was incubated in a boiling water bath for 10 minutes, cooled and then centrifuged at 3000 r pm for 10 minutes. Light absorbance of the clear supernatant was determined spectrophotometric ally at 535 nm against Blank. MDA concentration was calculated using a molar absorptivity coefficient $1.5 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$ (Sinhnber and LU)[11].

The results were expressed as n mol MDA/g tissue.

Glutathione (GSH) levels in tissue homogenized were determined according to the method of Ellman[12].

In which 0.5 ml of 4% sulphosalicylic acid was added to equal volume of tissue homogenate for precipitation of protein. After centrifugation 0.5 ml of cleer supernatant was mixed with 4.5 ml (DTNB) reagent [0.1Mm DTNB IN 0.1 m phosphate buffer pH8]. The light absorbance of the mixture was measured spectrophotometric ally at 412 nm after 2 minutes.

Statistically Analysis

The data of this study used statistical analysis of variance (ANOVA) test and least significantly difference (LSD) test by probability of less than 0.05 (P<0.05) according to [13].

Results

Table 1 Parameters of Thyroid Function Test (T3, T4, and TSH) in hypothyroidism syndrome are summarized in table 1

Parameters	Hypothyroidism (mean±SD)	Control (mean±SD)
Number	30	30
T4 n mol/L	39.78±22.98	105.88±9.88
T3 n mol/L	0.52±0.23	1.22±0.12
TSH mμ/L	35.98±17.21	2.65±1.03

The serum levels of thyroid hormones were significantly lower in Hypothyroidism patients compared to control, while the serum TSH levels were significantly higher ($P < 0.05$) in values as expected due to pituitary's response to the decreased hormone levels.

Parameters of Oxidative Stress

All the parameters of antioxidant enzymes and lipid peroxidation in plasma are summarized in Table1, the parameters of oxidative stress in Hypothyroidism syndrome compared with control.

Table 2 Parameters of oxidative stress in serum

Parameters	Hypothyroidism (mean±SD)	Control (mean±SD)
Number	30	30
MDA $\mu\text{mol/g}$	3.08±0.129	1.52±0.671
GSH $\mu\text{mol/g}$	1.89±0.224	5.07±0.583

The serum MDA was significantly higher ($P < 0.05$) in values in the Hypothyroidism groups compared with control group.

The serum GSH levels were significantly lower ($P < 0.05$) in values in the Hypothyroidism groups compared with control group

Table 3 Parameters of oxidative stress in tissue homogenate the parameters of oxidative stress in Hypothyroidism syndrome compared with control.

Parameters	Hypothyroidism (mean±SD)	Control (mean±SD)
Number	30	30
MDA $\mu\text{mol/g}$	6.97±0.228	1.277±0.168
GSH $\mu\text{mol/g}$	0.19±0.185	4.88±0.0311

The tissue homogenate MDA was significantly higher ($P < 0.05$) in values in the Hypothyroidism groups compared with control group.

The tissue homogenate GSH levels were significantly lower ($P < 0.05$) in values in the Hypothyroidism groups compared with control group

Discussion

There is no doubt that oxidative stress occurs in cells when the production of reactive oxygen species (ROS) exceeds intracellular antioxidant defenses [13]. At low concentrations, ROS can stimulate cell proliferation [14], but at higher concentrations, they damage cells by oxidizing proteins, DNA, and lipids[15], or by initiating apoptotic pathways [16].

Reverse T3 dominance, functional hypothyroidism also Known as Wilson's Syndrome is a condition that exhibits most hypothyroid symptoms although circulating levels of T3 and T4 are within normal test limits. This is a condition when T4 metabolism produces an excess of reverse T3 in relation to T3, thus being a problem with T4 activation rather than a lack of thyroid production.

Periods of prolonged stress may cause an increase in cortisol levels as the adrenal glands respond to the stress. The high cortisol levels inhibits the conversion of T4 into T3 thus reducing active T3 levels [17] In the present study we found this finding agree with other studies.

Proteinuria results in loss of thyroid hormones, most probably caused by loss of thyroxin-binding globulin along with T4 bound to it, thus stimulating TSH production [18].

In the Present Study we found that In hypothyroidism, thyroid hormone levels are very low, which suggests the

possible direct involvement of free radical scavengers and lipid peroxidation. Increased glutathione peroxidase activity could be a compensatory mechanism in response to increased oxidative stress these results agreed with [19].

The present study suggests that hypothyroidism syndrome is associated with an overall increase in oxidative stress. There is a concomitant statistically significant decrease in circulation of T4 and T3 levels and increase in serum TSH. The nature and severity of the original insult may possibly play role in contributing to the oxidative stress associated with hypothyroidism syndrome. Other than the decrease in thyroid function due to oxidative stress, loss of thyroid-hormone-binding proteins in urine could be another cause of the decrease in serum T3 and T4 levels these results agreed with other study [19].

Others study suggested an increase in lipid peroxidation and insufficient antioxidant defense in hypothyroidism syndrome patients. The author concluded that, during remission, there is a tendency for the GSH-GSSG redox system to normalize [20,21].

Conclusion

According to the present study we could conclude that hypothyroidism cause tissues undergo several biochemical changes that predispose them to oxidative damage. Therefore we suggest that hypothyroidism patients may benefit from supplements of antioxidants.

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