Oxidative Stress in Hypothyroidism

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Abstract

Free radical mediated oxidative stress has been implicated in the etio-pathogenesis of several autoimmune disorders. Hypothyroidism in humans is widely believed to impair health. The biochemical factors mediating decline in health, however, are poorly elucidated. Pathological consequences of hypothyroidism point to a high potential for antioxidant imbalance. The study population consisted of 60 subjects divided into two groups: 30 people with hypothyroidism and 30 age-matched healthy participants. This study examined the levels of total triiodothyronine (T3), total thyroxine (T4), thyroid stimulating hormone, (TSH), and some enzymatic antioxidant status. The mean TSH level was significantly higher in hypothyroid patients than in control group.

On the other hand, the levels of T3 and T4 were significantly lower in hypothyroid patients compared to control group. However, the activities of catalase (CAT), and glutathione –S- transfers (GST), and reduced glutathione (GSH) were significantly lower in hypothyroid patients than in healthy group. These results confirm the hypothesis that people with hypothyroidism have reduced anti-oxidative defense.

الخلاصة

تسبب الجذور الحرة العديد من الاضطرابات التأكدسية التي تعتبر عوامل مسببة في العديد من الامراض المناعية الذاتية ان قصور افرازات الغدة الدرقية في الانسان يعتبر عامل مهم في التأثير على الصحة العامة. إلا أن العوامل الكيموحيوية التي تؤثر في الصحة غير مفهومة بشكل واسع. ومن اهم المؤثرات هي الاضطرابات الناتجة في عدم التوازن في نظام مضادات الاكسدة.

تضمنت هذه الدراسة فحص ستون (60) ذكراً من المصابين بقصور افرازات الدرقية والذكور الاصحاء. حيث كان عدد المصابين (30) ذكراً وعدد الذكور الاصحاء (30) ذكراً ايضاً. بينت نتائج هذه الدراسة ارتفاعاً معنوياً بمستوى ($P<0.01$) في قيم الهرمون المحفز للدرقية (TSH). وانخفاضاً ($P<0.01$) في قيم الهرمون الدرقي ثلاثي اليود (T3) وهرمون الثايروكسين (T4) في الاشخاص المصابين مقارنة بالاشخاص الاصحاء. لوحظ من خلال هذه الدراسة بأن فعالية انزيم الكاتاليز (CAT) والكلتاثيون أس– ترانسفيريز (GST) والكلوتاثيون المختزل (GSH) قد اشرت انخفاضاً معنوياً بمستوى ($P<0.01$) عند مقارنتها بالاشخاص الاصحاء. ان النتائج المستحصلة من هذه الدراسة تبين بأن هبوط هرمونات الدرقية يختزل فعالية مضادات الاكسدة.

Introduction

Thyroid hormones are among the most important humoral factors involved in setting the basal metabolic rate on along term basis in target tissues such as liver, heart, kidney and brain (Guerrero *et al.*, 1999). Oxygen free radical can develop during several steps of normal metabolic events. Although free radicals have the potential to damage the organism, their generation is inevitable for some metabolic process. The main endogenous sources of free radicals are the microsomal membrane electron transport chain, reaction of oxidant enzymes, and auto-oxidation reactions (Hauck and Bartke, 2000; Yilmaz *et al.* 2003).

Both hydrogen peroxide and superoxide anion produce highly reactive hydroxyl radicals through the Huber-Weiss reaction. The hydroxyl radical can initiate lipid peroxidation, which is a free radical chain reaction leading to damage of membrane structure and function (Halliwell and Gutteridge, 1990). Variations in the levels of thyroid hormones can be one of the main physiological modulators of in vivo cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increases in reactive oxygen species induced by a deficiency of thyroid hormones can lead to an oxidative stress conditions in the liver and in the heart and some skeletal muscles with a consequent lipid peroxidative response (Yilmaz *et al.*, 2003).

Reactive oxygen species (ROS) including partially reduced forms of oxygen, i.e. super-oxide anion, hydrogen peroxide, and hydroxyl radical, as well as organic counter parts such as lipid peroxides, are produced as natural consequences of oxidative cell metabolism (Komosinska-Vassev *et al.*, 2000). Under physiological conditions, ROS generation is controlled by a large number of anti-free radical systems which acts as protective mechanisms. These systems consist anti oxidant enzymes such as super-oxide dismutase, catalase, glutathione peroxidase and glutathione reducates as well as non-enzymatic anti-oxidants, among which the most important are vitamins C and E, carotenoids, and glutathione. Disturbance of the prooxidant antioxidant balance results from the increased production of ROS, inactivation of detoxification systems, or excessive consumption of anti-oxidants. The disturbance is a causative factor in oxidative damage of cellular structures and molecules such as lipids, proteins, and nucleic acids (Kehrer, 1993).

Subclinical hypothyroidism is defined as a serum thyroid stimulating hormone (TSH) concentration above the statistically defined upper limit of the reference range when serum free T4 concentration is within its reference range. Greater sensitivity of assays and more frequent assessment of serum TSH levels have resulted in more patients requiring interpretation of abnormal thyroid function test results. However, controversy surround the definition, clinical importance, and necessity for prompt diagnosis and treatment of hypothyroid disease.

Previous review articles and position statement differ in their conclusion and recommendations, often a consequence of difficulties in interpreting inadequate and conflicting data (Surks *et al.*, 2004). Hypothyroidism-associated oxidative stress is the consequence of both increased production of free radicals and reduced capacity of the anti-oxidative defense (Das and Chainy, 2004; Sarandol *et al.*, 2005).

Hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria lead to accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radicals as well as lipid peroxides), which consequently leads o oxidative stress (OS). (Venditti *et al.*, 1997).

Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress (Carmeli *et al.* 2008).

Methods

Study of population

The study population consisted of 60 subjects (age and sex-matched) divided into two groups: hypothyroid patients (n=30) and healthy control subjects (n=30). All the patients and controls were recruited from Hilla teaching hospital during March to December of 2009. General healthy characteristics such as age, sex, smoking status, alcohol consumption, and dietary habits were investigated by a self-administered questionnaire.

Blood Collection and hemolysate preparation:

Blood samples were collected by venous puncture in plain tubes and the plasma was separated by centrifugation at 1000g for 15 minutes after centrifugation, the Buffy coat was removed and the packed cells were washed three times with physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer (pH 7.5). The hemolysate was separated by centrifugation at 2500g for 15 minutes.

Hormonal analyses

The levels of serum thyroid stimulating hormone (TSH), total triiothyronine (T3), and total thyroxine (T4) were measured by using enzyme immuno assay (EIA) methods (according by kits from Biocheck, Inc.).

Estimation of reduced glutathione

Reduced glutathione (GSH) content was determined by the method of Ellman's (Ellman, 1959). Plasma, 1.0ml, was treated with 0.5ml of Ellman's reagent (19.8 mg of 5.5=dithiobisnitro-benzoic acid [DTNB] in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412nm.

Assay of catalase

Catalase (CAT) was assayed colorimetrically at 620nm and expressed as μmol of H_2O_2 consumed min/mg/Hb as described by (Shina, 1972). The reaction mixture (1.5ml) contained 1.0ml of 0.01 mole pH 7.0 phosphate buffer, 0.1 ml of hemolysate, and 0.4 ml of 2mole H_2O_2. The reaction was stopped by addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Assay of glutathione-S-transfers:

Glutathione –S- transferase (GST) activity was determined spectrophotometrically using the method of (Hapig *et al.,* 1974). The reaction mixture contained 1.0ml of 0.3mmole phosphate buffer (pH 6.5), 0.1ml of 30mmole 1-chloro-2,4-dinitro benzene (CDNB), and 1.7ml of double distilled water. After preincubating the reaction mixture at 37°C for 5 minutes, the reaction was started by the addition of 0.1ml of hemolysate and 0.1ml of glutathione as substrate. The absorbance was followed for 5 minutes at 340nm. The activity of GST is expressed as: mole of CDNB-GSH conjugate formed/min/mg/Hb using an extinction coefficient of 9.6 mmole-$1cm^{-1}$.

Statistical analysis

All data were expressed as mean ±SD of number of experiments. The statistical significance was evaluated by student's t-test using SPSS version 10.0 (Daniel, 1999).

Results

The results of this study is shown in the following table. The mean age of hypothyroid patients was 43±14 years and of control subjects 47±13 years. The levels of TSH of hypothyroid patients show significant increase ($P<0.01$) in a comparison with healthy control. Hypothyroid patients also had significantly lower ($P<0.01$) levels of T4 and T3. For studying the deleterious consequence of hypothyroidism on antioxidant status, the activities of enzymatic antioxidants (CAT and G-ST) and non enzymatic antioxidants were measured. The activities of the enzymatic antioxidants (CAT, and GST) and GSH were significantly lower ($P<0.01$) in hypothyroid patients when compared with healthy subjects.

Table : The means of age, thyroid stimulating hormone (TSH), total triiodothyronine (T3), total thyroxine (T4), catalase (CAT), Glutathione-S-stransferase (GST), and reduced glutathione (GSH) in hypothyroid patients and control subjects.

Parameter	Control subjects	Hypothyroid patients
Age (years)	47±13	43±14
T3(ng/ml)	0.95±0.25	0.51±0.11**
T4(μg/dL)	8.021±2.10	1.86±0.91**
TSH(mIU/ml)	2.24±1.17	15.83±4.18**
CAT(U/mg Hb)	70.5±10.61	48.5±9.30**
GST(U/mg Hb)	2.52±0.45	1.05±0.40**
GSH (mg/dL)	41.11±7.52	25.36±4.91**

-Values are given as mean±SD

-Hypothyroid patients compared with control subjects (**P<0.01)

Discussion

Resh *et al.,* (2002) found that hypothyroidism was associated with enhanced oxidative stress and lipid peroxidation, and supposed that this might lead to the development and progression of atherosclerosis. Reactive oxygen species (ROS) have been reported to induce oxidative damage to membrane lipids, proteins, and DNA, and might in cell death by necrosis or apoptosis (Gamaley and Klyubin, 1999). Both glutathione peroxidase and catalase are major defenses against harmful effects of ROS in cells, and in cultured thyrocytes, both have a high capacity to degrade exogenous hydrogen peroxide (H_2O_2) (Bjorkman and Ekholm, 1995).

Specifically, observations indicate that glutathione peroxidase (GPX) is involved in the degradation of fairly low H_2O_2 levels, whereas catalase (CAT) is required to degrade H_2O_2 at a higher concentrations. It is thus possible that the lower activities of GPX and CAT lead to H_2O_2-induced apoptosis of thyroid cells in Hashimoto's thyroiditis patients. In an in vitro study by (Demelash *et al.,* 2004). Impaired capacity of GPX in degrading H_2O_2 in cultured thyroid pig cells aggravated the apoptic response. This data and presented results suggest the possibility that reduced GPX and CAT activities in hypothyroid patients might participate in initiation of the autoimmune process might lead to H_2O_2-induced damage of thyroid cells related to cystolic oxidative stress.

The mechanism linking hypothyroidism with oxidative stress and antioxidants is unknown. The effects of hypothyroidism on antioxidants parameters have been investigated in hypothyroid patients with intellectual disability (Yilmas *et al.,* 2003). Antioxidant deficiencies may lead to a failure to effectively combat extrinsic factors (i.e., weather, diet, drugs, and physical exercise) and intrinsic factors (i.e., injuries, weakness, and fatigue involved in oxidative stress. An extensive body of evidence now exists confirming that antioxidants are involved in the cellular defense against oxidative stress in a variety of pathological conditions.

It has been suggested that hypothyroidism lead to oxidative stress and to a reduction of antioxidant defenses. In addition, previous experimental studies have reported that hypothyroidism is characterized by endothelial dysfunction of blood vessels (Taddei *et al.,* 2003). In agreement with previous findings, thyroid hormones are involve in combating the toxicity of oxidative stress in animals (Petrovic *et al.,* 2005) and in humans (Gridilla *et al.,* 2001).

Thus, under normal conditions, the protective effect of thyroid hormone against oxidative stress can be explained by the function of antioxidants as a defense system. However a chronic state of hypothyroidism is characterized by impairments in the redox potential. This lead to free radical chain reactions and to metabolic suppression on antioxidant capacity. Results from this study support the suggestion that the hypothyroidism of patients with intellectual disability in some way is linked to the low levels of the major antioxidant molecules found in these patients. The depletion of antioxidants observed in hypothyroid individuals may reflected the increased free radical production in the electron transport chain in the mitochondrial inner membrane.

The increase of free radicals is not compensated, as one would expect, by a decrease of antioxidants. A high oxidative state in hypothyroid people has metabolic and biochemical characteristics such as increased mitochondrial enzyme activity.

Thus, it is likely that patient's cells are damaged by prolonged oxidative stress that far exceeds the capacity of the patient's organs to synthesize antioxidant molecules or to synthesize them from extra cellular sources (Komosinska-Vasser *et al.,* 2000).

Hypothyroidism is generally associated with decreased content of tissue protein. Hypothyroidism also specifically reduces most tissue's cellular thiol reserve and alters glutathione/GSH-PX content. Importantly, SOD is the first line of enzymetic defense against intracellular free radicals. Because of that, a decrease of SOD activity would expose the cell membrane and other components to oxidative damage. Catalase shares with GSH-Px its function of catalyzing the decomposition of H_2O_2 to water. A low level of catalase activity, then, could primarily damage the endoplasmic reticulum in the cells. Glutathione reductase was little affected by the presence of hypothyroidism (Venditti *et al.,* 1997).

In conclusion, the present study suggests a very high production of ROS and oxidative stress in patients with hypothyroidism, with enhanced lipid peroxidation and concomitant failure of antioxidant defense mechanism. Physical signs and symptoms in people with hypothyroidism are less reliable and there is a need for serum testing to determine the appropriate dosage of replacement thyroid hormones.

The purpose in this study was to provide evidence for, and to recommend, blood testing for hypothyroid patient's antioxidant system in order to monitor the progression of pathology and to prompt the consideration of medical care.

References

Bjorkman, U. and Ekholm, R. (1995). Hydrogen peroxide degradation and glutathione peroxidase activity in culture of thyroid cells. Mol. Cell Endocrinol., 111: 99-107.

Carmeli, E; Bachar; A.; Bachad, S.; Mora, M.; and Merrick, J.(2008). Anti oxidant status in the serum of persons with intellectual disability and hypothyroidism: A pilot study. Res. Development. Disab., 29: 431-438.

Daniel, W.W. (1999). Biostatistics: a foundation for analysis in health sciences. 7th. Ed. John Wiley. Philadelphia. P(83).

Das, K. and Chaing, G.B. (2004). Thyroid hormone influence antioxidant defense system in adult rat brain. Neurochem. Res. 29(9): 1755-1766.

Demelash, A.; Kalsson, J.O.; Nilsson, M.; and Bjorkman, U.S. (2004). Selenium has a protective role in caspase-3- dependent apoptosis induced by H_2O_2 in primary cultured Pig thyrocytes. Eur. J. Endocrinol., 150: 841-849.

Ellman, G.L. (1959). Tissue sulfhydral groups. Arch. Biochem. Biophys., 82(1): 70-77.

Gamaley, I.A. and klyubin, I.V. (1999). Roles of reactive oxygen species signaling and regulation of cellular functions. Intern. Rev. Cyto., 188: 203-255.

Gredilla, R.; Barja, G.; and Lopez. Torres, M. (2001). Thyroid hormone-induced oxidative damage on lipids, glutathione and DNA in the mouse heart. Free Rad. Res., 35(4): 417-425.

Guerrero, A.; Pamplona, R., Postero-otin, M.; Barja, G., and Lopez-Torres M. (1999). Effect of thyroid status on lipid composition and peroxidation in mous liver. Free. Rad. Biol. Med., 26: 73-80.

Habig, W.H., Pabst, M.J., and Jakoby, W.B. (1974). Glutathione-S-transfease, the first enzymatic step in mercapturic acid formation. J.B.C., 249: 7130-7139.

Halliwell, B., and Gutteridge, J.M.C. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol., 186: 1-85.

Hauck, J.S. and Bartke, A. (2000). Effects of growth hormone on hypothalamic catalase and Cu/Zn superoxide dismutase. Free. Rad. Biol. Med., 28: 970-979.

Kehrer, J.P. (1993). Free radicals as mediators of tissue injury and disease. Crit. Rev.; Toxicol., 23: 21-48.

Komosinska-Vassev, K.; Olczyk, K.; Kucharz, E.J.; Marciz, C.; and Kotulska, A (2000). Free radical activity and anti oxidants defense mechanisms in patients with hyperthyroidim due to Grave's disease during therapy. Clinical chimica Acta., 300: 107-117.

Petrovic, N.; Cuijic, G.; Dordjevic, J., and Davidovic, V. (2005). The activities of antioxidant enzymes and monoamine oxidase and uncoupling protein 1 content in brown fat of hypo-and hyperthyroid rats. Ann. N.Y. Acad. Sci. , 1040: 431-435.

Resh, U., Helsel, G., Tatzber, F., and Sinzinger, H. (2002). Anti-oxidant status in thyroid dysfunction. Clin. Chem. Lab. Med., 40: 1132-1134.

Sarandol, E.; Tas, S.; Dirican, M.; and Serdar, Z. (2005). Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: Effect of vitamin E supplementation. Cell. Biochem. Funct. 23(1): 1-8.

Sinha, A.K. (1972). Colorimetric assay of catalase. Anal. Biochem., 47(2): 389-394.

Surks, M.I.; Ortiz, E.; Daniels, G.H.; Sawin, C.T.; Col, N.F.; and Cobin, R.H. (2004). Subclinical thyroid disease: Scientific review and guidelines for diagnosis and management. J.A.M.A., 291(2): 228-238.

Taddei, S.; Caraccio, N.; and Viridis, A. (2003). Impaired endothelium dependent vasodilation in subclinical hypothyroidism: Beneficial effect of levothyroxine therapy. J.C.E.M., 88(8): 3731-3737.

Venditti, P., Balestrier, M., Dimeo, S. and Deleo, T. (1997). Effect of thyroid state on lipid peroxidation, antioxidants defenses, and susceptibility to oxidative stress in rat tissues. J. Endocrin., 155(1): 151-157.

Yilmaz, S.; Ozan, S., Benzer, F.; and Canatan, H. (2003). Oxidative damage and a ntioxidant enzyme activities in experimental hypothyroidism. Cell. Biochem. Funct., 21(4): 325-330.