Effect of increase thyroid hormone on antioxidant enzymes activity and their related metals in hyperthyroid patients in Basrah Governorate/Iraq.

تأثير زيادة هرمونات الغدة الدرقية على نشاط مضادات الاكسدة الانزيمية والفلزات المرتبطة بها في مرضى فرط الغدة الدرقية في محافظة البصرة العراق

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Abstract

The maintenance of optimal health requires an adequate supply of carbohydrates, proteins lipids, macronutrients, micronutrients, and trace elements. The present study evaluates the increase of thyroid hormones (hyperthyroidism) on antioxidant enzymes including superoxide dismutase, glutathione peroxidase **and** the levels of zinc, copper and selenium in serum of Iraqi patients with hyperthyroidism .The results showed a significant increase ($p \leq 0.05$) in erythrocyte superoxide dismutase (SOD) activity in patients as compared with control group. While there is a highly significant decrease ($p < 0.001$) in glutathione peroxidase activity in patients as compared with controls. The levels of zinc and selenium were highly significantly decrease in hyperthyroid patients compared with control with no significant change ($p > 0.05$) in concentration of copper in patients group compared with the control group. The results indicated that hyperthyroidism increased the free radicals due to decreasing the free radicals scavengers.

Keywords: Hyperthyroidism, ,Superoxide dismutase enzyme, Glutathione peroxidase, Zinc , ,Copper , Selenium.

الملخص

يحتاج الجسم الى كميات كافية من الكاربوهيدرات ,البروتينات ,الدهون والعناصر النزرة للحفاظ على الصحة .الدراسة الحالية تضمنت تأثير زيادة افراز هرمونات الغدة الدرقية على نشاط مضادات الاكسدة الانزيمية المتمثلة بفوق اوكسيد ديسميوتيز و الكلوتاثيون بيروكسيديزوعلى مستويات بعض العناصر(النحاس والزنك والسلينيوم) في دم المرضى الذين يعانون من فرط نشاط الغدة الدرقية. اظهرت النتائج زيادة معنوية في نشاط انزيم فوق اوكسيد ديسميوتيز المقاس في كريات الدم الحمراء للمرضى الذين يعانون من فرط الدرقية مقارنة بأشخاص اصحاء بينما اظهرت النتائج انخفاض عالي المعنوية في نشاط انزيم الكلوتاثيون بيروكسيديز في المرضى مقارنة بالاصحاء .كما اظهرت النتائج انخفاض معنوي عالي في تركيز الزنك والسلينيوم في مصل دم المرضى مقارنة بالاصحاء بينما لم يكن هناك اي تغير معنوي في تركيز النحاس لدى المرضى مقارنة بالاصحاء. الهدف من الدراسة الحالية تقييم نشاط كابحات الجذور الحرة في مرضى يعانون من فرط الغدة الدرقية وتقييم دور العناصر النزرة في عملية الايض سواء كانت مرافقات انزيمية او مغذيات اساسية وهذة النتائج اثبتت ان فرط نشاط الغدة الدرقية يزيد الجذور الحرة بسبب نقصان كابحات الجذور الحرة.

Introduction

Hyperthyroidism is an over functioning of thyroid gland which results in the production of excessive thyroid hormones .This speeds up most of the chemical reactions in the body and causes mental and physical changes (1).The hyperthyroidism increases the formation of reactive oxygen species (ROS) leading to oxidative damage to biomembrane lipid (2).In the aerobic cells active oxygen species generated by products of oxidative metabolism (3). Reactive oxygen species (ROS) including partially reduced form oxygen; i.e. superoxide anion, hydrogen peroxide and hydroxyl radical as well as organic counter parts such as lipid peroxides are produced as natural consequences of the oxidative cell metabolism .Under physiological conditions , ROS generation is controlled by a large number of anti-free radical systems which act as protective mechanisms. These systems consist of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, as well as non enzymatic antioxidants, such as vitamins C and E, glutathione, and uric acid (4).

Glutathione peroxidase one of the antioxidant systems remove free radicals from the environment (5), It functions to remove hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH) by coupling their reduction to water with the oxidation of glutathione (GSH) (6). Since the discovery of glutathione peroxidase as selenium-dependent enzyme in 1973, selenium has been identified as an essential cofactor for selenoproteins. (7).

Superoxide dismutase enzymes are catalyzes the reaction between two superoxide radicals to yield one molecule of oxygen and hydrogen peroxide (8). Two superoxide dismutase isoenzymes have so far been described in vertebrates i.e. Cu/Zn-SOD (Copper/Zinc Superoxide dismutase) and Mn-SOD (Manganese Superoxide dismutase). Cu/Zn- SOD has been demonstrated in the cytoplasm and also in the intermembrane space in mitochondria. Mn-SOD is found in the matrix space in mitochondria (9).

Trace elements serve a variety of functions including catalytic, structural and regulatory activities in which they interact with macromolecules such as enzymes, pro-hormones. Selenium is an essential mineral of pivotal importance for the human health (10).It occurs in the form of the amino acid selenocystein in selenoproteins (11).The selenoenzymes are the families of glutathione peroxidases (GPx), Thioredoxin reductases (TrxR) and iodothyronine deiodinases (DIO).These selenoenzymes are capable of modifying cell function by action as antioxidants and modifying redox status and thyroid hormone metabolism (12).

Zinc has important roles in thyroid metabolism (13); and a fundamental role in protein synthesis (14). It involves in triiodothyronine(T_3) binding to its nuclear receptor (15).While copper is necessary for proper iodine metabolism and synthesis (16),copper plays an important role in thyroid metabolism, especially in hormone production and consequently of proper thyroid hormone absorption. Copper stimulates the production of the thyroxine hormone (T_4) and prevents over-absorption of T_4 in the blood cells by controlling the body's calcium levels (Calcium is required for the stabilization of cell membranes and reduces) (17). Copper is a central component of the antioxidant superoxide dismutase molecule.

The present study aims to investigate the free radical scavenging activity, in hyperthyroid patients and the role of trace elements in much metabolic process either as essential nutrients or as cofactors.

Materials and methods

Subject:

Blood samples were collected from 47 patients with hyperthyroid (25 females and 22 males),Their age between (15-61)years and 52 euthyroid subjects(26females and 26 males) their age between (15-62) years .The patients who attended to the endocrinology center in AL-Mawani hospital/Basrah governorate in Iraq, were diagnosed depending on the results of the following

examinations clinical , examinations serum hormones level triiodothyronine (T_3),thyroxine(T_4),thyroid stimulating hormone(TSH). All individuals who were smokers, diabetes mellitus, pregnancy, liver or kidney disorders were excluding.

Samples Collection and Preparation:

Ten milliliters of venous blood from fasting subjects were drawn, part from blood allowed to clot and centrifuged at 4000 rpm for 10 minutes and sera were separated and stored at -20 °C until analysis, while the remaining blood was collected in heparinized tubes. Blood samples were centrifuged at 3000 rpm for 10 minutes; in order to separate the plasma. The remaining erythrocytes were washed three times with 0.9% NaCl, and lysate in 1:1 (v/v) of double-deionised water, which was lysates used for determination superoxide dismutase activity using a kit (RANSOD) from Randox Laboratories.U.K., based on Woolliams *etal* procedure (18). Activity of glutathione peroxidase in whole blood was measured using a kit (RANSEL) from Randox Laboratories, based on the method of Paglia and Valentine (19).

Serum thyroid hormones T_3, T_4 and TSH level were determined by mini-VIDS technique using kit supplied by Biomerieux / France. Serum zinc and copper were determined by Spectrophotometric method by using a kit from LTA. Italian and Spectrum, Egypt . Serum selenium was determined by spectrophotometric method .The method is based on the reaction of Se(IV) with indole in presence of concentrated phosphoric acid to form yellowish green water soluble compound.(20).

Statistical analysis

The results of the present study were analyzed by univarate analysis of variance using SPSS version 9 was used to analyse the result of this study. The data were expressed as mean ± SD. Least significant different test (LSD) was used to test the difference between groups.

Results

Under the recommended conditions (AL-Abichy *et al*. 2002) the linear calibration graph for selenium was obtained over the range (5-100)µg/L. Figure 1 shows the calibration graph , in series of Se(IV) standard the linear graph has regression coefficient (R^2) equal to(0.9937), the detection limit (2× noise) 1.52µg/l ,and the relative standard deviation (R.S.D%) for 5 replicates of 50 µg/L of Se(IV) was (0.2%).

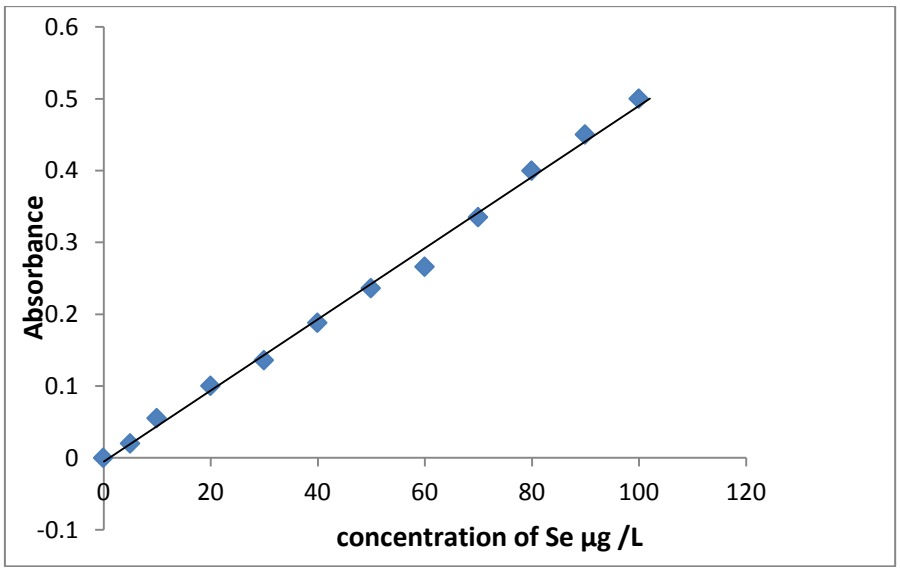

Figure (1) Standard calibration curve of selenium

The levels of Serum TSH,T_3 , T_4, Se, Zn, Cu ,erythrocyte superoxide dismutase activity (SOD) and whole blood glutathione peroxidase (GPX) active in subjects with hyperthyroidism and euthyroid are listed in table (1) which expressed as mean ±standard deviation.

Table (1): Biochemical characteristics in patients and control groups

Parameters	Hyperthyroidism n=47 (mean ±SD)	Control n=52 (mean ±SD)
T_3(nmol/L)	4.16***±2.58	1.77±0.44
T_4(nmol/L)	185.06***±84.62	89.21±11.5
TSH(µU/ml)	0.08***±0.05	1.55±0.69
GPx (U/L)	2009.82***±1007.05	4319.2± 1711.57
SOD(unit/ml)	221.4*±79.81	195.6±51.89
Serum Se (µg/ L)	37.19***±17.33	62.96±13.96
Serum Zn(µg/dl)	81.89***±30.24	101.26±15.05
Serum Cu(µg/dl)	144.81±36.94	131.11±52.51

*, *** significantly differences between patients and control group ($p\leq0.05$, $p<0.001$) respectively.

The results indicated that a highly significant increase in the levels T_3 and T_4 and decreased in TSH in hyperthyroidism patients($p<0.001$) comparing with control group respectively.

GPx shows a highly significant decrease in hyperthyroidism compared with control ($p<0.001$) while the activity of SOD enzyme shows a significant increase in patients compared with the control ($p\leq0.05$). Serum selenium and zinc levels of hyperthyroidism patients are significantly lower ($p<0.001$) than the levels in normal subjects, whereas there was not any significant difference in concentration of copper ($p>0.05$) when comparing with the control group.

Table (2) and figure (2) shows the correlation coefficient (r) between SOD and serum Zn and Cu in hyperthyroid patients. The results indicate that there is a significant positive correlation between erythrocyte SOD with serum Zn and Cu in the patients group.

Table (2) Correlation coefficient (r) among SOD, Zn and Cu in hyperthyroid patients.

Parameters	Correlation coefficient(r)
serum Zn(µg/dl)	0.324*
serum Cu(µg/dl)	0.401**

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

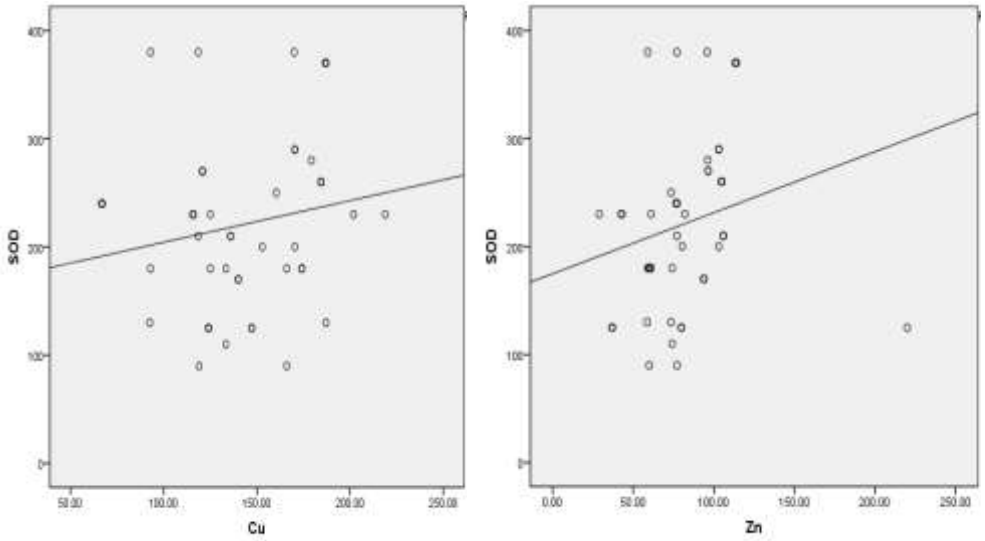

Figure (2) Correlation between SOD, Zn and Cu in hyperthyroid patients

Discussion

Thyroid hormones maintain the oxidant /antioxidant equilibrium to protect the cell. High levels of thyroid hormones are known to accelerate metabolic reactions, increase oxygen consumption and owing to oxidative reactions.

Free radical and disorders of the antioxidant defense system have pathogenic impact on human tissues and hence are seen as important factors in the development of various diseases (21).

The glutathione peroxidase (GPx) one of the antioxidant systems, remove free radicals from the environment (5).Our data showed significant decrease in the activity of GPX in hyperthyroidism patients in comparison to that of normal subjects are observed in other different researches (2; 22; 23; 24, and 25).The glutathione peroxides enzyme (GPx) catalyzes the reduction of hydrogen peroxide by glutathione (GSH). A reduction in GPx activity results in increased H_2O_2 levels and hence severe cellular damage is observed (26). GPx is a selenoezyme, human erythrocyte GPx does not show enzymatic activity in selenium deficiency, thus selenium deficit might be one of the causes of reduced GPx activity, and also another cause of reduced GPx might be explained by the increase degradation of this enzyme in the liver and muscles due to increased thyroid hormone levels (27). Superoxide dismutase enzymes (SOD) are the first line of enzymatic defense against intracellular free radicals which catalyzes the conversion of superoxide anion to H_2O_2 (28) The current results report a significant increase in the levels of SOD activity in hyperthyroid patients as compared to control subjects. These results are in agreement with the results previously reported by (27; 29; 30; and 31), reported the same results which confirmed the increase activity of SOD enzyme in hyperthyroid patients. The results of this study report that increase levels of SOD activity in hyperthyroid patients may be explained due to the presence of oxidative stress because of the increase of mitochondrial oxidation rate characterized by an overproduction of superoxide anion (32).

The effect of thyroid hormone on selenium, zinc and copper in hyperthyroidism was studied we found that mean concentration of serum selenium and zinc were significantly lower with hyperthyroidism patients than the control group, this result agree with (33; 34; 35) This decrease in serum level of zinc may be thought due to that (80%) of Zn in serum is known to be bound to albumin and thus it lowered Zn levels that accompanied low albumin level in hyperthyroid patients (36). The influence of TSH in the variation of the concentration of iodine, selenium and zinc in normal and altered human thyroid tissue can also be added as another factor (37).

The decrease in serum level of selenium in hyperthyroid patients may be thought to be due to selenium acts as cofactor for iodothyronine deiodinase that control the synthesis and degradation

of the biochemically active thyroid hormone T_3 (38).The utilization of selenium by this enzyme may lower the level of selenium in serum. As the enhanced hormone production is very pronounced in hyperthyroidism, deiodination of T4 is also increased ,since the body stores of selenium are limited deiodination is given preference over GPx to selenium supply (39) Thus ,selenium deficit might be one of the causes of reduced GPx activity ,it showed by (40).The current study revealed insignificant increase of serum copper in hyperthyroid patients as compared to the control group which is in agreement with that previously reported by (33 and 34) these results thought to be due to 93 % of plasma copper is bound to ceruloplasmin and 6 to 7 % fraction to albumin or is chelated to amino acids (less than 1%) , hence, the increase in serum copper may be due to the increase in ceruloplasmin . Plasma ceruloplasmin level was reported to be increased in patients with hyperthyroidism (33).On the other hand, Sugawara *et.al.* (41) Found that the SOD activity in thyroid tissues with hyperthyroidism was higher than normal thus, the increase activity of SOD in hyperthyroid patients could be related to the increase of serum copper levels.

Table (2) and figure (2) show a significant positive correlation between serum zinc and copper with erythrocyte SOD enzyme in hyperthyroidism, which suggest that antioxidant enzyme depends on their cofactor concentration .

In conclusion, the present study suggests a very high production of ROS and oxidative stress in patients with hyperthyroidism and failure of antioxidant defense mechanisms.

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