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Antioxidant Enzyme Activities and Selenium Status in Various Stages of Goiter

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Antioxidant Enzyme Activities and Selenium Status in Various Stages of Goiter

Summary : *The status of selenium (Se) and activities of antioxidant enzymes (AOE) [Glutathione peroxidase (GSHPx), superoxide dismutase (SOD), catalase (CAT)] were examined in goitrous children (n=45) living in the East Black Sea Region. Compared to a non-goitrous children group (n=41) selected from the same region and at the same age (15- 18 years) and gender, children with goiter had significantly lower erythrocyte AOE activities, plasma and erythrocyte Se levels and lower urinary iodine (UI) and plasma thyroxine (T_4) concentrations. These differences were not observed between subgroups of different goiter stages, suggesting that the development and not the progression of goiter may be related to the antioxidant status of the subjects evaluated.*

Keywords : *endemic goiter, iodine and/ or selenium deficiency, antioxidant enzymes*

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Farklı Guatr Evrelerinde Antioksidan Enzim Aktiviteleri ve Selenyum Statüsü

Özet : *Bu çalışmada Doğu Karadeniz Bölgesinde yaşayan guatrlı bir grup çocukda (n=45) selenyum (Se) statüsü ve antioksidan enzim (AOE) [Glutatyon peroksidaz (GSHPx), süperoksit dismutaz (SOD), katalaz (CAT)] aktiviteleri incelendi. Aynı yöreden gelen aynı yaş (15- 18 yaş) ve cinsiyetteki guatrsız çocuklara kıyasla, guatrlı çocukların önemli ölçüde düşük eritrosit AOE aktivitelerine, plazma ve eritrosit Se düzeylerine sahip oldukları; bu bulgulara düşük idrar iyot (UI) ve plazma tiroksin (T_4) konsantrasyonlarının eşlik ettiği bulundu. Bu farklar, farklı guatr evrelerinden oluşan guatr alt gruplarında gözlenmedi. Sonuçlar incelenen deneklerdeki AOE aktivitelerinin, guatrın ilerleme evreleri ile değil fakat guatr oluşumu ile ilişkili olabileceğini düşündürmektedir.*

Anahtar kelimeler : *Endemik guatr, iyot ve/veya selenyum eksikliği, antioksidan enzimler*

INTRODUCTION

Iodine deficiency is known to induce a variety of disorders of thyroid function, including endemic goiter, and is considered to be the greatest single cause of preventable brain damage and mental retardation all over the world[1]. The importance of iodine as an essential trace element arises from the fact that it is the integral component of the thyroid hormones T_4 and triiodothyronine (T_3), which are essential for normal growth, physical and mental development in man and animals[2]. The term "Iodine Deficiency Disorders (IDD)" is used to denote all the effects of iodine deficiency on growth and development which can be prevented by correction of iodine deficiency[1-5]. Goiter is the most visible and familiar consequence of IDD. Endemic goiter occurs when the prevalence of thyroid enlargement in the population of an area exceeds 10 %[1,4]. In mild iodine deficiency, both goiter itself and the nodules create clinical problems, but more serious complications occur with severe iodine deficiency[2,4]. The most severe problems caused by iodine deficiency are observed in fetuses, neonates and infants because of the irreversible changes that can

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occur during these periods of rapid structural and behavioral development. Cognitive impairment is the most common and serious finding seen with iodine deficiency. Even mild iodine deficiency has been reported to reduce intelligence quotients by 10-15 points. In severely affected areas the incidence of spontaneous abortion, stillbirths and infant mortality increase and worst of all, children with cretinism are born[1,2,4].

WHO estimates that a total global population of at least 1 billion is at risk of IDD, with 20 million suffering from varying degrees of preventable brain damage[6]. In fact, existing data indicate that endemic goiter prevails in all geographical regions of Turkey, and that there is no region having less than 2% of goiter prevalence[7]. The East Black Sea Region has long been recognized as one of the highest prevalence rate areas in the country[7-9].

It is now well recognized that, besides iodine, a second essential trace element Se is involved in the regulation of the thyroid hormone system[10,11]. T_4 is a prohormone requiring 5'-monodeiodination to produce the active thyroid hormone, T_3. 5'-monodeiodination of T_4 occurs practically in all tissues of the body and this reaction is catalyzed by three isozymes of iodotyhronine 5'-deiodinase, which are characterized by their tissue distribution, structure and biochemical properties. In recent years it has been shown that those isozymes catalyzing the deiodination of thyroid hormones are selenoenzymes[12-14]. Type I deiodinase (ID-I) in liver and kidney is the major source of plasma T_3, whereas type II deiodinase (ID-II), which occurs in the central nervous system, pituitary and brown adipose tissue, contributes little to circulating T_3, but is the major source of intracellular hormone in these tissues[10]. Type III deiodinase (ID-III) inactivates T_4 and T_3 by inner ring deiodination and is probably important for the clearance of plasma T_3 (15). The increase in plasma T_4 and decrease in plasma T_3 concentrations in severe Se deficiency are consistent with impairment of tissue deiodinase activities[10]. However the effects of Se in the regulation of this homeostatic and dynamic hormone system are complex and dual. Se is also an integral component of GSHPx which catalyses the reduction of reactive peroxides and protects cells from oxidative stress[16]. As the essential part of the antioxidant defense system, Se deficiency may increase the thyroid damage caused by iodine deficiency; however it may also provide sparing of iodine by decreasing the catabolism of prohormone, T_4, and, dependent on the degree of both deficiencies, may introduce either a deleterious or a protective effect on the consequences of iodine deficiency[17,18].

In this study, it was aimed to investigate the possible alterations of thyroid functions, Se status and AOE activities in goitrous children living in an endemic goiter area and to be able to evaluate the alterations of these parameters in different stages of goiter.

SUBJECTS AND METHODS

Chemicals

Selenium dioxide, cyclohexane, ethylenediamine tetra acetic acid, 2,3 diamino naphthalene, bromcresol purple, xanthine, xanthine oxidase, nitrobluetetrazolium, bovine serum albumin, and superoxide dismutase were obtained from Sigma (St Louis, USA). Ammonium sulphate, copper chloride, chloroform, sulphuric acid, sodium chloride, sodium carbonate, sodium bicarbonate, sodium hydroxide, sodium hydrogen phosphate, potasium dihydrogen phosphate, potasium iodate, and potasium cyanide were purchased from Merck (Darmstadt, Germany). Cerium amonium sulphate, potasium chlorate and arsenic trioxide were from Riedel (Seelze, Germany). Hydrochloric acid, ammonia, nitric acid and perchloric acid were obtained from BDH (Poole, Dorset, U.K.). Hydrogen peroxide was obtained from Aldrich (Gillingham-Dorset, U.K.). TSH, total and free T_4 and T_3 commercial kits were purchased from Roche Diagnostic (Mannheim, Germany) and RANSEL glutathione peroxidase kit was from RANDOX (Crumlin, U.K.)

Subjects

The study was conducted in the high schools of two towns in the East Black Sea Region. The whole student population of the schools (n=502) was screened for goiter by neck palpation, and classified as grades 1-3 according to size of the thyroid by the criteria rec-

ognized by WHO[19]. By simple random technique 45 goitrous children and 41 nongoitrous children of the same age[15-18] and gender were selected after the screening. Their plasma thyroid hormone parameters, erythrocyte AOE activities, urinary iodine (UI) concentrations and plasma and erythrocyte Se levels (P-Se, RBC-Se) were determined as described below. Dietary information, including the level and frequency of possible goitrogenic food intake including Brassicaceae family of vegetables was collected through a standard food-frequency questionnaire. The heights and weights of all subjects were also recorded.

The study was approved by the Ethical Review Board of Karadeniz Technical University, Faculty of Medicine, Trabzon. Written consent was obtained from the community school boards, as well as the approval of parents of the children involved.

Sampling

Venous blood samples were collected in tubes containing heparin in the morning after breakfast. Centrifugation was performed at 3000 rpm, plasma was separated, and erythrocyte packages, where the activities of AOE (GSHPx, SOD and CAT) and Se were measured, were prepared as recommended. Spot urine samples were collected at the same time. All samples were immediately aliquoted and stored in a freezer at –20 °C until analysis.

Analytical Measurements

Thyroid Hormones

The thyroid hormone status was determined by measuring the plasma total and free thyroxine (TT_4, FT_4), total and free triiodothyronine (TT_3, FT_3) and thyrotropin (TSH) concentrations by radioimmunoassay using commercial kits supplied by Elecysys.

Urinary iodine

UI concentrations were measured using a modification of the Sandell Kolkoff reaction as described by Dunn et al[20]. Urine was first digested with chloric acid in a heating block and iodine was determined from its catalytic reduction of ceric ammonium sulphate in the presence of arsenious acid.

GSHPx Activity

The activity of GSHPx was determined by using the RANSEL glutathione peroxidase kit, which is based on an enzymatic cycling assay as described by Paglia and Valentine[21]. The decrease in NADPH concentration, which is proportional to the enzyme, was measured spectrophotometrically by using cumene hydroperoxide as the substrate. The specific activity was expressed in units per gram of haemoglobin. One enzyme unit was defined as the amount of enzyme that transforms 1 µmol of NADPH to NADP per minute at 37°C.

SOD Activity

The activity of SOD (CuZn SOD) was measured according to the method of Sun et al[22]. The assay involves the inhibition of nitrobluetetrazolium reduction with the xanthine-xanthine oxidase system. The specific activity was expressed as units per mg haemoglobin. One unit was defined as the amount of enzyme required to inhibit the rate of reaction by 50 %.

CAT Activity

CAT activity was determined by the method of Aebi[23] so that the decrease in the absorbance of hydrogen peroxide was monitored at 240 nm in a spectrophotometer. The specific activity was expressed as K per g haemoglobin (K: rate constant of the first order reaction as defined by Aebi[23]).

Selenium

P-Se and RBC-Se levels were measured by a spectrofluorometric method as described by Lalonde et al[24]. Calibration of the spectrofluorometric method and the instrument; quality assessment of the analytical data; verification of precision, accuracy and sensitivity were accomplished by the direct use of Standard Reference Material (SRM) (Seronorm by Nycomed, Oslo, Norway). Results were in good agreement with certified values. Limit of detection of the method was 0.7 µg/L; within-day precision was 2.4% CV, between- day precision was 2.6%, and recovery was determined to be 98.10 ± 0.04%.

Statistical Analysis

Parameters showing a gaussian distribution were analysed with ANOVA followed by Duncan test. For parameters with nongaussian distribution (UI, TSH) Kruskal-Wallis and Mann-Whitney U tests were used. Student's t-test or Mann-Whitney U test (UI, TSH) were used when the group number was two. Statistical analysis was performed using SPSS Software version 9.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The prevalence of goiter was found to be (180/502) 35.9 % in the area of survey among the high school students. There was no significant difference among the groups studied with respect to physical development; and the data collected by a standard food-frequency questionnaire did not show any significant difference with respect to dietary habits, including highly consumed Brassica oleracea var. acephala. The haemoglobin levels of the subjects were within the reference limits. The overall goitrous group consisted of grade I, II and III subjects with a ratio of ~30% each.

As seen in Table I, 58 % of the goiter group and 32 % of the control subjects were moderately deficient. Only 22% of the control group had a normal iodine intake.

When the thyroid parameters, AOE activities, UI and P-Se concentrations of the three different stages of goiter groups were compared no difference was ob-

Table I. Distribution of the Study Group According to Severity of Iodine Deficiency

GROUPS	GOITER		CONTROL	
	n	%	n	%
Normal (UI≥10µg/dl)	–	–	9	22
Mildly Deficient (UI:5-9.9µg/dl)	–	–	16	39
Moderately Deficient (UI:2-4.9 µg/dl)	26	58	16	39
Severely Deficient (UI<2 µg/dl)	19	42	–	–
TOTAL	45	100	41	100

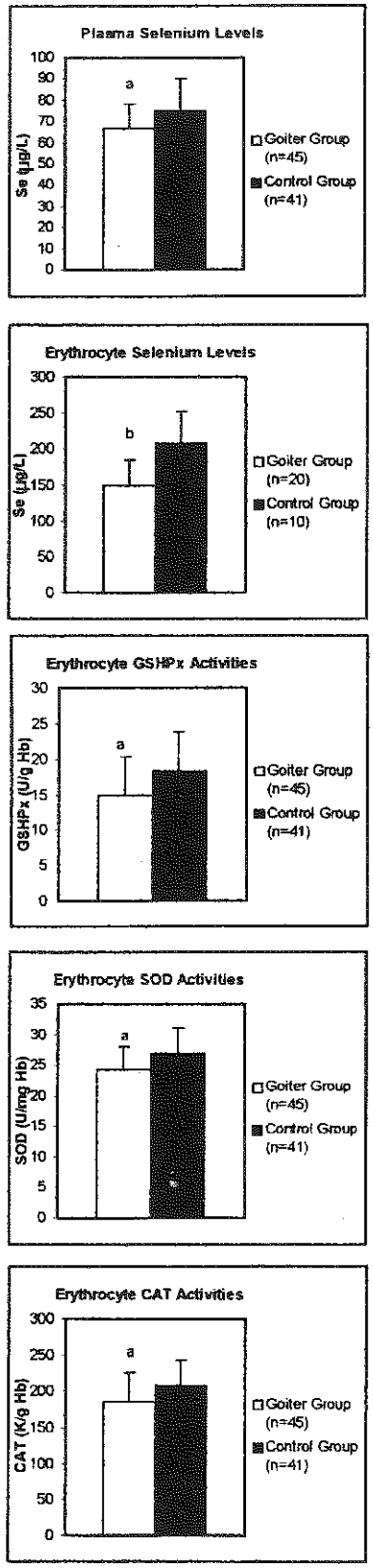

Figure 1. Selenium status and antioxidant enzyme activities in goitrous and healthy control groups. Results are mean ± SD, a $p<0.01$, b $p<0.001$

Table 2. Thyroid Hormone Concentrations, AOE Activities, UI and Plasma Se Levels Measured in Different Stages of Goiter in Goitrous High School Children

	UI µg/dl	TSH µIU/ml	FT_4 pmol/L	TT_4 nmol/L	FT_3 pmol/L	TT_3 nmol/L	Se µg/L	GSHPx U/gHb	SOD U/mgHb	CAT K/gHb
Stage I (n=12)	2.7±1.0	1.52±0.7	14.0±2.8	95.0±20.2	5.2±1.5	2.4±0.6	63.3±12.0	15.0±5.5	25.1±3.1	195.8±50.4
Stage II (n=15)	2.6±1.4	2.05±1.8	14.2±3.0	103.2±22.2	5.4±1.2	2.5±0.4	70.9±11.2	13.4±5.3	25.6±4.4	193.9±38.4
Stage III (n=18)	2.2±1.1	2.12±1.5	13.2±2.6	101.8±23.1	4.8±1.4	2.5±0.5	66.7±8.9	15.5±5.2	23.4±3.4	172.1±23.2

Values are given as mean ± SD.
Data which were normally distributed are analysed with ANOVA followed by Duncan test.
Parameters not normally distributed (UI, TSH) are compared by Kruskal-Wallis and Mann-Whitney U tests.

Table 3. Thyroid Hormone Concentrations and UI Levels Measured in Goitrous and Non-Goitrous Children

GROUPS	UI µg/dl	TSH µIU/ml	FT_4 pmol/L	TT_4 nmol/L	FT_3 pmol/L	TT_3 nmol/L
Goiter Group (n=45)	2.4±1.2[a]	2.0±1.5	13.8±2.8[a]	100.5±21.8[a]	5.2±1.2	2.5±0.5
Control Group (n=41)	8.3±7.2	2.0±1.2	16.2±3.0	119.3±22.2	5.4±1.0	2.3±0.4

Values are given as mean ± SD
Data which were normally distributed are analysed with Student's t-test.
Parameters not normally distributed (UI, TSH) are compared by Mann-Whitney U test.
[a] $p<0.001$

served for any parameter (Table II). However, as seen in Table III and Figure I, mean values of plasma TT_4, FT_4, P-Se, RBC-Se (measured in 20 goitrous and 10 non- goitrous children), UI concentrations and the activities of AOE's of the overall goiter group were significantly lower than the control group.

DISCUSSION

The results of this study indicated that there is a tendency to lower AOE' s activities and lower Se status in goitrous high school children coming from the East Black Sea Region compared to non-goitrous children at the same age and gender and from the same environment. As a common feature of endemic goiter, the goitrous group had lower thyroxin levels and UI concentrations, but TSH and T_3 levels did not differ from the control group. Therefore, all children with goiter were euthyroid. When comparison of different stages of goiter was made none of the parameters of thyroid hormones, and AOE or Se status differed from each other.

These results might be due to the possibility that the occurrence and development of goiter but not the progression is related to a lower AOE and Se status, or iodine deficiency and pathogenesis of goiter development introduces an oxidative stress and a depressing effect on the AOE and Se status. It is well-known that consistently lower iodine intake (lower than 50 µg/ day) usually results in goiter[25], but not all people with iodine deficiency develop goiter[26]. The first possibility, particularly lower AOE activities, might, therefore, be a contributory factor in goiter development. In fact, Hatemi and Urgancıoğlu[7] the investigators who have accomplished the most comprehensive epidemiological study on endemic goiter in Turkey, when reporting that all geographical regions of Turkey may be considered as having endemic goiter and the problem is more prominent in the Black Sea Region, added to their conclusion that the high prevalence in the Black Sea region suggests the role of hereditary familiar factors[7].

The second possibility which may also be connected

to the first one, might be related to the fact that in iodine deficient thyroid glands, the highly stimulated cells generate, under TSH control, large amounts of H_2O_2 as a cofactor to synthesise thyroid hormones[27]. H_2O_2 is toxic to the cell and can be the precursor of highly reactive peroxides[27,28]. The thyroid cell is protected by GSHPx enzymes, some of which are selenoenzymes, CAT and SOD. Hence, oxidative stress stimulated by iodine deficiency and lower GSHPx activity introduced by Se deficiency may cause degenerative alterations in the thyroid tissue as demonstrated by several investigators in various animal models[29-31]. In addition, Sugiwara et al[32] reported that there is a deficiency of SOD in endemic goiter tissue, which may cause more prolonged exposure to oxygen free radicals and possibly contributes to the degenerative changes of the thyroid tissue. However, since we have no direct evidence of thyroid status in this regard, no further conclusions can be made. Nevertheless, our results indicate that the goitrous children we evaluated have a different AOE status from the non- goitrous control children from the same environment and of the same age and gender. Furthermore, it seems that Se status of overall study group is not sufficiently high. In fact, when we estimated the daily intake of Se, from P-Se values, using an algorithm given by Longnecker et al[33], we found 42.2 ± 9.3 µg/ day Se (23.0- 59.6) for goitrous children and 49.2 ±13.2 µg/ day Se (22.6-83.8) for non-goitrous control children ($p<0.05$). This shows that not only goitrous children, but the overall study group have borderline if not deficient, Se intakes. When the RDA value of 50 µg/day Se for children aged 15-18 years is considered, it is revealed that more than 50 % of the overall group has inadequate daily Se intake. From these results it can be concluded that an inadequate dietary supply of Se is accompanying the well- known iodine deficiency in the area of our survey. Whereas it has been reported previously that iodine deficiency may produce an oxidant stress on the thyroid gland of rats increasing the requirement for Se to maintain selenoenzyme activity, and when dietary supplies of Se are limiting, to overcome the overall lack of micronutrient, compensatory mechanisms are needed to work efficiently[34]. Therefore, the balance between the two essential trace elements is very important for the homeostasis and regulation of thyroid hormone synthesis and functions.

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