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Status of Selenium and Antioxidant Enzymes of Goitrous Children Is Lower Than Healthy Controls and Nongoitrous Children with High Iodine Deficiency

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ABSTRACT

In order to investigate the relations of iodine deficiency and/or goiter with selenium (Se) and antioxidant enzyme (AOE) status, we determined the relevant parameters of goitrous high school children living in an endemic goiter area of Turkey. Subjects were selected by a simple random sampling technique after screening the whole population of the high schools of two towns by neck palpation. The results of the goitrous group (n = 48, aged 15–18 yr) were compared with those of nongoitrous control children (n = 49) from the same populations, and with an outside control group (n = 24) from a lower-goiter-prevalence area. The overall prevalence of goiter was 39.6% in the high school population of the area. Activities of erythrocyte AOE (glutathion peroxidase, catalase, and superoxide dismutase) and concentrations of plasma and erythrocyte Se and urinary iodine were found to be significantly lower in goitrous children than both in-region and out-region of the control groups. When the whole study group was reclassified according to the severity of iodine deficiency, it was found that the AOE and Se status of those control children without goiter but with high iodine deficiency was significantly higher than goitrous children, although they did not differ from nondeficient control group. This might be the result

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of the possibility that goitrous children are exposed of oxidative stress, which may introduce alterations to the antioxidant defense system and/or the antioxidant status is relatively lower in goitrous children than those children who are highly iodine-deficient but did not develop goiter. The results of this study seem to support the view that the risk of goiter development may be higher in highly iodine-deficient children with lower enzymatic antioxidant and Se status.

Index Entries: Antioxidant enzymes; goiter; iodine deficiency; selenium; thyroid hormones.

INTRODUCTION

Iodine deficiency is the principal ethiological factor in endemic goiter. The term *goiter* implies the enlargement of the thyroid gland and *endemic goiter* occurs when the prevalence of thyroid enlargement in the population of an area exceeds 10% *(1).* Goiter is the earliest and the predominant clinical sign of iodine deficiency, which, however, causes other significant health problems by inducing a variety of so-called "iodine deficiency disorders" (IDD), including increased rates of early and late pregnancy losses and perinatal and infant mortality, growth retardation, intellectual disability, and neonatal hypothyroidism and cretinism *(1–4).* It is considered as the world's single greatest cause of preventable brain damage and mental retardation *(4),* and WHO estimated earlier that a total of global population of at least 1 billion is at risk of IDD, with 20 million suffering from varying degrees of preventable brain damage *(5).* However, according to a more recent report of WHO, 2 billion people are at risk for IDD, based on the estimation of total goiter rate *(6).* Hence, at present, no less than 13% of the world's population has goiter and associated disorders, which may result in public health and socioeconomic problems of major proportions. In fact, existing data indicate that endemic goiter prevails in all geographical regions of Turkey *(7).* There is no region with less than 2% of goiter prevalence, and the East Black Sea region, which is a mountainous area with heavy rain and flooding, has long been recognized as one of the highest-prevalence-rate regions of the country *(7–9).*

On the other hand, in the last decade, it has been shown that the three isozymes of iodothyronine 5'-deiodinase catalyzing the deiodination of thyroid hormones are selenoenzymes *(10–12).* Thus, it is now well recognized that a second essential trace element, selenium (Se), is also involved in the regulation of this homeostatic and dynamic hormonal system. However, like iodine, Se is inadequately available for man and livestock in many parts of the world *(13),* and the relations between Se and thyroid function are complex and dual. On the one hand, Se is an essential part of the antioxidant defense system, being an integral component of glutathione peroxidase (GSHPx); the enzyme catalyzes the reduction of H_2O_2 and reactive peroxides and hence protects the cells from oxidative stress

(14). Therefore, when Se deficiency accompanies to severe iodine deficiency, it may increase the damage in thyroid tissue where large amounts of H_2O_2 are generated as a cofactor to synthesise thyroid hormones *(15).* On the other hand, Se, being an active component of type I deiodinase enzyme, is involved in thyroid hormone metabolism and thus may spare iodine by decreasing the catabolism of the prohormone thyroxin (T_4) when a shortage of iodine intake exists *(16).* Therefore, optimum levels of the two elements are very important.

The aim of this study was to determine thyroid hormone, Se, and antioxidant enzyme (AOE) status of goitrous children, in order to investigate the relations between them in iodine deficiency and/or goiter. Subjects were selected by a simple random technique after a screening by neck palpation in high schools of two towns in the East Black Sea region. Comparisons were made with those of nongoitrous healthy children from the same population and with an out-region control group from an area with a lower rate of goiter prevalence.

SUBJECTS AND METHODS

The study was carried out in two towns (Maçka and Şalpazari) in an area of endemic goiter in Trabzon province. The towns were approx 50 km distant from each other, approx 40–50 km away from the seacoast, and at an altitude of approx 300 m. All children (n = 502) in the high schools of the two towns were screened for goiter by inspection and palpation and were scored by two observers according to the WHO criteria *(1).* Two groups of goitrous children (Maçka-G, n = 25, girl/boy = 17/8; Şalpazari-G, n = 23, girl/boy = 12/11) and in-region healthy control groups (Maçka-C, n = 25, girl/boy: 16/9, Şalpazari-C, n = 24, girl/boy = 12/12) were selected by a simple random technique after the screening. An out-region control group (Ankara-C, n = 24, girl/boy = 12/12) was selected by the same technique among the students of a high school in Ankara (in Central Anatolia, where goiter prevalence was known to be relatively low) who were diagnosed as nongoitrous. 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 parents of the children involved.

Children were aged 15–18 yr. Dietary information, including the level and frequency of possible goitrogenic food intake, including Brassicaceae family vegetables, was collected through a standard food-frequency questionnaire. The heights and weights of all subjects were also recorded.

Venous blood samples (heparinized) were collected in the morning after breakfast. Centrifugation was performed at 800*g*, plasma was separated, and erythrocyte packages, where the activities of AOEs [GSHPx, superoxide dismutase (SOD) and catalase (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.

The thyroid hormone status was determined by measuring the plasma total and free thyroxin (TT_4, FT_4), total and free triiodothyronine (TT_3, FT_3), and thyrotropin (TSH) concentrations by radioimmunoassay using commercial kits supplied by Roche Diagnostic. Urinary iodine (UI) concentrations were measured using a modification of the Sandell–Kolkoff reaction as described by Dunn et al. *(17)* by catalytic reduction of ceric ammonium sulfate in the presence of arsenious acid. The iodine content of drinking water of the two towns was also measured by the same method.

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 *(18).* The decrease in NADPH concentration, which is proportional to the enzyme, was measured spectrofotometrically by using cumene hydroperoxide as the substrate. The specific activity was expressed in units per gram of hemoglobin. One enzyme unit was defined as the amount of enzyme that transforms 1 μmol of NADPH to NADP per minute at 37°C. The activity of SOD (CuZn SOD) was measured according to the method of Sun et al. *(19).* The assay involves the inhibition of nitrobluetetrazolium reduction with the xantine–xantine oxidase system, which is used as a superoxide generator. Specific enzyme activity was expressed as unit per milligram of hemoglobin. One unit was defined as the amount of enzyme required to inhibit the rate of reaction by 50%. CAT activity was determined by the method of Aebi *(20)*, in which the decrease in the absorbance of hydrogen peroxide was monitored at 240 nm in a spectrophotometer. The specific activity was expressed as *K* per gram of hemoglobin [*K* is rate constant of the first-order reaction as defined by Aebi *(20)*].

Plasma and erythrocyte Se (P–Se, RBC–Se) levels were measured by a spectrofluorometric method as described by Lalonde et al. *(21).* Calibration of the spectrofluorometric method and the instrument, quality assessment of the analytical data, and 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. The limit of detection of the method was 0.7 μg/L; within-day precision was 2.4% coefficient of variance, (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 analyzed by analysis of variance (ANOVA) followed by the Duncan test. For parameters with non-Gaussian distribution (UI, TSH), the Kruskal–Wallis and Mann–Whitney *U*-tests were used. The Student's *t*-test and Mann–Whitney *U*-test (UI, TSH) were used when the group number was 2. A *p*-value of 0.05 was considered significant.

Correlations between variables were evaluated by using Pearson's correlation coefficients or Spearman's rank correlation coefficients (for UI and TSH). Multiple correlation coefficients were also calculated, and relationships between two sets of variables were analyzed by canonical correlation analysis. Data processing and statistics were carried out using SPSS Software version 9.0 (SPSS Inc., Chicago, IL, USA), and Statistica version 5.0 (StatSoft, Inc., Tulsa, USA) is used for canonical correlation analysis.

RESULTS

The number of high school students in Maçka represented 18.5% of the age group of 15–18 yr of their town, whereas in Şalpazari, this figure was 64.5%. Screening by palpation showed that 54 of 250 students (21.6%) in Maçka and 126 of 252 students (50.0%) in Şalpazari high schools were goitrous. The overall prevalence was, thus, 35.9% in the area of survey.

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 hemoglobin levels of the subjects were in the reference limits. The overall goitrous group consisted of grade I, II, and III subjects with a ratio of approx 30% each.

When the thyroid parameters, AOE activities, and UI and P–Se concentrations measured in the two goiter populations were compared with those of respective in-region controls and the out-region control group, significant group differences were observed for all the parameters except TSH (Table 1). FT_4 values in both goiter groups were significantly lower than those of their respective controls and the out-region control group. However, in Maçka-G, significantly lower levels of TT_4, TT_3, and FT_3 than those of Şalpazari-G were observed, in contrast to the fact that the iodine content of drinking water was lower [2.3± 0.3 μg/L (2.0–2.5) in Maçka; 1.7± 0.4 μg/L (1.2–2.1) in Şalpazari; and 8.4± 0.6 μg/L (8.0–9.0) in Ankara in the vicinity that control children live] and the prevalence of goiter was higher in the latter group. Maçka-C also showed lower TT_4 and FT_3 values than those of Şalpazari-C, and lower TT_3 and FT_3 values even than those of Şalpazari-G. Ankara-C had the highest FT_4 value, but FT_3 and TT_3 did not differ from any other group.

Plasma Se levels in the two goiter groups were not different than each other, but only the level of Maçka-G was significantly lower than all the control values. Similarly, UI and AOE values of the two goiter groups were not different than each other, but they were lower than those of Ankara-C, whereas P–Se and AOE values in three control groups did not differ than each other.

Table 1
Thyroid Hormone Concentrations, AOE Activities, UI and Plasma Se Levels Measured in Two Towns in Goitrous Children and In-Region and Out-Region Control Groups

GROUPs	UI (μg/dl)	TSH (mU/L)	FT4 (pmol/L)	TT4 (nmol/L)	FT3 (pmol/L)	TT3 (nmol/L)	Plasma Se (μg/L)	GSHPx (U/g Hb)	SOD (U/mg Hb)	CAT (K/g Hb)
Maçka-Goiter (n=25)	3.2 ±2.8[a] (2.49)	1.8±1.3	13.3±2.4[a]	92.1±17.1[a]	4.8±1.2[a]	2.3±0.5[a]	66.0±11.3[a]	14.4±5.4[a]	24.8±3.8[a]	178.8±46.1[a]
Şalpazarı-Goiter (n=23)	2.7±1.7[a] (2.43)	2.0±1.6	14.6±3.2[ab]	109.1±22.6[b]	5.8±1.0[b]	2.6±0.4[b]	68.4±10.5[ab]	15.0±5.3[a]	24.2±3.9[a]	194.4±27.2[ab]
Maçka-Control (n=25)	7.6±7.7[b] (5.00)	1.9±1.0	15.3±2.5[bc]	111.4±18.0[b]	5.0±0.7[a]	2.2±0.4[a]	74.3±15.2[b]	18.2±5.4[b]	27.7±5.1[b]	203.5±37.9[bc]
Şalpazarı-Control (n=24)	6.6 ±6.3[b] (5.39)	2.1±1.3	16.6±3.1[c]	123.6±23.9[c]	5.7±1.0[b]	2.5±0.5[ab]	75.8±14.3[b]	18.9±5.6	26.3±3.1[ab]	213.3±27.1[bc]
Ankara Control (n=24)	8.0±4.5[c] (7.73)	1.8±0.7	18.5±3.8[d]	133.7±22.3[c]	5.3±1.0[ab]	2.4±0.5[ab]	75.3±12.8[b]	19.0±5.1[b]	27.4±2.6[b]	217.5±24.5[c]

Notes:
Values are given as mean ± SD; for UI, median values are given in parentheses.
Data that were normally distributed are analzed with ANOVA followed by the Duncan test.
Parameters not normally distributed (UI, TSH) are compared by Kruskal–Wallis and Mann-Whitney tests.
Values in columns not sharing a common superscript differ significantly, $p < 0.05$.

As an attempt to increase the statistical power of the analysis of the data, the two goiter groups and their respective controls were combined as "total goiter group" (Total-G) and total in-region control group. The comparison of the group parameters showed the presence of almost similar trends, but more obvious differences were obtained (Table 2). However, group differences for FT_3 and TT_3 were no longer available. The lowest FT_4 and TT_4 values were observed in Total-G, and the highest values were noted in out-region control group. UI and P–Se levels and AOE activities were also lower in Total-G, but there was no significant difference between the two control groups.

Considering that goiter surveys done by palpation can be inaccurate, especially when the goiters are not large, and the median UI concentration is a well-accepted indicator for iodine deficiency, a further attempt to reclassify the whole study group according to the degree of iodine deficiency that is based on UI levels as recommended by WHO *(22)* was made. In fact, as shown in Table 3, in both goitrous and nongoitrous groups, there were mildly to severely iodine-deficient individuals. Therefore, in order to better understand the effects of iodine deficiency and to assess the differences between those with goiter and without goiter, parameters of severely plus moderately iodine-deficient goitrous children (SMOID-G) were compared with those of nongoitrous control children with normal UI levels plus mildly iodine-deficient control children (NMID-C) and severely plus moderately iodine-deficient nongoitrous controls (SMOID-C) (Table 4). (Original statistical analysis was done including another control group, consisting only of nongoitrous children with normal UI, but none of the parameters differed from NMID-C; therefore, it was not included in the data shown in Table 4.) These comparisons revealed that SMOID-G had significantly lower plasma FT_4, TT_4, and Se levels and erythrocyte GSHPx, SOD, and CAT activities than those of NMID-C, but TSH, TT_3, or FT_3 values were not different. As seen in Fig. 1, the RBC–Se of goitrous children was lower as well. SMOID-C children also had significantly lower levels of TT_4 and FT_4 than NMID-C children, whereas, in contrast to SMOID-G, the P–Se level and the GSHPx, SOD, and CAT activities were not different than those of NMID-C, but significantly higher than SMOID-G.

Sex Differences

Except for lower mean values of CAT ($p < 0.05$) in females of Total-G, SMOID-G, and SMOID-C, there was no sex difference for any parameter within the goiter or control groups. When the males of the groups given in Table 4 were compared, no difference was observed for any parameter, whereas the females of SMOID-G had significantly lower AOE, Se, TT_4, and FT_4 values than SMOID-C and NMID-C, suggesting that group differences were mainly the result of female values.

When the Total-G was divided in subgroups according to plasma Se or UI levels, statistically significant differences of UI (2.4 ± 1.0 µg/dL vs 4.6

Table 2
Thyroid Hormone Concentrations, AOE Activities, UI and Plasma Se Levels Measured in Goitrous and Nongoitrous Children

GROUPS	UI (μg/dl)	TSH (mU/L)	FT_4 (pmol/L)	TT_4 (nmol/L)	FT_3 (pmol/L)	TT_3 (nmol/L)	Plasma Se (μg/L)	GSHPx (U/g Hb)	SOD (U/mg Hb)	CAT (K/g Hb)
Total Goiter Group (n=48)	2.9 ±2.3[a]	1.9±1.4	13.9±2.8[a]	100.3±21.5[a]	5.2±1.2	2.4±0.5	67.1±10.9[a]	14.7±5.3[a]	24.5±3.8[a]	186.3±38.0[a]
Total In-region Control Group (n=49)	7.1 ±7.0[b]	2.0±1.1	15.9±2.9[b]	117.3±21.8[b]	5.3±1.0	2.4±0.5	75.0±14.6[b]	18.5±5.5[b]	27.0±4.3[b]	208.3±33.1[b]
Out-region Control Group (n=24)	8.0 ±4.5[b]	1.8±0.7	18.5±3.8[c]	133.7±22.3[c]	5.3±1.0	2.4±0.5	75.3±12.8[b]	19.0±5.1[b]	27.4±2.6[b]	217.5±24.5[b]

Notes:
Values are given as mean ± SD.
Data that were normally distributed are analzed with ANOVA followed by the Duncan test.
Parameters not normally distributed (UI, TSH) are compared by Kruskal–Wallis and Mann-Whitney tests.
Values in columns not sharing a common superscript differ significantly, $p < 0.05$.

Table 3
Distribution of the Study Group According to Severity of Iodine Deficiency

IODINE DEFICIENCY DEGREE	Goitrous Children (n=48)		Non-goitrous children (n=73)	
	n	%	n	%
Severe iodine deficiency (UI< 2 μg/dl)	19	39.6	8	10.9
Moderate iodine deficiency (UI: 2- 4,9 μg/dl)	26	54.1	23	31.5
Mild iodine deficiency (UI: 5- 9,9 μg/dl)	2	4.2	24	32.9
Normal iodine levels (UI ≥ 10 μg/dl)	1	2.1	18	24.7

± 2.5 μg/dL, $p < 0.05$) or Se (62.8 ± 6.1 μg/dL vs 74.5 ± 6.6 μg/dL, $p < 0.01$) were revealed between females belonging to the lower and the upper Se (n = 7) or UI quartiles (n = 7). Such differences did not exist between the lower and upper quartiles of male children, and the difference of any parameter was not significant in the subgroups of either in-region or out-region control groups. When the respective female and male subgroups of Total-G were compared, none of them differed in UI level, but the following significant differences were observed: Between the lower UI quartiles of females and males, a significant difference of FT_4 (12.0 ± 2.5 pmol/L vs 16.5 ± 4.0 pmol/L, $p < 0.05$) existed; the upper UI quartiles of females and males differed in CAT (157.3 ± 32.2 *K*/g Hb vs 198.4 ± 15.0 *K*/g Hb, $p < 0.02$); the upper Se quartile of females (n = 7) differed from males (n = 5) in Se (77.4 ± 5.6 μg/L vs 85.4 ± 4.6 μg/L, $p < 0.05$) and FT_3 (4.9 ± 0.1 pmol/L vs 6.3 ± 0.9 pmol/L, $p < 0.01$).

When the whole study population (n = 121) was divided in subgroups as described earlier, similar differences of Se (67.1± 10.7 μg/L vs 79.2 ± 8.3 μg/L, $p < 0.01$) and UI (4.8 ± 6.6 μg/dl vs 10.4 ± 8.4 μg/dl, $p < 0.01$) were observed between the respective lower (n = 17) and upper quartiles (n = 17) of females, but not males. In females, the following significant differences were also observed: FT_4 was significantly low in the lower Se quartile than that of the upper Se quartile (14.4 ± 2.4 pmol/L vs 16.5 ± 3.3 pmol/L, $p < 0.05$); differences in SOD (24.1± 3.5 U/mg Hb vs 28.9 ± 4.3 U/mg Hb, $p < 0.01$) and CAT (175.5 ± 28.8 *K*/g Hb vs 209.7 ± 37.4 *K*/g Hb, $p < 0.01$) were observed between the lower and upper UI quartiles of females; and as expected, TT_4 and FT_4 values were significantly low in lower UI quartiles. However, when lower UI quartile of females were compared to lower UI quartile of males, significantly low levels of FT_4 (13.1 ± 2.4 pmol/L vs 15.7 ± 2.9 pmol/L, $p < 0.02$) and FT_3 (4.8 ± 1.1 pmol/L vs 5.8 ± 1.4 pmol/L, $p < 0.05$) were observed.

Table 4
Comparison of Parameters in Groups Formed According to Severity of Iodine Deficiency

GROUPS	UI (μg/dl)	TSH (mU/L)	FT4 (pmol/L)	TT4 (nmol/L)	FT3 (pmol/L)	TT3 (nmol/L)	Plasma Se (μg/L)	GSHPx (U/g Hb)	SOD (U/mg Hb)	CAT (K/g Hb)
Severely & moderately UI deficient goitre **(SMOID-G)** (UI < 5 μg/dl) (n =45)	2.4±1.2[a] (0.43-4.99)	2.0±1.5 (0.6-7.9)	13.8±2.8[a] (8.4-20.8)	100.5±21.8[a] (53.5-150.3)	5.2±1.2 (3.4-7.9)	2.5±0.5 (1.6-3.7)	67.0±11.1[a] (47.5-89.9)	14.9 ± 5.4[a] (3.0-25.1)	24.3 ± 3.8[a] (17.3-34.0)	186.6 ± 39.0[a] (102.0-172.3)
Severely & moderately UI deficient control **(SMOID-C)** (UI < 5 μg/dl) (n =31)	2.8±1.0[a] (1.32±4.99)	1.8±0.8 (0.7-4.1)	15.2±2.7[a] (8.9-21.1)	113.6±23.8[b] (67.1-166.5)	5.2±0.9 (3.4-6.7)	2.3±0.4 (1.6-3.5)	75.5±14.4[b] (42.0-98.8)	18.9±5.0[b] (8.0-28.5)	27.0±4.3[b] (18.6-40.1)	215.7±30.8[b] (165.0-284.6)
Normal & mildly UI deficient control **(NMID-C)** (UI ≥ 5 μg/dl) (n =42)	10.9±6.4[b] (5.07-31.60)	2.1±1.1 (0.7-5.9)	17.9±3.4[b] (11.6-26.0)	129.8±20.0[a] (91.8-172.6)	5.4±1.1 (3.7-8.0)	2.4±0.5 (1.6-3.5)	74.8±13.8[b] (48.6-114.4)	18.5 ± 5.6[b] (5.1-29.1)	27.3 ± 3.4[b] (20.2-34.5)	207.9 ± 30.5[b] (136.1-281.2)

Notes:
Values are given as mean ± SD.
Data that were normally distributed are analzed with ANOVA followed by the Duncan test.
Parameters not normally distributed (UI, TSH) are compared by Kruskal–Wallis and Mann-Whitney tests.
Values in columns not sharing a common superscript differ significantly, $p < 0.05$.

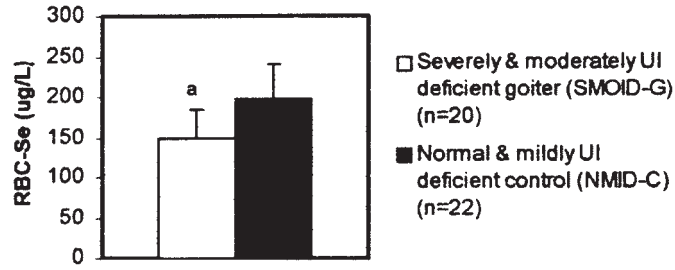

Fig. 1 Erythrocyte selenium (RBC–Se) concentrations in goitrous and control children; [a] $p < 0.05$.

Correlation Studies

Either Pearson's or Spearmen's correlation coefficients were determined for all parameters in various classification groups (Table 5). Significant correlations existed between TSH and FT_4 [($r = -(0.32–0.55)$], and TT_4 and TT_3 ($r = 0.29–0.62$) in most of the groups analyzed. In addition, the following correlations were noted reflecting the relations between thyroid parameters or iodine status and Se level or AOE activities: In SMOID-G, CAT was correlated with TT_4 ($r = 0.34$) and with SOD ($r = 0.44$); in the "severely deficient goiter group" (SID-G), SOD and TT_3 ($r = –0.59$) and CAT and TT_4 ($r = 0.64$) were correlated; in "all severely deficient children" (All-SID) correlations of UI and Se ($r = 0.42$), UI and GSHPx ($r = 0.52$), UI and CAT ($r = 0.38$), CAT and TT_4 ($r = 0.64$), and CAT and FT_4 ($r = 0.50$) were observed.

Multiple correlation analysis of the data revealed that AOEs were correlated with TT_4 in the SMOID-G, SID-G, and All-SID groups ($r = 0.44$, 0.78, and 0.66, respectively), with TT_3 in SID-G ($r = 0.66$), and with UI in All-SID ($r = 0.58$). Se plus iodine showed correlation with only TT_3 in the normal control group ($r = 0.58$) and with GSHPx in All-SID ($r = 0.49$). However, when canonical analysis was performed, a high correlation ($r = 0.90$, $p < 0.05$) was found to exist between Se plus iodine and AOEs in the All-SID group (Table 6).

DISCUSSION

Endemic goiter prevails in all regions of Turkey. The high prevalence is attributed to the low level of iodine content of drinking water, soil, and food; the overall rate is reported to be 30% *(7,23).* There is no region having less than 2% prevalence *(7),* and the highest rate is encountered in the Black Sea region, reaching over 50% particularly in high-altitude mountain villages *(7–9,24).* Iodization of salt was started in Turkey, in 1968, but the level of utilization has been far from sufficient to improve the iodine sta-

Table 5
Pearson's or Spearman's Correlation Coefficients for the Parameters of the Various Classification Groups

	Groups classified by UI levels and presence of goiter								Groups classified by UI levels			
	CONTROL GROUP				GOITER GROUP							
	Total (n=73)	Normal (n=18)	Normal & Mildly Deficient (n=42)	Severely & Moder. Deficient (n=31)	Total (n=48)	Severely & Moder. Deficient (n=45)	Severely Deficient (n=19)	Moderat. Deficient (n=26)	All-Normal (n=19)	All-Mildly Deficient (n=26)	All-Moderat. Deficient (n=49)	All-Severely Deficient (n=27)
1. TSH& FT4	-	-0.49*		-0.55•	-0.32*	-0.37**	-	-0.44*	-	-	-.38***	-
3. TSH & TT4	-	-			-	-	-	-	-	-	-	-
4. FT4 & FT3	0.27**	-		0.45***	0.31*	-	0.46*	-	-	-	0.30*	-
5. FT4 & TT3	0.27*	-	0.33*			-		-	-	-	-	-
6. TT4 & TT3	0.44•	0.59***	0.47***	0.40*	0.32*	0.34*	0.62***	-	0.60***	-	0.29*	0.43*
7. TT4 & FT3	0.31***	-		0.45***	-	-	-	-	-	-	0.39***	-
8. UI & FT4	0.33***	-			-	-	-	-	-	-	-	-
9. UI & TT4	0.28**	-			-	-	-	-	-	-	-	-
10.UI & CAT	-	-			-	-	-	-	-	-	-	0.38*
11.UI & Se	-	-			-	-	-	0.49**	-	-	0.30*	0.42*
12.UI & GPx	-	-	-		-	-	-	-	-	-	-	0.52***
13.GPx & Se	-	-			-	-	-	-	0.49*	-	-	-
14.SOD & TT3	-	-			-	-	-.59***	-	-	-	-	-0.39*
15.SOD & TT4	-	-			-	-	-	-	-	-0.48**	-	-
16.SOD & CAT	-	-			0.40***	0.44***	-	0.48**	-	-	0.55•	-
17.SOD & GPx	-	0.49*			-	-	-	-	0.50*	-	-	-
18.CAT & FT4	-	-			-	-	-	-	-	-	-	0.50**
19.CAT& TT4	-	-			0.29*	0.34*	0.64***	-	-	-	-	0.64•

Note:
Only statistically significant associations and correlation coefficients are shown.
*$p<0.05$, **$p<0.02$, ***$p<0.01$, •$p<0.001$.

Table 6
Multiple Correlation Coefficients (R) and Canonical Correlation Coefficients (r_c) in Various Groups

	Multiple Correlation Coefficients (R) Independent variables		Canonical Correlation Coefficients (r_c)
GROUP	**Se+ UI**	**AOE's**	**Se+ UI**
Normal Control (n=18)	TT3 (0.58) *		
Severely&Moderat. Deficient Goiter **(SMOID-G)** (n=45)		TT4 (0.44)*	
Severely Deficient Goiter **(SID-G)** (n=19)		TT4 (0.78) *** TT3 (0.66) **	
All Severely Deficient **(All-SID)** (n=27)	GSHPx (0.49) *	TT4 (0.66) ** UI (0.58) *	AOE's (0.90)*

Note:
Only statistically significant associations are shown and correlation coefficients are given in parentheses.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

tus. Recently, the Ministry of Health introduced a national salt iodization program to reinforce the nationwide production and consumption. On the other hand, existing data show that the Se status of Turkey is, generally, not deficient *(25–28).* Average P–Se levels determined in 122 subjects (aged 16–61 yr) from four main regions were in a range 58–75 µg/L *(25).* In an age group similar to the present study, living in Ankara, serum Se levels were measured as 80.0± 17.2 µg/L *(26).* However, for an isolated rural area, as low as 23 µg/d Se intake was reported *(28).*

The goiter prevalence observed in this survey was in accordance with above-mentioned results. All goitrous children were in the state of euthyroid: TSH levels did not differ, but were in agreement with the general feature of endemic goiter, FT_4 and TT_4 concentrations were lower than those of in-region and out-region controls. However, there were significant differences with respect to AOE activities and Se levels. These differences were more obvious when the groups were reclassified according to the severity of iodine deficiency. Hence, SMOID-G children had significantly lower mean values of both P–Se and RBC–Se levels and activities of AOEs, in addition to the lower FT_4 and TT_4 concentrations. This classification was necessary, because the diagnosis of goiter had been done by inspection and palpation, which can be inaccurate, especially in children and when the

goiter is not large, but is still used almost as a routine screening procedure in endemic areas of developing countries. Furthermore, because Turkey is considered a region of endemic goiter, cases of iodine deficiency cannot be excluded in advance in any group investigated. Therefore, in studies of this nature, classification of both goitrous and nongoitrous groups according to UI concentration will provide more accurate information, and comparisons of better characterized groups will, therefore, be more meaningful.

Our results clearly showed that SMOID-G children had relatively lower enzymatic antioxidant and Se status. This may be the result of the possibility that goitrous children are exposed to oxidative stress, which may introduce alterations on the antioxidant defense system, and/or the antioxidant status is relatively lower in goitrous children in contrast to their counterparts who are highly iodine deficient but did not develop goiter. The first possibility might be related to the fact that in iodine-deficient thyroid glands, the highly stimulated cells synthesize, under TSH control, an increased amount of H_2O_2, the electron acceptor for thyroperoxidase reaction in the synthesis of thyroid hormones *(29).* However, H_2O_2 is toxic to the cell and can be the precursor of highly reactive peroxides *(30).* The thyroid cell, as other cells, is protected by several enzymes of GSHPx family, some of which are selenoenzymes, and SOD and CAT. The thyroid gland contains mechanisms for superoxide radical ($O_2^{\cdot -}$) production, such as xanthine oxidase *(31)* and NADPH oxidase *(32).* Dismutation of $O_2^{\cdot -}$ by SOD produces H_2O_2. The net effect of SOD is to decrease the steady state level of $O_2^{\cdot -}$, and GSHPx and CAT do the same for H_2O_2 *(33).* Sugawara et al. *(34)* reported that endemic goiter tissue contains significantly lower SOD activity and concentration compared to normal thyroid tissue, and the SOD protein does not differ from the normal. They found the same lower SOD activity in patients previously treated with iodized oil injection and hence concluded that there is a deficiency of SOD in endemic goiter tissue, which may cause more prolonged exposure to oxygen free radicals possibly contributing the degenerative changes of the tissue. On the other hand, Se deficiency leads to a GSHPx deficit and, consequently, to a lack of H_2O_2 reduction. Se deficiency coupled to iodine deficiency through an increased availability of H_2O_2 and a decrease in thyroid GSHPx activity might be responsible for a greater exposure of the stimulated thyroid gland to H_2O_2 and, in turn, highly reactive peroxides *(15).* In fact, Contempre et al. *(35)* showed that Se deficiency increases necrosis, induces fibrosis, and impedes compensatory epithelial cell proliferation in the same conditions in rats. Although Se deficiency was marginal in our goitrous group, it had effects on the antioxidant status of the individuals, as evidenced by low GSHPx activities of erythrocytes. Nevertheless, we did not examine directly the thyroid gland status; therefore, we cannot discuss these mechanisms any further. However, we assessed the second possibility by comparing SMOID-G with SMOID-C and NMID-C. These comparisons revealed that the status of AOE and Se in those control chil-

dren without goiter but with high iodine deficiency was significantly higher than goitrous children, although they did not differ from those of NMID-C children. In addition, their FT_4 and TT_4 values were lower than for the latter group, in agreement with the degree of their iodine deficiency.

These findings seem to support the hypothesis of this study that the risk of goiter development is higher in those highly iodine-deficient children who have lower enzymatic antioxidant and Se status. However, in order to reach a better understanding, it is certain that large-scale studies including all of the other parameters of antioxidant status would be needed.

On the other hand, the results of a simple correlation or multiple regression and canonical analysis of our study provided supportive evidence for the association of iodine deficiency and the status of AOE and Se. In those children, either goitrous or nongoitrous but severely iodine deficient, there were high correlations ($r \geq 0.44$) among AOEs, Se plus UI, and thyroid hormone concentrations.

We have further analyzed our data in order to examine the possibilities of sex differences between parameters. In fact, Zagrodzki et al. *(36)* reported statistically significant elevations in FT_4 and TSH concentrations in relation to Se deficiency in a goitrous population from Poland. The relationship existed only for females ($n = 90$) and it was considered by the authors as suggesting a sex-linked hormonal response to concomitant Se and iodine deficiency. We have not observed such differences between lower and upper Se quartiles of either gender. However, there were important qualitative and quantitative differences between the two study groups: Our group sizes were very much lower than those of Zagrodzki et al. *(36).* However, more importantly, the mean value and the ranges of UI of that study were higher than our goiter group (and even higher than those of our in-region control group); hence, the severity of iodine deficiency was highly lower than ours, whereas their mean P-Se concentrations were lower than those of all our groups. It appears that when a high level of Se deficiency accompanies an even mild or moderate degree of iodine deficiency, it affects the thyroid hormone metabolism significantly in females but not in males. However, what degree of Se deficiency causes such effects is not clear from that study, because the mean values and ranges of Se are not given for any of the gender.

In spite of the above-mentioned limitations, we have observed an association between Se and UI levels in females, but not in males, when we analyzed our data for lower and upper quartile differences by dividing various classification groups according to either Se or UI levels. In all groups where such analysis was possible, females with a high degree of iodine deficiency had lower Se levels and vice versa. However, male children had higher thyroid hormone levels than females of various groups. In addition, lower UI quartile of all females (including both goitrous and nongoitrous children) had lower SOD and CAT activities than males. All these results, therefore,

suggested that females are more affected by the deficit of either iodine or Se. In fact, it is well established that goiter is seen more frequently in females and frequently develops in puberty, especially in girls *(37).*

In conclusion, various types of analysis of our data consistently showed that goiter at the age of puberty is associated with low enzymatic antioxidant and Se status.

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