CLINICAL STUDY

Selenium and goiter prevalence in borderline iodine sufficiency

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Abstract

Design: Selenium (Se) is required for the biosynthesis of selenocysteine-containing proteins. Several selenoenzymes, e.g. glutathione peroxidases and thioredoxin reductases, are expressed in the thyroid. Selenoenzymes of the deiodinase family regulate the levels of thyroid hormones. For clinical investigators, it is difficult to determine the role of Se in the etiology of (nodular-)goiter, because there are considerable variations of Se concentrations in different populations as reflected by dietary habits, bioavailability of Se compounds, and racial differences. Moreover, most previous clinical trials which investigated the influence of Se on thyroid volume harbored a bias due to the coexistence of severe iodine deficiency in the study populations.

Methods: Therefore, we investigated the influence of Se on thyroid volume in an area with borderline iodine sufficiency. First, we investigated randomly selected probands for urinary iodine (UI) and creatinine excretion in spot urine samples and determined the prevalence of goiter and thyroid nodules by high-resolution ultrasonography. After this, we determined urinary Se excretion (USE) in probands with goiter as well as in matched probands without goiter. Adjustments between the two compared groups were made for age, gender, history of thyroid disorders, smoking, and UI excretion.

Results: The mean USE and UI rates of all 172 probands were 24 µg Se/l or 27 µg Se/g creatinine and 96 µg I/l or 113 µg I/g creatinine indicating borderline selenium (20–200 µg/l) and iodine (100–200 µg/l) sufficiency of the study population. Probands with goiter ($n=89$) showed significantly higher USE levels than probands with normal thyroid volume ($n=83$; $P<0.05$). USE rates were not influenced by present smoking or pregnancy.

Conclusions: In our investigation, USE was not an independent risk factor for the development of goiter. The higher USE in probands with goiter in comparison with probands with normal thyroid volume is most likely a coincidence. Se does not significantly influence thyroid volume in borderline iodine sufficiency because the iodine status is most likely the more important determinant.

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Introduction

Selenium (Se) is an essential micronutrient and as a component of selenocysteine, Se is involved in the catalysis of all known selenoenzymes e.g., iodothyronine deiodinases. The iodothyronine deiodinases are required for the activation and the inactivation of the thyroid hormones T₄ and T₃ respectively (1–5). Since the human thyroid contains the highest concentration of Se in comparison with all other organs (3) even in the case of Se-deficient nutrition (6), an important role of Se for thyroid function was suggested. For clinical investigators, it is difficult to determine the role of Se in the etiology of (nodular-)goiter, because there are considerable variations of Se concentrations in different populations as reflected by dietary habits, bioavailability of Se compounds, and racial differences (4, 7–12). Moreover, previous clinical trials investigated populations with concurrent moderate to severe Se and iodine deficiency (7, 10, 13, 14) or failed to describe the

iodine status of the investigated population (15). For these reasons, heterogeneous results with no differences for thyroid volume and Se concentration have been described for 140 males with goiter in comparison with 140 healthy probands (10), whereas other investigators (7) found a significantly negative relationship between thyroid volume and Se concentration in 73 school children. Many previous clinical trials (7, 10, 13, 16) which investigated the influence of Se on thyroid volume harbored a bias due to the coexistence of severe iodine deficiency in their study populations. Since the iodine status is likely to be the key factor in the maintenance of normal thyroid volume and function (17–20), trials in severely iodine-deficient areas could not clearly evaluate the role of Se deficiency in the etiology of goiter. Clinical investigators suggested that a coexistent Se deficiency increases thyroid-stimulating hormone (TSH) levels in severely iodine-deficient areas thereby subsequently contributing to the development of goiter (13, 16). Up to now, there is only sparse data

about the influence of Se on thyroid volume in mildly iodine-deficient areas. Derumeaux *et al.* (21) found an inverse association between Se status and thyroid volume in women in France. Their study design (21) included assessment of biological specimen at inclusion but thyroid ultrasonography 1 year later. Middle Europe is an area of only borderline Se and iodine deficiency (9, 17–20, 22–24). In contrast to the previous studies which investigated how the Se status modifies the effects of severe iodine deficiency on thyroid volume, we set out to investigate how the Se status of probands with mild iodine deficiency/borderline iodine sufficiency influences thyroid volume. The design of our prospective clinical trial also aimed to investigate the role of Se as an independent factor in the etiology of (nodular-)goiter.

Materials and methods

We conducted a prospective clinical trial in 2002. The study protocol was approved by the local ethics committee. Eight hundred and five randomly selected students and employees of the University of Leipzig participated in the study. The probands were contacted by posters, university press, and the university intranet website. All participants lived in the area of Leipzig, Saxony and all volunteers were German. Probands were asked to give a spot urine sample and to fill in a questionnaire. All probands gave written informed consent.

First, we investigated randomly selected probands ($n=805$) for urinary iodine (UI) and creatinine excretion and determined the prevalence of goiter and thyroid nodules by high-resolution ultrasonography at the time of inclusion (17). After this, we determined urinary Se excretion (USe) in a subsample of probands with (nodular-)goiter ($n=89$) as well as for matched probands without goiter ($n=89$). Only a setting which eliminates other goitrogenic factors is able to investigate Se as an independent risk factor for goiter. Therefore, adjustments were done for age, gender, history of thyroid disorders, smoking, and UI excretion between the compared groups by a matching procedure. Iodine concentration was measured manually according to the protocol described by Sandell & Kolthoff, which is based on the catalytic role of iodine in the reduction of ceric ammonium sulfate in the presence of arsenious acid (25, 26). Creatinine was measured as described by Jaffe (27).

Urinary Se concentration was determined by a spectrofluorimetric assay after oxidative digestion of the sample, subsequent reduction to Se-IV, and formation of piasezenol complexes using the 2,3-diaminonaphthalene reagent (28). A commercially available standard serum and a standard urine (Sero AS, Billingstad, Norway) were used to verify the method, which has a detection limit of 7.5 µg Se/l urine. The coefficient of variation in the Se range of human urine was below 21%. Recovery of added Se in the form of selenite was between 88 and 112%. The

total recovery of absorbed selenate and selenite in the urine is about 95% (12).

Selenium and iodine excretions were correlated with creatinine excretion in every spot urine sample to minimize bias through kidney function and variable 24-h urinary volume as previously recommended (29, 30).

Thyroid volume determination was performed using a high-resolution real-time instrument (7.5 MHz). Thyroid volumes were calculated according to the spherical ellipsoid formula: volume = $\pi/6 \times$ anteroposterior diameter (cm) $\times$ width (cm) $\times$ length (cm) (31, 32). Thyroid volumes >18 ml were considered to be enlarged in adult women and thyroid volumes >25 ml were considered to be enlarged in adult men, which corresponds to the mean $\pm$ 3s.d. in iodine-sufficient populations (33) and guaranteed, that gender-specific values for goiter are above the 97th percentile of thyroid volumes found in iodine-replete control population (34). Hypo echoic thyroid glands detected by ultrasound, thus suggesting thyroid autoimmunity, were excluded from the further statistical evaluation.

Statistical analyzes were performed using SPSS software, version 10.0 (SPSS GmbH Software, Munich, Germany). Multivariable comparison between the groups was performed with ANOVA (endpoint USe). A value of $P < 0.05$ was considered statistically significant.

Six urine samples of probands with normal thyroid volume were excluded from further statistical analysis due to partial evaporation during oxidation with nitric acid and 34 probands failed to give an answer about their smoking behavior.

Results

We investigated USe in the overall study population, in the two groups with and without goiters, and in females and males separately due to the possible influence of sex on USe (19, 21, 35). The mean Se and iodine excretion rates of all 172 individuals were 24 µg Se/l or 27 µg Se/g creatinine and 96 µg I/l or 113 µg I/g creatinine indicating borderline selenium (20–200 µg/l, (36)) and iodine (100–200 µg/l, (34)) sufficiency of the study population. There was a slightly positive correlation between USe (µg Se/g creatinine) and thyroid volume in females (Pearson correlation coefficient 0.196 significant at the 0.05 level (t -test, two-tailed)), whereas we found a negative correlation between USe (µg Se/g creatinine) and thyroid volume in males (Pearson correlation coefficient -0.297 significant at the 0.05 level (t -test, two-tailed)). Probands with goiter (♂+♀) showed significantly higher USe levels than probands with normal thyroid volume due to the higher USe rates in females (88% of the study population) with goiter (Table 1). Males with goiter ($n=11$) had lower USe than males with normal thyroid volume ($n=10$) without reaching statistical significance ($P=0.124$, t -test,

Table 1 Urinary selenium and I excretions of the study population.

	Urinary selenium excretion ^a ($\mu\text{g Se/g creatinine}$)	Urinary iodine excretion ^a ($\mu\text{g I/g creatinine}$)	Thyroid volume ^a (ml)
Normal thyroid volume ($n=83$)	$23 \pm 12^*$	118 ± 87	10 ± 3
Goiter ($n=89$)	$32 \pm 28^*$	108 ± 75	29 ± 12
Males with goiter ($n=11$)	18 ± 3	77 ± 19	45 ± 18
Females with goiter ($n=78$)	$34 \pm 29^\dagger$	112 ± 79	26 ± 9
Males with normal thyroid volume ($n=10$)	22 ± 8	84 ± 27	13 ± 3
Females with normal thyroid volume ($n=73$)	$23 \pm 12^\dagger$	122 ± 96	10 ± 3
Present smoker ($n=30$) (87% females)	31 ± 27	110 ± 73	24 ± 12
Nonsmoker ($n=108$) (87% females)	26 ± 17	111 ± 84	18 ± 13

Selenium and iodine excretions were expressed as creatinine ratios in every spot urine sample to minimize bias through kidney function and variable 24-h urinary volume. Symbols (* vs *, $\dagger$ vs $\dagger$) mark the corresponding differences at the significance level $P < 0.05$ level, (t -test, two-tailed).

^aMean $\pm$ S.D.

two-tailed; Table 1). We found no differences in USe ($P > 0.05$, t -test, two-tailed) between smokers and nonsmokers or pregnant females ($n=9$) versus no pregnancy. There was no correlation between USe and age. Females showed higher UI ($P < 0.05$, t -test, two-tailed) than males (Table 1). The mean age of our probands ($n=172$) was 43 ± 11 years.

Figure 1 represents the normal distribution of USe of the study population.

We additionally compared the selenium excretion of the two groups (goiter yes versus goiter no) by ANOVA. Covariates are sex and age. UI excretion, history of thyroid disorders, and smoking behavior were not different between the compared groups at baseline in our initial matching procedure.

ANOVA revealed a significant dependence of USe on the presence of goiter (two compared groups: goiter yes versus goiter no). Multivariate ($P=0.012$) as well as

univariate ($P=0.04$) tests showed significantly higher selenium levels in probands with goiter. This was also the case for the t -tests (Table 1).

Moreover, we investigated (ANOVA) a correlation between thyroid volume and selenium excretion to evaluate the possibility to predict thyroid volume by selenium excretion of individuals of our study population.

To avoid statistical bias by analyzing correlation between USe and thyroid volume in the highly pre-selected matching group, we described below dependence of USe from thyroid volume of the individuals of our study population especially in the unselected group of probands with goiter ($n=89$).

ANOVA failed in multivariate ($P=0.28$) as well as univariate ($P=0.28$) tests to describe a dependence of USe from thyroid volume of the individuals of our overall study population, especially in the unselected group of probands with goiter, $P \geq 0.46$ (multivariate as well as univariate tests).

In conclusion, there were significant differences for USe between the compared groups: goiter versus no goiter. However, there was no possibility to predict thyroid volume by selenium excretion in individuals of our overall study population as also shown by the contrary interclass (male vs female) Pearson correlation coefficients.

Discussion and conclusions

In our population, USe was not an independent risk factor for the development of goiter (Fig. 1, Table 1). The most likely explanation for this finding is that our probands with goiter showed borderline iodine and selenium sufficiency. Concurrent Se and iodine deficiency lead to increases in thyroid volume and/or thyrotropin (7, 10, 13, 14, 37). Combined severe deficiency of selenium and iodine can result in a more significant rise of TSH and thereafter increased thyroid gland weight/volume than produced by isolated iodine deficiency as demonstrated in rats (38). Moreover, severe selenium deficiency decreases the glutathione peroxidase enzyme and increases free radicals in the thyroid

Figure 1 The X-axis represents the Se excretion ($\mu\text{g/g}$) and the Y-axis represents the total number of probands. Three specimen with higher excretion Se rates (132–188 $\mu\text{g Se/g creatinine}$) were excluded for scale reasons.

(3, 4, 15, 39). Most likely, Se status modulates thyroid volume in severe iodine deficiency areas (7, 10, 13, 14) rather than acting as an independent risk factor for goiter in (borderline) iodine-sufficient areas especially if the selenium status is borderline sufficient. In severely iodine-deficient areas, the antioxidant defence system/antioxidant status as reflected by the Se status of probands seems to be lower in subjects with goiter than in subjects without goiter (13, 14). In line with our findings, Ozata *et al.* (10) did not find a correlation between thyroid volume and selenium status (plasma) in a moderately iodine-deficient Turkish population with endemic goiter. However, there still exists some uncertainty regarding the exact definition of 'Se status'. Often used measurements of 'Se status' include serum Se, plasma glutathione peroxidase activity (GPx), erythrocyte GPx, hair or toenail Se and USe. However, the exact interrelationships have not been determined for all these parameters. Urinary and plasma Se concentrations are more likely to reflect the present Se bioavailability of the human body than hair or toenail Se content that rather represent an integral over a longer period (11, 21, 36). Regarding Se supplementation, there are significant differences in bioavailability between selenate and selenomethionine. Selenomethionine showed a strong relationship between 24 h urine and plasma Se (11). A survey in France (21) found an inverse correlation between serum Se levels and thyroid volume in 1108 females. Mean serum Se level in these women was 1.1 mol/l (≈ 87 g/l) and clearly higher than in our study population (Table 1). One possible explanation for these finding is that the mean age of the investigated French cohort ranged from 45 to 60 years and was higher than in our study group. This is an important age difference, since Se excretion increases with older age (22, 29), but also different dietary Se intake cannot be ruled out. A 24-h USe of 200 μ g is regarded as normal (36), but clearly depends on the dietary habits of the study population. The mean UI of the French population (81–98 g/l, (21)) was indicative for borderline adequate iodine nutrition as in our investigation. However, in the French study, thyroid volumes were determined 1 year after the measurement of selenium. Finally, goiter prevalence increases with increasing age (18). Therefore, different goiter prevalences in the French and in our study population are a further explanation for the different findings. The increased excretion of USe in probands with goiter (Table 1) was an unexpected finding in our study. The thyroid gland can accumulate Se even under conditions of dietary Se deficiency (4, 6, 40, 41). The specific mechanisms of selective Se retention are poorly understood (3), but recent evidence in selenium-deficient transgenic mice has demonstrated that the thyroid is to a large extent independent from circulating plasma Se levels (42). Possibly, Se uptake or retention is impaired in patients with goiter. For example, USe could increase in animals and humans lacking the Se transport protein selenoprotein P (SePP) and it has

been reported that several conditions adversely impact on SePP production (43), possibly increasing urinary Se loss. Thus, it would be interesting to determine whether goiter or other thyroid conditions impact on circulating SePP levels. An alternative explanation could be that the higher USe in probands with goiter in comparison with probands with normal thyroid volume is a coincidence and that Se does not significantly influence thyroid volume in (borderline) iodine sufficiency. The iodine status is likely to be the key factor in the maintenance of normal thyroid volume and function. Iodine sufficiency can probably prevent the contributing effects of deficiencies for Se, or copper (10). Investigations in mildly iodine-deficient areas (UI > 95 mcg/l) demonstrated high frequencies of thyroid disorders. Up to 30% of probands harbored thyroid nodules and up to 15% showed goiter (17, 44). Even minor differences in the ambient iodine supply seem to be reflected in the prevalence of thyroid abnormalities. Knudsen *et al.* (45) found a difference in goiter prevalence with 15% in mild versus 22.6% in moderately deficient areas in Denmark. Also the nodule size is increased by moderate iodine deficiency (45). Our probands with goiter showed mild iodine deficiency or rather borderline iodine sufficiency. However, the exact level of iodine intake that produces the lowest prevalence of thyroid disorders has still to be determined (17, 20, 44) and might show large interindividual variability. Some authors (46) found higher (plasma/hair-) Se levels in men than in women, whereas other investigators found no influence of gender on (plasma/tissue-) Se levels in humans (47). Therefore, the influence of sex on Se excretion needs further evaluation. Also, the influence of present smoking on Se levels is discussed controversially. Some authors (48) described lower Se levels in smokers than in nonsmokers, whereas most investigators failed to find any correlation between smoking and Se levels of probands (29, 47) as in our investigation. However, we cannot exclude an information bias due to the fact that 34 probands failed to give an answer about their smoking behavior.

In conclusion, Se did not significantly influence thyroid volume in borderline iodine sufficiency and borderline-sufficient USe was not an independent risk factor for the development of goiter. The higher USe in probands with goiter in comparison with probands with normal thyroid volume is most likely a coincidence. Therefore, the iodine status is most likely the more important determinant for thyroid volume. Under conditions of only borderline iodine deficiency, there is probably a marginal interplay between iodine and other goitrogenic factors (e.g., Se). However, it remains to be elucidated why moderate to severe Se deficiency was a risk factor for developing goiter in concurrently severely iodine-deficient areas in previous reports. Moreover, further research is needed to understand if there is a sex-specific Se excretion and how factors such as selenium interact with the genetic susceptibility to develop a goiter.

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