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Clinical studies

Trace elements status in multinodular goiter

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ABSTRACT

Importance of iodine and selenium in thyroid metabolism is well known, but the roles of other essential trace elements including copper, zinc, manganese and iron on thyroid hormone homeostasis remain unclear. The aim of this study was to investigate the status of those trace elements in benign thyroid diseases and evaluate possible links between trace element concentrations and thyroid hormones.

The study group was composed of 25 patients with multinodular goiter. Concentrations of thyroid hormones (plasma-free thyroxine, FT_4; free triiodothyronine, FT_3; and thyrotropin, TSH), selenium, copper, zinc, manganese and iron in plasma, and urinary iodine were determined. The results were compared with those of a healthy control group (n=20) with no thyroid disorder.

A mild iodine deficiency was observed in the patients with multinodular goiter whereas urinary iodine levels were in the range of "normal" values in healthy controls. All patients were euthyroid, and their thyroid hormone concentrations were not significantly different from the control group. Plasma selenium, zinc and iron concentrations did not differ from controls, while copper and manganese levels were found to be significantly higher in the patients with multinodular goiter indicating links between these trace elements and thyroid function and possibly in development of goiter. Besides iodine, there was a significant correlation between plasma copper concentration and FT_3/FT_4 ratio.

Introduction

Thyroid hormones are essential for the maintenance of normal metabolic function in living organisms, and regulate development, growth and differentiation in many systems and physiological processes. The prohormone thyroxine (T_4) is produced exclusively in the thyroid gland, and it may be converted to the metabolically active hormone 3,5,5′-triiodothyronine (T_3) by the iodothyronine 5′-deiodinases in the tissue [1,2]. Thyroid diseases occur when the thyroid gland does not supply the proper amount of hormones needed by the body. If the thyroid is overactive, it releases too much hormone resulting in hyperthyroidism. Whereas hypothyroidism is usually a primary process, in which the thyroid gland produces insufficient amounts of thyroid hormone. Goiter is the enlargement of thyroid and insufficient daily dietary iodine intake is generally considered to be the most common cause of goiter. Multinodular goiter disease is a common condition, and characterized by soft nodules that grow slowly within the thyroid [3–6].

The synthesis, metabolism and action of thyroid hormones require availability of two essential trace elements, iodine and selenium. Selenium has important roles on normal thyroid hormone metabolism and regulation as the integral component of the iodothyronine deiodinases as well as the major antioxidant enzyme glutathione peroxidase (GPx) and several other selenoproteins that participate in the protection of thyrocytes from oxidative damage [7–9]. Zinc is also considered important for normal thyroid homeostasis [10], and the potential link between zinc and thyroid metabolism is based on the hypothesis that T_3 receptors, like other nuclear receptors, include nuclear zinc-binding proteins [11]. The roles of iron, manganese and copper in the thyroid are less well defined, however, it was demonstrated that sub- or supra-optimal dietary intakes of all these elements could adversely affect thyroid hormone metabolism [10]. Well-known essential components of antioxidant defense are copper, zinc, selenium and manganese, while iron acts as an important mediator in cell injury accompanying oxidative stress. Since all have important roles in the regulation of various critical cellular processes, modification of transcription factors and receptors, or function as cofactors of critical proteins, alterations in mineral status and distribution may lead to various patho-physiological states. Their inadequate dietary intakes are also the cause of numerous diseases affecting circulatory, respiratory, nervous, endocrine, immune and reproductive systems [12].

This study was undertaken with the aim to investigate the status of essential trace elements including iodine, selenium,

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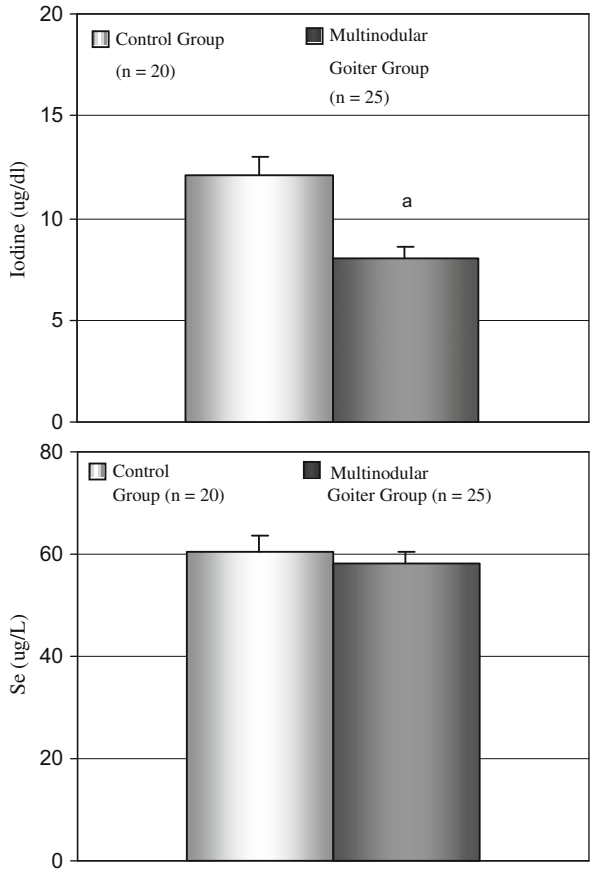

Fig. 1. Urinary iodine and plasma selenium concentrations in the patients with multinodular goiter and control group; $^{a}p < 0.05$.

manganese, copper, zinc and iron in patients with multinodular goiter and to evaluate possible links between mineral concentrations and thyroid hormone parameters

Subjects and methods

Subjects and sampling

The study group was composed of 25 patients with multinodular goiter (n=25); (21 women and 4 men), 29–65 years of age (mean 45.2 ± 9.2 years) undergoing thyroid surgery at Hacettepe University Hospital, Department of Surgery. The control group was composed of 20 healthy subjects of comparable age and sex (16 women and 4 men), with no history of thyroid disease chosen from university staff. The age range of the controls was 30–60 years with a mean age of 41.6 ± 6.1 years. Dietary information, including the level and frequency of goitrogenic food intake was collected through a standard food-frequency questionnaire. The study was approved by the Hacettepe University's Ethics Committee. All subjects voluntarily participated in the study and informed consents were obtained before blood samples were drawn

Venous blood samples were collected into heparinized trace element free tubes for the determination of thyroid hormone concentrations and mineral levels. Centrifugation was performed at 800*g* and plasma samples were separated. Spot urine samples were collected at the same time. All samples were immediately aliquoted and stored in a freezer at −20 °C until analysis.

Analysis of thyroid hormones and trace elements

The thyroid hormone status was determined by measuring plasma-free T_4 (FT_4), free T_3 (FT_3) and thyrotropin (TSH) concentrations by radioimmunoassay using commercial kits supplied by Abbott Laboratories (Illinois, USA). Urinary iodine concentrations were measured using a modification of the Sandell–Kolkoff reaction as described by Dunn et al. [13], which is based on catalytic reduction of ceric ammonium sulfate in the presence of arsenious acid.

Plasma concentrations of manganese, iron and selenium were determined by electrothermal atomic absorption spectrometry (Perkin Elmer Model 3030, with a Zeeman background correction and a HGA 600 graphite furnace). Flame atomic absorption spectrophotometry was used for the measurement of zinc and copper levels in plasma (Perkin-Elmer Model 560, Überlingen, Germany). Utak and Seronorm trace element serum samples were used for analytical quality control. The daily intake of selenium was estimated by using an algorithm given by Longnecker et al. [14] and a regression curve calculated by Haldimann et al. [15].

Statistical analysis

Results were expressed as mean ± standard error (SEM). Parameters showing a Gaussian distribution were analyzed by Student's *t*-test. Mann–Whitney *U*-test was used for the parameters with non-Gaussian distribution (selenium, iodine, manganese, FT_4, TSH). A p value of 0.05 was considered statistically significant. The correlations between variables were evaluated by using Pearson's correlation coefficients or Spearman's rank correlation coefficients (for selenium, iodine, manganese, FT_4, TSH) using SPSS Software version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Whilst the control group was found to have normal urinary iodine concentrations (12.1 ± 0.93 μg/dL);, a mild iodine deficiency, based on the criteria of the World Health Organization (WHO), was observed in patients with a mean urinary iodine concentration of 8.7 ± 0.57 μg/dL (Fig. 1). However, all patients were euthyroid, none of the thyroid hormone parameters were significantly different from those of controls, and although the mean FT_3/FT_4 ratio of patients was lower (∼30%); the difference was not statistically significant (Fig. 2).

The mean plasma selenium level of patients was not different than that of controls (Fig. 1), and the daily dietary intake of selenium calculated by two estimation methods [14,15] showed slightly lower intake values in patients (32.6 vs. 36.15 μg/kg/day), but the difference was not significant (Table 1).

Neither zinc nor iron levels of patients were different compared to the control group (Fig. 3). Plasma copper was found slightly (∼10%) but significantly elevated in the patients, however, the copper/zinc ratio did not change. There was a major difference found in plasma manganese concentrations of patients with a mean elevation of 32% compared to the control subjects (Fig. 4).

Evaluations of correlations showed that the only significant correlation existed between thyroid parameters and trace element levels of goiter group were FT3/FT4 ratio and urinary iodine (r= −0.47) and plasma copper (r=0.50).

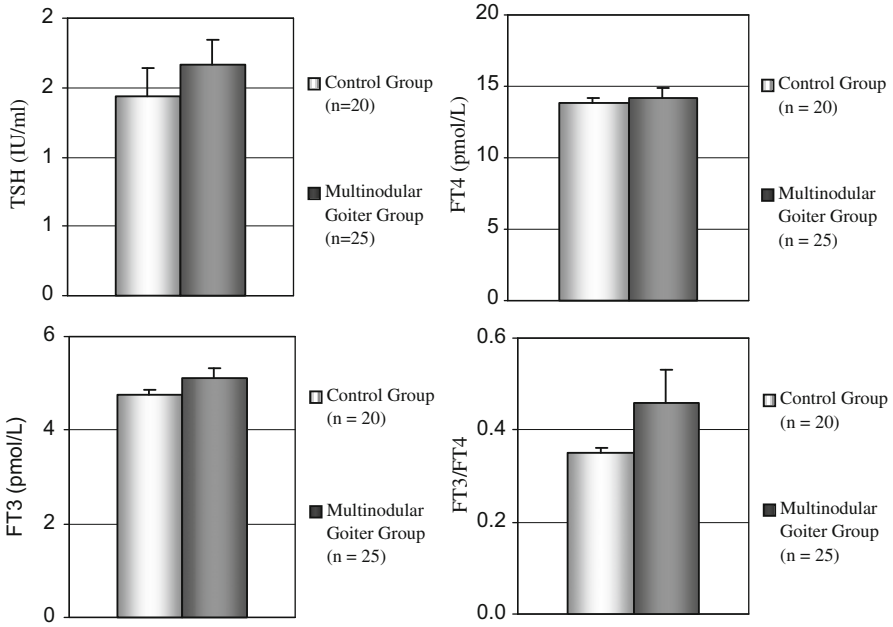

Fig. 2. Thyroid hormone parameters in the patients and control groups.

Table 1
The daily intake of selenium in the subjects.

	Daily intake[a] (μg/day)	Daily intake[b] (μg/day)
Control group (n=20)	33.2 ± 13.2	39.1 ± 18.7
Multinodular goiter group (n=25)	31.5 ± 11.4	33.6 ± 14.1

[a] Estimated as described by Longnecker et al. [14].
[b] Estimated as described by Haldimann et al. [15].

Discussion

Iodine, as a structural component, is the primary requirement for thyroid hormone synthesis and regulation. Iodine deficiency is a worldwide problem and its earliest and predominant clinical sign is goiter. Deficiency of iodine leads to diminished thyroid hormone production resulting in cell proliferation, thus, goiter development is related to the increased TSH secretion. TSH stimulates the growth and metabolic activity of thyroid follicular cells, and stimulates each cell to increase iodine uptake and thyroid hormone synthesis and secretion. This process tends to conserve iodine stores and help to maintain normal thyroid function (euthyroidism). Therefore, enlargement of the thyroid gland begins as an adaptive hyperplasia to low iodine intake [5,16–18]. In fact, our patient group was mildly iodine deficient as shown by low urinary iodine levels and although their mean FT_3/FT_4 ratio was higher than the control group, the difference was not statistically significant and patients were euthyroid.

Selenium is the second essential trace element for thyroid function as being the essential part of antioxidant defense system and due to the fact that the iodothyronine deiodinases that catalyze the deiodination of thyroid hormones are selenoenzymes. The effects of selenium are complex and dual in the thyroid hormone system. As the essential component of antioxidant defense, selenium deficiency may increase the thyroid destruction caused by iodine deficiency, but may also provide sparing of iodine by decreasing the catabolism of prohormone, thyroxin. The selenoenzyme families of glutathione peroxidases and thioredoxin reductases protect the thyrocytes from oxidative damage and selenium supplementation seems to modify the immune response in patients with autoimmune thyroiditis [19]. Available data indicate that Turkish daily dietary intake of selenium, in general, is at the borderline of deficiency, if not considered as deficient [20]. However, in the present study both

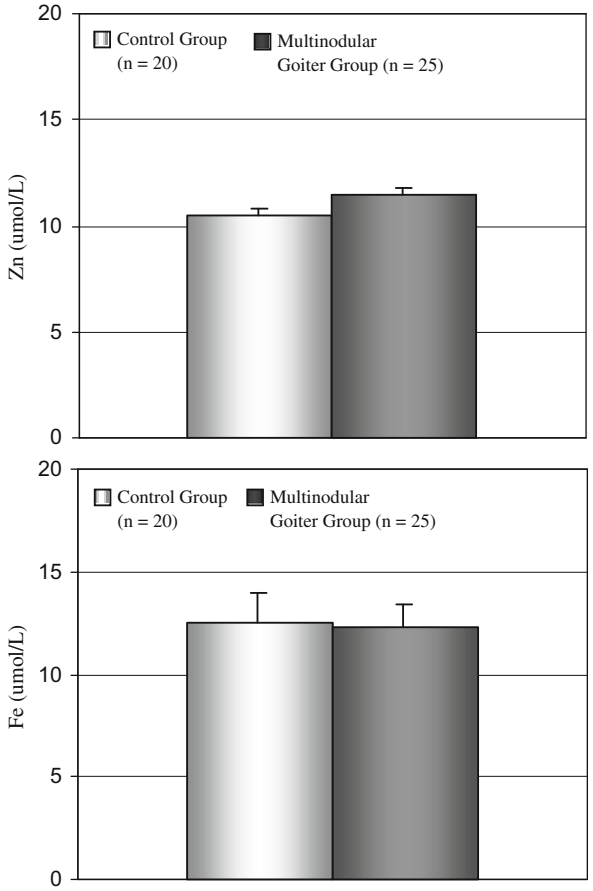

Fig. 3. Plasma zinc and iron concentrations in the patients with multinodular goiter and control group; [a]$p < 0.05$.

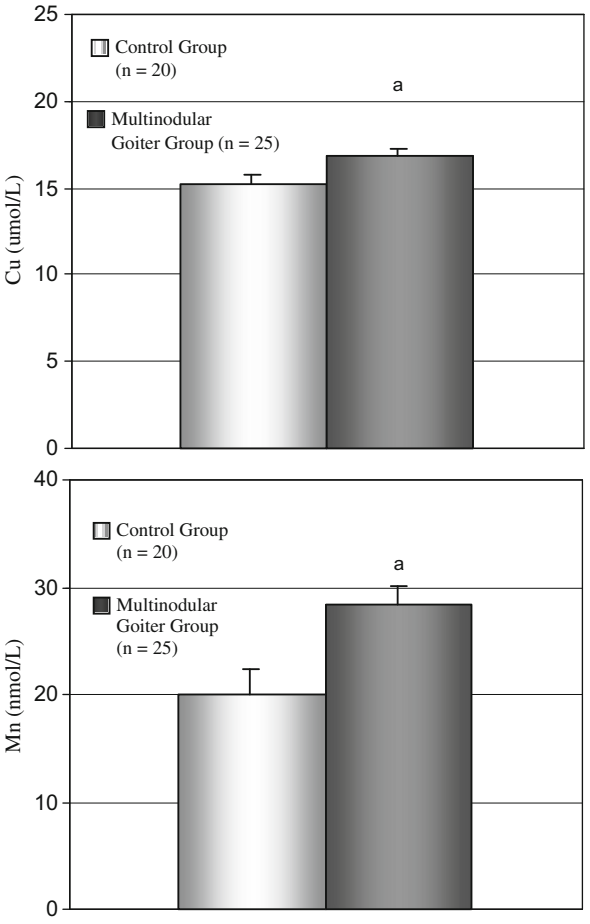

Fig. 4. Plasma copper and manganese concentrations in the patients with multinodular goiter and control group; $^{a}p < 0.05$.

patients with multinodular goiter and control subjects had similar low plasma selenium levels, which correspond to considerably lower daily dietary selenium intake than RDA recommendation of 55 μg/day [21]. It is possible that those low levels of selenium contributed to the development of goiter in iodine-deficient individuals.

Manganese is an integral component of numerous enzymes such as arginase, cholinesterase, phosphoglucomutase, pyruvate carboxylase and the intra-cellular antioxidant enzyme, superoxide dismutase (MnSOD). It plays an important role in various physiological processes, including bone mineralization, protein and energy metabolism, metabolic regulation and cellular protection from damaging free radical species. However, manganese is potentially toxic at high levels, and may cause adverse health effects including neurocognitive deficits, and manganism, which is characterized by motor deficits, damage to basal ganglia and decreased dopamine levels [22–24]. Dopamine, the major neurotransmitter, is also known as an inhibitory modulator of TSH secretion. Considering these facts, Soldin and Aschner [22] recently suggested that manganese may affect thyroid hormone homeostasis and neurodevelopmental processes because of both direct dysregulation at the level of thyroid gland and thyroid hormones, or indirectly via alterations in dopaminergic control of the thyroid gland and its hormones. They also suggested that manganese interferes with deiodinase activity and, thus, affects circulating thyroid hormone concentrations [22]. Further data indicating a link between manganese and thyroid function include the observation of significant correlation between erythrocyte manganese and plasma thyroid hormone concentrations in men [25]. In mice fed with excessive amounts of manganese, thyroid follicular hyperplasia and dilatation was observed [28] demonstrating that manganese uptake by the thyroid is controlled by serum TSH and thyroid hormone concentrations in mice [26] and is involved in goiter development [27]. Although we did not find a correlation between thyroid hormone parameters and plasma manganese concentrations, our results show significantly higher (32%) plasma concentrations of manganese in goiter patients indicating a possible link between manganese and multinodular goiter development. The increase in manganese concentrations possibly due to imbalance in dopaminergic control of thyroid gland or direct dysregulation of thyroid hormones might cause the development of thyroid disorders.

Zinc is involved in a multitude of diverse catalytic, structural and regulatory functions and several studies indicate a potential link between zinc and thyroid function [29]. Both thyroid hormones and zinc play important roles in growth and development. Thyroid hormone-binding transcription factors, which are essential for modulation of gene expression, were reported to contain zinc bound to cysteine residues [30]. However, the direct effect of dietary zinc deficiency on thyroid hormone metabolism is not clear. The results of animal studies are controversial and human data are not consistent and conclusive [31–36]. Although there was a trend toward decreased thyroid hormone concentrations in zinc deficiency, no evidence of hypothyroidism was reported [34,35]. Normal zinc concentrations were generally observed in patients with either hyperthyroidism or hypothyroidism [25,37] although in one recent study lower serum zinc levels were reported [38]. There is no conclusive data on the role of zinc in goiter development. The results of our study also did not support the role of zinc in goiter development, showing no difference between patients and controls. It is, however, possible that plasma zinc is a poor indicator of the zinc status causing inconsistent results seen in the literature. In addition, the zinc concentrations observed in this study were not in the range of deficiency [39].

Copper acts both as an antioxidant and a prooxidant in the body [40], and copper deficiency has been linked to decreased plasma T_3 levels in animals and in man [41]. However, whether these changes are the results of copper deficiency directly or nonspecific response to poor health is not clear [10]. In a previous study, we had observed 20% increase in plasma copper levels in iodine-deficient rats compared to controls, but possibly due to high biological variations the difference was not significant [42]. In the present study, plasma copper concentrations of goiter patients showed significant elevation, which correlated well with FT_3/FT_4 ratio. Our results are in agreement with those of Kazi et al. [43], however, contrary to those of Aihara et al. [25] who reported high levels of copper in only hyperthyroid patients but no alterations with other thyroid diseases.

We also examined the plasma concentrations of iron in our study groups along with other trace elements, considering that iron deficiency with or without anemia can have adverse effects on thyroid metabolism. None of the patients was anaemic, and there was no difference between patients and controls in terms of iron concentrations. However, it is possible that conclusive data cannot be obtained in this way because the measurement of plasma iron concentrations is of very little value in the investigation of iron metabolism.

In conclusion, the most important finding of this investigation was the high elevation (almost 1.5 fold of the control levels) of plasma manganese in patients with benign multinodular goiter supporting the link between manganese and thyroid hormone function. Plasma iron, selenium, and zinc levels of patients were similar to the healthy control subjects, and both patients and controls had marginal levels of selenium suggesting a predisposition to the consequences of insufficient selenium including its

thyroid-modulating effects. Copper levels of patients were also found to be significantly but slightly higher than control levels, and correlated significantly with FT_3/FT_4 ratio indicating the role of copper in the thyroid system. However, it is certain that further studies are needed to investigate other indicators of trace element status for better understanding of their contribution and/or importance in the benign thyroid diseases.

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