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Selenium Concentration in Korean Patients with Thyroid Disease: a Preliminary Report

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Background and Objectives: Selenium is an important trace element for thyroid hormone metabolism, and its deficiency can cause hypothyroidism. Serum selenium concentration is the best biomarker to reflect selenium intake and reserve, although other markers can reflect. Therefore, we preliminarily assessed serum and urine selenium concentrations in patients with thyroid disease compared to those of a healthy population. We also investigated the correlation between serum and urine selenium concentration, thyroid hormone and urinary iodine concentration (UIC). **Materials and Methods:** A total of 97 patients (32 men, 65 women, 52.4±14.7 years) with benign thyroid nodules or thyroid dysfunction who visited the Samsung Medical Center between 2008 and 2013 were included. Data for 175 healthy subjects provided by Lee et al. were used as the control. Serum T3, free T4, and thyroid stimulating hormone (TSH) were measured using commercialized RIA or IRMA kits. Serum/urine selenium and UIC were measured by inductively coupled plasma-mass spectrometry (ICP-MS). **Results:** Median serum selenium concentration was 110 μg/L (95% CI, 73-156). Median urine selenium concentration was 66.3 μg/gCr (95% CI, 28.7-283.5). Compared to 175 healthy subjects (serum 84 μg/L (95% CI, 30-144), urine 34.5 μg/gCr (95% CI, 0.8-107.2)), serum and urine selenium concentrations of patients with thyroid disease were significantly higher than those of healthy subjects ($p<0.001$). Serum selenium concentration was significantly correlated with urine selenium concentration after log transformation ($r=0.88$, $p=0.022$), but was not significantly correlated with UIC, T3, free T4 and TSH. **Conclusion:** Selenium concentrations of patients with thyroid disease were significantly higher than those of healthy subjects. Serum selenium concentration was significantly correlated with urine selenium concentration.

Key Words: Serum selenium, Urine selenium

Introduction

Understanding of the essential role of selenium in human health and thyroid disease has progressed in recent decades.[1-4] The thyroid gland contains exceptionally high selenium content. Selenium is important for biosynthesis of a small number of selenocysteine-containing selenoproteins. The thyroid gland expresses several specific selenoproteins for thyroid hormone metabolism and antioxidant properties.[2] Selenoproteins protect the thyroid gland from excess hydrogen peroxide generated from thyroid hormone sproduction.[5-7] Selenium-dependent iodothyronine deiodinases make active thyroid hormone, tri-iodothyronine (T3), from its inactive precursor, thyroxine (T4).[7,8] A randomized controlled trial of selenium supplementation in 368 euthyroid elderly patients with low

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to moderate selenium status in the United Kingdom showed no evidence of any effect of selenium on thyroid function or on the ratio of T4 to T3.[9] No clinical data on the effect of selenium intake on thyroid function have demonstrated an apparent relationship between deiodinase expression/activity and selenium status. Several studies have evaluated the effects of selenium supplements on thyroid function in different population groups of industrialized countries. Four studies showed significantly increased plasma selenium concentrations in patients taking selenium supplementation compared with control groups. However, selenium supplementation only affected thyroid hormone concentrations in two studies.[9-13] Therefore, the association between serum selenium concentration and thyroid hormone remains controversial. Several studies have suggested the immunological benefit of selenium in thyroid disease. Numerous studies have demonstrated by ultrasonography that selenium supplementation decreased anti-thyroid peroxidase (TPO) antibody titers and improved morphological changes in patients with Hashimoto's thyroiditis.[14-18] Negro et al.[19] also reported that selenium supplementation in pregnant women significantly decreased the prevalence of postpartum thyroiditis and definitive hypothyroidism. A few studies showed that selenium supplementation resulted in achieving euthyroid status more rapidly in patients with Graves' disease and appeared to have a beneficial effect on mild ophthalmopathy.[20,21] However, increased risk of type 2 diabetes mellitus and dyslipidemia has been reported following long-term, high- dose selenium supplementation.[22-24]

As mentioned in several papers, selenium have protective effect of thyroid disease. But there is no data to demonstrate comparison of selenium concentration between patient with thyroid disease and normal persons, directly. So we preliminarily assessed serum and urine selenium concentrations in patients with thyroid disease compared to those of a healthy normal population.

Serum or plasma selenium concentration is the best biomarker of selenium intake and reserve.[25] A strong correlation has been established between selenium intake and urinary selenium excretion in a wide range of populations with different selenium intake.[26] However, there are no clinical data showing a correlation between serum selenium concentration and urinary selenium excretion. Therefore, we also investigated the correlation between serum selenium concentration and urine selenium concentration, thyroid hormone levels and urinary iodine concentration (UIC).

Materials and Methods

Patients and Study Design

A total of 134 patients with benign thyroid nodules or thyroid dysfunction who visited the thyroid clinic at Samsung Medical Center between January 2008 and December 2013 were initially screened in this study. Among them, seven patients with serious infection or malignancy and 30 patients taking levothyroxine were excluded. After exclusion, 97 patients were finally enrolled in this study. Data of 175 healthy subjects provided by Lee et al.[27] were used as the control. The Institutional Review Board of Samsung Medical Center approved this study (SMC 2016-03-160).

Measurements

Serum T3 and free T4 concentrations were measured using commercialized radioimmunoassay kits (Immunotech, Marseille, France). Serum TSH concentration was measured using an immunoradiometric assay kit (Immunotech, Marseille, France). All samples were run in duplicate. The reference ranges of T3, free T4, and TSH were 1.1-2.9 nmol/L, 10-23 pmol/L, and 0.40-5.0 mU/L, respectively. Serum titers of anti-TPO antibody and anti-thyroglobulin (Tg) antibody were measured using a commercialized radioimmunoassay kit (BRAHMS AG, Hennigsdorf, Germany). Titers less than 60 U/mL were considered negative. TSH-receptor antibody titer was measured by a radioreceptor assay using the TRAK human kit (Brahms GmbH, Hennigsdorf, Germany). Values less than 1.0 mU/L were considered negative.

Serum/urine selenium and UIC were measured by inductively coupled plasma-mass spectrometry (ICP-MS) using the Agilent 7500 series instrument (Agilent

Technologies, Inc., Tokyo, Japan), which is extremely accurate for measuring UIC.[28] The intraday coefficient of variation for UIC ranged from 0.3 to 1.2%, and the interday coefficient of variation ranged from 1.4 to 3.3%. UIC was expressed as μg/L from spot urine samples.

Statistical Analysis

Statistical analysis was performed using SPSS statistics 21.0 (SPSS Inc., Chicago, IL, USA). A Mann-Whitney test and t test were used to assess continuous variables. A multiple regression test was used to examine association of serum selenium concentration with urine selenium concentration and thyroid hormone values. Selenium concentration and thyroid hormone values were not normally distributed even after log transformation. Therefore, median values were compared using the Kruskal-Wallis test for selenium concentrations (serum or urine) and thyroid hormone values. With normally distributed parameters, mean±standard deviations (SD) were compared using a non-paired t test between the two groups. P values less than 0.05 were considered statistically significant.

Results

Clinical Characteristics of Enrolled Patients and Healthy Subjects

The clinical characteristics of 97 patients with thyroid disease are provided in Table 1. Of the 97 patients, 32 (33%) were men and 65 (67%) were women. Mean age was 52 years with a range from 20 to 83 years. Of the 97 patients, 53 patients (55%) had euthyroid benign thyroid nodules, 5 patients (5%) had Graves' disease, 17 patients (18%) had hypothyroidism, and the remaining 22 patients (23%) had subclinical thyroid dysfunction (4 had subclinical thyrotoxicosis and 18 had subclinical hypothyroidism). Of the 84 patients with measured anti-TPO or anti-Tg antibodies, 51 patients (61%) had either positive antibody titer (>60 U/mL) while 9 patients (11%) had a positive TSH-receptor antibody titer (>1.0 mU/L).

Selenium (serum and urine) Concentration and Urine Iodine Concentration

The median serum selenium concentration of 97 patients with thyroid disease was 110 μg/L (95% CI, 73-156) with a range from 67 to 169 μg/L. Compared to 103 healthy subjects (median, 84 μg/L; 95% CI, 30-144) with a range from 26 to 172 μg/L, the serum selenium concentrations of patients with thyroid dis-

Table 1. Clinical characteristics of 97 patients with thyroid disease

	All	Men	Women	p value
Number	97	32	65	
Age (years)	52.4±14.7	52.6±17	52.3±13.6	NS
Selenium, serum (μg/L)	110 (73-156)	113 (67-150)	109 (73-153)	NS
Selenium, urine (μg/gCr)	66.3 (28.7-283.5)	59.7 (33.5-117)	68.0 (21.3-420.5)	NS
UIC (μg/L)	713 (31-4061)	678 (53-4000)	847 (27-4000)	NS
T3 (nmol/L)	1.6 (0.9-3.0)	1.6 (0.8-2.5)	1.6 (1.0-3.4)	NS
Free T4 (pmol/L)	15 (1-34)	15 (5-24)	14 (0.9-43)	NS
TSH (mU/L)	4.48 (0.01-80.6)	3.27 (0.01-59)	4.53 (0.01-92.92)	NS
Anti-TPO Ab (U/mL)	30 (6-9, 600)	21 (1-5, 100)	31 (6-12, 291)	NS
Anti-Tg Ab (U/mL)	73 (18-8, 315)	58 (18-4, 100)	74 (22-6, 559)	NS
TRAb (mU/L)	0.1 (0.1-10.8)	0.1 (0.1-6.5)	0.1 (0.1-16.8)	NS

Data were expressed as median (95% confidence intervals) or mean±SD
Ab: antibody, NS: not significant, Tg: thyroglobulin, TPO: thyroid peroxidase, TRAb: TSH-receptor antibody, TSH: thyrotropin, UIC: urinary iodine concentration

ease were significantly higher than those of healthy subjects ($p<0.001$). The median urine selenium concentration of 69 patients with thyroid disease was 66.3 μg/gCr (95% CI, 28.7–283.5) with a range from 14.4 to 489.0 μg/gCr. Compared to 175 healthy subjects (median, 34.5 μg/gCr; 95% CI 0.8–107.2), the urine selenium concentrations of patients with thyroid disease were also significantly higher than those of healthy subjects ($p<0.001$, Fig. 1). Men had higher serum selenium levels (113 vs. 109 μg/L) and lower urine selenium levels (59.7 vs. 68.0 μg/gCr) than women, but the differences were not significant ($p=0.099$ and $p=0.168$, respectively). When 97 patients were classified into 3 groups (hyperthyroidism, hypothyroidism, and benign thyroid nodule), and compared to 175 healthy subjects, the serum and urine selenium concentrations of patient groups were not different from each other, but they were significantly higher than those of healthy subjects ($p<0.001$, Figs. 2, 3).

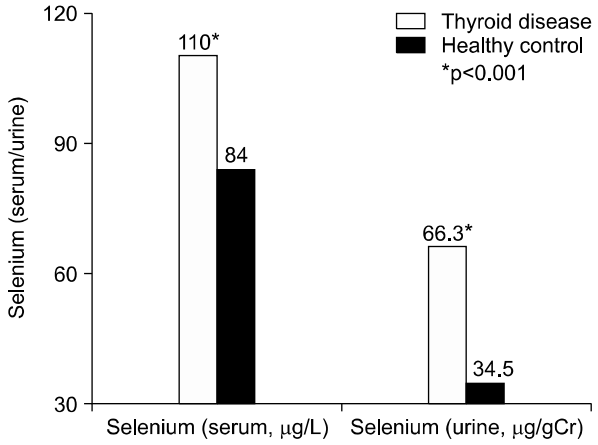

Fig. 1. Comparison of serum and urine selenium concentrations of patients with thyroid disease to those of healthy subjects.

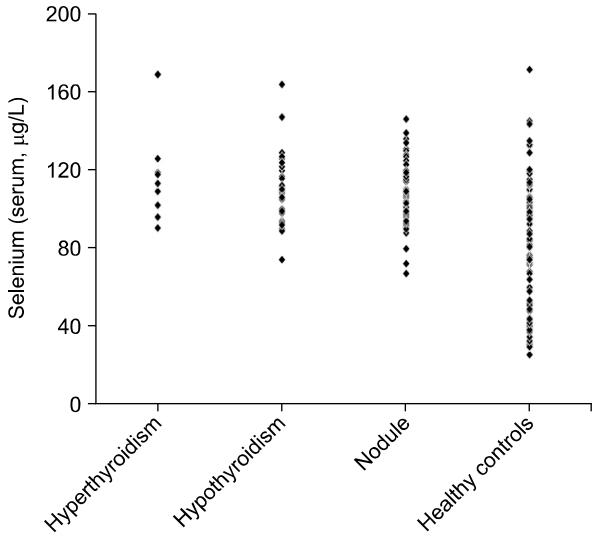

Fig. 2. Comparison of serum selenium concentrations of patients with various thyroid diseases to those of healthy subjects. Hyperthyroidism vs. controls; hypothyroidism vs. controls; nodule vs. controls, $p<0.001$.

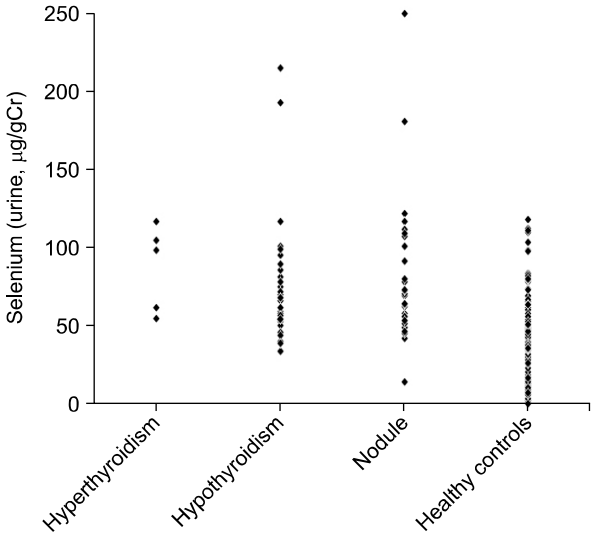

Fig. 3. Comparison of urine selenium concentrations of patients with various thyroid diseases to those of healthy subjects. Hyperthyroidism vs. controls; hypothyroidism vs. controls; nodule vs. controls, $p<0.001$.

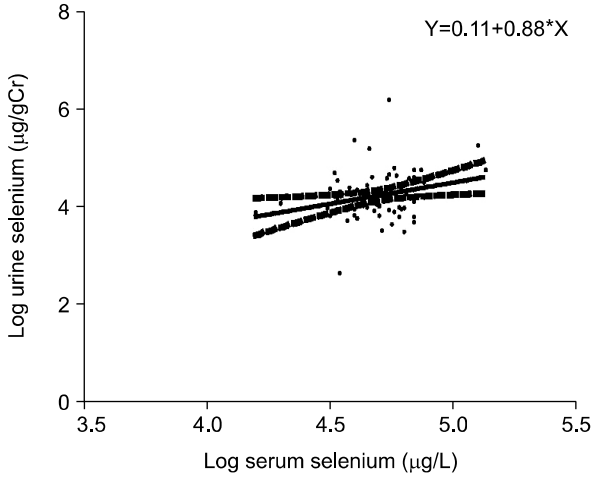

Fig. 4. Relationship between serum selenium and urine selenium after log transformation.

Correlation of Serum Selenium with Urine Selenium, Urinary Iodine, and Thyroid Hormone Levels

Correlations of serum selenium with urine selenium, urinary iodine, and thyroid hormone concentrations were evaluated using multiple regression tests. Serum selenium concentration was significantly correlated with urine selenium concentration after log transformation (r=0.88, p=0.022) but was not significantly correlated with UIC, T3, free T4 and TSH concentrations (Fig. 4).

Discussion

Selenium is an essential trace element in humans that has both antioxidant and anti-inflammatory effects.[2,3] Selenium is present in specific selenoproteins as selenocystein. The major selenoproteins include glutathione peroxidase, deiodinase, and thioredoxin reductase, which are expressed in the thyroid gland. Glutathione peroxidase plays an important role in protection against damage caused by oxygen free radicals. The main function of deiodinase is conversion of T4 into its active form, T3. Thioredoxin reductase plays a role in antioxidant processes and the regulation of some transcription factors/gene expression.[2,5-8]

The body's selenium pool body depends on its dietary intake. Meat, fish, eggs, and cereals have high selenium content.[29] A combined deficiency of selenium and iodine can cause severe hypothyroidism, called 'myxoedematous cretinism,' which is characterized by persistence of hypothyroidism despite iodine supplementation.[30] Selenium supplementation without prior iodine repletion may aggravate hypothyroidism.[31] Plasma selenium concentrations of the healthy population ranges between 60 and 120 μg/L. It is not recommended to measure plasma or serum selenium levels in routine practice because selenium deficiency is very rare. However, plasma or serum selenium measurement may be useful for screening patients who may be undernourished.[29] In this study, we compared the median serum selenium concentration of 97 patients with thyroid disease with 103 healthy subjects. Interestingly, the serum selenium concentrations of 30% of healthy subjects were less than 60 μg/L, but no patients with thyroid disease had serum selenium concentrations less than 60 μg/L. Because of an unequal distribution, the serum selenium concentrations of patients with thyroid disease were significantly higher than those of healthy subjects ($p < 0.001$).

Daily selenium dose recommendations vary by country (55-75 μg/day). Also selenium intakes are different by country because food and the nature of the soil is different. Choi et al.[32] reported that selenium intake of Korea 128.2 μg, which dose are higher than recommended dose of 50 μg/day. And this study was conducted from 2006 to 2007. Global variation in selenium status can be expressed by plasma or serum selenium concentration. Maximal selenoprotein expression is associated with plasma selenium concentration around 80 μg/L. Selenium intake is variable in many countries. Populations in some areas of China, Egypt, New Zealand, and many European countries are likely to have sub-optimal selenium levels, with average plasma or serum selenium concentrations less than 100 ng/mL. On the contrary, populations in Venezuela and Guatemala have average selenium concentrations of more than 150 μg/L.[33] High selenium intake has an anti-tumorigenic effect, but selenium doses should not exceed 400 μg/day. Extremely high selenium intake of more than 1000 μg/day can cause acute intoxication.[29,34] Therefore, it is important to assess selenium status by measuring selenium biomarkers such as plasma/serum or urine selenium concentrations. Plasma or serum selenium concentrations are the best biomarkers to reflect selenium intake and reserve. Combs et al.[35] found that plasma or serum selenium concentration increased when selenium intake increased in non-deficient Americans. Aside from two studies, thyroid hormone concentration did not increase after selenium supplementation in most studies.[9-13,36,37] In other studies, urine selenium concentration also increased when selenium intake increased.[38-41] However, no study has investigated the direct relationship between plasma/serum and urine selenium concentrations. We analyzed the relationship between serum and urine se-

lenium concentrations via linear regression after log transformation because values did not have a normal distribution. Significant positive correlation was found between serum and urine selenium concentrations (r=0.88, p=0.022). This is the first report to describe the relevance of serum and urine selenium concentrations.

In this study, there was no significant correlation between selenium and thyroid hormone concentrations. Type 1 and type 2 deiodinases convert T4 into T3, the active thyroid hormone in humans.[29,42] Deiodinase shows maximal expression at a lower concentration than other selenoproteins, such as glutathione peroxidase or selenoprotein P.[25,43] Maximal expression of glutathione peroxidase 3 may occur at lower selenium concentrations of around 66 μg/L compared to maximal selenoprotein P expression.[44,45] In this study, selenium might not affect deiodinase expression because the serum selenium concentrations of most patients with thyroid disease were more than 70 μg/L. However, additional research is needed to clarify relation between thyroid hormone and selenium concentration.

But the this study has several limitations. First, investigation period of healthy control with thyroid patients was different. The data of healthy control was surveyed in 2002. But our data of patients with thyroid disease was investigated between 2008 and 2013. Selenium concentration was strongly influenced by the selenium concentration of soils and food which may be differed over time. Second, our data did not investigate medication, consumed food and glucose contraction. Patients with thyroid disease may take medication including selenium because of concern for health. And because foods could include selenium, whether or not for the meal could influence serum and urine selenium concentration. Although our study represented that patients with thyroid disease had higher serum and urine concentration of selenium, further prospective study is needed in this area. But in spite of this limitation, there is a further significance because of first study to compare healthy control's selenium concentration with patients of thyroid disease and to describe the relevance of serum and urine selenium concentrations.

In conclusion, selenium concentrations of patients with thyroid disease were significantly higher than those of healthy subjects. Serum selenium concentration was significantly correlated with urine selenium concentration.

References

1) Duntas LH. *Selenium and the thyroid: a close-knit connection. J Clin Endocrinol Metab 2010;95(12):5180-8.*
2) Schomburg L. *Selenium, selenoproteins and the thyroid gland: interactions in health and disease. Nat Rev Endocrinol 2012; 8(3):160-71.*
3) Rayman MP. *Selenium and human health. Lancet 2012; 379(9822):1256-68.*
4) Effraimidis G, Wiersinga WM. *Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. Eur J Endocrinol 2014;170(6):R241-52.*
5) Howie AF, Walker SW, Akesson B, Arthur JR, Beckett GJ. *Thyroidal extracellular glutathione peroxidase: a potential regulator of thyroid-hormone synthesis. Biochem J 1995;308(Pt 3): 713-7.*
6) Schmutzler C, Mentrup B, Schomburg L, Hoang-Vu C, Herzog V, Kohrle J. *Selenoproteins of the thyroid gland: expression, localization and possible function of glutathione peroxidase 3. Biol Chem 2007;388(10):1053-9.*
7) Schomburg L, Kohrle J. *On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health. Mol Nutr Food Res 2008;52(11):1235-46.*
8) Salvatore D, Bartha T, Harney JW, Larsen PR. *Molecular biological and biochemical characterization of the human type 2 selenodeiodinase. Endocrinology 1996;137(8):3308-15.*
9) Rayman MP, Thompson AJ, Bekaert B, Catterick J, Galassini R, Hall E, *et al*. *Randomized controlled trial of the effect of selenium supplementation on thyroid function in the elderly in the United Kingdom. Am J Clin Nutr 2008;87(2):370-8.*
10) Olivieri O, Girelli D, Azzini M, Stanzial AM, Russo C, Ferroni M, *et al*. *Low selenium status in the elderly influences thyroid hormones. Clin Sci (Lond) 1995;89(6):637-42.*
11) Duffield AJ, Thomson CD, Hill KE, Williams S. *An estimation of selenium requirements for New Zealanders. Am J Clin Nutr 1999;70(5):896-903.*
12) Thomson CD, McLachlan SK, Grant AM, Paterson E, Lillico AJ. *The effect of selenium on thyroid status in a population with marginal selenium and iodine status. Br J Nutr 2005;94(6): 962-8.*
13) Hawkes WC, Keim NL, Diane Richter B, Gustafson MB, Gale B, Mackey BE, *et al*. *High-selenium yeast supplementation in free-living North American men: no effect on thyroid hormone metabolism or body composition. J Trace Elem Med Biol 2008;22(2):131-42.*
14) Gartner R, Gasnier BC, Dietrich JW, Krebs B, Angstwurm MW. *Selenium supplementation in patients with autoimmune*

thyroiditis decreases thyroid peroxidase antibodies concentrations. J Clin Endocrinol Metab 2002;87(4):1687-91.

15) Duntas LH, Mantzou E, Koutras DA. *Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. Eur J Endocrinol 2003;148(4):389-93.*

16) Turker O, Kumanlioglu K, Karapolat I, Dogan I. *Selenium treatment in autoimmune thyroiditis: 9-month follow-up with variable doses. J Endocrinol 2006;190(1):151-6.*

17) Mazokopakis EE, Papadakis JA, Papadomanolaki MG, Batistakis AG, Giannakopoulos TG, Protopapadakis EE, *et al*. *Effects of 12 months treatment with L-selenomethionine on serum anti-TPO levels in patients with Hashimoto's thyroiditis. Thyroid 2007;17(7):609-12.*

18) Nacamulli D, Mian C, Petricca D, Lazzarotto F, Barollo S, Pozza D, *et al*. *Influence of physiological dietary selenium supplementation on the natural course of autoimmune thyroiditis. Clin Endocrinol (Oxf) 2010;73(4):535-9.*

19) Negro R, Greco G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. *The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. J Clin Endocrinol Metab 2007;92(4): 1263-8.*

20) Bacic Vrca V, Skreb F, Cepelak I, Mayer L. *Supplementation with antioxidants in the treatment of Graves' disease: the effect on the extracellular antioxidative parameters. Acta Pharm 2004;54(2):79-89.*

21) Marcocci C, Kahaly GJ, Krassas GE, Bartalena L, Prummel M, Stahl M, *et al*. *Selenium and the course of mild Graves' orbitopathy. N Engl J Med 2011;364(20):1920-31.*

22) Bleys J, Navas-Acien A, Guallar E. *Serum selenium and diabetes in U.S. adults. Diabetes Care 2007;30(4):829-34.*

23) Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, *et al*. *Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern Med 2007;147(4):217-23.*

24) Stranges S, Laclaustra M, Ji C, Cappuccio FP, Navas-Acien A, Ordovas JM, *et al*. *Higher selenium status is associated with adverse blood lipid profile in British adults. J Nutr 2010; 140(1):81-7.*

25) Combs GF Jr. *Biomarkers of selenium status. Nutrients 2015;7(4):2209-36.*

26) Sanz Alaejos M, Diaz Romero C. *Urinary selenium concentrations. Clin Chem 1993;39(10):2040-52.*

27) Lee SY, Oh HJ, Choi YH, Kim JW, Kim SH. *Trace metal analysis using inductively coupled plasma-mass spectrometry (ICP-MS). Korean J Lab Med 2004;24(6):362-70.*

28) Lee JH, Ji OJ, Song MJ, Park HD, Kim HK, Kim SW, *et al*. *Determination of urinary iodine concentration by inductively coupled plasma-mass spectrometry in thyroid cancer patients on low-iodine diet. Korean J Lab Med 2010;30(4):351-6.*

29) Drutel A, Archambeaud F, Caron P. *Selenium and the thyroid gland: more good news for clinicians. Clin Endocrinol (Oxf) 2013;78(2):155-64.*

30) Contempre B, Duale NL, Dumont JE, Ngo B, Diplock AT, Vanderpas J. *Effect of selenium supplementation on thyroid hormone metabolism in an iodine and selenium deficient population. Clin Endocrinol (Oxf) 1992;36(6):579-83.*

31) Contempre B, Dumont JE, Ngo B, Thilly CH, Diplock AT, Vanderpas J. *Effect of selenium supplementation in hypothyroid subjects of an iodine and selenium deficient area: the possible danger of indiscriminate supplementation of iodine-deficient subjects with selenium. J Clin Endocrinol Metab 1991;73(1): 213-5.*

32) Choi MK, Kang MH, Kim MH. *The analysis of copper, selenium, and molybdenum contents in frequently consumed foods and an estimation of their daily intake in Korean adults. Biol Trace Elem Res 2009;128(2):104-17.*

33) Combs GF Jr. *Selenium in global food systems. Br J Nutr 2001;85(5):517-47.*

34) Bleys J, Navas-Acien A, Guallar E. *Selenium and diabetes: more bad news for supplements. Ann Intern Med 2007;147(4): 271-2.*

35) Combs GF Jr, Watts JC, Jackson MI, Johnson LK, Zeng H, Scheett AJ, *et al*. *Determinants of selenium status in healthy adults. Nutr J 2011;10:75.*

36) Hess SY. *The impact of common micronutrient deficiencies on iodine and thyroid metabolism: the evidence from human studies. Best Pract Res Clin Endocrinol Metab 2010;24(1):117-32.*

37) Olivieri O, Girelli D, Stanzial AM, Rossi L, Bassi A, Corrocher R. *Selenium, zinc, and thyroid hormones in healthy subjects: low T3/T4 ratio in the elderly is related to impaired selenium status. Biol Trace Elem Res 1996;51(1):31-41.*

38) Thomson CD, Robinson MF, Campbell DR, Rea HM. *Effect of prolonged supplementation with daily supplements of selenomethionine and sodium selenite on glutathione peroxidase activity in blood of New Zealand residents. Am J Clin Nutr 1982;36(1):24-31.*

39) Thavarajah D, Vandenberg A, George GN, Pickering IJ. *Chemical form of selenium in naturally selenium-rich lentils (Lens culinaris L.) from Saskatchewan. J Agric Food Chem 2007;55(18):7337-41.*

40) Pedrosa LF, Motley AK, Stevenson TD, Hill KE, Burk RF. *Fecal selenium excretion is regulated by dietary selenium intake. Biol Trace Elem Res 2012;149(3):377-81.*

41) Hargreaves MK, Liu J, Buchowski MS, Patel KA, Larson CO, Schlundt DG, *et al*. *Plasma selenium biomarkers in low income black and white americans from the southeastern United States. PLoS One 2014;9(1):e84972.*

42) Salvatore D, Tu H, Harney JW, Larsen PR. *Type 2 iodothyronine deiodinase is highly expressed in human thyroid. J Clin Invest 1996;98(4):962-8.*

43) Moreno-Reyes R, Suetens C, Mathieu F, Begaux F, Zhu D, Rivera MT, *et al*. *Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. N Engl J Med 1998;339(16):1112-20.*

44) Xia Y, Hill KE, Byrne DW, Xu J, Burk RF. *Effectiveness of selenium supplements in a low-selenium area of China. Am J Clin Nutr 2005;81(4):829-34.*

45) Neve J. *Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity. J Trace Elem Med Biol 1995;9(2):65-73.*