THE ASSOCIATION OF CIRCULATING SCLEROSTIN LEVEL WITH MARKERS OF BONE METABOLISM IN PATIENTS WITH THYROID DYSFUNCTION

VEZA CIRKULIŠUĆEG NIVOA SKLEROSTINA SA MARKERIMA METABOLIZMA KOSTIJU KOD PACIJENATA SA POREMEĆAJEM RADA ŠTITASTE ŽLEZDE

Olgica Mihaljević¹, Snežana Živančević-Simonović¹, Aleksandra Lučić-Tomić², Irena Živković³, Rajna Minić⁵, Liljana Mijatović-Teodorović⁴, Zorica Jovanović¹, Manja Andjelković⁵, Marijana Stanojević-Pirković⁵

¹University of Kragujevac, Kragujevac, Serbia, Faculty of Medical Science, Department of Pathophysiology
²University of Kragujevac, Kragujevac, Serbia, Faculty of Medical Science, Department of Internal Medicine
³Department of Scientific Research, Institute of Virology, Vaccines and Sera, Torlak, Belgrade, Serbia
⁴University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Nuclear Medicine
⁵University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Biochemistry

Summary

Background: The aim of this study was to compare serum sclerostin concentrations in patients with thyroid dysfunction with euthyroid control subjects and to assess the relationship between sclerostin and markers of bone metabolism (osteocalcin and beta-cross-laps).

Methods: The study included 30 patients with thyroid dysfunction (hypothyroidism, hyperthyroidism and subclinical hyperthyroidism) and ten euthyroid controls. Free thyroxine (FT4) was measured by radioimmunoassay, while thyroid stimulating hormone (TSH) concentration was determined immunoradiometrically. We used an ELISA kit to determine the sclerostin level. The electrochemiluminescence method was applied for measuring the bone markers.

Results: Sclerostin levels were significantly lower in hypothyroid patients (p=0.009) and significantly elevated in hyperthyroid patients (p=0.008) compared to control values. Hyperthyroid patients also had higher sclerostin than patients with subclinical hyperthyroidism (p=0.013). Sclerostin concentrations were negatively correlated with TSH levels (r=-0.746, p<0.001), but positively with FT4 (r=0.696, p<0.001). Moreover, sclerostin was positively associated with osteocalcin (r=0.605, p=0.005) and beta-

Kratak sadržaj

Uvod: Cilj ove studije bio je da uporedimo serumске концентрације склеростина код пацијената са дисфункцијом штитасте Ѽезде у односу на еутиroidне контролне испитанке, и да проценимо однос између склеростина и маркера коштаног метаболизма (остеокальцина и beta-cross-lapsа) у истој популацији.

Методе: Студијом је обухваћено 30 пацијената са дисфункцијом штитасте Ѽезде (гипотиреоидизам, хипертриоидизам и субклинички хипертриоидизам) и 10 еутиroidних контрола. Свободни тироксин (FT4) meren je radioimmunološkom metodom, dok je концентрација тиреостимулирајућег хормона (TSH) одре|dana имунорадиометријски. За меренje нивоа склеростина користили smo ELISA-test. Метода електрохемилуминисценциje премешена je за меренje концентрације коштаних маркера.

Резултати: Ниво склеростина bio je značajno niži kod bolesnika sa hipotireoizom (p=0,009) odnosno značajno viši kod bolesnika sa hipertireoizom (p=0,008) u poređenju sa vrednostima kod eutiroidних kontrolnih ispitanika. Pacijenti sa hipertireoizom su takođe imali statistički značajno viši nivo склеростина u односу на боснеке са субклиничким хипертриоизом (p=0,013). Покaza je negativna korelacija koncentracije склеростина и TSH (r=-0.746, p<0.001), односно pozitivna korelacija sa FT4 (r=0.696, p

List of abbreviations: DTC, differentiated thyroid carcinoma; GD, Graves’ disease; HT, Hashimoto’s thyroiditis
Conclusions: Serum sclerostin is significantly affected in subjects with thyroid dysfunction. Both sclerostin and thyroid status affect bone homeostasis, which is reflected through the significant correlations with osteocalcin and beta-cross-laps.

Keywords: beta-cross-laps, bone metabolism, osteocalcin, sclerostin, thyroid dysfunction

Introduction

Bone homeostasis is based on the balance between two coupled processes: bone resorption and bone formation, so any removed damaged bone must be replaced by an equal amount of healthy tissue (1). There are many influences involved in bone homeostasis, such as genetic factors, growth factors, mechanical load, hormonal and metabolic status.

Thyroid hormones are known to play a key role in the regulation of metabolism and development of different organs, including bones. They are necessary for the maintenance of bone structure and strength and the achievement of peak bone mass (2). Thyroid hormones have been shown to change the duration of the bone remodelling cycle and to alter relations between bone formation and bone resorption (3). During bone growth, thyroid hormones have mainly anabolic effects, while they predominantly exert catabolic action in adults (4).

Thyroid-stimulating hormone (TSH) can also influence skeletal development, as the expression of TSH receptors has been demonstrated in both osteoblasts and osteoclasts (5, 6). It may have a direct effect on bone tissue by inhibition of osteoclastogenesis (7). Certainly bone homeostasis is influenced by the balanced actions of thyroid hormones and TSH (5).

Sclerostin is an osteocyte-derived protein, encoded by the SOST gene. This regulator of skeletal metabolism acts as an inhibitor of bone formation (8) by stimulating apoptosis of osteocytes and osteoblasts (9). Sclerostin also reduces bone mineral content and cortical thickness, and thus makes bones less resistant (10). Previous studies indicated that serum sclerostin is negatively correlated with parathormone and cortisol but positively correlated with calcitonin (11–13). However, published data on the relationship between sclerostin and thyroid hormones are scarce and mostly relate to findings obtained in animal studies (14, 15).

In humans, sclerostin has been analyzed in hyperthyroid patients before and during antithyroid therapy (10, 16). The cross-sectional study of Engler et al. (17) established significant correlations between thyroid hormone excess and faster bone turnover, observed through an increase of bone degradation markers in overt hyperthyroidism. Regarding patients with subclinical hyperthyroidism, various studies have indicated either normal or elevated bone resorption markers (18, 19).

Likewise, available results about sclerostin and markers of bone metabolism also diverge. Some authors demonstrated that sclerostin correlates negatively with both markers of bone formation and bone resorption, while others affirmed a positive correlation only with bone resorption markers (20–22).

This study aimed to compare serum concentrations of sclerostin in patients with abnormal thyroid function (hypothyroidism, hyperthyroidism and subclinical hyperthyroidism) with values for healthy euthyroid individuals. Possible mutual relationships of serum sclerostin levels and thyroid status, and both sclerostin and thyroid status with bone metabolism markers (osteocalcin and β-cross-laps) were analyzed in all study subjects.

Materials and Methods

Study population

The study population included 30 patients with thyroid dysfunction: 23 (79.3%) females and 7 (20.7%) males of mean age 48.31±18.23 yrs. Patients with thyroid gland disorders were divided into three groups: 10 patients with thyroid gland disorders were divided into three groups: 10 patients with thyroiditis (HT), 10 patients with hyperthyroidism due to Graves’ disease (GD) and 10 patients with subclinical hyperthyroidism due to thyroxine suppressive therapy (differentiated thyroid carcinoma, DTC). Hashimoto’s thyroiditis and Graves’ disease were diagnosed based on clinical presentation, high levels of antithyroid antibodies (561.94±1022.1 U/mL for TgAbs and 5523±4070 U/mL for TPOAbs in HT and 18.47±18.14 U/mL for TSHAbs in GD) and TSH concentrations. DTC was diagnosed histologically, according to the main principles of the current WHO classification of thyroid tumours (23). All DTC patients included in this study had undergone total thyroidectomy and were then treated with fixed nominal activities of sodium (131-I)
iodide administered orally according to the EANM guidelines (24). In all DTC patients, subsequent whole-body scintigraphy detected no regional or distant metastases.

The control group comprised 10 healthy subjects, 9 (90%) females and 1 (10%) male of mean age 46.5±10.23 yrs. All control subjects were evaluated for thyroid function and absence of thyroid antibodies.

None of the study participants had acute infections or other conditions that could affect the tested parameters. After venipuncture, blood samples (5 mL) from patients and control subjects were collected in tubes (Vacutainer) without anticoagulants. Approximately 30 min after venipuncture, serum samples were obtained by centrifugation at 2000 rpm for 15 min and frozen at -20 °C until required for biochemical analysis.

The study was planned according to ethical guidelines following the Declaration of Helsinki. The institutional review committee of Clinical Center Kragujevac approved our study protocol according to local biomedical research regulations. All patients and control subjects gave informed consent prior to enrolment in the investigation.

**Measurement of free thyroxine (fT4) and thyroid-stimulating hormone (TSH)**

Free thyroxine (fT4) concentration was measured by radioimmunoassay (RIA, OCFD03-FT4, Cis-Biointernational, France), with a reference range of 7–18 pg/mL. Thyroid-stimulating hormone (TSH) concentration was determined immunoradiometrically (IRMA TSH, INEP, Zemun, Serbia), with a reference range of 0.3–5.5 mU/L. All measurements were made on a Wallac Wizard 1470 Automatic gamma counter (PerkinElmer Life Sciences, WallacOy, 2005, Finland).

**Measurement of serum sclerostin**

Serum sclerostin was determined by quantitative sandwich enzyme immunoassay (DSST00 Human SOST Immunoassay, R&D Systems, USA). A monoclonal antibody specific for human sclerostin was pre-coated onto a microplate. Aliquots of standards and samples were pipetted into the wells for any sclerostin present to be bound by the immobilized antibody. After washing away all unbound substances, an enzyme-linked polyclonal antibody specific for human sclerostin was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, substrate solution was added for colour to develop in proportion to the amount of sclerostin bound in the initial step. After stopping further development, the colour intensity was measured at 540 nm. Intraassay and interassay coefficients of variance (CV) were 2% and 9.5%, respectively.

**Determination of serum osteocalcin and β-cross-laps**

Serum osteocalcin and β-cross-laps were measured by electrochemiluminescence on a Cobas e 411 analyzer (Roche Diagnostics). The reference range for osteocalcin and β-cross-laps were 11–43 ng/mL and 0.010–5.940 ng/mL, respectively.

**Statistical analysis**

All values were expressed as mean ± standard deviation (SD). The commercial SPSS version 13.0 for Windows was used for statistical analysis. The significance of differences in the determined parameters between various groups was analyzed by the Independent samples T-test or Mann-Whitney U-test (depending on the distribution). Association between variables was evaluated by the bivariate correlation test and Pearson/Spearman coefficient. Chi-square tests were used to evaluate categorical data. Probability (p) values less than 0.05 were considered to be statistically significant.

**Results**

We determined sclerostin concentrations in sera samples from groups of patients with thyroid dysfunction (hypothyroidism, hyperthyroidism and subclinical hyperthyroidism) and compared them with each other and with those for control subjects. Also, we analyzed the relationships of circulating serum sclerostin levels with osteocalcin and β-cross-laps concentrations. There were no significant differences in age or gender distribution among the patients subgroups and control group (hypothyroid group: c²=18.0, p=0.324 for age and c²=1.25, p=0.264 for gender; hyperthyroid group: c²=12.98, p=0.449 for age and c²=1.25, p=0.264 for gender; sub-hyperthyroid group: c²=15.33, p=0.356 for age and c²=1.05, p=0.305 for gender).

Peripheral serum concentrations of sclerostin, free thyroxine and thyroid-stimulating hormone in our study groups are given in Table I, mean serum sclerostin, osteocalcin and β-cross-laps concentrations are shown in Figure 1 (A, B, C). There were significant differences in the levels of serum sclerostin (Kruskal-Wallis test, p<0.001), osteocalcin (One way ANOVA, p=0.014) and beta-cross-laps (One way ANOVA, p<0.001) between the study groups. We found that patients with hypothyroidism had much lower serum sclerostin concentrations than control subjects (66.54±48.13 pg/mL vs 145.91±81.36 pg/mL) (Mann Whitney test, p=0.009). In contrast, concentrations of sclerostin were significantly higher
Table I  Concentrations (geometric mean) of sclerostin, fT4 and TSH in the study groups.

| Concentrations | Experimental group | Control group | Sig. |
|----------------|--------------------|---------------|------|
|                | Hypothyroid group  | Hyperthyroid group | Subhyperthyroid group | Physiologically healthy |
| Sclerostin (pg/mL) | 54.52 (33.79–79.44) | 317.12 (188.90–476.50) | 175.39 (147.28–226.21) | 125.31 (87.36–177.41) | p < 0.05 |
| fT4 (pg/mL)    | 0.89 (0.450–1.689) | 59.54 (36.45–103.97) | 16.56 (14.12–20.04) | 9.58 (8.65–10.64) | p < 0.05 |
| TSH (mU/L)     | 93.63 (62.22–136.83) | 0.13 (0.11–0.156) | 0.24 (0.19–0.30) | 1.67 (1.45–1.89) | p < 0.05 |

The values in parentheses represent the 95% confidence interval of geometric mean, and the differences were considered statistically different if p-value was < 0.05.

Figure 1  Differences in serum sclerostin (A), osteocalcin (B) and beta-cross-laps (C) concentrations between thyroid patients and control subjects.
in patients with hyperthyroidism than in control sub-
jects (377.49±197.44 pg/mL vs 145.91±81.36 pg/mL) (Independent samples t-test, p=0.008) and
patients with subclinical hyperthyroidism (Mann
Whitney test, p=0.013). Hyperthyroid patients had
higher concentrations of osteocalcin and beta-cross-
laps in relation to patients with hypothyroidism
(p=0.018 for osteocalcin; p=0.030 for beta-cross-
laps), patients with subclinical hyperthyroidism
(p=0.044 for osteocalcin; p=0.005 for beta-cross-
laps) and control subjects (p=0.034 for osteocalcin;
p=0.041 for beta-cross-laps).

Analysis of the relationship between sclerostin
and FT4 showed a strong positive correlation of scle-
rostin concentrations and free thyroxine in the sub-
jects with thyroid dysfunctions (Bivariate correlation
test, Spearman r=0.696, p<0.001) (Figure 2A). On
the contrary, serum sclerostin was negatively correlat-
ed with TSH in the same participants (Bivariate corre-
lation test, Spearman r=-0.746, p<0.001) (Figure
2B). In the group of control subjects, the correlations
of sclerostin and parameters of thyroid status tended
in the same directions as for the patients, i.e. positive
with FT4 and negative with TSH, but did not reach
the level of statistical significance (rTSH=-0.490,
P_{TSH}=0.150; rFT4=0.382, P_{FT4}=0.386).

When considering the relationship of bone
turnover markers and thyroid status, statistical analy-
sis showed that osteocalcin and beta-cross-laps posi-
tively correlated with FT4 (Spearman r = 0.641, p =
0.003 for osteocalcin; Spearman r=0.522, p=0.022
for beta-cross-laps) but negatively correlated with
TSH (Spearman r= -0.735, p<0.001 for osteocalcin;
Spearman r=-0.612, p=0.004 for beta-cross-laps)
in the patients with altered thyroid function.

Finally, sclerostin concentrations were positively
associated with both markers of bone metabolism
(osteocalcin and beta-cross-laps) in the patients with
abnormal thyroid function (Spearman r=0.605,
p=0.005 for osteocalcin; Spearman r=0.573,
p=0.008 for beta-cross-laps). The relationship
between serum sclerostin and osteocalcin (Spearman
r=0.131, p=0.779) as well as sclerostin and beta-
cross-laps (Spearman r=0.337, p=0.459) in control
subjects was not statistically significant probably due
to the small number of subjects (n=10) in this group.
Discussion

The aim of our study was to evaluate sclerostin concentrations in patients with abnormal thyroid functions (hypothyroidism, hyperthyroidism and subclinical hyperthyroidism) and healthy euthyroid subjects, as well as to analyze the relationship between sclerostin concentrations and markers of bone metabolism (osteocalcin and beta-cross-laps).

Thyroid hormones are considered important regulators of bone metabolism, so any deficiency or excess may result in disturbance of bone turnover. Current knowledge about the action of thyroid hormones on the skeleton has mainly been obtained from animal models (5). Since bone remodelling determines skeletal integrity, thyroid hormones might change the rate of bone turnover (leading to an acceleration in hyperthyroidism or deceleration in hypothyroidism) and bone density (3). On the other hand, it is accepted that TSH can suppress osteoclast formation and stimulate osteoblast differentiation (6). Abe and coworkers (5) hypothesized that TSH, rather than thyroxine and triiodothyronine, is a direct negative regulator of skeletal development. It seems that skeleton maintenance is set by the concerted activity of thyroid hormones and TSH.

Sclerostin is a glycoprotein product of the SOST gene expressed mainly in osteocytes (8). It has a negative effect on bone mineralization because it reduces bone mineral content and has been the subject of many studies. Thus, increased serum sclerostin levels were found in patients with chronic kidney disease (25), diabetes mellitus (26), prostate cancer (27), liver dysfunction (28) and obesity (29). Its expression is regulated mostly by mechanical load, although many data indicate the great importance of humoral factors (10, 12, 13). Thus, thyroid hormones were demonstrated to increase the number of sclerostin-positive osteocytes in hyperthyroid mice (14, 15). Here we attempted to clarify the relationship of sclerostin and thyroid hormones in humans and are the first to show differences in circulating sclerostin concentrations in patients with three different types of thyroid dysfunction (hypothyroidism, hyperthyroidism and subclinical hyperthyroidism).

In earlier studies on sclerostin concentrations in subjects with hyperthyroidism before and/or after medicament therapy, Skwrońska-Jóżwiak et al. (10) and Sanetkin et al. (16) found no statistically significant correlation with thyroid status. Unlike them, we have compared serum sclerostin concentrations in patients with different abnormalities of thyroid function both between the groups of patients and with healthy euthyroid individuals.

We showed that serum sclerostin concentrations were the highest in the group of hyperthyroid patients, significantly higher than in control subjects. On the contrary, patients with hypothyroidism had lower serum concentrations of sclerostin than the other groups. These results are in line with data obtained by Tsourdi et al. (15) in male mice. It seems that hypothyroidism, although this may not be expected, affects bone homeostasis and reduces skeletal turnover.

Our study also included DTC patients with subclinical hyperthyroidism caused iatrogenically by thyroxine suppressive therapy. Subclinical hyperthyroidism is characterized by circulating TSH levels below the reference range and normal serum concentrations of thyroid hormones. The small difference in sclerostin levels between these patients and control subjects was not statistically significant, although the mean concentration was slightly higher in patients with subclinical hyperthyroidism. These results are in agreement with those of Lee et al. (30), who concluded that TSH suppression in DTC patients has no effect on bone health, so suppressive levothyroxine therapy could be rated safe.

A close positive correlation of sclerostin concentrations with thyroxine and a strong negative correlation with TSH in sera samples from our three groups of patients were observed. The same correlation trend of sclerostin and thyroid status (FT4 and TSH) was seen in control subjects but did not reach statistical significance, probably due to the small number of subjects in the control group.

Analysis of the relationship with bone metabolic markers showed that serum sclerostin was positively associated with osteocalcin and beta-cross-laps levels in patients with thyroid dysfunctions. Moreover, osteocalcin and beta-cross-laps were positively associated with concentrations of FT4 and negatively with TSH in all patients.

Osteocalcin is the most common non-collagenous protein of the bone extracellular matrix. Synthesized by mature osteoblasts (31), it is considered a marker of bone formation. Thus, serum osteocalcin level correlates with osteoblast activity, although fragments can be released into the blood during bone resorption. In contrast, beta-cross-laps is a collagen-degradation product and a marker of bone resorption (32).

In an analysis of osteocalcin concentrations in patients with abnormal thyroid function (hyperthyroidism, hypothyroidism and subacute thyroiditis), Kojima et al. (33) found a positive correlation with thyroxine and triiodothyronine levels, suggesting that osteoblast activity was increased in hyperthyroidism and decreased in hypothyroidism. Moreover, thyroid suppressive therapy significantly reduced osteocalcin concentrations in subjects with hyperthyroidism (34). Our results correspond with these findings, but we have also demonstrated that both osteocalcin and beta-cross-laps are associated with thyroid status in patients with different thyroid dysfunctions. Furthermore, we have examined the relationship of sclerostin with other bone metabolism markers, which was not
the subject of previous research. Earlier results about the association of sclerostin and markers of bone metabolism mostly concerned a healthy elderly population or immobilized subjects. Thus some authors demonstrated that sclerostin correlates negatively with markers of bone formation and bone resorption, while others affirmed a positive correlation only with bone resorption markers (20–22). Unlike Santekin et al. (16), our examination indicated positive relationships of serum sclerostin with both osteocalcin and beta-cross-laps in patients with abnormal thyroid function. The differences in sclerostin concentration in our study participants reflect changes in the level of osteocalcin and beta-cross-laps and vice versa. As a marker of bone formation, osteocalcin showed the same distribution among our study groups as sclerostin and beta-cross-laps (the highest values in hyperthyroid patients, the lowest in hypothyroid patients). We assume that is a consequence of a compensatory reaction (as increased osteoblast activity) in patients. We assume that is a consequence of a compensatory reaction (as increased osteoblast activity) in patients with abnormal thyroid functions. Thus, patients with hyperthyroidism had the highest level of sclerostin and patients with hypothyroidism the lowest. There were positive relationships between circulating concentrations of sclerostin and markers of bone metabolism, i.e. osteocalcin a marker of bone formation and beta-cross-laps marker of bone resorption in all thyroid patients. Thyroid status affects bone homeostasis which is reflected through its significant association with osteocalcin and beta-cross-laps but even more closely with sclerostin.

In conclusion, serum concentrations of sclerostin are significantly affected in subjects with different abnormal thyroid functions. Thus, patients with hyperthyroidism had the highest level of sclerostin and patients with hypothyroidism the lowest. There were positive relationships between circulating concentrations of sclerostin and markers of bone metabolism, i.e. osteocalcin a marker of bone formation and beta-cross-laps marker of bone resorption in all thyroid patients. Thyroid status affects bone homeostasis which is reflected through its significant association with osteocalcin and beta-cross-laps but even more closely with sclerostin.

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Conflict of interest statement

The authors state that they have no conflicts of interest regarding the publication of this article.

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