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ORIGINAL RESEARCH ARTICLE

Evaluation of Bone Turnover Markers Such as Osteoprotegerin, Sclerostin and Dickkopf-1 in Subclinical Hyperthyroidism

Ayşe Elverdi Özbek[1] · Hüseyin Korkmaz[2] · Mehmet Sözen[3,8] · Süleyman Hilmi İpekçi[4] · Sedat Abuşoğlu[5] · Cem Onur Kıraç[6] · Ali Ünlü[5] · Levent Kebapçılar[7]

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Abstract

In this study, it was aimed to assess effects of subclinical hyperthyroidism (SH) on bone metabolism using osteoprotegerin (OPG), sclerostin, Dickkopf-1 (DKK1) and biochemical parameters. This cross-sectional prospective study included 40 patients with SH and 40 euthyroid controls. Serum OPG, sclerostin, DKK-1, type-1 procollagen, C-terminal polypeptide (CTx) and 24-hours urine N-terminal telopeptide (NTx) were measures using ELISA kit. Bone mineral density measurements were performed using dual energy X-ray absorptiometry (DEXA). Risk for 10-years hip and major fracture was estimated by Turkish version of FRAX. No significant difference was detected in age, gender, body mass index, smoking and menopause rates between SH and control groups. The risk for 10-years hip fracture and major osteoporotic fracture were estimated as 4.45% and 0.55% in SH group, respectively. The OPG levels were significantly lower in patients with SH than controls ($P = 0.017$). No significant difference was detected in other bone formation and degradation parameters. No significant correlation was detected between OPG level and risk for major osteoporotic fracture ($P > 0.05$); however, a negative correlation was detected between OPG level and risk for hip fracture (rho = 0.233; $P = 0.038$). Serum OPG is markedly affected in patients with SH. In addition, OPG seemed to be associated with osteoporotic fracture risk. Available data show that SH is significantly associated with risk for fracture; thus, it is important to assess risk for fracture in patients with SH.

Keywords Subclinical hyperthyroidism · Bone metabolism · Osteoprotegerin · Sclerostin · DKK-1

Introduction

There are many causes of osteoporosis and, in particular, hyperthyroidism is among major causes of secondary osteoporosis. The overt hyperthyroidism has been linked to increased bone turnover, osteoporosis and risk for fracture [1]. The thyrotropin (TSH) can directly affect both osteoclastic bone resorption and osteoblastic bone formation through distinct intracellular signaling pathways [2].

However, due to marked controversies and variations in available studies, the net effect of subclinical hyperthyroidism (SH) on bone turnover and osteoporosis has not been clearly known yet. In previous studies, the effects of SH on bone metabolism and osteoporosis was investigated using biochemical parameters such as carboxy-terminal telopeptide of type 1 collagen (CTx), N-terminal telopeptide of type 1 collagen (NTx) and type 1 procollagen, radiology studies and bone mineral density (BMD) measurements. However, in recent years, it was shown that several endogenous mediators including osteoprotegerin (OPG), a member of tumor

✉ Mehmet Sözen
mehmetsozen07@gmail.com

1 Department of İnternal Medicine, Selcuk University, Konya, Turkey

2 Department of Gastroenterology, Selçuk University Faculty of Medicine, Konya, Turkey

3 Department of Endocrinology and Metabolism, Kocaeli University Faculty of Medicine, Kocaeli, Turkey

4 Department of Endocrinology and Metabolism, Atlas University Faculty of Medicine, İstanbul, Turkey

5 Department of Biochemistry, Selcuk University Faculty of Medicine, Konya, Turkey

6 Department of Endocrinology and Metabolism, Necip Fazil City Hospital, Kahramanmaraş, Turkey

7 Department of Endocrinology and Metabolism, Selçuk University Faculty of Medicine, Konya, Turkey

8 Department of Endocrinology and Metabolism, Kocaeli University Faculty of Medicine, Prof. Baki Komsuoglu avenue, Kocaeli 41000, Turkey

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necrosis factor (TNF) receptor family known to influence on bone metabolism, Wnt signal, sclerostin and Dickkopf-1 (DKK1) play important role in the control of osteoblastogenesis and bone formation [3].

In the present study, the effects of SH on bone resorption and formation was investigated for the first time using mediators OPG, sclerostin and DKK1, which are thought to have major effect on bone mineral density measurements and bone turnover, in addition to conventional parameters such as CTx, NTx, calcium (Ca), vitamin D (VitD), parathormone (PTH) and others used to assess bone metabolism in previous studies.

Materials and Methods

Study Setting and Sample

This cross-sectional, prospective study was conducted at Department of Endocrinology and Metabolism, Selçuk University Faculty of Medicine between 2017 and 2018. The study included 40 patients with SH and 40 euthyroid controls in same period. The control group was selected among patients who did not have any disease and applied to the internal medicine outpatient clinic for check-up. Those who met the exclusion criteria were informed about the study. Written consent was first sought from those who agreed to participate in the study. All subjects gave written informed consent for their clinical data to be used in accordance with the Declaration of Helsinki.

Exclusion Criteria

Patients received high-dose VitD supplementation within prior 6 months, patients on steroid therapy, patients with history of cancer, those with chronic hepatic and renal disease, those with heart failure, those using drugs that may affect thyroid function tests, those with finding active infection, those with history of previous fracture and those with subacute thyroiditis were excluded.

Data Collection and Blood Sample Analysis

The sociodemographic characteristics and exclusion criteria were questioned in the patients. Urinary Ca levels were analysed via Abbott Architect (USA) aoutoanalyzer. Total serum DKK1, osteoprotogerin, CTx, type 1 procollagen, sclerostin and urinary NTx were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Sunred, Shangai, China) kit according to the manufacturer's recommendations.

Bone Densitometry Measurement and Fracture Risk Estimation

BMD was measured by GE Lunar device using dual energy x-ray absorptiometry (DEXA). Lumbar spine 1–4, femur total and femoral neck T-scores were classified as normal (SD>-1), osteopenia (SD:-1 to -2.5) and osteoporosis (SD<-2.5). Ten-year hip and major fracture risks were estimated by Turkish Version of Frax Risk Assessment Tool (FRAX) accepted by WHO (https://www.sheffield.ac.uk/FRAX/tool.aspx?lang=tu).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 20.0. Variables were compared between groups using the Chi-square test. Student's t test was used to compare variables with normal distribution while Mann Whitney U test was used to compare variables with skewed distribution. Spearman's correlation test was used to assess relations between osteoprotegerin, sclerostin, DKK1 and 24-hours urine NTx values and other parameters. Data with normal distribution are presented as mean ± standard deviation while those with skewed distribution as median (min-max). A P value < 0.05 was considered as statistically significant.

Results

The study included 40 SH and 40 controls. Mean age, gender distribution, body mass index (BMI) and smoking rate were comparable between groups. No significant difference was detected in menopause rate between groups. No significant differences were detected in biochemical parameters between groups. As expected, there were significant differences in TSH, fT3 and fT4 levels between groups (Table 1). The 10-year fracture risk estimation and BMD values calculated with FRAX in SH patients are given in Table 2.

OPG levels was found to be significantly lower in patients with SH ($P = 0.017$). Other bone formation and resorption parameters were similar between the groups ($P > 0.05$) (Table 3).

In patients with SH, a significant, positive correlation was detected between OPG levels and serum TSH, DKK1, serum CTx and type 1 procollagen levels ($P < 0.05$). No significant correlation was detected between OPG level and risk for major osteoporotic fracture ($p > 0.05$) while a negative correlation was detected with risk for hip fracture (rho=-0.233; $P = 0.038$). Again, a significant negative correlation was detected between sclerostin and urinary NTx levels (rho=-0.252; $P = 0.024$). A significant, positive

Table 1 Demographic characteristics and laboratory data of the individuals included in the study

	Control	SH	*P*
Female/Male	26/14	24/16	0.818
Age (Year)	50.6 ± 10.6	52.9 ± 13.5	0.399
BMI (kg/m^2)	28.06 (18.69–33.30)	28.02 (20.05–43.56)	0.479
Postmenopause	11/26	12/24	0.777
Smoker	8/40	5/40	0.546
Graves' disease		19	
Toxic adenoma		15	
Toxic MNG		6	
Parathormone (ng/L)	57.14 ± 19.70	51.34 ± 19.98	0.195
Albumin (g/dL)	4.21 ± 0.25	4.18 ± 0.40	0.677
Calcium (mg/dL)	9.49 ± 0.28	9.51 ± 0.37	0.812
Phosphorus (mg/dL)	3.39 ± 0.50	3.55 ± 0.60	0.220
Magnesium (mg/dL)	2.00(1.40–2.36)	1.96(1.66–2.25)	0.538
Alkaline phosphatase (U/L)	68.5(28–147)	80(42–142)	0.077
Vitamin D (ng/mL)	16.79(5.25–46.20)	20.05(2.00-48.17)	0.765
TSH (mlU/L)	1.66(0.53–3.80)	0.02(0.001–0.39)	< 0.001
fT4 (ng/dL)	1.26 ± 0.19	1.37 ± 0.26	0.030
fT3 (ng/dL)	3.16 ± 0.41	3.55 ± 0.57	0.001
Anti-TPO (+) (IU/mL)	4/40	8/40	0.348
Anti-Tg (+) (IU/mL)	4/40	10/40	0.139

BMI: Body mass index; MNG: multinodular goiters; TSH: Thyrotropin; fT4:Free T4; fT3:Free T3; Anti-TPO:Anti-thyroid peroxidase; Anti-Tg:Anti-thyroglobulin

Table 2 Bone densitometry measurements and FRAX calculations of SH patients

	SH
L1-4 T-score	-0.70 [(-5.4)-(2.8)]
L1-4 Zcore	-0.35 [(-3.8)-(2.6)]
Femur neck T-score	-1.00 [(-3.8)-(0.8)]
Femur neck Z-score	-0.40 [(-2.0)-(1.5)]
L1-4 BMD (g/cm^2)	1.116 ± 0.206
Femur neck BMD (g/cm^2)	0.912 ± 0.166
FRAX - Major frakture risk (%)	4.45 (1.10–1.90)
FRAX - Hip frakture risk (%)	0.55 (0.10–6.80)

BMD: Bone mineral density; FRAX: Fracture Risk Assessment Tool

correlation was detected between DKK1 and serum CTx level (rho = 0.375; $P = 0.001$). Again, there was a significant, positive correlation between DKK1 and type 1 procollagen (rho = 0.247; $P = 0.027$).

Discussion

Our study showed that SH was numerically associated with fracture risk. The OPG is a member of TNF receptor family that inhibits nuclear factor-κB ligand (RANKL)-mediated osteoclastic bone resorption receptor activator, which was found to be lower in patients with SH. This finding will help to understand the hypothesis proposing that SH is a risk factor for decreased bone mineral density and increased bone turnover.

Table 3 Comparison of bone formation and destruction parameters

	Control	SH	*P*
Sclerostin (pmol/L)	3211.73 (89.53-5698.37)	3537.38 (128.43-3834.08)	0.866
DKK1 (pg/mL)	894.59 (215.4-3626.8)	1122.54 (248.3–3764.0)	0.893
Osteoprotegerin (pg/mL)	5.61 (0.89–7.20)	3.90 (0.56-7.00)	0.017
Serum CTx (pg/mL)	0.195 (0.065–0.935)	0.160 (0.090–1.170)	0.501
Type 1 Procollagen (ng/mL)	840.53 (19.31-1394.41)	686.63 (13.63-1304.09)	0.154
Urine NTx (nmol/mmol)	204.06 (12.39-761.01)	222.41(3.06-781.88)	0.264
Urine Ca (mg/day)	133.28 (40.99-363.52)	145.21 (26.62–475.00)	0.855

DKK1: Dickkopf-1; CTx: carboxy-terminal telopeptide of type 1 collagen; NTx: N-terminal telopeptide of type 1 collagen; Ca: Calcium

There is an increased bone turnover in patients with apparent hyperthyroidism and such patients are at risk for osteoporosis. However, the physiological effects of SH hasn't been fully elucidated since the symptoms and findings are not as apparent as those in clinical hyperthyroidism in SH [1, 4]. In recent years, SH has become focus of interest and it is thought that SH is a potential risk for decreased BMD and increased bone turnover. However, there is limited number of studies about effects of SH on bone with contradictory outcomes. In our study, in addition to BMD, we, for the first time used novel parameters such as sclerostin, DKK1 and OPG in order to evaluate bone metabolism.

In a meta-analysis evaluating fracture risk, SH was linked to increased risk for fractures at hip and other regions, particularly in those with TSH level < 0.10 mIU/L [5]. In a study on postmenopausal women, TSH levels were found to be lower in women with osteoporosis and a positive correlation was observed between TSH and BMD [6]. In previous studies, osteoporosis seemed to have an independent association with serum TSH level.

The OPG and its ligand, RANKL receptor activator, are important factors that mediate paracrine signaling between osteoblasts and osteoclasts. OPG act as trapping receptor that binds RANKL; thus, inhibits interaction between RANKL and surface receptors on osteoclasts [7]. The key importance of OPG in bone metabolism was shown by severe osteoporosis due to increased osteoclastogenesis in OPG-knockout adult mice [8]. Jørgensen et al. found that serum OPG and gene polymorphism at OPG promoter region was associated to increased risk for osteoporosis and fracture risk [9]. In a cohort including patients with hyperthyroidism, OPG concentrations were evaluated before and after metamizole (MMI) therapy and serum OPG levels were found to be higher in patients with hyperthyroidism when compared to controls. Moreover, OPG levels were found to be higher in patients with Graves' disease (GD) than those with toxic MNG. On month 6 after MMI therapy, a significant decrease was observed in toxic MNG group but not in GD group. On month 12, OPG was significantly decreased in both groups [10]. In our study, we found a significantly decreased OPG level in SH patients when compared to controls. Suppression of osteoprotegerin, which acts as an osteoclastogenesis inhibitor, by thyroid hormones may increase bone resorption. These results suggest that thyroid hormones may have effects on bone metabolism via OPG.

The current guidelines on diagnosis and management of osteoporosis recommends estimation of absolute fracture risk by FRAX. The FRAX predicts 10-years risk for major osteoporotic fractures and osteoporotic hip fractures by combination of age and gender with 7 clinical risk factors independently from BMD [11]. Traditionally, the BMD is predictive factor for osteoporotic fractures. The studies indicate that the addition of specialized FRAX models to current DEXA-based assessment will improve identifying patients at high-risk for fracture [12]. In a study on patients with SH, major osteoporotic fracture and osteoporotic hip fracture risk scores by FRAX were significantly higher in the patient group when compared to controls [13]. In our study, a negative correlation was found between OPG level and hip fracture risk. The decreased OPG level in SH patients may suggest an increased risk of hip fracture in these patients.

In recent years, it was shown that Wnt signal pathway plays an important role in the control of osteoblastogenesis and bone formation. The Wnt signaling is modulated by various endogenous mediators including sclerostin and DKK1 [3]. The regulation of Wnt signaling pathway by thyroid hormones has been investigated in vivo manner. The decreased DKK1 and increased sclerostin levels were detected in rats with hyperthyroidism while both sclerostin and DKK1 levels were found to be increased in rats with hypothyroidism [14]. The DKK1 depletion was not sufficient to reverse effects of thyroid hormone on bone mass and bone turnover in rats with global or osteocyte-specific DKK1 deletion [15]. Thus, it is unlikely that DKK1 blockade can be an effective strategy to prevent pathological changes in bone tissue in patients with thyroid dysfunction. In a study on sclerostin levels, no significant difference was detected between patients with hyperthyroidism and controls [16]. In our study, no significant association was shown between SH and DKK1 or sclerostin involved in Wnt signaling pathway.

In recent years, specific markers for bone turnover have been developed, which may help for assessment and monitoring bone turnover. Type 1 procollagen is mainly synthesized by osteoblasts. CTx and NTx are degradation products resulting from type 1 collagen [17]. The serum CTx levels were found to be significantly higher in patients with GD and toxic MNG when compared to controls. In addition, CTx showed a negative correlation with TSH whereas a positive correlation with fT3 and fT4 [18]. In a study in SH patients, CTx was found to be higher when compared to controls [19]. On contrary, no significant correlation was detected between SH and CTx; thus, SH was not identified as a predictive factor for hip fracture [20]. In our study, type 1 procollagen, CTx, NTx and 24-hour urine Ca levels were found to be comparable among SH and control groups. However, heterogeneities such as variations in SH duration or mean age may lead differences in clinical outcome.

This study has some limitations: (1) the smaller sample size; (2) lack of BMD data in control group; (3) lack of clear data regarding duration of subclinical thyroid dysfunction in the patients since it was established that there was a negative interaction between duration of thyroid dysfunction and bone metabolism in previous studies [18]. However, this study is valuable since it is the first study evaluating effects of novel parameters such as OPG, sclerostin and DKK1 on bone metabolism in SH and one of the limited studies evaluating effects of SH on bone metabolism in the literature.

Conclusions

In this study, no significant difference was observed between the SH and control groups with sclerostin, DKK1, serum CTx, urine NTx and urine Ca levels. However, it was seen

that serum OPG concentration was significantly decreased in SH and a negative correlation was found between OPG level and hip fracture risk. This supports the hypothesis that OPG contributes to hyperthyroidism-related reduction in bone mineral density. There is need for further studies with larger sample size and subgroups according to TSH levels, in which disease duration can be monitored.

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Authors' Contributions AE: Conceptualization, Project administration, Data curation, Investigation, Methodology, Writing – original draft. HK: Conceptualization, Project administration, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. MS: Supervision, Validation, Writing – review & editing SHI: Methodology, Resources, Writing – review & editing. SA: Visualization, Formal Analysis, Writing – original draft. COK: Investigation, Methodology, Software. AU: Visualization, Formal Analysis, Writing – original draft. LK: Conceptualization, Project administration, Methodology, Supervision.

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Data Availability The datasets used are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate The study was approved by Ethics Committee of Selçuk University Medicine School (Approval number 2017/131-19.04.2017).

Consent for Publication Written informed consent was obtained from all individual participants included in the study.

Conflict of Interest Ayşe Elverdi Özbek, Hüseyin Korkmaz, Mehmet Sözen, Süleyman Hilmi İpekçi, Sedat Abuşoğlu, Cem Onur Kıraç, Ali Ünlü and Levent Kebapçılar declare that they have no confict of interest.

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