Bone mineral density and its correlation with serum 25-hydroxyvitamin D levels in patients with hyperthyroidism

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Abstract
Objective: This study aimed to determine the association between 25-hydroxyvitamin D (25(OH)D) levels and bone mineral density (BMD) in patients with hyperthyroidism after undergoing treatment.

Methods: A total of 120 patients with hyperthyroidism were selected as participants. Methimazole tablets were provided to all of the participants, with an initial dose of 20 mg/day and a maintenance dose of 2.5 mg/day for 1 year. Blood calcium, phosphate, parathyroid hormone (PTH), and thyroid hormone levels were assayed using an automatic biochemical analyzer. Levels of 25(OH)D and bone alkaline phosphatase (ALP) in serum were determined by enzyme-linked immunosorbent assay. BMD was measured using dual energy X-ray absorptiometry.

Results: Serum phosphorus, PTH, and 25(OH)D levels in patients with hyperthyroidism were significantly higher, and bone ALP and 24-hour urinary calcium levels were significantly lower after treatment compared with before treatment. BMD in patients with hyperthyroidism was significantly improved after treatment. In logistic regression analysis of BMD-related risk factors, bone ALP, PTH, and 25(OH)D levels were risk factors of BMD.

Conclusion: Treatment for hyperthyroidism should be supplemented with vitamin D and calcium, which have important clinical significance for adjusting bone metabolism and delaying the process of osteoporosis.

Keywords
Hyperthyroidism, bone mineral density, 25-hydroxyvitamin D, thyroid function, calcium, parathyroid hormone

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Introduction

Hyperthyroidism is a common disease of the endocrine system. Although the etiology of hyperthyroidism is largely unknown, hyperthyroidism is an autoimmune disease. On the basis of heredity, hyperthyroidism is induced by stress factors, such as infection or mental trauma, followed by dysfunction of inhibitory T lymphocytes.1 A total of 80% to 85% of hyperthyroidism is caused by Graves’ disease. Graves’ disease is characterized by hyperthyroidism caused by positive thyroid-stimulating hormone (TSH) receptor antibody. Thyroid hormone plays an important role in an increase in osteoblast activity, which accelerates bone turnover, promotes bone metabolism, accelerates bone loss, and leads to osteoporosis.2 Osteoporosis is a systemic bone disease,3 which is characterized by increased bone fragility and a risk of fracture due to reduced bone mass and damage to bone microstructure. Osteoporosis can be divided into two major categories of primary and secondary osteoporosis. The latter is usually caused by endocrine or metabolic diseases or systemic diseases, whereas hyperthyroidism is a common endocrine disease that causes secondary osteoporosis.4 Although the number of studies is limited in this field, Yoon et al. reported that chronic TSH suppression therapy improved lower BMD of the spine and total hip in postmenopausal women.5 Arnautovic-Halimic et al.6 reported that mean follicle-stimulating hormone levels were significantly higher in the group of women with osteoporosis. Additionally mean TSH and free triiodothyronine (FT3) levels were not significantly different in the group of women with osteoporosis compared with the control group. Mean free thyroxine (FT4) levels in women with osteoporosis were significantly lower compared with those in the control group.6

Vitamin D is an essential vitamin in the human body. This vitamin cannot be synthesized by the human body itself. There are two sources of vitamin D. One source is food and the other is production by 7-dehydrocholesterol through sunlight. Vitamin D in the human body is mainly derived from sunlight. 25-hydroxyvitamin D (25(OH)D is produced by vitamin D through the action of 25 hydroxylase in the liver and then 1, 25(OH) 2D3 with biological action is produced through action of mitochondrial 1α hydroxylase in renal tubular epithelial cells.7 Levels of 25(OH) D reflect the reserve of vitamin D in the body and they are the best indicator for evaluating the level of vitamin D in the body. In addition to the role of regulating calcium and phosphorus metabolism, vitamin D also plays a role in regulating cell proliferation, differentiation and apoptosis, and regulating development of the immune system and the central nervous system. The lack of vitamin D is associated with many diseases, such as coronary heart disease, cancer, inflammation, obesity, and autoimmune diseases7–9

This study aimed to determine the changes in serum biochemical markers, hormones, and bone mineral density (BMD) in patients with hyperthyroidism after treatment with oral methimazole. We also examined the relationship between BMD and 25(OH)D levels in these patients.

Materials and methods

Participants

Patients with typical clinical symptoms who were diagnosed with hyperthyroidism by a laboratory examination and color Doppler examination were selected as participants. All participants were patients with hyperthyroidism who had never taken hormones, vitamin D, calcium, or other drugs.
Patients who had any of the following diseases were excluded: patients with diabetes, tumors, bone metastases, or other diseases that cause abnormal bone metabolism; patients with a history of hormone medication; and patients with hyperthyroidism accompanied by cardiovascular, digestive, respiratory, or other primary diseases. All experiments and procedures were approved by the Institutional Ethics Committee of Weifang People’s Hospital. The participants provided written informed consent.

**Treatment methods**

Methimazole tablets (specification: 10 mg × 50 tablets, approval number: H20140405; Merck Serono Co., Ltd., Darmstadt, Germany) were provided to all participants. The dose of the drug was gradually reduced, with an initial dose of 20 mg/day orally. Thyroid function was reviewed after 1 month following continuous medication. The amount of the drug was gradually reduced on the basis of results of a review and the patient’s condition. The dosage of the maintenance period was 2.5 mg/day orally for 1 year.

**Observational indicators**

Venous blood (8 mL) was collected from an empty stomach in the early morning and added to a serum tube. The serum was separated to measure blood levels of calcium, phosphate, and thyroid hormones. An automatic biochemical analyzer was used to perform these measurements (Siemens Dimension RxL analyzer, Siemens Healthcare Diagnostic, Beijing, China). Serum levels of 25(OH)D and bone alkaline phosphatase (ALP) were determined by enzyme-linked immunosorbent assay (Jieshikang Biotechnology, Qingdao, China). The reference values for 25(OH)D recommended for the Chinese population were determine according to the Application Guideline for Vitamin D and Bone Health in Adult Chinese: levels <30 nmol/L in serum indicate a lack of 25(OH)D; levels of 30 to 49.9 nmol/L in serum indicate deficiency of 25(OH)D; and levels ≥50 nmol/L in serum indicate adequacy of 25(OH)D.

**BMD detection**

BMD of the lumbar spine (L1–L4), femoral neck, and total hip of each subject was measured using DPX-L dual energy X-ray absorptiometry (Lunar Corp., Madison, WI, USA) (expressed as g/cm²). The coefficient of variation of accuracy of the machine was 1%. A self-test was performed by professionals using a quality control phantom and BMD measurements were performed on all subjects. In accordance with the 2012 International Osteoporosis Foundation’s Recommendations for the Diagnosis of Osteoporosis in Young and Middle-Aged Hyperthyroidism Patients, Z values were the standards of healthy young people and T values were the standards of patients with hyperthyroidism. T standards were divided into normal bone mass (T > −1.0), reduction in bone mass (T > −2.5 and ≤−1.0), and osteoporosis (T ≤ −2.5). All subjects were divided into two groups of normal bone mass and abnormal bone mass (including osteoporosis and osteopenia).

**Statistical analysis**

All data were analyzed using the statistical software SPSS 20.0 (IBM Corp. Armonk, NY, USA). Measurement data that conformed to a normal distribution are expressed as mean ± standard deviation. The groups were compared using the independent sample t test or the F test. Measurement data that were not normally distributed are expressed as the median (25th percentile, 75th percentile).
Comparison among the groups was performed by the independent sample non-parametric rank sum test. Count variables are expressed by rate. Rates were compared by the \( \chi^2 \) test. Correlation analysis among the variables and BMD was performed by using a logistic regression model along with the Wald test. A \( p \) value <0.05 indicates a statistically significant difference.

**Results**

There were 120 patients (35 men and 85 women) who were aged 20 to 53 years old, with a mean age of 34.6±7.65 years. Body mass index was 23.89±4.11 kg/m\(^2\). The FT3 level was 14.98±6.61 pmol/L (6.21–46.08 pmol/L), FT4 level was 42.77±16.55 pmol/L (16.56–69.52 pmol/L), TSH level was 0.47 (0.01, 2.69) uIU/mL, and thyrotropin receptor antibody level was 9.65 (4.02, 22.22)U/L. After treatment for 1 year, the patients’ clinical symptoms and signs returned to normal, the thyroid gland had shrunk back to normal and FT3, FT4, and TSH levels had returned to normal levels. Adverse drug reactions in cases of hyperthyroidism were rash and liver function damage after 1 year of treatment. The incidence of rash was 5% (4/84) and the incidence of liver function damage was 5% (4/84).

Mean serum phosphorus levels in patients with hyperthyroidism were significantly higher after treatment than before treatment \((p<0.05)\). Mean bone ALP and 24-hour urinary calcium (UCa) levels were significantly lower after treatment than before treatment \((both \ p<0.01)\). Mean serum PTH and 25(OH)D levels were significantly higher after treatment than before treatment \((both \ p<0.01)\). There was no significant difference in mean serum calcium levels between before and after treatment \((Table 1)\).

After 1 year of treatment, BMD of L1, the femoral neck, and the total hip was significantly improved compared with before treatment \((Table 2, all \ p<0.05)\). In logistic regression analysis of BMD-related risk factors, phosphorus, ALP, PTH, 25(OH)D, and 24-hour UCa levels, which showed a significant difference in Table 1, were used as independent variables. BMD of the total hip was used as the dependent variable \((1/0, \ respectively)\). ALP, PTH, and 25(OH)D levels were significant risk factors of BMD \((all \ p<0.05)\ \((Table 3)\).

**Discussion**

Hyperthyroidism is a systemic disease caused by increased secretion of thyroid hormone. Thyroid hormone not only results in hypermetabolism if its secretion is increased, but is also closely related to bone growth and development;\(^{12}\) thyroid hormone levels in the normal range in the

**Table 1. Levels of various indicators before and after treatment.**

| Parameters            | Before treatment \((n=120)\) | After treatment \((n=120)\) | \( t \)  | \( p \)  |
|-----------------------|------------------------------|-----------------------------|--------|--------|
| Calcium (mmol/L)      | 2.43±0.19                    | 2.35±0.15                   | −0.987 | 0.3761 |
| Phosphorus (mmol/L)   | 1.32±0.19                    | 1.52±0.16                   | 3.121  | 0.0265 |
| ALP (U/L)             | 144±21                       | 75±14                       | −6.905 | 0.0011 |
| PTH (pg/mL)           | 26.55±8.56                   | 35.96±11.33                 | 5.112  | 0.0045 |
| 25(OH)D (ng/mL)       | 36.33±2.76                   | 47.28±3.67                  | 6.127  | 0.0022 |
| 24-hour UCa (mg)      | 328±36.5                     | 225±25.3                    | −4.523 | 0.0088 |

ALP: alkaline phosphatase; PTH: parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D; UCa: urinary calcium.
human body promote bone formation and development. Excessive thyroid hormone is likely to cause an increase in bone resorption, resulting in osteoporosis. In this study, a decrease in the number of bone trabecula in patients with hyperthyroidism indicated that osteogenesis and osteolysis were simultaneously enhanced, but enhancement of osteolysis was more obvious. Thyroid hormone can directly participate in and accelerate bone turnover, enhance bone metabolism, and cause bone loss, thus leading to osteoporosis.

At present, there are two types of commonly used drugs for treating hyperthyroidism as follows: representative drugs for thioureas (propylthiouracil and methylthiouracil) and representative drugs for imidazoles (methimazole and carbazole). We treated our patients with methimazole. We found that serum levels of phosphorus, PTH, and 25(OH)D in patients with hyperthyroidism were significantly higher after treatment than before treatment. Additionally, serum levels of bone ALP and 24-hour UCa were significantly lower after treatment than before treatment. Calcium levels were not different between before and after treatment. Serum calcium levels before and after treatment were within the normal range. This finding is likely related to the increase in basal metabolic rate and acceleration of protein decomposition in patients with

| Table 2. Changes in BMD before and after treatment. |
|---------------------------------|
| Groups | L1 | L2 | L3 | L4 | L1–L4 | Femoral | Total |
|--------|----|----|----|----|-------|---------|-------|
| Before treatment (n=120) | 1.04 ± 0.09 | 1.15 ± 0.12 | 1.22 ± 0.41 | 1.17 ± 0.28 | 1.16 ± 0.27 | 0.88 ± 0.17 | 0.91 ± 0.36 |
| After treatment (n=120) | 1.09 ± 0.11 | 1.17 ± 0.08 | 1.24 ± 0.39 | 1.22 ± 0.32 | 1.20 ± 0.35 | 0.93 ± 0.22 | 0.99 ± 0.16 |
| t     | 2.343 | 1.887 | 0.765 | 1.011 | 1.233 | 1.987 | 2.895 |
| p     | 0.031 | 0.063 | 0.564 | 0.420 | 0.267 | 0.042 | 0.016 |

BMD: bone mineral density.

| Table 3. Logistic regression analysis of risk factors of BMD. |
|-----------------|----------------|----------------|----------------|
| Variables       | β value | SE  | Wald value | p   | OR  | 95% CI         |
|-----------------|---------|-----|------------|-----|-----|----------------|
| Phosphorus (mmol/L) | 0.742   | 0.094 | 1.275 | 0.136 | 1.126 | 1.232–5.986 |
| ALP (U/L)       | 1.147   | 0.386 | 1.856 | 0.012 | 2.847 | 0.757–1.904 |
| PTH (pg/mL)     | 0.361   | 0.462 | 3.614 | 0.028 | 1.638 | 1.769–3.846 |
| 25(OH)D (ng/mL) | 2.126   | 0.827 | 4.288 | 0.007 | 1.573 | 1.423–2.359 |
| 24-hour UCa (mg) | 1.325   | 0.137 | 2.334 | 0.262 | 0.894 | 2.213–4.568 |

SE: standard error; OR: odds ratio; CI: confidence interval; ALP: alkaline phosphatase; PTH: parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D; UCa: urinary calcium.
hyperthyroidism, and related to the decrease in capability of protein in serum binding to calcium.\textsuperscript{13} ALP is an important indicator\textsuperscript{14,15} of bone formation. The activity of osteoblasts is enhanced with occurrence and development of hyperthyroidism, which increases the amount of ALP secreted by osteoblasts. Sclerostin, which is a glycoprotein produced by osteocytes, reduces the formation of bones by inhibiting the Wnt signal pathway. There is a positive correlation between sclerostin and ALP levels.\textsuperscript{16} In the present study, patients with hyperthyroidism showed a significant reduction in bone ALP levels after treatment compared with before treatment, which indicated an improvement in osteoporosis.

After 1 year of treatment, BMD of L1, the femoral neck, and the total hip in patients with hyperthyroidism was significantly improved compared with before treatment. This finding is consistent with a study by Segna et al who reported that, among adults, subclinical thyroid dysfunction was associated with increased femoral neck bone loss, potentially contributing to an increased risk of fracture.\textsuperscript{17} In logistic regression analysis of BMD-related risk factors, we found the bone ALP, PTH, and 25(OH)D levels were risk factors of BMD.

In conclusion, bone metabolism and BMD in patients with hyperthyroidism are improved by different degrees after treatment with methimazole. In treatment of patients with hyperthyroidism combined with osteoporosis, administration of the basic drugs should be supplemented with vitamin D and calcium, which have important clinical significance for adjusting bone metabolism and delaying the process of osteoporosis.

**Author contributions**

HL and QM performed the experiments; XH carried out data analysis; WH designed the study and wrote the manuscript. All authors approved the submission.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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