Changes in bone mineral density, 25-hydroxyvitamin D_3 and inflammatory factors in patients with hyperthyroidism

YALI ZHOU[1], XIXIA WANG[2], MAOYUAN XIN[1] and HAITING ZHUANG[3]

[1]Department of Traumatic Orthopaedics, Weifang People's Hospital, Weifang, Shandong 261000;
[2]Department of Internal Medicine, Zhucheng BaiChiHe Hospital, Zhucheng, Shandong 262217;
[3]Department of Anesthesiology, Weifang People's Hospital, Weifang, Shandong 261000, P.R. China

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Abstract. The present study aimed to evaluate changes in bone mineral density, 25-hydroxyvitamin D_3 [25-(OH)D_3] and inflammatory factors in patients with hyperthyroidism, in order to determine the correlations with the pathogenesis of hyperthyroidism. A total of 55 patients with hyperthyroidism (observation group) and 53 healthy patients (control group) enrolled at Weifang People's Hospital from March 2017 to February 2018 were randomly enrolled. The thyroid function, bone mineral density, 25-(OH)D_3 and inflammatory factors were measured and compared between the two groups. The measurement data are presented as mean ± standard deviation (SD), and Student t-test was performed for the comparison between two groups. Chi-square test was used for enumeration data regarding sex. Pearson correlation analysis was performed for two-variable analysis on L1, 25-(OH)D_3, interleukin (IL)-2, IL-6 with FT3, respectively. In regards to the results, no difference in sex, age and body mass index (BMI) between the two groups were found but the thyroid function was markedly enhanced in the observation group compared to the control group. Bone mineral density index and 25-(OH)D_3 in the observation group were significantly lower than those in the control group ($P<0.05$). There were significant differences in the inflammatory factors between the two groups ($P<0.05$). The L1, 25-(OH)D_3 and IL-2 levels were significantly negatively correlated with thyroid function index and free triiodothyronine (FT3) while a statistically positive correlation was found between IL-6 and FT3 ($P<0.05$). In conclusion, abnormal levels of bone mineral density, 25-(OH)D_3 and inflammatory factors are observed in patients with hyperthyroidism, and there are correlations between L1, 25-(OH)D_3, IL-2, IL-6 and FT3 in the pathogenesis of hyperthyroidism, which provides new insight for the diagnosis of hyperthyroidism.

Correspondence to: Dr Haiting Zhuang, Department of Anesthesiology, Weifang People's Hospital, 151 Guangwen Street, Kuiwen District, Weifang, Shandong 261000, P.R. China
E-mail: minglongli2kz@163.com

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Introduction

Hyperthyroidism represents a type of autoimmune disease (1-3). Previous studies have revealed that bone mineral density (BMD) is frequently decreased in patients, which potentially leads to osteoporosis (4,5). In general, BMD is associated with the metabolism of vitamin D in the body. The reduction in vitamin D may result in the decrease in bone mineral (6). A previous study suggested the involvement of vitamin D status, parathyroid hormone and BMD in the pathogenesis of osteoporosis in inflammatory bowel disease (IBD) and chronic inflammation was found to cause a reduction in BMD, leading to osteopenia and osteoporosis (7). However, the relationship of vitamin D, BMD and inflammatory factors with hyperthyroidism remains poorly understood. The aim of the present study was thus to investigate the clinical change in BMD, vitamin D and inflammatory factors in patients with hyperthyroidism, and their correlations with the pathogenesis of hyperthyroidism.

Patients and methods

General data. A total of 55 patients with hyperthyroidism (observation group) (male/female: 12/43) and 53 healthy patients (control group) (male/female: 13/40) at Weifang People's Hospital from March 2017 to February 2018 were enrolled. General data such as age, sex, weight and body mass index (BMI) showed no significant differences between the observation group and the control group. All subjects provided informed consent before enrollment into the study, and the study protocol was approved by the Ethics Committee of Weifang People's Hospital (Approval no. SPH20170206E) (Weifang, Shandong, China).

Inclusion criteria were: i) patients who met the diagnostic criteria of hyperthyroidism (8), ii) patients with good compliance to health-care workers during examination and treatment, iii) patients who had not previously received treatment with antithyroid medicines or drugs that affect bone metabolism, and iv) patients who did not suffer from major injury to organs, including the heart, liver and kidney as kidney or heart disease may affect inflammatory factor levels (9,10).

Exclusion criteria were: i) patients in pregnancy, ii) patients with other immunologic diseases, such as autoimmune

Table I. Comparisons of the general clinical data between the two groups.

Group	n	Sex (male/female)	Age (years)	BMI (kg/m^2)
Control group	53	27/26	56±6	21.21±2.54
Observation group	55	28/27[a]	59±7[a]	20.98±2.32[a]

A total of 55 patients with hyperthyroidism (observation group) and 53 healthy patients (control group) were enrolled. [a]P>0.05 vs. the control group, Student t-test. BMI, body mass index.

Table II. Comparison of thyroid function between the two groups.

Group	FT3 (pmol/l)	FT4 (pmol/l)	TSH (mIU/l)	TT3 (nmol/l)	TT4 (nmol/l)
Control group	18.23±1.86	25.32±2.96	1.99±2.08	1.43±0.14	92.56±9.99
Observation group	25.21±2.88[a]	49.98±5.43[a]	0.53±0.09[a]	5.69±0.67[a]	409.23±42.54[a]

A total of 55 patients with hyperthyroidism (observation group) and 53 healthy patients (control group) were enrolled. [a]P<0.05 vs. the control group, Student t-test. All data are expressed as the mean ± standard deviation. TT3, total triiodothyronine; TT4, total thyroxine; FT3, free T3; FT4, free T4; TSH, high-sensitive thyroid stimulating hormone.

hepatitis or primary sclerosing cholangitis, and iii) patients with bone development disorders, such as rickets, osteomalacia and osteogenesis imperfecta.

Methods. All patients did not receive antithyroid therapy at the time of sample collection. Dual energy X-ray absorptiometry was applied to measure the BMD at lumbar vertebrae L1-L4, the femoral neck, the total hip and the Wards triangle of the patients with hyperthyroidism (11).

The fasting venous blood of the patients was collected to detect inflammatory factors, interleukin-2 (IL-2), IL-6 and transforming growth factor-β (TGF-β) via immunoassay (cat. no. 03-0051-00; SMC™ Human Interleukin 2 (IL-2) Immunoassay kit; cat. no. K-03-0089-01; SMC™ Human Interleukin 6 (IL-6) Immunoassay kit; cat. no. RAB0460; Human TGF-β 1 ELISA kit; Merck KGaA), All the operations were conducted in strict accordance with the instructions in the kits. Enzyme-linked immunoassay was performed to determine the content of serum 25-hydroxyvitamin D_3 [25-$(OH)D_3$] according to the manufacturer's instructions (cat. no. ab213966; 25(OH) Vitamin D ELISA kit; Abcam).

The thyroid function indices in the serum, including total triiodothyronine (TT3), total thyroxine (TT4), free T3 (FT3), free T4 (FT4) and high-sensitive thyroid stimulating hormone (TSH) were measured using a chemiluminescent analyzer as previously described (12).

Statistical analysis. Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS, Inc.) was adopted for data analysis. The measurement data are presented as mean ± standard deviation (SD), and the Student t-test was performed for the comparison between two groups. Chi-square test was used for enumeration data. Pearson correlation analysis was performed for two-variable analysis. A level of statistical significance was defined at P<0.05.

Results

No difference in sex, age and BMI between the observation group and control group. There were no statistical differences in general clinical data between the observation group and control group, such as sex, age and body mass index (BMI) (P>0.05; Table I).

Thyroid function is enhanced in patients with hyperthyroidism. The comparison of thyroid function between the control group and observation group indicated that the levels of FT3, FT4, TT3 and TT4 in the observation group were significantly elevated compared to these levels in the control group, with a significant reduction in TSH level (P<0.05; Table II).

Bone mineral density was decreased in patients with hyperthyroidism. Compared with the control group, BMD in the observation group was significantly decreased at L1, L2, L3, L4, the femoral neck, the Wards triangle as well as the total hip (P<0.05; Table III).

25-$(OH)D_3$ is reduced in patients with hyperthyroidism. Our ELISA result showed a significantly decreased level of 25-$(OH)D_3$ in the observation group compared with the control group (P<0.05; Fig. 1).

Inflammatory factors are altered due to hyperthyroidism. In the observation group, the levels of TGF-β and IL-6 were significantly upregulated and the IL-2 level was significantly decreased, compared with these levels in the control group (P<0.05; Table IV).

Correlations of FT3 with L1, 25-$(OH)D_3$, IL-2 and IL-6. FT3 had a significantly negative correlation with L1 (r=-0.7435; P<0.001), 25-$(OH)D_3$ (r=-0.8802; P<0.001) or IL-2 (r=-0.7854;

Table III. Comparison of bone mineral density (BMD) between the two groups.

Index	Control group	Observation group
L1	0.89±0.05	0.80±0.09[a]
L2	0.99±0.08	0.85±0.08[a]
L3	0.98±0.08	0.90±0.09[a]
L4	0.99±0.09	0.97±0.08
L1-4	0.97±0.09	0.91±0.09[a]
Femoral neck	0.90±0.08	0.80±0.09[a]
Femoral great trochanter	0.69±0.08	0.67±0.08
Wards triangle	0.70±0.07	0.60±0.07[a]
Total hip	0.89±0.09	0.80±0.09[a]

A total of 55 patients with hyperthyroidism (observation group) and 53 healthy patients (control group) were enrolled. All data are expressed as the mean ± standard deviation. [a]P<0.05 vs. the control group, Student t-test. L1-L4, lumbar vertebrae.

Table IV. A total of 55 patients with hyperthyroidism (observation group) and 53 healthy patients (control group) were enrolled.

Group	TGF-β (ng/l)	IL-6 (ng/l)	IL-2 (ng/l)
Observation group	2.32±0.22	64.95±6.9	1.53±0.11
Control group	1.34±0.11[a]	18.31±1.78[a]	4.92±0.54[a]

[a]P<0.05 vs. the observation group, Student t-test. All data are expressed as the mean ± standard deviation. IL, interleukin; TGF-β, transforming growth factor-β. Comparisons of inflammatory factors between the two groups.

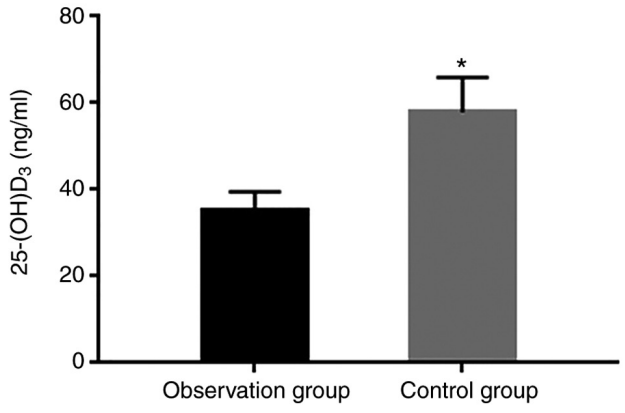

Figure 1. Comparison of 25-(OH)D_3 level between the patients with hyperthyroidism (observation group) and the healthy patients (control group). *P<0.05 vs. the observation group. 25-(OH)D_3, 25-hydroxyvitamin D_3.

P<0.001) and a statistically positive correlation with IL-6 (r=-0.5420; P<0.001). This suggests that the aberrant enhancement of thyroid function is associated with the reduction in L1, 25-(OH)D_3, IL-2, and an increase in IL-6 (Fig. 2).

Discussion

Hyperthyroidism is characterized as an endocrine disorder, which results in abnormality of hormone secretion and triggers various complications (13). In clinical practice, abnormal bone metabolism is found in patients with hyperthyroidism, and a majority of cases are accompanied with osteoporosis, thus it is proposed that hyperthyroidism is associated with bone mineral density (BMD) (14,15). In addition, hyperthyroidism is an autoimmune disease, and the immune function is impaired with abnormal secretion of inflammatory factors (16-18). Previous research demonstrated changes in BMD, vitamin D and inflammatory factors in hypothyroid patients (19), so as to provide clinical evidence for the treatment and diagnosis of patients with hyperthyroidism. In the present study, we further determined changes in BMD, 25-hydroxyvitamin D_3 [25-(OH)D_3] and inflammatory factors in patients with hyperthyroidism, to explore potential correlations with the pathogenesis of hyperthyroidism.

It has been demonstrated that the excessive secretion of thyroid hormone can accelerate the process of bone metabolism (20). Thyroid hormone is able to stimulate osteocytes and to enhance activity of these cells (21). The overactivation of bone metabolism suppresses the osteogenic function and the imbalance between bone formation and resorption functions finally resulting in osteopenia (22). Furthermore, excessive thyroid hormone can induce the decomposition of proteins in the body, retard the accumulation of calcium in the body and decrease BMD (23). At the molecular level, it has been revealed that thyroid hormone interacts with interleukin (IL)-6, thus further improving the production and secretion of osteoclasts and impairing the osteogenic function (24). Moreover, BMD is also associated with vitamin D in the body. 25-(OH)D_3 serves as an important indicator to evaluate the vitamin D level *in vivo*. Meanwhile, this factor functions to maintain the stable states of calcium and phosphorus in body and protect bone formation (25).

IL-6, a type of glycoprotein secreted from immune cells such as T lymphocytes and B lymphocytes, mainly participates in multiple inflammatory responses. It can promote the generation and production of immune cells, induce the differentiation of lymphocytes and favor the activity of immune cells at the same time (26). Transforming growth factor-β (TGF-β) is a type of cytokine with immunomodulatory functions, which is involved in inflammation and tissue repair. According to clinical research findings, this factor exerts relevant regulatory effects mainly through suppressing differentiation of immune cells, as well as generation of immunologic factors (27). IL-2 is produced by T lymphocytes and is able to promote the differentiation and proliferation of B lymphocytes, facilitating the immune response in the body (28). Previous study illustrated that the content of IL-2 is decreased in patients with hyperthyroidism (29), which plays a protective role against autoimmune reactions. Clinically, it was discovered that the IL-2 level is elevated in patients with hyperthyroidism after treatment. It is also found that IL-6 can promote excessive proliferation of B cells, induce hypersecretion of immunoglobulin G (IgG) in the body and improve humoral immunity (30). Consistent with the present study, previous data have demonstrated that the IL-6 level is significantly increased and the IL-2 level is significantly reduced in patients with hyperthyroidism (31).

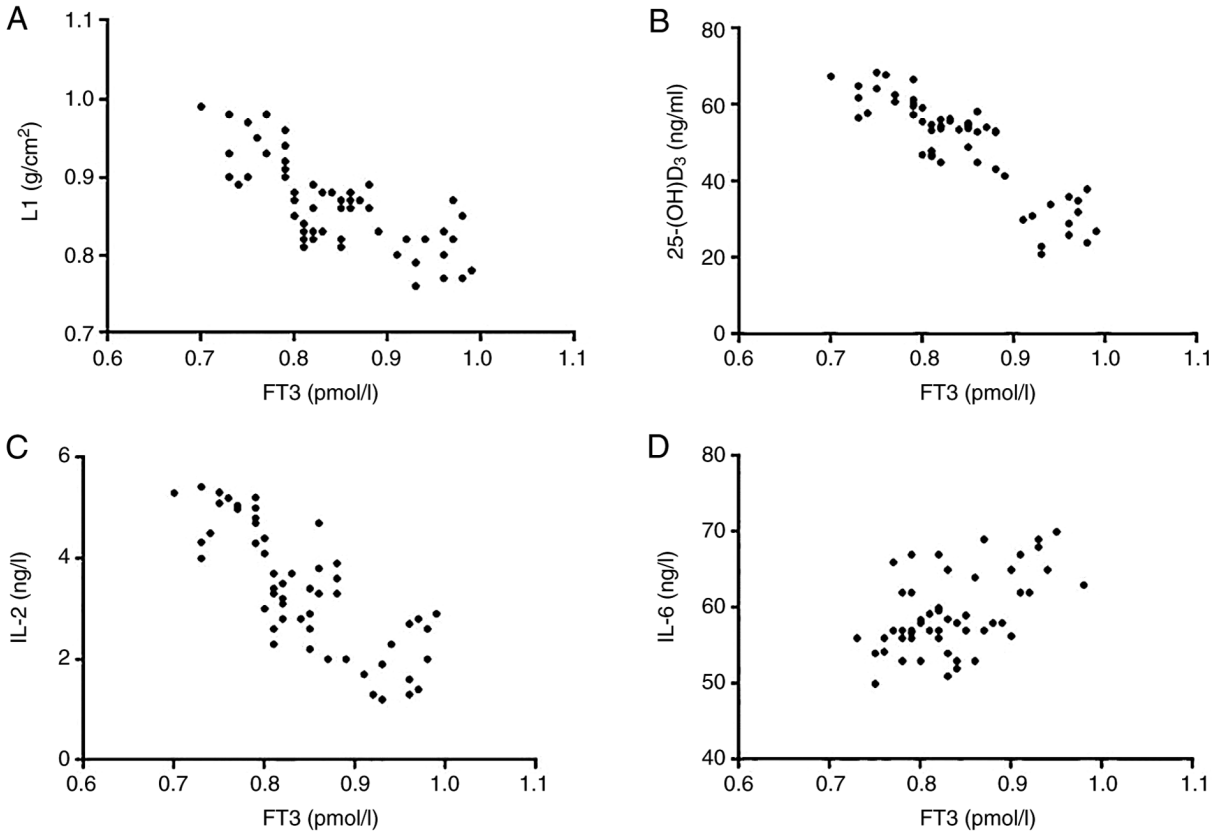

Figure 2. Correlation of L1, 25-(OH)D_3, IL-2 and IL-6 with FT3. (A) Analysis of the correlation between (A) FT3 and L1, (B) FT3 and 25-(OH)D_3, (C) FT3 and IL-2 and (D) FT3 and IL-6. L1, lumbar 1; 25-(OH)D_3, 25-hydroxyvitamin D_3; FT3, free T3; IL, interleukin.

Body mass index (BMI) is a person's weight in kilograms divided by the square of height in meters. A high BMI can be an indicator of high body fat. Consistent with a previous study, no significant difference in BMI was found between the patients with untreated hyperthyroidism and normal individuals (32). Of note, in this study, compared with the control group, it was demonstrated that the levels of FT3, FT4, TT3 and TT4 in the observation group were significantly increased, with significantly decreasing level of TSH, indicating that the thyroid function in patients with hyperthyroidism was abnormally enhanced. Moreover, the BMD in the observation group was significantly lower than that in the control group, which was in line with previous findings (14,15), indicating that in the case of excessive secretion of thyroid hormone, both the BMD and osteogenic function in the body are impaired. Importantly, in the observation group, the level of inflammatory factor IL-2 was significantly lower than that in the control group, while the levels of IL-6 and TGF-β were significantly higher, suggesting that the immune function of patients with hyperthyroidism is inhibited. It was also found that FT3 had negative correlations with L1, 25-(OH)D_3, IL-2 and IL-6, which implies that the bone formation of the patients with hyperthyroidism was suppressed. However, one limitation of this study was that the thyroid antibody was not detected in our present study. Our initial study only focused on BMD and vitamin D in hyperthyroidism. In the future, the expression of the thyroid antibody will be evaluated to identify its relationship with BMD and vitamin D in patients with hyperthyroidism. Importantly, unexpectedly, in a study regarding the detection of serum levels of osteotrophic cytokines in patients with various hyperthyroid states, IL-6 was higher in the euthyroid control group, which was in contrast with our data (33). But another study showed that serum IL-6 values were significantly higher in hyperthyroid patients when compared to a control group (34). Therefore, a large number of hypothyroid patients from different geographic regions ought to be involve to further validate our result and 'cocktail' indicators of the occurrence and development of hyperthyroidism require additional evaluation. In addition, further investigation may focus on specific therapy and evaluate its effect in clinical practice.

In conclusion, our preliminary data demonstrated that the abnormal enhancement of thyroid function is related to a decrease in BMD, vitamin D and aggravated inflammation in patients with hyperthyroidism, which provides insight into the development of new markers for predicting the severity of hyperthyroidism.

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Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request..

Authors' contributions

YZ substantially contributed to the experimentation and acquisition of data, designed experiments, performed data analysis and wrote the manuscript. XW contributed to the conception of the study. MX helped perform the analysis with constructive discussions. HZ contributed significantly to the data analysis and manuscript preparation.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Weifang People's Hospital (Approval no. SPH20170206E) (Weifang, Shandong, China) and informed consent from the subjects was obtained prior to the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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