**Study the Relationship between FT3, FT4, and TSH with Some Bone Resorption Indexes in Hypothyroidism Men**

**Asma'a H. Mohamed\*1, Noor S. Naji2, Ali H. Al-Saadi3**

1Radiology Techniques Department/ Al-Mustaqbal University College/ Hilla/ Iraq

,2,3Department of Biology-College of Science-University of Babylon/Iraq

1E-mail : [Asmaa\_Hassan@mustaqbal-college.edu.iq /ORCID 0000-0001-8638-0291](mailto:Asmaa_Hassan@mustaqbal-college.edu.iq%20%20%20/ORCID%200000-0001-8638-0291)

**Abstract**

The current study was aimed to find the effect of hypothyroidism in men on metabolism and the density of some bone mineral. The study included patients group of 90 men suffering from hypothyroidism and 120 healthy subjects as control group. the study comprised the estimation the concentration of Blood free triiodothyronine (FT3), free thyroid hormone (FT4), thyroid stimulating hormone (TSH), bone resorption index type I collagen C-terminal peptide (CTX-1), the serum calcium (Ca2+), serum phosphorus (Pi3+), the bone mineral density of lumbar spine and femoral neck. **The results showed that** In the hypothyroidism men group, 1. The bone mass was lower than the control group in significant differences . 2. The level of bone resorption index CTX-1 was significantly higher than that in control group; calcium and phosphorus were not differing from those in healthy control subjects. 3) TSH is positively correlated with CTX-1. male TSH and CTX-1 were positively correlated. **Conclusions:** There is bone loss in men with hypothyroidism, which may be related to increased bone resorption.

**Keywords**

Hypothyroidism, bone metabolism, bone mineral density

**Introduction**

Recent studies have shown that thyroid-stimulating hormone (TSH) have major regulatory role in bone remodeling independently of thyroid hormone [1-2]. Patients with subclinical hypothyroidism have elevated serum TSH and normal value of thyroid hormone, so it is a suitable model to study the role of TSH in regulating bone metabolism. At present, there is still controversy about the impact of TSH on bone mineral density and bone metabolism in domestic and foreign studies. The study by Jin Yunyun et al[3-4] showed that hypothyroidism may cause bone loss and decrease in bone mineral density. An investigation pointed out that the bone mineral density of patients with hypothyroidism is affected by serum TSH. However, a multicenter study in the United States did not observe a significant correlation between TSH and bone mass [5]. Therefore, in this study, by measuring the bone mineral density and bone metabolism indexes of cases with hypothyroidism, and comparing them with the normal control group, this study explored the impact of TSH on bone metabolism indexes and bone mineral density in hypothyroidism men.

**Materials and Methods**

A bout 210 cases who were examined in Marjan teaching hospital from October 2020 to October 2021 were selected. Among them, 120 healthy men were the control group with an average age of (52.97 ± 1.55) years old, BMI (23.50 ± 1.05) kg/m2; 90 patients men with hypothyroidism, with age mean (51.66 ± 1.64) years old, the BMI mean was (23.59 ± 1.12) kg/m2. There was no significant changing in the smoking, exercise, diabetes, and hypertension composition ratios between the two groups. The criteria of hypothyroidism diagnoses which depended were : TSH > 4.2 mIU/L, FT3 and FT4 are within the normal range. Exclusion criteria include: patients with thyroid disease after application of antithyroid drugs, levothyroxine tablets or surgical treatment, long-term of drugs utilized affecting bone metabolism, liver and kidney dysfunction. The serum TSH level in the hypothyroidism group was raised than control group, and the difference was statistically significant (P<0.05). The concentration FT3 and FT4 in the hypothyroidism group were lower than the control group (P<0.05),Table 1.

**1. Collection of Fasting Blood Samples**

About 5 ml of blood was collected for serum separated, then it stored at -20°C for later use. A biochemical analyzer was used to reveal the serum levels of FT4, FT3and TSH.

Table (1). Basic data of control and hypothyroidism men groups.

|  |  |  |  |
| --- | --- | --- | --- |
| **Basic Information** | **Control group**  **n = 120** | **Hypothyroidism men group**  **n = 90** | **P Value** |
| **Age (Year)** | 52.97 ± 1.55 | 51.66 ± 1.64 | 0.22 |
| **BMI (kg/m2)** | 23.50 ± 1.05 | 23.59 ± 1.12 | 0.29 |
| **Smoking n/%** | 43/ 35.9 | 41/45.5 | 0.23 |
| **Exercise n/%** | 32/26.6 | 30/33.3 | 0.25 |
| **Diabetes n/%** | 20/16.6 | 26/28.9 | 0.70 |
| **Hypertension n/%** | 55/45.9 | 74/82.2 | 0.63 |
| **FT3 pmol. L-1** | 4.82 ± 0.08 | 4.20 ± 0.06 | 0.04\* |
| **FT4 pmol. L-1** | 15.20 ± 0.05 | 13.54 ± 0.48 | 0.04\* |
| **TSH pmol. L-1** | 2.34 ± 0.45 | 5.14 ± 1.23 | 0.01\* |

**\* Signification at P value < 0.05**

**2. Detection of bone metabolism and resorption indexes**

The serum levels of calcium and phosphorus measured by colorimetric method, whereas CTX-1 levels were detected by ELISA.

**3. Detection of Bone Density**

The Dual energy X-ray absorptiometry was utilized for estimate the density of bone of the lumbar spine (L1-4) and left femoral neck, and calculate the T value. According to the diagnostic criteria of WHO for osteoporosis [6]: T value ≥ -1.0 referred to normal bone mass, T value (-2.5 to -1.0) referred to osteopenia, and T value ≤ -2.5 referred to bone mass. Note: As long as there is 1 decrease in T value in 2 parts, it is regarded as osteopenia or osteoporosis.

**4. Data statistical analysis**

Data analysis using SPSS 23.0 software. Continuous quantitative data are represented by M±S. The t test was used to compare the means among independent samples, the χ test was used to compare the rate and frequency, and the Mann-Whitney U test was used to contrast the rank data. The correlation between bone metabolism indexes and thyroid function was analyzed by Pearson correlation. The test level for overall comparison was α=0.05, and the test level after adjustment for subgroup comparison was α=0.0167.

**Results**

**Comparison of bone mass distribution in each group**

The bone mass in hypothyroidism group was significantly decreased compared to control group in significant changes (P<0.05), Table 2.

Table (2).Distribution of bone mass in each group

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study groups** | **Total Number** | **Normal** | **Bone Mass Reduce** | **Bone loss** | **Z** | **P Value** |
| **Control** | 120 | 86 | 33 | 1 | 2.28 | 0.01\* |
| **Hypothyroidism** | 90 | 53 | 36 | 2 |

**\* Signification at P value < 0.05, The corrected inspection level α/3=0.0167**

**Comparison of bone metabolism indexes in each group**

The level of bone resorption index CTX-1 in hypothyroidism men was significant raised than the control group table 3. According to correlation analysis, male TSH and CTX-1 were positively correlated (r=0.17, P=0.01), table 4.

Table (3). Comparison of bone metabolism indexes in each group

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Indexes** | **Control subject**  **(M±S.D)** | **Hypothyroidism men**  **(M±S.D)** | **t** | **P Value** |
| **Ca2+ (mmol. L-1)** | 2.45 ± 0.13 | 2.41 ± 0.14 | 0.033 | 0.87 |
| **Pi 3+ (mmol. L-1)** | 1.24 ± 0.26 | 1.29 ± 0.20 | 1.002 | 0.29 |
| **CTX-1(pg.ml-1)** | 1.41 ±1.01 | 2.43 ± 1.90 | 7.80 | 0.00\* |

Table (4). Correlation analysis of bone metabolism and hypothyroidism indexes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Indexes** | **FT3** | | **FT4** | | **TSH** | |
| **r** | **P** | **r** | **P** | **r** | **P** |
| **Ca2+** | 0.20 | 0.15 | 0.15 | 0.19 | - 0.11 | 0.35 |
| **Pi3+** | -0.01 | 0.52 | -0.16 | 0.39 | 0.04 | 0.85 |
| **CTX-1** | 0.05 | 0.61 | - 0.06 | 0.58 | 0.17 | 0.01\* |

**\* Signification at P value < 0.05**

**Discussion**

The dynamic balance between bone formation and bone resorption is an important for normal bone metabolism., and is regulated by a variety of factors. In recent years, endocrine osteoporosis is increasing day by day, and thyroid disease is one of the important causes of secondary osteoporosis. In the past, it was believed that osteoporosis caused by abnormal thyroid function was mainly due to thyroid hormones accelerating bone turnover, and bone resorption was greater than bone formation, thus manifesting osteoporosis, which had nothing to do with thyroid-stimulating hormone [7-8]. Other studies have shown that TSH itself have regulation ability in bone metabolism independently of thyroid hormones[9-10]. TSH is regulated by the thyroid-stimulating hormone receptor (TSHR) acting on the cell surface [11-12]. TSHR is not only expressed on the thyroid follicular cell membrane, but in recent years, scientists have also detected the RNA and protein expression of TSHR in many extrathyroid tissues, such as thymocytes, cardiomyocytes, hematopoietic stem cells, lymphocytes, testicular cells, kidneys, brains, and humans. Rat osteosarcoma cells, mouse osteoblasts, etc. [13-14]. Animal experiments have shown that TSH prevent bone remodeling by binding to TSHR on the membrane of osteoblasts and osteoclasts, resulting in decreased osteogenesis and osteoclast effects, resulting in bone loss in mice. Studies on animal models showed that the bone mineral density of TSHR knockout mice was notably lower than that of wild-type mice [5-16]. When the expression of TSHR is reduced by 50%, it can lead to large areas of osteoporosis and focal bone sclerosis in knockout mice [17]. Regarding the mechanism of TSH regulating bone metabolism, in vitro studies have shown that TSH acts on TSHR and mainly regulates the expression of bone metabolism markers such as IL-11, OPN, and ALP through the Gs-cAMP pathway [18].

A small molecule ligand of TSHR, D3-βArr, can enhance the TSHR-mediated β-arrestin1 molecular pathway to promote osteoblast differentiation [19-20]. Therefore, the TSH-TSHR pathway have major role in the regulation of bone metabolism.

In this paper, the bone mass loss of the two groups was compared by measuring the dual-energy X-ray bone mineral density of the research subjects, and it was observed that the bone mass in the hypothyroidism men group was significantly lower the control group. control group, suggesting the presence of bone in men with hypothyroidism amount is lost. Hao Xiaoyun[21-22] conducted a study on hypothyroid men in Taiyuan and found that their lumbar spine and hip bone mineral density were decreased than in the control group.

The study of 22 patients with hypothyroidism by Liang Libo et al. [23] showed that the serum calcium and bone mineral density concentration of men and women in the hypothyroidism group were lower than in the control group, and the serum phosphorus level of men with hypothyroidism increased. Another study found that the blood phosphorus and iPTH of residents in the subclinical hypothyroidism group were higher than control group, and 25(OH)D was decreased compared to control group, and there was no significant difference in serum calcium between the two groups [24]. However, there was no significant difference in calcium, phosphorus, between the study groups. It is generally believed that in hypothyroidism, due to the decrease in thyroid hormone levels, the blood calcium level decreases and the blood phosphorus level increases. However, the thyroid hormone levels in patients with hypothyroidism are in the normal range, only the TSH level is elevated, and there is no epidemiological data on the changes of calcium and phosphorus levels.

CTX-1 is a group of specific peptides at the carboxyl terminus of type I collagen, and it is one of the most widely used markers of collagen degradation, is commonly used indicator to assess the level of bone turnover. In this paper, by measuring the levels of CTX-1 in the research subjects, the results suggest that the level of bone resorption index CTX-1 is increased in hypothyroid men, and the difference is statistically significant. Histomorphological studies of adult bone remodeling by some scholars [25-26] showed that the rate of bone remodeling slowed down in hypothyroidism, the time of osteoblast-mediated bone formation was extended to 2 times, and the osteoclast-mediated bone resorption was increased. Extended to 4 times, the overall performance of bone mass and bone mineralization increased. In hyperthyroidism, both bone formation and bone resorption are accelerated, and the level of bone resorption is higher than the level of bone forming, showing high-transformation osteoporosis [27].

In this research, we further analyzed the correlation between TSH, FT3, FT4 and bone metabolism indexes, and found that TSH was positively correlated with CTX-1, suggesting that in men with hypothyroidism, the level of bone turnover may be accelerated, and the level of bone resorption is higher than the level of bone formation. This can lead to osteopenia or even osteoporosis. However, the analysis of FT3, FT4 and bone metabolism indexes did not find a significant correlation. This may be due to the fact that the levels of T3 and T4 in our observed objects are in the normal range, which is not enough to cause changes in the biochemical indexes of bone metabolism. This research did not measure osteocalcin, vitamin D3, parathyroid hormone and other indicators, so the results have certain limitations. In the next step, we can increase the detection indicators to comprehensively evaluate the bone metabolism status of patients with hypothyroidism, and further explore the metabolism status of hypothyroidism men. Downstream mechanisms for increased absorption. In addition, this study focused only on analyzing the correlation between the research factors and this cannot explain its causal relationship. The results of the study have certain limitations. More large-sample cross-sectional studies or cohort studies are required to explore the relation between bone metabolism and bone metabolism. related mechanisms of thyroid function system.

I. Ethical approval:

The manuscript is written in original and all the data, results pertaining to this manuscript are original according to the research performed. The authors followed academic integrity and have not copied any content/results from another source.

II. Funding details (In case of Funding):

The authors of this manuscript did not receive any funding to perform the present research

III. Conflict of interest

The authors of the study do not have any conflict of interest

IV. Informed Consent:

The authors of the manuscript agrees to publish this research in the journal if it’s considerable by the editors of the journal. The authors provide full consent for reviewing and publishing this manuscript.

V. All the authors of this study contributed equally in terms of performing the research as well as in preparing the manuscript. All the authors of the study followed the guidelines of the corresponding author. Any query/suggestion related to the manuscript can be reached to the corresponding author

**References**

[1] Bassett JH, Williams GR. Role of Thyroid hormonesin Skeletal development and development [J]. Endocr Rev, 2016, 37(2): 135-187. DOI: 10.1210/er. 2015-1106**.**

**[**2] Baliram R, Latif R, Berkowitz J, et al. Thyroid-stimulating hormone induces a Wnt-dependent, feed-forward loop for osteoblast oogenesis in embryonic stem cell cultures[J]. ProcNatl A cad Sci USA, 2011, 108(39): 16277-16282. DOI: 10.1073/pnas. 1110286108.

[3] van Vliet NA, Nordam R, van Klinken JB, et al. Thyroid Stimulating Hormone and Bone Mineral Density: Evidence From a Two Sample Mendelian Randomization Study and a Candidate Gene Association Study[J]. J Bone Miner Res, 2018, 33(7): 1318-1325. DOI: 10. 1002/jbmr. 3426.

[4] Jin Yunyun, Ding Xuesuo. Correlation between hypothyroidism and bone mineral density in middle-aged and elderly women Correlation analysis [J]. Marker Immunoassay and Clinical, 2018, 25(4): 454-456, 486. DOI: 10.11748/bjmy. issn. 1006-1703. 2018.04.003.

[5] Waring AC, Harrison S, Fink HA, et al. A spective study of thyroid function, bone loss, and fractures in older men: The Mr OS study[J]. J Bone Miner Res, 2013, 28(3):472-479. DOI: 10.1002/jbmr.1774.

[6] Jamal SA, Leiter RE, Bayoumi AM, et al. Criticality Of laboratory testing in women with host eoporosis [J]. Osteoporos Int, 2005, 16(5): 534-540. DOI: 10.1007/ s00198-004-1718-y.

[7] Reid IR. Short-term and long-term effects of osteoporosis Therapies[J]. Nat Rev Endocrinol, 2015, 11(7): 418- 428. DOI: 10.1038/nrendo.2015.71.

[8] Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide fol- low-upstudyin16, 249patients[J]. Thyroid, 2002, 12 (5): 411-419. DOI: 10.1089/105072502760043503

[9] Mohamed AH, ALKHAFAJI RS, Al-Saadi AH. Correlation between vitamin D deficiency and its receptor (FokI-rs2228570) gene polymorphisms in anemic men. Iran. J. Ichthyol. (Special issue 2022): 98-103.

[10] Segna D, Bauer DC, Feller M, et al. Association between subclinical thyroid dysfunction and change in bone mineral density in prospective cohorts[J]. J Intern Med, 2018, 283(1):56-72. DOI: 10.1111/joim.12688.

[11] Moon JH, Kim KM, Oh TJ, et al. The effect of TSH suppression on vertebral trabecular bones coresin patients With differentiated thyroid carcinoma [J]. J Clin Endocrinol Metab, 2017, 102(1): 78-85. DOI: 10.1210/jc. 2016-2740.

[12] Liao Eryuan. Endocrinology and Metabolism [M]. Beijing: People's Health Publishing House, 2012:1628-1706.

[13] Zaidi M, Davis TF, Zallone A, et al. Thyroid-stimulating hormone, thyroid hormones, and bone loss [J]. CurrOs-teoporos Rep, 2009, 7(2): 47-52.

[14] Busuttil BE, Frauman AG. TSH receiver expression Cardiac muscle tissue[J]. J Clin Endocrinol Metab, 2002, 87(6): 2994.

[15] Zaidan HK and Mohamed AH (2018) Neurotrophic factors determination of type II diabetic peripheral neuropathy patients. Biochem. Cell. Arch.,18( 2): 2051-2059.

[16] Stadlmayr W, Spitzweg C, Bichlmair AM, et al. TSH receptor transcripts and TSH receptor-like immune-reactivity in orbital and pretibial fibroblasts of patients with Graves' ophthalmopathy and pretibial myxedema [J].Thyroid, 1997, 7(1):3-12. DOI: 10.1089/thy. 1997.7.3.

[17] AbeE, Marians RC, YuW, et al. TSH is a negative regulator of skeletal remodeling [J]. Cell, 2003, 115(2):151-162. DOI: 10.1016/s0092-8674(03)00771-2.

[18] Boutin A, Neumann S, Gershengorn MC. Multitrans-division pathways mediate ethyrotropin receptor signaling In preosteoblast-like cells[J]. Endocrinology, 2016, 157(5): 2173-2181. DOI: 10.1210/en. 2015-2040.

[19] Neumann S, Eliseeva E, Boutique A, et al. Discovery of a Positive all osteric modulator of the rotropin receptor potentiation of thyrotropin-mediated pre osteoblast differentiation in vitro[J]. J Pharmacol Exp Ther, 2018, 364(1): 38-45. DOI: 10.1124/jpet. 117.244095.

[20] Hao Xiaoyun. Subclinical hypothyroidism and bone marrow in Taiyuan residents Metabolic correlation research [D]. Taiyuan:Shanxi Medical University, 2018.

[21] Zaidan HK, Mohammed AH. Glucagon like peptide-1 and C peptide level and their relationship with some physiological and biochemical variables of non-insulin dependent diabetes mellitus type 2 patient. Australian Journal of Basic and Applied Sciences. 2014 ; 8:1-10.

[22] Segna D, Bauer DC, Feller M, et al. Association between subclinical thyroid dysfunction and change in bone mineral density in prospective cohorts[J]. J Intern Med, 2018, 283(1):56-72. DOI: 10.1111/joim.12688.

[23] Liang Libo, Wang Youjuan, Zhang Mei, et al. subclinical hypothyroidism Correlation study with bone mineral density and bone metabolism indexes[J]. Sichuan University Chinese Journal of Medical Sciences, 2014, 45(1): 66-69, 83.

[24] Wang Jiadan, Zhang Qiao, Shi Lixin, et al. subclinical hypothyroidism Residents' serum 25-hydroxyvitamin D levels and cardiovascular risk factors Changes [J]. Chinese General Medicine, 2016, (22): 2671-2675. DOI: 10.3969/j. issn. 10079572.2016.22.012.

[25] Chinese Medical Association Osteoporosis and Bone Mineral Disease Branch. bone metabolism Guidelines for clinical application of chemical markers. Chinese osteoporosis and bone mineral Journal of Diseases, 2015, (4): 283-293. DOI: 10.3969/j. issn. 1674-2591.2015.04.001.

[26] Mosekilde L, Eriksen EF, Charles P. Effects of Hormones on bone and mineral metabolism[J]. Endocrinol Metab Clin North Am, 1990, 19(1): 35-63.

[27] Liu C, Zhang Y, Fu T, et al. Effects of magnetic Fields on bonne loss in hyperthyroidism rat model[J]. Bioelectronics, 2017, 38(2): 137-150. DOI: 10.1002/bem.22022.