The Influence of Thyroid State on Biochemical Markers of Bone Metabolism

Farida Mammadkhanova[1,2*], Gulnara Azizova[3]

[1]PhD student at Khazar University, Department of Life Sciences
[2]Doctor of Laboratory medicine at Bona Dea International Hospital.
Baku, Azerbaijan
[3]Biochemistry Department of Azerbaijan Medical University,
Head of Biochemistry Department. Baku, Azerbaijan.
[*]Corresponding author email: faridamamedkhanova@gmail.com

Abstract

Thyroid hormones are essential for normal development and function of human skeleton. Both excess as well as deficiency of thyroid hormones lead to disturbance of bone metabolism. Consequently, the disorder of bone formation and bone resorption phases can cause loss of bone mass and fractures. The aim of this study is to investigate better markers of bone metabolism pathologies during thyroid disfunction.

Keywords: Thyroid hormones, thyroid disease, osteoporosis, osteocalcin (OSC)

Introduction

For normal functioning of bone metabolism, harmonious roles of different factors are vital, such as genetic condition, hormonal and metabolic homeostatis, balanced diet, and mechanical load (Raisz, 2005). The disturbance of these systems can lead to serious and dangerous consequences, the prevention of which requires a correct adjustment (Raisz, 2005). Any abnormalities and irregularities of bone mineralization lead to significant changes for the human body such as bone length reduction and deformations and fractures (Raisz, 2005). The rising number of diagnosed osteoporosis cases in society makes it important to study all secondary conditions which can lead to reduced bone mineral density (Raisz, 2005).

The thyroid hormone plays a pivotal role in normal skeletal development and is essential for bone remodeling processing (Harvey *et al.,* 2002). More than 120 years

ago, Von Recklinghausen described a correlation between multiple fractures and the state of hyperthyroidism, and these studies and discussions are still ongoing as thyroid dysfunctions are widespread on a global scale (Nicholls *et al.,* 2012).

The thyroid hormone receptors TRα1, TRβ1 and TRβ2 regulate expression of target genes in response to T3 (Forrest *et al.,* 1990). TRα1 and TRβ1 are present in growth plate chondrocytes, bone marrow stromal cells, bone-forming osteoblasts, coordinating bone formation and bone resorption in the skeleton. Additionally, TRα1 and TRβ1 mediate the action of the biologically active hormone 3,5,3′-L-triiodothyronine (T3) in the bone, which demonstrates a close relationship between thyroid dysfunction and bone metabolism (Forrest *et al.,* 1990; Robson et al., 2000; Gauthier et al., 2000).

Both cases of thyroid disfunction, hyperthyroidism and hypothyroidism, disturb bone metabolism and change the balance of bone formation and bone resorption markers (Williams and Bassett, 2018; Bakos *et al.,* 2018).

The excess of thyroid hormones in childhood can lead to premature growth of plates, closure of cranial sutures, causing low growth and deformation of the skeleton. In adults, hyperthyroidism is characterized by accelerated bone renewal and a loss of mineral density in 10-20% of a study group. The bone remodeling cycle is reduced in almost 50%, dropping from 200 to 113 days, and the proportions between bone formation and its resorption are inhibited (Basset & Williams, 2003; Harvey et al., 2002; Basset & Williams, 2008; Greenspan & Greenspan, 1999; Stevens et al., 2003; Williams, 2009). The bone formation phase decreases by 2/3, which effects a loss of more than 10% of the mineralized bone in one cycle. As a result, thyrotoxicosis leads to increased risk of fractures (Lakatos, 2003; Kosińska *et al.,* 2005).

On the contrary, deficiency of thyroid hormones in childhood leads to growth retardation or even growth arrest, disturbances of endochondral ossification, delayed bone age and persistent short stature (Basset & Williams, 2003; Harvey et al., 2002; Basset & Williams, 2008; Greenspan and Greenspan, 1999; Stevens et al., 2003; Williams, 2009). Hypothyroidism causes general hypometabolism. Bone formation processes are slowed in 50% in hypothyroid patients and bone resorption processes in 40%, although their mechanism remains unclear (Lakatos, 2003; Kosińska *et al.,* 2005).

Biochemical markers released during bone remodeling by osteoblasts and osteoclasts can be evaluated in the blood (Greenblatt *et al.,* 2017; Ale *et al.,* 2018). In our study, we investigate osteocalcin as a bone turnover marker and beta-CrossLaps as a bone resorption marker.

Osteocalcin (OSC) is the most important non-collagen bone matrix protein and accounts for about 1% of the total bone protein. Osteocalcin is synthesized in osteoblasts and is widely known as a marker of osteoblastic bone activity (Greenblatt *et al.,* 2017; Cancela *et al.,* 1990; Ale *et al.,* 2018). After releasing from the osteoblasts, osteocalcin is not only assimilated into the bone matrix, but also secreted into the blood stream and is therefore termed a bone turnover marker and is used for this purpose (Gouveia *et al.,* 2001; Varga *et al.,* 1997; Tsevis *et al.,* 2018).

More than 90 % of organic bone matrix consists of type I collagen, which is preferentially synthesized in bone. There is regulated anabolism and catabolism of the basic substance in bone (Greenblatt *et al.,* 2017; Markus, 2005). During normal bone metabolism, mature type I collagen is degraded, and small fragments pass into the bloodstream and are excreted via the kidneys. In physiologically or pathologically elevated bone resorption (e.g. in old age or as a result of osteoporosis), type I collagen is degraded to an increased extent, and there is a commensurate rise in the level of collagen fragments in the blood (Greenblatt *et al.,* 2017; Markus, 2005). By determining these bone resorption markers, the activity of osteoclasts can be detected. Especially relevant collagen type I fragments are the β-isomerized C-terminal telopeptides (β-CrossLaps or β-CTx). These isomerized telopeptides are highly specific for the degradation of type I collagen dominant in bone (Greenblatt *et al.,* 2017). Elevated serum levels of isomerized C-terminal telopeptides of type I collagen have been reported for patients with increased bone resorption and return to normal levels during antiresorptive therapy (Markus, 2005).

Materials and methods

Clinical samples for the study were collected during a one-year period from January to November 2019 at the Central Laboratory of the Surgical Clinic Azerbaijan Medical University, Baku, Azerbaijan. Suitable patients attending the Outpatient Department for evaluation of thyroid status were enrolled in the study.

Twenty hyperthyroid (16 female, 4 male) and twenty hypothyroid (17 female, 3 male) patients aged 20-60 years were selected for the study. The control group also included 20 healthy patients with no history of chronic disease from ages 25-35 years. The patients were diagnosed as Graves disease, multinodular goiter and Hashimoto disease. Patients with co-morbidity, such as vitamin D deficiency, renal disease, alcoholism and patients on medication influencing bone turnover were excluded from the study.

The diagnosis was based on their detailed medical history and thyroid profile analysis (TSH, free T3 and free T4). Fasting venous blood samples were collected from patients and control subjects under the sterile condition. The samples were centrifuged to obtain serum on the same day of collection for further clinical analysis. All samples were stored at -20ºC.

Quantitative determination of serum osteocalcin (N-MID Osteocalcin) and beta-CrossLaps (β-CrossLaps/serum) bone markers were analyzed by electrochemiluminescent (ECLIA) enzyme immunoassay for in-vitro diagnostic using a Roche cobas e 411 analyzer (Delmas *et al.,* 2000; Christgau *et al.,* 1998; Markus, 2006). For the thyroid profile (TSH, freeT3, freeT4) analysis the Roche cobas e 411 analyzer (ECLIA method) was also used.

The results of all variables are reported as the mean±S.D. Independent t-test and Pearson correlation were used to compare the difference of the values between hyper- and hypo-thyroid state and control patients. The analysis was performed by using GraphPad Prism software. The p-value <0.05 was considered as statistically significant and p<0.0001 as statistically highly significant.

Results

The identification criteria for diagnosis of hyperthyroid patients were applied for elevated levels of active thyroid hormones and lowered level of TSH. Free Trijodthyronin (fT3) (6.57±2.22 vs. 3.49±0.53, p-value <0.0001), free Thyroxine (fT4) (22.45±5.53 vs. 13.62±1.61, p-value <0.0001) in the serum were higher and the TSH level in hyperthyroid state was significantly lower than that of controls (0.134±0.22 vs. 1.45±0.68 p-value <0.0001).

Table 1. Serum bone turnover markers and thyroid hormones of patients in hyperthyroid state and controls.

| Parameters	Hyperthyroid state (n=20) Mean±SD	Control (n=20) Mean±SD	p-value
Osteocalcin (ng/mL)	25.42±11.57	15.44±3.49	0.0025
β-CrossLaps (ng/mL)	0.477±0.309	0.244±0.07	0.0063
TSH (μIU/mL)	0.134±0.22	1.45±0.68	0.0001
Free Trijodthyronin (pmol/L)	6.57±2.22	3.49±0.53	0.0001
Free Thyroxine (pmol/L)	22.45±5.53	13.62±1.61	0.0001

Results are expressed as a Mean±Standart Deviation (SD). Statistical analysis was performed by unpaired t-test. $p < 0.05$ Statistically Significant, $p < 0.0001$ Statistically highly significant, $p > 0.05$ Statistically not significant.

Hypothyroidism is associated with low levels of thyroid hormones, high level of TSH, and the signs and symptoms as well. Free Trijodthyronin (fT3) (1.58 ± 0.65 vs. 3.49 ± 0.53, p-value < 0.0001) and free Thyroxine (fT4) (8.15 ± 1.54 vs. 13.62 ± 1.61, p-value < 0.0001) were highly decreased in hypothyroid patients, while serum TSH (19.3 ± 17.54 vs. 1.45 ± 0.68, p-value < 0.0001) was increased in relation to the control group.

Table 2. Serum bone turnover markers and thyroid hormones of patients in hypothyroid state and controls.

Parameters	Hypothyroid state (n=20) Mean±SD	Control (n=20) Mean±SD	p-value
Osteocalcin (ng/mL)	20.43±6.05	15.44±3.49	0.0010
β-CrossLaps (ng/mL)	0.430±0.194	0.244±0.07	0.0001
TSH (μIU/mL)	19.3±17.54	1.45±0.68	0.0001
FreeTrijodthyronin(pmol/L)	1.58±0.65	3.49±0.53	0.0001
Free Thyroxine (pmol/L)	8.15±1.54	13.62±1.61	0.0001

Results are expressed as a Mean±Standart Deviation (SD). Statistical analysis was performed by unpaired t-test. $p < 0.05$ Statistically Significant, $p < 0.0001$ Statistically highly significant, $p > 0.05$ Statistically not significant.

BONE METABOLISM MARKER: Osteocalcin

BONE METABOLISM MARKER: β-CrossLaps
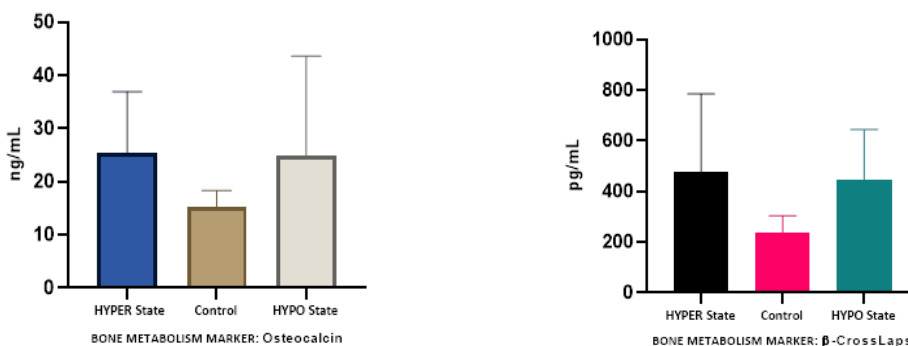
Serum bone marker levels, osteocalcin and β-CrossLaps, were higher in hyperthyroidism in relation to the control group (25.42 ± 11.57 vs. 15.44 ± 3.49 ng/mL,

p=0.0025 and 0.477±0.309 vs. 0.244±0.07 ng/mL, p=0.0063, respectively). In our study, the osteocalcin level in hypothyroid patients does not have a large deviation in comparison with the control group (20.43±6.05 vs.15.44±3.49 p=0.0010). However, the bone resorption marker β-CrossLaps in hypothyroidism was significantly increased in comparison to the control patients (0.430±0.194 vs. 0.244±0.07 p=0.0001).

In both hyperthyroid and hypothyroid states, we found a strong correlation between OSC and β-CrossLaps levels, with r=0.805 p<0.01 and r=0.672 p<0.01 respectively, which were statistically significant (p less than 0.001). These results of our investigation suggest that osteoblastic activity is enhanced in hyperthyroidism and suppressed in hypothyroidism. Consequently, the analysis of both biochemical bone metabolism markers has/plays an important role in thyroid patients. In the hypothyroid state, bone resorption processes proceed more intensively, than during bone remodeling processes, so the β-CrossLaps marker is a better marker for the detection of osteoporosis in hypothyroidism (Varga *et al.,* 2010; Murphy & Williams, 2004; Vestergaard and Bassett, 2002).

Discussion

Recent studies reveal that thyroid status has a significant role in bone metabolism regulation (Murphy and Williams, 2004). The excess of thyroid hormones leads to increased bone resorption and formation activity (De Leo *et al.,* 2016; Bassett & Williams, 2016; Reddy *et al.,* 2012; Svare *et al.,* 2009). A meta-analysis by Vestergard *et al.* (2003) noted that bone mass density was significantly decreased in patients with untreated hyperthyroidism.

The expedited bone remodeling and reduced bone density in hyperthyroidism increases the risk of fracture and osteoporosis, which can be detected by elevated levels of bone markers (Vestergaard & Bassett, 2002; Fitzpatrick, 2002; Gorka *et al.,* 2013; Reddy *et al.,* 2012; Svare *et al.,* 2009).

In our investigation, the hyperthyroid patients had highly significant elevated osteocalcin and β-CrossLaps. Kisakol *et al.* (2003), also showed a high level of osteocalcin in their study as a marker increasing during osteoblastic activity. A similar study by Barsal *et al.* (2004), reported that bone formation and bone resorption marker levels are elevated in hyperthyroid patients, which confirms the high bone turnover state.

Thyroid hormones bind to nuclear receptors and regulate gene transcription via interaction with thyroid hormone response elements of specific genes (Waung *et al.,* 2011; Gauthier *et al.,* 1999). The action of active thyroid hormone formed in bone are complex and partially understood (Waung *et al.,* 2011). Thyroid hormones stimulate both bone formation and resorption. During bone formation, fT3 stimulates osteoblast proliferation, differentiation and apoptosis, and increases the expression of osteocalcin, type 1 collagen, alkaline phosphatase, metalloproteins, IGF-1 and its. It also amplifies the effects of interleukin-1, interleukin-6, and prostaglandin E2 during bone resorption receptor (Basset and Williams, 2003; Harvey *et al.,* 2002; Williams, 2009; Bassett & Williams, 2016).

TSH acts as a direct negative regulator of bone turnover, that hinders osteoblast differentiation and repress osteoclast formation (Abe *et al.,* 2003).

Contrasting with hyperfunction of thyroid hormones, hypothyroidism leads to a low bone turnover, with a reduction of osteoclast bone resorption and of osteoblast formation (Bassett *et al.,* 2007; Fitzpatrick, 2002; Gallford *et al.,* 2005; Waung *et al.,* 2011). The hypothyroid state slows the remodeling process and increases the time taken in the remodeling cycle. This is due to the prolongation of the mineralization phase (Bassett *et al.,* 2007; Fitzpatrick, 2002; Vestergaard, 2002; Gallford *et al.,* 2005). Vestergaard (2002) histomorphometry data demonstrated that hypothyroidism is associated with a two- to three- fold increased risk of fracture, which is related mainly with hypometabolism, "metabolic depression" and tissue hypoxia. Impaired growth and differentiation cells and tissues, physiological regeneration slows down, but bone resorption passes faster and because of this/as a result/as its consequence, the bone resorption marker β-CrossLaps (p-value<0.0001) becomes significantly elevated.

Therefore, based on our study and recent studies, early management of thyroid disorders is essential for the prevention bone related complication.

Conclusion

Thyroid hormones are essential for normal development and function of the human skeleton. In hyper- and hypo-thyroidism, the conditions observed increased fracture risk (Fitzpatrick, 2002; Vestergaard and Mosekilde, 2003; Leger *et al.,* 1997). Our recent study suggests that osteocalcin and β-CrossLaps markers are non-invasive markers, which can be used in the investigation and prevention pathologies of bone metabolism during thyroid disfunction.

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