Serum Osteocalcin Levels in Hyperthyroidism before and after Antithyroid Therapy

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Hyperthyroidism is characterized by accelerated bone turnover, caused from direct stimulation of bone cells by increased thyroid hormones. In this study, we aimed to investigate serum osteocalcin levels as a bone formation marker, before antithyroid (propylthiouracil) therapy at hyperthyroid stage and after antithyroid therapy at euthyroid stage of the patients. Twenty four hyperthyroid patients (18 females, 6 males) and 20 (13 females, 7 males) healthy controls were included into this study. Blood and urine samples were taken before medical treatment at hyperthyroid state, and after the antithyroid therapy until the patients reached the euthyroid state. Serum alkaline phosphatase, osteocalcin, calcium, phosphorus, Free T3, Free T4, TSH and urine calcium/creatinine levels were assessed. We found a significant decrease in serum osteocalcin (p=0.006), urinary calcium/creatinine (p=0.004), and serum phosphorus (p=0.038) levels in euthyroid state in comparison to hyperthyroid state. The increases in serum bone formation marker osteocalcin and bone resorption marker urinary calcium/creatinine levels in hyperthyroid state compared to euthyroid state in our study confirmed that hyperthyroid patients have high bone turnover. We conclude that, hyperthyroid patients has high bone turnover of formation and resorption even after attainment of euthyroidism. Osteocalcin and urine calcium/creatinine are sensitive markers in documenting bone remodeling during treatment of hyperthyroidism.

Bone turnover; hyperthyroidism; osteocalcin

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Hyperthyroidism is characterized by accelerated bone turnover, which is caused from direct stimulation of bone cells by increased thyroid hormone concentrations (Rizzoli et al. 1986; Pandolfi et al. 1992; Abu et al. 1997; Pantazi and Papapetrou 2000). It has been demonstrated that increased bone turnover results in osteoporosis (Rosen and Adler 1992). The biochemical markers of bone formation and bone resorption, such as osteocalcin (OC), alkaline phosphatase (ALP), bone specific ALP, and urinary collagen pyridinoline (Upyr) or deoxypyridinoline (Udpd) cross-links were elevated in hyperthyroid patients, indicating increased bone turnover in favor of osteoclastic bone resorption (Garrel et al. 1986; Meunier 1995). Because of the increased mobilization of bone mineral, a tendency to hypercalcemia and hyperphosphatemia, hypercalciuria and hyperphosphaturia often occur in hyperthyroid patients (Mosekilde et al. 1990). After treatment of hyperthyroidism, serum calcium falls, sometimes enough to cause tetany. This hypocalcemia is ascribed to the healing of the metabolic bone disease and increased calcium deposition to bone. Calcium and phosphorus balance was negative during the hyperthyroid status and was converted to positive soon after euthyroidism was attained (Meunier 1995). The effect of antithyroid therapy on treatment on calcium and phosphorus metabolism in hyperthyroidism was studied and the initially high serum ALP level increased further to a maximum after 8 weeks of antithyroid treatment. Serum calcium and the 24 hour urinary calcium excretion decreased. These findings were suggestive of decreased bone resorption and increased bone formation with deposition of bone mineral after antithyroid treatment (Meunier 1995). Cooper et al. (1979) showed that the rise of serum ALP observed after treatment of hyperthyroidism is due mainly to bone isoenzyme. Recently, Siddiqi et al. (1997) also observed a fall of the bone resorption markers Upyr and Udpd and a further rise of the bone formation markers B-ALP and OC during treatment of hyperthyroid patients with antithyroid drugs. In this study, we aimed to investigate the effects of high serum thyroid hormones on calcium-phosphorus metabolism and on serum osteocalcin levels during antithyroid therapy, before and after attainment of euthyroidism.

**Materials and Methods**

Twenty four hyperthyroid patients (18 female, 6 male) with recent onset hyperthyroidism were enrolled from the out-patient clinic of endocrinology department. Twenty age and sex matched healthy controls (13 female, 7 male) were included in the study. The mean age and standard deviation (S.D.) of the patients and controls were 45.5±22.3 years and 41.5±15.2 years, respectively. The patients were diagnosed as Graves’ disease (n=9), multinodular goiter (n=13), and toxic nodular adenoma (n=2). Informed consents of the patients were obtained before the study. The study protocol was approved by the local ethics Committee. The postmenopausal women had not received hormone replacement therapy or any other treatment for osteoporosis in the past. Throughout the period of the study, the patients were not taking any other medication apart from the antithyroid drug. None had a history of hepatic or renal disorders, alcoholism, early menopause or any other major medical condition. After the initial examination (week 0) antithyroid medication was initiated. The patients were examined every 2 weeks between 09:00-11:00 hours and venous blood samples were obtained. At this period only serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) levels were assessed to evaluate whether the patients reached the euthyroid state. The patients were followed up for a period of 2-8 months with antithyroid therapy until each patient attains the euthyroid state.

Blood and urine samples were obtained before medical treatment at hyperthyroid state, and after antithyroid treatment at euthyroid state. Propylthiouracil was used as antithyroid therapy for a range of 2-8 months in patients. In hyperthyroid patients serum thyroid hormones (Free
T3, Free T4 and TSH) ALP, calcium, phosphorus, creatinine, osteocalcin and urine calcium/creatinine levels were assessed before the propylthiouracil treatment was initiated and after treatment at euthyroid state of the patients. Fasting, venous blood samples of the control subjects were obtained at 09:00-11:00 hours. Serum Free T3, Free T4, TSH and osteocalcin levels were assessed by enzyme-amplified chemiluminescence assay on Immulite One analyzer (IMMULITE, Diagnostic Products Co., Los Angeles, CA, USA). Serum ALP, creatinine, albumin, calcium and phosphorus levels were analyzed on Olympus AU 5200 analyzer (Olympus System Reagents, Southall Middlesex, Ireland). Serum calcium levels are corrected with serum albumin levels using the formula: corrected total calcium (mg/100 ml)=total Ca+0.84(4-albumin g/100 ml). Urinary calcium/creatinine is calculated using the formula: urinary calcium/creatinine (mg/100 ml glomerular filtrate)=urine calcium (mg/100 ml)x(serum creatinine (mg/100 ml) / urinary creatinine (mg/100 ml) (Burtis and Edward 1999). A urinary calcium/creatinine value exceeding>0.16 mg/100 ml usually implies an increase in osteoclastic bone resorption. Sera were stored at −20°C until assessments and urine samples were assessed instantly. The results of all variables are reported as the mean±s.d. The significance of the differences of the values between hyperthyroid and euthyroid state was evaluated by paired t-test, and Pearson correlation analysis and p<0.05 were considered as statistically significant.

**RESULTS**

Results of the bone turnover markers and thyroid hormones of the study groups are summarized in Table 1. Serum OC, ALP, calcium and phosphorus levels are 10.80±5.29 ng/ml, 144.65±42.33 U/litter, 9.28±0.35 mg/100 ml and 3.56±0.54 mg/100 ml, respectively in the control group. Serum TSH, FT4 and FT3 levels are 1.48±1.16 μIU/ml, 1.26±0.20 ng/ml and 2.60±0.57 pg/ml in control group.

Serum osteocalcin levels were significantly increased in hyperthyroid state in relation to euthyroid state (28.60±18.73 vs. 15.20±14.73 ng/ml, p=0.006), and in hyperthyroid state in relation to control group (28.60±18.73 vs. 10.80±5.29 ng/ml, p=0.0001). Serum ALP levels were slightly higher in hyperthyroid state than in euthyroid state but this elevation was statistically insignificant (p>0.05). Serum ALP levels were high in hyperthyroid and euthyroid state compared to control group (269.20±96.77 vs. 144.65±42.33 U/litter, p=0.0001) and (227.87±90.47 vs. 144.65±42.33 U/litter, p=0.004), respectively. Serum

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hyperthyroid State (n=24)</th>
<th>Euthyroid State (n=24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>28.60±18.73</td>
<td>15.20±14.73</td>
<td>0.006</td>
</tr>
<tr>
<td>Serum ALP (U/litter)</td>
<td>269.20±96.77</td>
<td>227.87±90.47</td>
<td>n.s.</td>
</tr>
<tr>
<td>Serum calcium (mg/100 ml)</td>
<td>9.35±0.49</td>
<td>9.39±0.73</td>
<td>n.s.</td>
</tr>
<tr>
<td>Serum phosphorus (mg/100 ml)</td>
<td>4.09±0.89</td>
<td>3.56±0.64</td>
<td>0.038</td>
</tr>
<tr>
<td>Urine calcium/creatinine (mg/100 ml glomerular filtrate)</td>
<td>0.1187±0.1006</td>
<td>0.0840±0.0388</td>
<td>0.004</td>
</tr>
<tr>
<td>TSH (μIU/ml)</td>
<td>0.0009±0.002</td>
<td>0.7±0.42</td>
<td>0.006</td>
</tr>
<tr>
<td>Free T3 (pg/ml)</td>
<td>11.43±2.26</td>
<td>3.27±0.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Free T4 (ng/ml)</td>
<td>3.99±0.59</td>
<td>1.52±0.48</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are shown as mean±s.d. n.s., non significant.
Statistical analysis was performed by paired t-test.
calcium levels were 9.35±0.49 mg/100 ml in hyperthyroid state, 9.39±0.73 mg/100 ml in euthyroid state, and 9.28±0.35 mg/100 ml in control subjects. We did not find any significant changes in serum calcium levels between the groups. Serum phosphorus levels decreased significantly between hyperthyroid and euthyroid state (4.09±0.89 vs. 3.56±0.64 mg/100 ml, \( p=0.038 \)) and between hyperthyroid state and controls (4.09±0.89 vs. 3.56±0.54, mg/100 ml, \( p=0.046 \)). Urinary calcium/creatinine excretion was higher in hyperthyroid state than in euthyroid state (\( p=0.004 \)). The statistical differences were found as \( p=0.006 \) for TSH and \( p=0.001 \) for Free T3 and \( p=0.001 \) for Free T4 between the hyperthyroid and euthyroid states of the patients. Before antithyroid treatment mild correlation was found between OC and FT_3 (\( r=0.491, p<0.05 \)) and FT_4 levels (\( r=0.431, p<0.05 \)) using Pearson correlation analysis. We found a moderate correlation between OC and ALP levels both in hyperthyroid state (\( r=0.479, p<0.05 \)) and euthyroid state (\( r=0.492, p<0.05 \)).

**DISCUSSION**

Recent investigations reveal that hyper secretion of thyroid hormones results in an increase in resorption and formation activity of bone metabolism (Siddiqi et al. 1997; Isaia et al. 2000; Akalin et al. 2002; Kısakol et al. 2003). In our study, we found elevated OC levels in hyperthyroid state, significantly higher than the control group. When these patients were in euthyroid state serum OC levels decreased still being higher than the control group. Our results point to the increase in osteoblastic activity in bone metabolism and thus we believe that the serum OC level is a good marker to detect the osteoblastic activity of bone metabolism in hyperthyroidism. There are similar results with our study showing high levels of OC as a marker pointing to the increase of the osteoblastic activity (Kobe et al. 1999; Akalin et al. 2002; Kısakol et al. 2003). In our study group, all patients had clinical symptoms where as in Engler et al.’s study (1999), the subclinical hyperthyroid patients were studied and the same increase in bone activity was also found. Siddiqi et al. (1997), found that after 4-8 weeks of antithyroid treatment their patients were in euthyroid state and the osteocalcin and bone-ALP levels increased to a peak level and then decreased. However, Ross et al. (1991) thought that OC is not a useful marker in showing the increased bone turnover in subclinical hyperthyroidism. These conflicting results may be due to the inconsistent OC levels in the beginning of the euthyroid state. Also it is important to determine the time to analyze the OC levels because it has been shown that the OC has a circadian secretion such as low serum OC in the morning, starting to increase in the afternoon and peak in the night (Gundberg et al. 1985). This phenomenon may result in the conflicting results in literature.

Kobe et al. (1999), studied the acute effects of T3 on healthy volunteers, and they studied the same parameters in 7 days period and they also conclude that OC increase is a better marker of than the other conventional bone markers in pointing the effects of thyroid hormone on bone metabolism.

In recent studies of hyperthyroidism, as a result of bone formation, serum ALP increases and after therapy declines to it’s original levels. However, if the bone formation continues serum ALP levels may remain at high levels. In our study we exclude the patients with hepatic diseases to eliminate the possible interferences on serum ALP levels. However we could not find a statistical difference in serum ALP levels of hyperthyroid state and euthyroid state. We found a moderate correlation between OC and ALP levels both in hyperthyroid state and euthyroid state as in accordance with the literature (Roiter et al. 1990). Siddiqi et al. (1997), showed that after 4-8 weeks of antithyroid therapy, serum OC had lower sensitivity than serum bone specific ALP. Although, there are many studies that demonstrate a correlation between serum OC and ALP levels (Leon et al. 1990; Roiter et al. 1990; Siddiqi et al. 1997; Isaia et al. 2000; Jodar et al. 2001) some
contradictory data also exists (Vamos and Balazs 1997; Isaia et al 2000). In our study, serum ALP levels were slightly higher in hyperthyroid group than in euthyroid group but this elevation was statistically insignificant. Serum ALP levels were high in hyperthyroid group and euthyroid group compared to the control group. In our study urinary calcium/creatinine excretion decreased after antithyroid therapy. In hyperthyroid patients as the bone resorption normalizes urinary calcium/creatinine excretion decreased as can be seen in similar studies (Roiter et al. 1990; Vamos and Balazs 1997).

Recent studies (Akalin et al. 2002; Kisakol et al. 2003) reveal that hyperthyroidism leads to osteoporosis and both bone formation and resorption rates are increased in case of elevated thyroid hormones, but the increases in the resorption is more prominent. In the present study, we found a decrease in serum osteocalcin, serum phosphorus, and urinary calcium/creatinine excretion parallel to the decrease of thyroid hormones in euthyroid state of the subjects. The increases in serum bone formation marker osteocalcin and bone resorption marker urinary calcium/creatinine levels in hyperthyroid state compared to euthyroid state in our study confirmed that hyperthyroid patients have high bone turnover. These results confirmed previous data that in hyperthyroid patients have high bone turnover and negative calcium and phosphorus balance. We are of the opinion that, being secreted only from the bone and a bone-specific protein, OC, is a non-invasive marker that can be used in investigating the pathologies of bone metabolism. The failure of bone turnover markers to totally normalize even after attainment of euthyroidism indicates relatively high rate of bone turnover of both resorption and formation.

References


