Carboxy-Terminal Propeptide of Type 1 Procollagen (P1CP) and Carboxy-Terminal Telopeptide of Type 1 Collagen (1CTP) as Sensitive Markers of Bone Metabolism in Thyroid Disease

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Abstract. We measured serum levels of the carboxy-terminal propeptide of type 1 procollagen (P1CP) as a marker of bone formation and the carboxy-terminal telopeptide of type 1 collagen (1CTP) as a marker of bone resorption by RIA in sera from 40 Graves' disease patients and 14 Hashimoto's disease patients before and during treatment. The serum P1CP levels of the untreated Graves' disease were significantly higher than in the controls (176.8 ± 93.5 vs. 107 ± 35 ng/ml, P<0.01), and these levels decreased significantly during treatment with antithyroid drugs. There was a significant statistical correlation between serum P1CP levels and serum total alkaline-phosphatase activity (r=0.61, P<0.01) in the patients with Graves' disease and Hashimoto's disease as a whole. 1CTP levels were also significantly increased in untreated Graves' patients (6.5 ± 2.8 compared with 2.7 ± 1.1 ng/ml in normal subjects, P<0.01). The P1CP/1CTP ratio, which reflects the relative ratio of bone formation to bone resorption, was lower than normal in untreated Graves' disease, but increased following the normalization of thyroid function. The results of this study suggest that the measurement of serum P1CP and 1CTP levels may be useful in evaluating bone metabolism in thyroid disease.

Key words: Carboxy-terminal propeptide of type 1 procollagen (P1CP), Carboxy-terminal telopeptide of type 1 collagen (1CTP), Graves' disease, Hashimoto's disease

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TYPE 1 collagen is the most abundant collagen type in many soft tissues and accounts for over 90% of the organic matrix of bone [1, 2]. It is synthesized in the form of a larger protein, type 1 procollagen, which contains additional sequences at both ends [3]. These sequences are removed by specific proteinases before the collagen molecules are assembled into fibers [4]. The part removed from the carboxy-terminal end of the molecule, known as the carboxy-terminal propeptide of type 1 procollagen (P1CP), is released into the circulation, and serum P1CP levels have been shown to provide a useful indicator of type 1 collagen synthesis, and consequently of bone formation [5]. On the other hand, the 1CTP (pyridinoline cross-linked telopeptide domain of type 1 collagen) is released in the process resolving of collagen fiber. Since 1CTP is not resolved any further, it has been proposed as a good marker of bone resorption [6]. Both serum PICP and 1CTP concentrations have been determined in a variety of physiological states [7, 8] and disorders associated with altered bone metabolism [7–11]. Increased serum levels of PICP and 1CTP have been reported in hyperthyroid patients with increased bone loss, suggesting increases in both bone formation and bone resorption [12–14], but there are few data on the simultaneous determination of serum PICP and 1CTP in the same patients with thyroid disorders before and during
treatment.
In the present study, we measured the serum PICP and ICTP levels of patients with Graves' disease and Hashimoto's disease before and after treatment, and investigated correlations with thyroid function and other markers of bone metabolism.

Patients and Methods

Patients

Forty patients with untreated Graves' disease (7 men and 33 women; mean age: 42.3 yr, range: 19–51 years) and fourteen hypothyroid patients with Hashimoto's disease (all women; mean age: 42.9 yr, range: 30–52 years) were included in this study. These patients were all pre-menopausal women because bone metabolism is different from that in post-menopausal women. Age-matched normal subjects (20 females and 10 males; mean age: 41.6 years) served as controls. Graves' disease was diagnosed on the basis of the clinical findings, high serum thyroid hormone (T4 and T3) levels with suppressed TSH levels, and positive TRAb (TSH-receptor antibody). Sixteen of the 40 patients were tested for serum PICP and ICTP levels before and after treatment with methimazole for 6 months when they became euthyroid, and another twenty-five patients with Graves' disease were also tested during treatment (20 females and 5 males; mean age: 43.6 years). The 14 patients with Hashimoto's disease associated with hypothyroidism were diagnosed on the basis of subnormal serum thyroid hormone levels and high TSH levels (higher than 5 µU/ml), and high titer of microsomal antibody (MCHA) and/or thyroglobulin antibody (TGHA). Six of the 14 patients with Hashimoto's disease were also tested for serum PICP and ICTP levels before and after treatment with thyroid hormone (Thyradin S; 50 to 100 µg/day). The mean duration of treatment was 6.2 ± 1.3 months. None of these patients had liver or renal dysfunction. The thyroid function data for all groups are summarized in Table 1.

Methods

Serum concentrations of PICP and ICTP were determined with commercially available radioimmunoassay kits (PICP and ICTP RIA kit, Farmos Diagnostica, Oulunsalo, Finland). The intra- and interassay coefficients of variation were both below 5%, and the detection limit was 6 ng/ml for PICP and 0.25 ng/ml for ICTP. The source of the antigen was type 1 procollagen precipitated from the medium of cultured human skin fibroblasts [5]. Serum T3 and T4 were measured with commercial radioimmunoassay kits (Eiken Corp., Tokyo, Japan and Johnson & Johnson Clinical Diagnostics Ltd., Amersham, England, respectively). Serum TSH concentrations were measured by a sensitive immunoradiometric method (CIS Bio International, France). Serum TSH receptor antibody (TRAb) was measured with a kit (Johnson & Johnson Clinical Diagnostics Ltd., Amersham, England). Serum total alkaline-phosphatase was measured with a multichannel analyzer. All results are expressed as means ± SD.

Statistical analysis was performed by using Wilcoxon non-parametric tests for paired and unpaired data, as indicated. Relationships between variables were tested by using Spearman's rank correlation test. P values <0.05 were considered significant.

Table 1. Thyroid function data for thyroid disease patients

<table>
<thead>
<tr>
<th></th>
<th>T3 (ng/dl)</th>
<th>T4 (µg/dl)</th>
<th>TSH (µU/ml)</th>
<th>TRAb (%)</th>
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</thead>
<tbody>
<tr>
<td>Untreated Graves</td>
<td>377.5 ± 158.8</td>
<td>14.7 ± 5.4</td>
<td>0.1&gt;</td>
<td>55.5 ± 23.9</td>
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<tr>
<td>(n=40)</td>
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<tr>
<td>Treated Graves</td>
<td>133.3 ± 19.7</td>
<td>8.5 ± 1.4</td>
<td>1.2 ± 1.4</td>
<td>18.9 ± 21.6</td>
</tr>
<tr>
<td>(n=25)</td>
<td></td>
<td></td>
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<tr>
<td>Hashimoto's</td>
<td>116.0 ± 41.2</td>
<td>6.6 ± 3.7</td>
<td>146.1 ± 181</td>
<td>6.4 ± 18.2</td>
</tr>
<tr>
<td>disease</td>
<td></td>
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<tr>
<td>(n=14)</td>
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Results are the mean ± SD.
Results

Figure 1 shows serum P1CP concentrations in patients with untreated and treated euthyroid Graves’ disease and in Hashimoto’s disease patients with hypothyroidism. P1CP levels in age-matched normal subjects were 107 ± 35 ng/ml. As shown in the figure, the mean serum concentration in the patients with untreated Graves’ disease (176.8 ± 93.5 ng/ml) was significantly (P<0.01) higher than in normal subjects, although the values in two thirds of the patients were in the normal range. The values in treated Graves’ disease patients who became euthyroid (95.2 ± 30.8 ng/ml) were significantly (P<0.01) reduced and not different from normal values. The serum P1CP levels (99.3 ± 33.7 ng/ml) of the hypothyroid Hashimoto’s disease patients were in the normal range except in one case.

The serum P1CP concentrations of the 16 Graves’ disease patients before treatment and after 6 months of treatment with methimazole were compared (Fig. 2 left). The P1CP levels of every patient were 20–30% lower after they became euthyroid. The P1CP value after treatment was 89.3 ± 33.5 ng/ml, and significantly (P<0.005) lower than before treatment. Serum P1CP levels were also determined before and after treatment in patients with Hashimoto’s disease. As shown in Fig. 2 (right), serum P1CP levels increased significantly, from 106.4 ± 37.6 ng/ml to 172.8 ± 70.6 ng/ml, in all patients after treatment. As shown in Fig. 3, P1CP levels were positively correlated with serum Al-P when both Graves’ disease and Hashimoto’s disease patients were included (r=0.61, P<0.001). There was also a positive correlation between these two variables in the Graves’ disease patients alone, but there were no correlations between serum P1CP and thyroid hormones (Fig. 4), TRAb or serum Ca levels in any of the patients.

Serum 1CTP levels were also high in the majority of untreated Graves’ disease patients (Fig. 5). The mean value was 6.5 ± 2.8 ng/ml, and significantly (P<0.001) higher than normal (2.7 ± 1.1ng/ml). The 1CTP level in hypothyroid Hashimoto’s disease was 2.0 ± 1.0 ng/ml, which was not different from the value in normal subjects. The differences between serum 1CTP levels before and after treatment were examined in sixteen patients with Graves’ disease and six patients with Hashimoto’s disease (Fig. 6). As with P1CP levels, 1CTP levels decreased in all patients with Graves’
Serum 1CTP levels in Hashimoto's disease were within the normal range both before and after treatment. As shown in Fig. 7, there were positive correlations between serum 1CTP and both T3 and T4 levels in the patients with Graves' disease and Hashimoto's disease after treatment (2.7 ± 1.0 ng/ml, P<0.005). Serum 1CTP levels in Hashimoto's disease were within the normal range both before and after treatment. As shown in Fig. 7, there were positive correlations between serum 1CTP and both T3 and T4 levels in the patients with Graves' disease and Hashimoto's disease after treatment (2.7 ± 1.0 ng/ml, P<0.005).
Hashimoto's disease (r=0.72, P<0.01 and r=0.64, P<0.01, respectively). Since serum T3 and T4 levels were not correlated with P1CP levels, 1CTP appears to be more sensitive to thyroid hormones.

Bone turnover is regulated by both bone formation and bone resorption. We therefore calculated the serum P1CP/1CTP ratio in patients with Graves' disease during treatment (Fig. 8). The mean P1CP/1CTP value in untreated Graves' disease was 25.1 ± 24.0, and it increased to 37.8 ± 21.5 after 6 months of treatment with antithyroid drugs. The difference was significant (P<0.05), but the ratio was still lower than the control levels (73.4 ± 25.9). Although both bone formation and bone resorption were accelerated in hyperthyroidism, as evidenced by the increase in P1PC and 1CTP, the lower P1CP/1CTP ratio in the hyperthyroid state suggests that bone resorption predominates over bone formation.

Discussion

The data presented here demonstrate significantly increased serum concentrations of P1CP and 1CTP in patients with untreated Graves' disease, which was in agreement with earlier reports [8, 12-14]. We also showed that high P1CP levels returned to the normal range after 6 months of treatment with anti-thyroid drugs. Serum P1CP levels have been shown to be correlated with the iliac bone formation rate assessed histomorphometrically [15], with the mineral apposition rate, and with the bone formation rate [16]. Charles et al. [17], on the other hand, reported that serum P1CP levels were not different from those of normal subjects, in spite of increased skeletal mineralization and increased of serum concentrations of osteocalcin (OC), a marker of bone formation, and there was no correlation between serum Al-P activity and P1CP levels in their study. Their data conflict with the highly significant positive correlation between serum P1CP and Al-P in our study (regression coefficient; 0.61, P<0.001). This discrepancy may be due to differences between thyroid function, duration of hyperthyroidism, or menstrual state in the two groups, because, for example, Franklyn et al. (1994) reported a difference between the response to thyrotoxicosis to the skeletons of pre- and post-menopausal women, and a previous history of thyrotoxicosis rather than exogenous thyroxine therapy is a risk factor for bone loss in post-menopausal women [18].
Serum P1CP levels have never been reported in hypothyroid patients before. The values in hypothyroid patients were similar to normal values in our study, but increased within the normal range in all patients after treatment with thyroxine, indicating that hypothyroidism decreases serum P1CP in individual patients. Several reports have correlated serum P1CP levels with other markers of bone formation in metabolic bone diseases. A positive correlation was reported between serum P1CP and OC levels in hyperthyroid patients [12]. Charles et al. [17] reported that OC is a reliable marker of mineralization, whereas Al-P and P1CP displayed disease-specific differences with respect to bone mineralization. They suggested that P1CP reflects matrix formation alone, but a fraction of newly synthesized osteocalcin is incorporated into bone again, and the circulating serum osteocalcin is cleared by the kidneys, and thus its clearance is affected by renal function [19]. In contrast, P1CP is too large to be filtered by the renal glomeruli, and is removed from the blood by an endothelial receptor mechanism [20]. Since P1CP is produced in a constant molar ratio to type I collagen and is probably not incorporated into bone again, serum P1CP may be a better marker of bone formation than serum OC.

We demonstrated a positive correlation between serum Al-P and P1CP in patients with thyroid diseases, including both Graves’ disease and hypothyroid Hashimoto’s disease. A positive correlation between Al-P and P1CP in thyroid diseases suggests that both bone mineralization and matrix formation are closely coupled.

A number of markers for bone resorption have been developed. Urinary excretion of pyridinoline and deoxypyridinoline, in particular, have been shown to be useful markers of bone resorption. Increased excretion of these markers has been reported in patients with breast cancer, tumor-associated hypercalcemia [21, 22], and hyperthyroidism [23], but these markers include the cross-linked peptides not only of type I but type II collagen. On the other hand, ICTP is the pyridinoline cross-linked telopeptide of type I collagen, and is more specific for bone resorption of type I collagen. Serum ICTP levels have recently been shown to be increased in association with localized bone destruction in multiple myeloma [24] and osteolytic bone metastases. Our results showing that serum ICTP levels were increased in the hyperthyroid Graves’ disease patients are compatible with previous observations [14, 25, 26]. A positive correlation between serum ICTP and thyroid hormone and the significant reduction in ICTP levels after treatment suggests that serum ICTP levels reflect a peripheral action of thyroid hormones on bone.

Thus, both serum P1CP and ICTP levels were increased in hyperthyroidism, suggesting that bone resorption and bone formation were accelerated, but serum P1CP/ICTP ratio, which reflects the relative rate of bone resorption and formation, was lower in the hyperthyroid state rather than the euthyroid state, indicating that bone resorption is more accelerated than bone formation in hyperthyroidism. It has been suggested that normalization of bone formation takes longer than that of resorption, according to the coupling theory [27]. Hyperthyroidism has been recognized as a risk factor for osteoporosis [18, 28]. In fact, histological studies have shown that thyroxine stimulates both osteoblast and osteoclast activity in cortical and trabecular bone, with resorptive surfaces exceeding formative surfaces, resulting in net bone loss [29, 30]. This was supported by a report by Rosen et al. [31] stating that total bone mass measured by DEXA decreased in thyrotoxic patients, and returned to normal after nine months of treatment. Our results are compatible with these previous observations.

In summary, we have confirmed and extended previous findings showing that both bone resorption and formation, predominantly the former, are accelerated in hyperthyroid patients. RIAs for these markers provide useful information not only on bone metabolism, but on the effectiveness of anti-thyroid drug therapy.

Acknowledgments

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SERUM PICP AND ICTP LEVELS IN THYROID DISEASE

References


