Serum carboxy-terminal propeptide of human type I procollagen in patients with primary hyperparathyroidism: studies in basal conditions and after parathyroid surgery

Salvatore Minisola, Elisabetta Romagnoli, Liliana Scarnecchia, Rossana Rosso, Maria T Pacitti, Antonella Scarda and Gianfranco Mazzuoli

Institute of 2nd Medical Clinic. Chair of Internal Medicine. University of Rome "La Sapienza", Rome, Italy


This study was carried out in order to evaluate serum carboxy-terminal propeptide of human type I procollagen (PICP) in patients with primary hyperparathyroidism and to examine its changes following parathyroidectomy. Seventeen patients (four males and 13 females, aged 53.8 ± 3.1 SEM years) were studied in basal conditions; six patients also were investigated after successful parathyroid surgery. Mean serum PICP values of patients with primary hyperparathyroidism (194.5 ± 27 SEM µg/l) were significantly higher (p < 0.001) with respect to those found in normal subjects. However, deviations from the norm (Z score values) were significantly less with respect to deviations of serum osteocalcin, alkaline phosphatase and urinary hydroxyproline/creatinine ratio. Following parathyroidectomy, it was possible to observe a discrepancy between markers of bone resorption and those of bone formation. The former tend to decrease, while the latter either do not show any significant change (serum alkaline phosphatase and serum osteocalcin) or increase (serum procollagen). The results of our investigation indicate that in basal conditions the assay of serum procollagen may be of clinical value but it would be better to use it in combination with other biomarkers of skeletal remodelling. The results obtained after parathyroidectomy are the opposite of those obtained following parathyroid hormone infusion and should be ascribed to the effect of acute hormone deficiency on collagen synthesis. The positive biochemical uncoupling following surgery might lend support to the rise of bone mineral density consistently reported in the first few months following parathyroidectomy.

S Minisola. Istituto di II Clinical Medica. Policlinico Umberto I. Via del Policlinico 155, 00161 Rome, Italy

Type I collagen is the predominant protein in bone: during the conversion of type I procollagen to type I collagen, a relatively large part of the molecule splits off from both ends in a stoichiometric manner and is released into the extracellular fluids (1). The extension cleaved off from the carboxy terminus, known as the carboxy-terminal propeptide of type I procollagen (PICP), is a glycoprotein with a molecular weight of about 100 000 (2). Its concentration in serum has been suggested as a possible new marker of bone formation (3).

Although a number of studies have appeared so far concerning serum levels of this propeptide in a variety of metabolic bone diseases (4–6), no investigation has been published regarding the possible clinical usefulness of this marker in patients with primary hyperparathyroidism. This study was therefore carried out in order to evaluate serum procollagen levels in patients with this disorder and to examine their changes following parathyroidectomy.

Patients and methods

Seventeen patients (four males and 13 females, aged 53.8 ± 3.1 SEM years) with primary hyperparathyroidism were studied in basal conditions. Clinical diagnosis was confirmed in 12 cases by histological examination at surgery (removal of a parathyroid adenoma); in the remaining five cases, diagnosis was based on clinical and laboratory criteria, including a history of at least 2 years of prolonged hypercalcaemia without evidence of a non-parathyroid aetiology and increased serum levels of immunoreactive parathyroid hormone (PTH). Furthermore, these latter patients did not show reduced calcium clearance to creatinine clearance ratio or a positive family history for familial benign hypercalcaemia. Of the patients studied, seven had a history of one or more kidney stones and four complained of skeletal symptoms: in the remaining six patients, increased serum calcium values were found by chance following routine biochemical screening.

Metabolic tests included 24-h urine collection in order to evaluate calcium, phosphorus and creatinine, followed by a spot urine collection (from 08.00 to 11.00 h) after a 12-h overnight fast in order to determine the calcium and hydroxyproline/creatinine ratio. Halfway through the latter collection, a blood
sample was taken for the measurement of serum total and ionized calcium, phosphorus, creatinine, PTH, osteocalcin (BGP), total alkaline phosphatase activity (ALP), tartrate-resistant acid phosphatase activity (TRAP) and PICP.

Six patients (three males and three females, mean age 51.8 ± 4.6 years) also were investigated after successful parathyroid surgery. They were studied while on a gelatin-free diet in basal conditions and then at 24-h intervals for 5 days after parathyroidectomy. Blood samples were collected at the same time of the day, in order to evaluate serum levels of PICP, ALP, BGP and TRAP (this last marker in five patients); hydroxyproline and creatinine were measured in concomitant 24-h urine collections in four patients. In addition, in four patients (two males and two females, mean age 49 ± 6.6 years) undergoing surgery for benign thyroid cysts, serum levels of PICP also were measured according to the same protocol.

Serum concentrations of PICP were measured in duplicate 100-μl serum samples with a recently developed equilibrium radioimmunoassay supplied by Farmos Diagnostica (Turku, Finland) (7). The sensitivity of the test was 1.2 µg/l. The intra-assay coefficient of variation was 2.8% in the concentration range of our study. The corresponding interassay variation was about 5.5%.

The reference interval (mean ±2 sd) for 103 normal subjects (47 males and 56 females of 25–74 years of age) is 56–215 µg/l. All these subjects were ambulatory, none had a history of any medical disease and none were using drugs, including oestrogens, known to affect bone metabolism. Finally, no normal subject over 50 years of age had evidence of vertebral compression on radiographs of the lumbar and thoracic spine. Biochemical parameters of mineral metabolism were measured according to methods described previously (8, 9).

Statistical analyses

For each biochemical index measured, data are presented as mean ± sem. In order to compare the results of various biochemical markers of bone remodelling, individual values were expressed as standard deviation units (sd unit or Z transform), the distribution of which is centred on a mean of 0 with an sd of 1. Standard units are used here as a means of simplifying the comparison of different variables.

Relations between variables were tested by linear regression analysis. Significance between means was assessed using Student's t-test for paired or unpaired data, whenever appropriate. The biochemical data following surgery were analysed with one-way analysis of variance (ANOVA) using repeated measurements.

Results

Mean preoperative values of total serum calcium and intact PTH levels of patients with primary hyperparathyroidism were 2.87 ± 0.03 mmol/l and 130 ± 7.3 ng/l, respectively.

Mean serum PICP values (194.5 ± 27 µg/l) were significantly higher (p < 0.001) with respect to those

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Absolute values</th>
<th>Standardized values</th>
<th>Normal values</th>
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<tbody>
<tr>
<td>PICP (µg/l)</td>
<td>194.5 ± 27</td>
<td>1.6 ± 2.9</td>
<td>135 ± 40</td>
</tr>
<tr>
<td>BGP (µg/l)</td>
<td>9.7 ± 1.4</td>
<td>6.4 ± 1.4***</td>
<td>3.3 ± 1.0</td>
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<tr>
<td>ALP (U/l)</td>
<td>192.5 ± 38</td>
<td>6.5 ± 2.1**</td>
<td>75 ± 18</td>
</tr>
<tr>
<td>TRAP (U/l)</td>
<td>18.0 ± 1.0</td>
<td>2.1 ± 0.4</td>
<td>12.9 ± 2.4</td>
</tr>
<tr>
<td>OHPr/Cr (mg/g)</td>
<td>92.5 ± 20.6</td>
<td>6.3 ± 2.1*</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>Ca/Cr (mg/g)</td>
<td>255 ± 21</td>
<td>2.3 ± 0.3</td>
<td>113 ± 62</td>
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*PICP: carboxy-terminal propeptide of human type I procollagen (PICP) levels in the seventeen patients with primary hyperparathyroidism studied. Horizontal lines represent normal values for serum PICP in our laboratory.

Fig. 1. Individual values of serum carboxy-terminal propeptide of human type I procollagen (PICP) levels in the seventeen patients with primary hyperparathyroidism studied. Horizontal lines represent normal values for serum PICP in our laboratory.

Table 1. Biochemical markers of bone turnover in patients with primary hyperparathyroidism.
found in normal subjects; individual values are reported in Fig. 1. Mean absolute and standardized values, i.e. Z score values, of all the biomarkers investigated are reported in Table 1: it can be observed that the mean Z score values of serum BGP, ALP activity and the fasting urinary hydroxyproline/creatinine ratio were significantly higher with respect to the mean procollagen Z score value. On the contrary, no significant differences were noted with respect to Z score values of serum TRAP and the urinary calcium/creatinine ratio.

Table 2 illustrates the correlation matrix between biomarkers of bone remodelling investigated. No correlation was found between serum PICP and each of the remaining markers; however, it is important to underline the good correlations found among the traditional biomarkers considered.

Individual percentage variations of serum procollagen levels observed following adenoma removal in the six patients investigated are reported in Fig. 2; serum PICP levels changed significantly following surgery in primary hyperparathyroid patients (p < 0.001 by ANOVA, for repeated measurements) but not in the four patients undergoing surgery for reasons unrelated to parathyroid hyperactivity (Fig. 3); therefore, absolute values observed in hyperparathyroid patients on the 5th postoperative day were significantly higher with respect to preoperative values (176 ± 24 vs 307 ± 37, p < 0.024, by Student’s t-test). None of the control patients showed reduced serum ionized calcium values and hyperparathyroid patients did not take vitamin D therapy during the entire postoperative period. Finally, percentage changes of five markers of bone turnover observed following parathyroidectomy in the hyperparathyroid patients studied are reported in Fig. 4. It is possible to observe a discrepancy between markers of bone resorption: urinary hydroxyproline and serum TRAP both tend to decrease; changes in markers of bone formation are either not significant, as in the case of serum ALP and BGP, or increase significantly, as in the case of serum procollagen.

![Fig. 2. Individual percentage variations of serum procollagen levels observed following adenoma removal: preoperative values are shown in parentheses.](image1)

![Fig. 3. Mean ± SEM postoperative percentage changes of serum carboxy-terminal propeptide of human type I procollagen (PICP) levels observed in six primary hyperparathyroid (PHPT) patients (●) and in four patients operated on for benign thyroid cysts (○).](image2)
Discussion

In this study we evaluated serum levels of PICP along with the most widely used markers of bone remodelling in a series of patients with primary hyperparathyroidism. Data obtained in basal conditions indicate that, considered as a whole, patients with this disorder are characterized by high mean serum PICP levels with respect to the control population, even though there was a large overlap with normal values. This finding is consistent with the variability of bone turnover reported in patients with primary hyperparathyroidism (10, 11).

In the assessment of the clinical utility of serum procollagen assay, deviations from the norm were significantly less with respect to deviations of serum BGP, ALP and the urinary hydroxyproline/creatinine ratio. Furthermore, there were no significant correlations between serum PICP and each of the remaining biomarkers considered. This last finding should not be ascribed to the relatively small number of patients studied, because conventional markers were highly correlated among them.

The reason for the relative insensitivity of the serum PICP assay could be due to the fact that circulating concentrations of procollagen do not reflect the true bone collagen synthesis rate accurately. This may be the consequence of both immunochemical heterogeneity and technical problems related to the assay. In fact, it has been suggested that the polyclonal antiserum raised against the products of bacterial collagenase digestion may recognize epitopes different from those present in the native PICP (5). However, it should also be kept in mind that the smaller deviation from normal with respect to the other markers (reflecting a limited increase in type I collagen production) is in accordance with the relative reduction in osteoblastic function reported in histomorphometric studies in hyperparathyroid patients (4, 12).

When analysing serum procollagen levels following surgery for primary hyperparathyroidism, an increase can be observed after parathyroidectomy; even though only six cases have been studied, this increase does not seem to be related to the preoperative value. Furthermore, the rise in serum procollagen levels observed following the cessation of parathyroid gland hyperfunction seems to be a specific event, because no significant changes were observed in patients treated surgically for other reasons.

It is noteworthy to emphasize that, in this respect, our results are the counterpart to those obtained both in vitro and in vivo following PTH administration. In fact, as has been reported by Simon and co-workers (13), PTH infusion in humans induces a significant decrease of serum procollagen levels. Therefore, our results could be expected when considering this PTH-induced decrease in new bone collagen synthesis. In some aspects, the behaviour of this marker following parathyroidectomy is similar to that of a very old one related to a very early phase of collagen synthesis, i.e. the non-dialysable urinary hydroxyproline: in fact, its excretion has been shown to increase after successful parathyroid surgery (14).

Finally the postoperative changes of the biomarkers considered indicate strongly that in hyperparathyroid patients there is a positive uncoupling of the two processes of bone remodelling immediately after surgery. In this respect it is noteworthy that post-parathyroidectomy changes of both urinary hydroxyproline and serum TRAP are similar to those observed by Stepan and co-workers (15). The finding of an increase of serum PICP levels, while serum BGP and ALP do not change significantly, strongly supports the hypothesis that these markers do not reflect necessarily the same aspect of the bone formation process. Indeed, both serum ALP and BGP seem to be related mainly to the calcification process and may not reflect matrix synthesis (4, 16).

In conclusion, the results of our study indicate that in basal conditions the assay of serum procollagen may be of clinical value but, owing to the lack of correlations with other biomarkers of bone remodelling, it would be better to use it in combination with them. The results obtained after parathyroidectomy are the opposite of those observed following PTH infusion and should be ascribed to the effect of acute hormone deficiency on collagen synthesis. Finally, the positive biochemical uncoupling following parathyroidectomy might lend support to the rise of bone mineral density reported consistently in the first few months following parathyroidectomy (11, 17).

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