Parathyrin Measured Concurrently with Free or Total Calcium in the Differential Diagnosis of Hypercalcemia

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We assessed the diagnostic utility of measuring C-terminal immunoreactive parathyrin (i-PTH) concurrently with free (Cafr) or total (Caf) calcium in four groups of patients: (1) 91 patients with histologically proven primary hyperparathyroidism; (2) seven patients without histological evidence of hyperparathyroidism; (3) 70 patients with non-parathyroid malignancies with hypercalcemia; and (4) 28 similar patients without hypercalcemia. In patients in Group 1, the use of either calcium measurement along with i-PTH increased diagnostic accuracy from 95 to 99%. In Group 2, values consistent with primary hyperparathyroidism were obtained for 21% of samples with i-PTH assay alone, 29% with i-PTH and Caf, and 12% with i-PTH and Cfr. Surprisingly, 36% of samples from both malignancy groups (3 and 4) had above-normal i-PTH values. In patients with malignancy and hypercalcemia (Group 3) the following percentages were classified as possibly being hyperparathyroid: 26% with i-PTH assay alone; 31% with i-PTH and Cfr, and 24% with i-PTH and Cfr. Our data indicate that (a) about a fourth of patients with nonparathyroid malignancies also have evidence of hyperparathyroidism as judged by this (and probably all other) i-PTH methods based on C-terminal antibody specificity and that (b) measuring Caf and i-PTH rather than Caf and i-PTH gives a small increase in diagnostic accuracy.

Additional Keyphrases: hyperparathyroidism • cancer • free and total Cs in serum • C-terminal immunoreactive parathyrin

Hypercalcemia is a relatively common finding in both general and hospital practice (1, 2). It can have any of several etiologies, but the three most common are malignancy, primary hyperparathyroidism, and thiazide administration (2). In the case of a patient being treated with thiazides, the therapy can be stopped and the patient retested; thus it usually presents no problem in differential diagnosis of disease involving hypercalcemia. Rather, the usual problem is to distinguish patients with primary hyperparathyroidism from euparathyroid subjects with (e.g.) renal stones or peptic ulcer, and to distinguish primary hyperparathyroidism from malignancy as a cause of hypercalcemia. The latter is especially important because there appears to be a relatively high coincidence (16 to 33%) of primary hyperparathyroidism with malignancy (3, 4). Data on immunoreactive parathyrin in blood presumably can help the clinician make these differential diagnoses.

Interpretation of the results of parathyrin assays is complicated by the immunoheterogeneity of parathyrin in peripheral blood, which leads to different results with different antibodies (5). This immunoheterogeneity is thought to be the result of metabolism of the hormone in the parathyroids, liver, and (or) kidney (6). Although the biological activity of parathyrin resides in its N-terminal amino acid sequence (7, 8), the metabolites containing the C-terminals of the hormone have the longer biological half-life. Accordingly, their concentrations in the peripheral blood are greater (9) and patients with primary hyperparathyroidism can be sensitively distinguished from normal subjects when parathyrin is assayed by using antibodies directed to the C-terminus fragments as compared to assays involving antibodies directed toward the N-terminus (7, 10–15).

Results vary for assays of parathyrin in the serum of patients with malignancies. Some workers rarely find above-normal concentrations in hypercalcemic patients with nonparathyroid malignancies (16–19), others find the reverse (20–25). Differences in antibody specificity (20) or alterations in the metabolism of parathyrin (26), or both, may explain these discrepancies. So few patients are represented in the published studies that conclusions are difficult.

Many workers believe diagnosis is more accurate if the parathyrin value is interpreted concurrently with Caf, whether in distinguishing primary hyperparathyroidism from euparathyroidism (7, 20, 21, 27–30) or from non-parathyroid malignancies (17, 21, 22, 30). Although data on Caf have been shown to be superior to data on Cfr in helping to define hypercalcemia (31), the simultaneous measurement of i-PTH and Caf has not been systematically evaluated as a diagnostic aid.

As part of a comprehensive study to determine which tests are most effective for the differential diagnosis of hypercalcemia, we report here our results in applying a parathyrin assay involving an antibody with specificity predominantly for the C-terminus (i-PTH) to a large, well-documented group of patients with hypercalcemia. We also assess the diagnostic value of measuring Cfr or Caf together with i-PTH.

Materials and Methods

Patient Data

The patients studied were part of a large retrospective study of 1633 patients, who have been described in detail previously (31, 32).

The subjects studied here had all had concurrent analyses

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6 Nonstandard abbreviations used: Caf, free calcium; Caf, total calcium; and i-PTH, C-terminal immunoreactive parathyrin.
for CaF, CaT, and i-PTH, and surgical exploration of the parathyroid glands or a histological diagnosis of malignancy. These patients were divided into four groups as follows:

**Group 1:** 91 patients (130 pre-operative measurements) with primary hyperparathyroidism as confirmed by histological examination of surgically excised parathyroid glands.

**Group 2:** seven patients (24 pre-operative measurements) who had no histological evidence of hyperparathyroidism at surgical exploration for suspected primary hyperparathyroidism.

**Group 3:** 70 patients (84 sets of measurements) with histologically established non-parathyroid malignancies, for whom at least one value for CaF exceeded 104 mg/L (2.60 mmol/L). This above-normal CaF value was not necessarily from a sample in which i-PTH and CaF had also been determined.

**Group 4:** 28 patients (36 sets of measurements) with histologically established non-parathyroid malignancies who had no CaT values above 104 mg/L (2.60 mmol/L) and whose values for CaT were all within normal limits.

Only preoperative data from Groups 1 and 2 were considered. We analyzed sera from 60 ostensibly healthy laboratory personnel during the same 27-month period as the patient groups, as controls.

** Analytical Methods **

When we measured i-PTH, CaT, and CaF in serum from the same sample of blood, the blood specimen was placed on ice, transported to the laboratory, allowed to clot for 1 h at room temperature, and then centrifuged. Serum was anaerobically transferred to a syringe for the analysis for CaF and CaT, and an aliquot was quickly frozen for use in analysis for i-PTH, which was measured by radioimmunoassay, with use of an antibody (CH9) raised in cockerels that is predominantly sensitive to the C-terminal of parathyrin (33-35). Serum from a patient with secondary hyperparathyroidism was used as a standard and the values were reported in microliter equivalents per milliliter (μL equiv./mL). CaT was measured by automated atomic absorption spectroscopy (36), CaF by direct potentiometry with use of a modified Orion 99-20 (Orion Research Inc., Cambridge, MA 02139) membrane electrode (31, 32).

In addition to CaF, CaT, and i-PTH, some other biochemical and hematological variables were also measured by continuous-flow analyses (SMA 6/60 and SMA 12/60; Technicon Instruments Corp., Tarrytown, NY 10591) and Coulter Model S (Coulter Diagnostics, Hialeah, FL 33010). All CaT measurements that were performed concurrently with CaF and i-PTH were made by atomic absorption spectroscopy; non-concurrent measurements were by atomic absorption spectroscopy or by reaction with cresolphthalein complexone (SMA 12/60).

**Statistical Methods**

Data are expressed as mean ± SEM throughout this paper; \( t \)-values, chi-square values, correlations, and their significance were calculated by standard statistical techniques (37). We established reference intervals nonparametrically, using the central 95% of values found in the normal control group. Bivariate 95% confidence ellipses (the elliptical region within which 95% of the points are found) and discriminant functions

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7 This system of reporting results has been used by many other workers (e.g., references 7, 10, 21). It consists of setting the standard sera to an arbitrary value of 1000 μL equiv/mL and then using various amounts of the standard sera (0.5-4 μL) to prepare the standard curve. For example if 200 μL of unknown sera gives the same antibody binding as 1 μL of standard serum the final result is (1/200) × 1000 = 5 μL equiv/mL.

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**Fig. 1. Distribution of parathyrin values in the normal and patient groups**

The dashed horizontal line represents the upper limit of normal. Twenty-two samples with i-PTM values > 20 μL equiv./mL were not diluted and reassayed because this was not necessary for clinical use. Such samples are plotted as open circles at the parathyrin values the result was reported to be greater than. Only the parathyrin values with a concurrent increase in free calcium are shown for patients in Group 3 and with normal free calcium values for Group 4. The mean parathyrin values in Groups 3 and 4 were not statistically different.
Fig. 2. Parathyrin vs free calcium (A) or total calcium (B) for normal persons and patients with primary hyperparathyroidism
Open circles represent values for the normal controls. Closed triangles represent pre-operative values from patients with surgically confirmed primary hyperparathyroidism. Ellipses representing 95% confidence regions for the normal persons and the hyperparathyroid patients are shown as solid lines. The dashed lines passing through the points of intersection of these ellipses is the quadratic discriminant function. Twenty-eight samples were excluded in deriving the confidence ellipses and discriminant function, owing to lack of a definitive parathyrin value (see Methods) and have been excluded from these plots (38) were established in plots of i-PTH vs CaT and i-PTH vs CaP for the normal controls and patients with primary hyperparathyroidism after logarithmically transforming the i-PTH values. The logarithmic transformation was necessary to obtain a normal distribution of the i-PTH values. Since derivation of the confidence ellipses and discriminant functions required the assumption of an underlying bivariate normal data distribution, we excluded 22 samples for which i-PTH had not been diluted and re-assayed to give a final answer and six samples with i-PTH values greater than three standard deviations from the mean of the computation of the confidence ellipses and discriminant functions.

Results

Figure 1 shows the i-PTH values for the control group and the four patient groups. i-PTH was detectable in 58 of 70 samples (85%) from the normal subjects. Above-normal i-PTH values were found in 123 of 130 samples (95%) from patients with primary hyperparathyroidism (Group 1). The seven samples with normal i-PTH values were from six patients, five of whom had shown above-normal values for samples obtained at other times. Increased i-PTH values were found in 21% of samples from patients who had negative results for neck explorations (Group 2); an increased i-PTH concentration was found at least once in five of the seven patients in this group. Sixty-four samples with increased CaP were available from patients with hypercalcemia and malignancy (Group 3); i-PTH was above normal in 23 (36%) of these samples. Moreover, 36% of the i-PTH values were above-normal for patients with malignancy for whom CaP values were normal (Group 4).

To further investigate the similarity in the percentage of elevated i-PTH values in Groups 3 and 4, we compared all the other available biochemical and hematological data between these two patient groups. The only differences found were in values for calcium (by definition) and urea nitrogen. The urea nitrogen values were slightly higher in Group 3 patients [372 ± 56 mg/L (13.3 ± 2.0 mmol/L)] than in the Group 4 patients [261 ± 10 mg/L (9.0 ± 0.35 mmol/L)] (p < 0.05), but the i-PTH values did not correlate with either the urea nitrogen or creatinine values in either group. Furthermore, we found no differences (by chi-square test) in the frequency of various histological types of primary tumors or in the overall incidence of metastatic disease between Groups 3 and 4.

When the i-PTH values were plotted vs either the CaP
values (Figure 2A) or CaF values (Figure 2B) measured for the same blood samples, grouping of values was noted for the normal subjects and for the patients with histologically proven primary hyperparathyroidism. By reference to the confidence ellipses derived for these groups, we misclassified only one sample from the hyperparathyroid group as normal from data on CaF (Figure 2A) or CaT (Figure 2B). Interestingly, these two misclassified samples (one with CaF as the criterion, one with CaT) represented two different samples from the same patient; values for two other samples from this patient clearly fell within the hyperparathyroid region for i-PTH with either CaF or CaT.

The values for i-PTH and CaF (Figure 3A) and CaT (Figure 3B) from patients with negative histological findings (Group 2) were assessed by using the 95% confidence ellipses and discriminant function derived from Figures 2A and 2B. Three samples from three patients in Group 2 fell inside the 95% confidence ellipse for primary hyperparathyroidism when CaF was used (Figure 3A). When i-PTH and CaT were used (Figure 3B), these same three samples plus four other samples from four other patients fell within the primary hyperparathyroid area. Two of the subjects with negative histological findings who had a sample classified as consistent with primary hyperparathyroidism according to i-PTH and CaT values, but not according to i-PTH and CaF values, had other samples clearly classified as normal according to either calcium value. Thus, the use of values for CaF and i-PTH gave fewer apparent sample misclassifications (12%) for this group of patients than did the use of CaT and i-PTH (29%).

Figures 4A and 4B show data from patients who had a histologically proven non-parathyroid malignancy and at least one above-normal CaT value (Group 3). The samples were classified into three categories by reference to the 95% confidence ellipses shown in Figure 2; samples with values falling inside the two ellipses composed the normal and possible primary hyperparathyroidism categories; samples with values falling outside of the ellipses were the "distinct" category. Using i-PTH with CaF (Figure 4A) or CaT (Figure 4B), we classified similar numbers of patients' samples as distinct (51% and 48%, respectively). However, using i-PTH with CaF, we saw fewer samples classified as primary hyperparathyroidism (29% vs 39%). An analogous classification for patients rather than samples was also derived. Only samples with increased CaF or CaT values (Figure 4B) were considered and patients having multiple samples that could be classified differently were eliminated. Of these 50 patients, 13 had above-normal values for i-PTH. Twelve of 50 patients (24%) were classified as possible hyperparathyroid and 35 of 50 (70%) were classified as distinct with use of data i-PTH and CaT; the corresponding percentages were 31% and 63% with use of i-PTH and CaT.

No explanation was apparent for the different patterns of i-PTH and calcium values observed in the hypercalcemic patients with malignancy. Various biochemical, hematological, and physical data were compared between the malignancy patients classified as primary hyperparathyroid and distinct on the basis of the PTH and CaF values (Figure 4A). For this comparison we used only the values for various biochemical data obtained when the CaT was >104 mg/L (2.60 mmol/L). Above-normal CaF values could not be used as a criterion, because few of the other biochemical data were determined simultaneously with CaT, whereas many were determined simultaneously with CaF. When CaF values were unrelated, but only alkaline phosphatase [possible hyperparathyroid, 100 (SEM 8) U/L; distinct, 193 (SEM 31); p < 0.01] and phosphate [possible hyperparathyroid, 31 (SEM 1) mg/L (1.0 SEM 0.03 mmol/L); distinct 34 (SEM 1) mg/L (1.1 SEM 0.03 mmol/L); p < 0.05] gave significant differences, but examination of the data showed marked overlap. There were no differences in the frequency of tumor type between the two subgroups of hypercalcemic malignancy patients. These data will be presented in greater detail elsewhere. In patients who were autopsied there was insufficient examination of the parathyroids to be of value.

Discussion

The i-PTH assay used in this study detected i-PTH in 83% of samples from normal subjects. Increased values were found for 95% of samples from patients with confirmed primary hyperparathyroidism (Figure 1). In this regard, the assay was similar to other heterologous (5, 7, 10, 13–15, 29) or homologous (11, 21, 39) assay systems in which antibodies with predominant C-terminal specificity are used.

When data from the i-PTH assay were interpreted together with a concurrent CaF value, the distinction between patients with primary hyperparathyroidism and normal subjects was virtually complete, a finding consistent with that of other workers (7, 20, 21, 27–30). However, interpretation of i-PTH together with CaF did not decrease the misclassification of samples from patients with negative histological findings at
parathyroidectomy (21% for i-PTH alone, 29% for i-PTH and CaP). Patients with primary hyperparathyroidism could also be almost completely distinguished from normal subjects when i-PTH values were interpreted with a concurrently obtained CaP value. In addition, interpretation of i-PTH values with CaP decreased the number of samples classified as consistent with primary hyperparathyroidism from patients with negative histological findings at parathyroidectomy (21% for i-PTH alone, 12% for i-PTH and CaP). Examination of the data in Figure 3 indicates that diagnostic accuracy was enhanced when i-PTH and CaP data were used because fewer patients were considered hypercalcemic, in keeping with the increased accuracy of CaP in defining hypercalcemia previously reported (31).

A prominent finding in this study was the relatively high percentage of patients with non-parathyroid malignancies whose i-PTH values, interpreted alone or together with CaP or CaF, were consistent with hyperparathyroidism. Data from some other studies show a similar percentage of patients with cancer having evidence of hyperparathyroidism from i-PTH assays with specificity for the C-terminal (22), N-terminal (17, 25, 30), or intact hormone (28). In contrast some workers using polyvalent antisera (18, 40) or an assay of undefined specificity (19) find normal or undetectably low i-PTH values in virtually all patients with non-parathyroid malignancies. A recent comparison of four commercially available i-PTH assays (41) showed considerable variation among the different assays in the values they gave for i-PTH for patients with non-parathyroid malignancies.

It would appear from the above that many i-PTH assays with which one can easily distinguish patients with primary hyperparathyroidism from normal individuals (and probably all such assays with C-terminal specificity) will also give results consistent with hyperparathyroidism for a relatively large number of patients with non-parathyroid malignancies. Whether these elevated i-PTH results reflect co-existing true primary hyperparathyroidism, ectopic hyperparathyroidism, or an as-yet-undiscovered molecular species that cross reacts in i-PTH assays is not known. The other biochemical data we obtained on patients with malignancies classified as possibly hyperparathyroid or as distinct did not help distinguish these alternatives. Analysis for nephrogenous cyclic AMP is also unlikely to be of help, because both primary hyperparathyroidism and ectopic hyperparathyroidism should lead to increased nephrogenous cyclic AMP values and patients with non-parathyroid malignancies with undetectable i-PTH values by some assays have had markedly elevated cyclic AMP values (19).

The question of whether these patients with malignancy have primary hyperparathyroidism is controversial. Two studies reveal a high incidence, 16% (3) and 33% (4), of hyperparathyroidism in patients with non-parathyroid malignancy. Bradley et al. (42) found that five patients with increased CaF and i-PTH (C-terminal) all had primary hyperparathyroidism, as shown by neck exploration. On the other hand, because our study shows the incidence of increased i-PTH values to be as high in normocalcemic patients with malignancy as it is in hypercalcemic patients with malignancy (Figure 1), our data would not support the idea that primary hyperparathyroidism is the cause of increased i-PTH in malignancy.

Until prospective studies with use of various i-PTH assays of defined specificity can be performed, increased i-PTH values in patients with malignancy must be interpreted cautiously. The best currently available diagnostic test for patients with malignancy and an i-PTH and calcium value suggestive of hyperparathyroidism is probably i-PTH measurements in blood from the neck veins. This technique correctly identified the presence of primary hyperparathyroidism in 10 patients with non-parathyroid malignancy (43).

References


