THE VALUE TO THE SURGEON OF PARATHYROID HORMONE ASSAYS IN PRIMARY HYPERPARATHYROIDISM

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The role of various parathyroid hormone (PTH) radio-immunoassays in the diagnosis of primary hyperparathyroidism (PHP) is controversial. A series of 204 patients with surgically proven PHP was studied. Serum total calcium, serum ionized calcium, amino (*N*)-terminal PTH and carboxyl(*C*)-terminal PTH were assessed in relation to the volume and weight of adenomatous or hyperplastic parathyroid tissue excised at operation. *N*-terminal PTH was elevated above the normal laboratory range in only 24% of patients and correlated relatively poorly with the volume of abnormal parathyroid tissue ($r = 0.20$, $P = 0.05$). *C*-terminal PTH was elevated above the normal range in 91% of patients and had a strong correlation with the volume of abnormal parathyroid tissue ($r = 0.63$, $P < 0.001$). The correlation coefficients between *C*-terminal PTH and serum total calcium and serum ionized calcium were both 0.63 ($P < 0.001$). In contrast, there was no correlation between *N*-terminal PTH and serum total calcium ($r = -0.02$), serum ionized calcium ($r = -0.04$) or *C*-terminal PTH ($r = 0.09$).

A combination of hypercalcaemia and elevated *C*-terminal PTH can be regarded as strong diagnostic evidence of PHP. Furthermore, the level of *C*-terminal PTH can assist the surgeon by approximately predicting the amount of adenomatous or hyperplastic parathyroid tissue that may be expected at surgical exploration.

Key words: parathyroid hormone radio-immunoassay, primary hyperparathyroidism, surgery.

Introduction

Persistent hypercalcaemia is the characteristic biochemical abnormality in primary hyperparathyroidism (PHP). While other causes of hypercalcaemia should be considered and excluded by appropriate investigation, the availability of parathyroid hormone (PTH) assays permits a confident preoperative diagnosis of PHP to be made in most patients.

Nevertheless, since the discovery of a radioimmunoassay for PTH in 1963, the role of PTH assays in diagnosis has been controversial.[1,2] The failure of many assays to reveal increased levels of PTH in some patients has led to reluctant acceptance of the combination of hypercalcaemia and 'inappropriately normal' PTH as a diagnostic feature of PHP. This unsatisfactory criterion and much of the confusion is accounted for by the multiplicity of available assays and variable laboratory techniques.[3,4]

PTH is secreted as a single chain polypeptide of 84 amino acids. The biological activity is believed to be contained in the 1–34 amino acids at the amino(*N*)-terminal end, this fragment having a biological half-life of a few minutes. The remainder of the molecule containing amino acids 35–84 is referred to as the carboxyl(*C*)-terminal portion and has a half-life of approximately 1 h.[5]

The study reported here assessed the diagnostic value of standardized assays of *C*-terminal PTH (*C*-PTH) and *N*-terminal PTH (*N*-PTH) in a series of 204 patients with surgically and histologically proven PHP.

Methods

The 204 patients in this series were operated on by one surgeon (NWT) using a standardized technique[6] over a 5-year period to April 1984. All patients had surgically and histologically proven PHP and had been evaluated pre-operatively with either or both *C*-PTH and *N*-PTH radio-immunoassays according to the methods of Hawker *et al.* (Smith Kline Clinical Laboratories, Creve Coeur, Missouri).[7] The PTH assay results were interpreted with reference to a normal range for *C*-PTH of 60–450 pg/ml and for *N*-PTH of 163–347 pg/ml. Serum total calcium was interpreted with reference to a normal range of 8.5–10.5 mg/dl and serum ionized calcium was interpreted with reference to a normal range of 3.8–

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4.8 mg/dl. Patients who had elevated serum creatinine or PTH assayed by other methods were not included in this series.

The three diameters (d_1, d_2 and d_3) of the parathyroid adenomas were measured and the volume of the gland (which approximates a spheroid in shape) calculated according to the formula $\pi/6 \times d_1 \times d_2 \times d_3$ and expressed in mm^3. The volume of hyperplastic parathyroid tissue, when more than one gland was enlarged in an individual patient, was calculated for each gland and then the volumes were added. For each of the 204 patients, data were available for parathyroid volume and serum total calcium, and either or both *C*-PTH and *N*-PTH, on specimens taken at the same time. Serum ionized calcium data were available for 104 patients.

ANALYSIS

Initial analysis determined that logarithmic transformation of all the data for serum total calcium, serum ionized calcium, *C*-PTH, *N*-PTH and parathyroid volume provided a better approximation of a Gaussian (normal) distribution than did the raw data, and the following calculations were therefore performed with logarithmically transformed data. Correlation coefficients testing the degree of association between each of these variables were performed by regression analysis using the method of least squares. Analyses of variance were performed to assess the extent to which each variable independently predicted parathyroid volume.

Results

Data from 204 patients were analysed. All of these patients had surgically and histologically proven PHP; 196 patients had a single adenoma, three had two adenomas, and five had multiple gland hyperplasia. The volume of adenomatous or hyperplastic parathyroid tissue excised ranged from 28 mm^3 to 18849 mm^3 and postoperatively all patients were normocalcaemic.

There were 150 females (age range 12–87 years, mean = 56.4 years, s.d. = 15.3) and 54 males (age range 13–86 years, mean = 49.6 years, s.d. = 15.8), the age difference being significantly different (Student's *t* test, $t = 2.75$, $P < 0.01$). There was no difference in serum total calcium levels between the sexes: in the 150 females mean total calcium was 11.3 mg/dl, s.d. = 1.02, and in the 54 males mean total calcium was 11.4 mg/dl, s.d. = 1.00 ($t = 0.65$, $0.6 > P > 0.5$). Similarly, there was no difference in parathyroid volume between the sexes: in 150 females mean volume was 1451 mm^3, s.d. = 2406, and in 54 males mean volume was 1820 mm^3, s.d. = 3297 ($t = 0.75$, $0.5 > P > 0.4$).

C-PTH was assayed in 185 patients. It was normal in 17 and elevated above the normal range in 168 (91%). Again, there was no difference in *C*-PTH between the sexes: in the 138 females mean *C*-PTH was 1664 pg/ml, s.d. = 2984 and in the 47 males mean *C*-PTH was 1357 pg/ml, s.d. = 1036 ($t = 1.04$, $0.3 > P > 0.2$). *N*-PTH was assayed in 95 patients and was normal in 71, low in one and elevated above the normal range in 23 (24%). *N*-PTH was elevated in only two of the patients in whom *C*-PTH was normal.

The value of *C*-PTH in patients with marginal hypercalcaemia (serum total calcium of 10.7 mg/dl or less) was examined and was found to be normal in 12 and increased in 46 (79%). *C*-PTH in patients with serum total calcium of 10.8 mg/dl or more was normal in five and increased in 122 (96%). This difference was significant ($\chi^2 = 13.38$, $P < 0.001$).

All of the patients had elevated serum total calcium on at least one test, but on the sample of blood on which the simultaneous total calcium and PTH was measured, 47 had serum total calcium levels in the normal range (that is, 77% were elevated). There were 104 patients who had both total and ionized calcium measured simultaneously and of these serum ionized calcium was normal in 25 and elevated in 79 (76%). Thirteen patients had normal levels of both total and ionized calcium, 18 had normal total calcium and elevated ionized calcium, 12 had elevated total calcium and normal ionized calcium, and 61 had elevated levels of both.

There was a strong positive correlation between the level of *C*-terminal PTH and the volume of abnormal parathyroid tissue ($r = 0.63$, $P < 0.001$). In contrast, the correlation between the level of *N*-terminal PTH and the volume of abnormal parathyroid tissue was relatively weak ($r = 0.20$, $P = 0.05$). There was good correlation between *C*-PTH and serum total calcium (185 patients) and *C*-PTH and serum ionized calcium (89 patients), the values being the same for both ($r = 0.63$, $P < 0.001$). In contrast, there was no correlation between *N*-PTH and serum total calcium ($r = -0.02$), *N*-PTH and serum ionized calcium ($r = -0.04$) or *N*-PTH and *C*-PTH ($r = 0.09$). Table 1 shows a correlation matrix of logarithmically transformed data in 51 patients in whom total calcium, ionized calcium, *C*-PTH and *N*-PTH were all available from the same blood specimen.

Analyses of variance were performed to assess the extent to which each of the other three variables

Table 1. Correlation matrix of logarithmically transformed data

	Total calcium	Ionized calcium	*N*-PTH
Ionized calcium	0.65		
N-PTH	−0.02	−0.04	
C-PTH	0.52	0.62	0.09

predicted volume independently of *C*-PTH. The correlation between log volume and log total calcium, serum ionized calcium and *N*-PTH, after adjusting for *C*-PTH (fitting a linear model to *C*-PTH) was 0.01, 0.05 and 0.09 respectively. None of these was significantly different from zero and therefore none could be used to improve the prediction of volume based on *C*-terminal PTH. The relationship of log *C*-PTH to log volume is shown (Fig. 1). The upper and lower lines indicate the 95% confidence limits for volume for any known value of *C*-PTH.

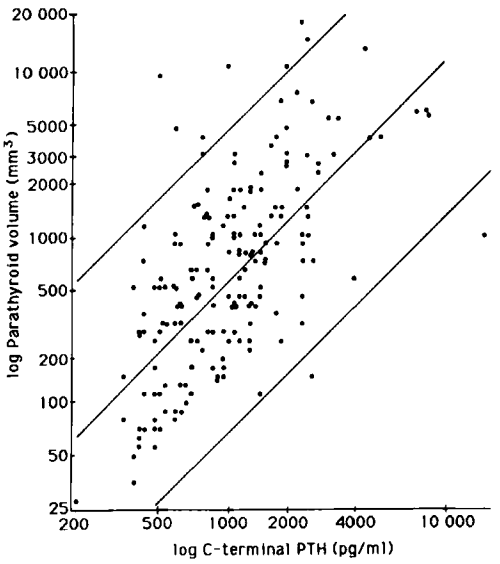

Fig. 1. Correlation between log C-terminal PTH and log volume of adenomatous or hyperplastic parathyroid tissue ($r = 0.63$, $P < 0.001$). 95% confidence limits are indicated by the upper and lower lines.

The weight of abnormal parathyroid tissue was available in 48 patients and the range was 65–20000 mg (mean = 1841 mg, s.d. = 3557). The volume of abnormal parathyroid tissue in these patients correlated very strongly with the weight ($r = 0.90$, $P << 0.001$).

The number of false negative and false positive diagnoses which may have occurred during the time this series was collected was not accurately known and therefore the sensitivity and specificity of the diagnostic tests could not be calculated. Three additional patients with hypercalcaemia and elevated *C*-PTH during the time this series was collected probably had PHP but were not included in the analysis because their treatment and its outcome were not finalized.

Discussion

The results of this study show that the *C*-PTH assay was a valuable aid to diagnosis in patients with suspected PHP. In contrast, the *N*-PTH assay was of little diagnostic value and the results of this assay may even confuse the diagnosis.

The results of previously published clinical studies of PTH assays are summarized in Tables 2 and 3. In these studies, *C*-PTH assays were elevated above the normal range in 87% of patients, and *N*-PTH assays were elevated in 67%. Four of the studies involved comparisons of *C*- and *N*-PTH assays and in each of these the *C*-PTH assay appeared to be superior.[9,10,13,14]

There are several methods of assay for *C*- and *N*-PTH, and there are, in addition, other assays towards different aspects of the PTH molecule. Some of these assays have not been well-characterized. Heterologous PTH assays directed towards both *C*- and *N*-PTH have been reported to be elevated in 11 of 19,[22] 41 of 56,[23] and 18 of 34[20] patients with PHP, but these assays have not been widely used.

Table 2. *C*-terminal PTH assays in primary hyperparathyroidism

Year	Author	Number of patients	Number with elevated PTH
1968	Reiss and Canterbury[8]	32	32
1973	Silverman and Yalow[9]	13	11
1974	Arnaud *et al.*[10]	38	35
1974	Conaway and Anast[11]	29	27
1978	Di Bella *et al.*[12]	73	70
1979	Raisz *et al.*[13]	45	29
1980	Simon and Cuan[14]	29	13
1981	Kao *et al.*[15]	112	100
1981	Roos *et al.*[16]	16	14
1981	Freaney *et al.*[17]	51	49
		438	380

Table 3. *N*-terminal PTH assays in primary hyperparathyroidism

Year	Author	Number of patients	Number with elevated PTH
1973	Silverman and Yalow[9]	13	3
1974	Arnaud *et al.*[10]	35	21
1974	Woo and Singer[18]	22	19
1976	Samaan *et al.*[19]	82	62
1979	Raisz *et al.*[13]	15	6
1980	Simon and Cuan[14]	18	6
1980	Papapoulos *et al.*[20]	34	28
1981	Lafferty[21]	100	64
		301	203

Other assays directed towards the mid-region of the PTH molecule have been described and the early results appear to be encouraging.[16,24] More recently, apparently sensitive and specific double antibody assays for the 'intact' PTH molecule have been developed. It seems likely that progressive improvement in assays will occur and the former unsatisfactory status of PTH assays will be resolved favourably.

The present study represents the largest series of patients with proven PHP in which the diagnostic value of *C*-PTH and *N*-PTH assays has been evaluated. The results indicate that *C*-PTH was elevated in 91% of patients, while *N*-PTH was elevated in only 24%. *C*-PTH was strongly positively correlated with the volume of adenomatous or hyperplastic parathyroid tissue, but *N*-PTH was only weakly correlated with parathyroid volume. Furthermore, *N*-PTH was negatively correlated with serum total calcium and serum ionized calcium and did not correlate with *C*-PTH. It can therefore be concluded that the *N*-PTH assay used in this study was of little diagnostic value, and if it had been the only assay used would have been falsely negative in about 75% of patients. Although *N*-PTH assays have limited value in the diagnosis of PHP, they may still have a potential role in selective venous catheterization for the location of parathyroid adenomas not found at the first operation, in the evaluation of secondary hyperparathyroidism in renal failure and in the assessment of parathyroid autograft function.

It would be expected from the demonstrated correlations that *C*-PTH levels would tend to be lower in patients with lower levels of serum total calcium. It is well-recognized that some patients with asymptomatic PHP and even some of those with symptoms or complications such as renal calculi may have mild or intermittent hypercalcaemia. It was therefore of interest to examine more closely the *C*-PTH results in patients with serum total calcium of 10.7 mg/dl or less on the simultaneous blood specimen. Although there was a significantly greater proportion of false negative results, the fact that 79% of results were abnormally elevated indicates that the *C*-PTH assay should be of diagnostic value in the majority of even this difficult group of patients. It should therefore be rarely necessary in the future to base a diagnosis of PHP on the combination of hypercalcaemia and an inappropriately normal PTH assay.

In this series the ratio of females:males was 2.8:1, compared with ratios of 2:1 and 2.6:1 in other large series.[25,26] The females, as would be anticipated, were on average 6 years older than the males, but there were no other differences regarding serum total calcium, serum *C*-PTH or parathyroid volume, indicating that the *C*-PTH assay is likely to be useful in males and females of all ages. Single adenomas were found in 96% of patients compared with an incidence of 80% in a previous series from the University of Michigan Hospitals,[27] and incidences of approximately 76% in other large series.[25,26] This pattern of pathology would not influence the results of the study because the data were analysed using the volume of adenomatous or hyperplastic parathyroid tissue as the standard.

Although serum ionized calcium has been reported to be helpful in the diagnosis of PHP when the serum total calcium levels are near the upper limit of normal,[28] it provided no additional information in the present study.

C-PTH was shown to correlate strongly with parathyroid volume and therefore it was of interest to plot the volume of adenomatous or hyperplastic parathyroid tissue relative to levels of *C*-PTH. A similar exercise has been reported previously relating the mass of excised phaeochromocytoma tissue to levels of urinary vanillyl mandelic acid excretion.[29] A recent study of pre-operative serum calcium and PTH levels concluded that identification of the pathological parathyroid glands in PHP could not be based on the demonstrated relationship between the results of these tests and the size of the enlarged glands because the correlations were not sufficiently strong.[30] These authors reviewed 92 patients and found correlations between serum calcium and parathyroid weight ($r = 0.14$, $P < 0.001$)

and volume ($r = 0.144$, $P < 0.001$). They also found stronger correlations between immuno-PTH levels by three different assays and parathyroid weight ($r = 0.25$, 0.4, 0.44, all $P < 0.001$) and volume ($r = 0.25$, 0.26, 0.44, all $P < 0.001$).

The most important factor in determining the success of parathyroid surgery is the thoroughness of the dissection by an experienced surgeon. However, the level of a reliable PTH assay, as shown in the present study, can be of some assistance to the surgeon. Volume in mm^3 and weight in mg are approximately equal and can be regarded as equivalent for practical purposes. The surgeon can obtain some guidance by reference to data such as in Fig. 1. If the minimum volume (or weight) is not accounted for by the tissue identified then surgical exploration should probably continue. For example, if the patient had a *C*-PTH level of 2000 pg/ml there would be a 95% probability that at least 150 mg of abnormal parathyroid tissue was present and a 90% probability that at least 200 mg was present.

This study showed that a *C*-PTH assay was elevated in 91% of patients with PHP. The *C*-PTH assay correlated well with the amount of adenomatous or hyperplastic parathyroid tissue excised at operation. The combination of hypercalcaemia and elevation of a sensitive *C*-PTH assay is therefore strong diagnostic evidence of PHP, and the levels in an individual patient can give the surgeon some guidance by approximately predicting the amount of abnormal parathyroid tissue which should be identified at operation.

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