Serum Calcitonin in Thyroid Disorders and in Pheochromocytoma Kindred*

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ABSTRACT

Serum calcitonin was determined by RIA in 59 healthy subjects (Group 1), 49 randomly selected patients with treated or untreated thyroid disorders (Group 2), and in 12 kindred of a pheochromocytoma index case (Group 3). Although most subjects in Group 2 had normal calcitonin levels, there were significant (p < 0.001) differences between all three groups. Of the five patients in Group 2 with high serum calcitonin, one had medullary cancer of the thyroid, one had multiple endocrine neoplasia, one had acromegaly, and two remained undiagnosed. Increased serum calcitonin levels were also found in seven of 12 normotensive relatives of a patient with pheochromocytoma. It is therefore concluded that high serum calcitonin levels in patients with thyroid disorders strongly suggest the presence of C-cell neoplasia or medullary cancer of the thyroid.

Introduction

Elevated human calcitonin (hCT) levels in the thyroid and in the systemic circulation are frequently associated with medullary thyroid cancer (MTC).7,8,9,10,12
It was also reported that elevated calcitonin levels could be associated with non-thyroid carcinomas. Nonetheless, the normal serum calcitonin range is not well defined, owing to wide calcitonin fluctuations ranging from undetectable levels to 580 pg per ml. For instance, basal serum calcitonin levels measured by radioimmunoassay (RIA) in a large group of healthy adults were less than 250 pg per ml in 25 percent, and below 100 pg per ml in 75 percent of tested subjects. The immunological heterogeneity of hCT probably accounts for some of the reported variances in healthy humans. Therefore, additional data contributing to the definition of normal hCT levels are useful. In this communication are reported the results of hCT determinations by RIA in normal human sera, in sera of patients with thyroid disorders, and in relatives of a patient with pheochromocytoma.

Materials and Methods

Fifty-nine healthy human volunteers (Group 1), 49 patients with thyroid disorders (Group 2), and 12 healthy relatives of a patient with pheochromocytoma (Group 3) were used in this study. Serum calcitonin levels were measured in an endocrine survey. The endocrine indices in the healthy kindred of the pheochromocytoma patient included: thyroxine, triiodothyronine, oral glucose tolerance test, follicle stimulating hormone, thyroid stimulating hormone, luteinizing hormone, prolactin, insulin, growth hormone, gastrin, catecholamines, and vanillylmandelic acid.

Eight- to ten-pound albino rabbits were purchased. Synthetic hCT, labeled human calcitonin (sp. act 0.3 mCi per pg [1.3 × 10^-7 Bq per pg]), and kallikrein trypsin inhibitor (Trasylo) were obtained. Antibodies to hCT were supplied. Calculitine free sera, Norit “A” charcoal, and Dextran 250 were purchased.

Antibody Production

Antibodies to hCT were induced in rabbits using conventional procedures. Briefly, rabbits received weekly subcutaneous injections of 0.5 mg synthetic hCT dissolved in 0.001 N HCl and emulsified with an equal volume of Freund's adjuvant, until sufficiently high titer (1:10000) of antibodies was detected by RIA.

Radioimmunoassay for Human Calcitonin in Sera

Radioimmunoassay of hCT was performed using standard RIA method as follows: 100 µl of standards, ranging from 10 to 1500 pg per ml of synthetic hCT, and serial dilutions of samples were pipetted into ice-cold tubes. Following the 100 µl addition of the antibody, the tubes were vortexed and then refrigerated for three days. Thereafter, 100 µl of 125I-labeled hCT were added to the tubes. The solutions were mixed and the tubes refrigerated for an additional three days. The antigen-antibody complex was separated from the labeled antigen-antibody by dextran-charcoal suspension as described elsewhere. Briefly, a five percent w/v Norit “A” charcoal and 0.5 percent w/v dextran in 0.13 M borate buffer were diluted 1:25 with a 0.05 M tris HCl buffer. The tris-HCl buffer (pH-7.5) contained 10 percent human serum from an athyrotic or normal patient (i.e., one with undetectable

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hCT), HCl, tris (hydroxymethyl) aminomethane, and 5000 KI units of Trasylol per 10 ml.

The diluted dextran-charcoal suspension was stirred for one hour and then 1.0 ml of the suspension was pipetted into each tube. All tubes were then centrifuged at 4°C for at least 30 minutes at 3000 rpm. The radioactivity of the supernatants and precipitates was measured in a gamma scintillation counter for a time sufficient to ensure a counting accuracy of more than 2 percent. The percent of bound calcitonin in each sample was then calculated as follows:

\[
\text{% bound calcitonin} = \left[ 1 - \frac{\text{cpm of precipitate}}{\text{total cpm of precipitate} + \text{supernatant}} \right] \times 100\%
\]

From these data, a standard curve was plotted (10 1000 pg per ml) and the calcitonin concentrations in the test samples determined. Calcitonin levels ranging from undetectable to 200 pg per ml were considered to fall within the normal range.

### Statistical Analysis of Data

Statistical significance of RIA data was evaluated by conventional methods (Wicoxon's, Kruskal-Wallis', and Dunn's tests). Non parametric methods were used on account of the markedly skewed data, and a high degree of differences in variances (Bartlett's test; p < 0.001). Only the employment of logarithmic transformation\textsuperscript{50} stabilized the variances and resulted in an approximately normal distribution of data points (Kolmogorov-Smirnov "goodness of fit test"; p < 0.025).

### Results

Serum calcitonin (hCT) levels were measured by RIA in healthy male and female adults, in patients with a variety of thyroid disorders, and kindred of a patient with pheochromocytoma. As shown in figure 1, the average hCT levels for these groups were 40, 116, 108, and 248, while the median values were 12, 15, 48, and 255 pg per ml, respectively. The difference in hCT levels between males (n = 17) and females (n = 42) were not statistically

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**Figure 1.** Serum hCT levels were measured in four groups of subjects. Each data point is the mean of three values. The average and median of these points is shown by --- and ----, respectively. *Exclusive of propositus.
significant, although significant differences between the young males and females and the old men and women have been reported. Therefore, calcitonin values from the normal subjects of both sexes were pooled to comprise the control group. Comparison of these three groups of subjects indicated significant differences between hCT levels of all groups. (Kruskal-Wallis one-way classification; p < 0.001). The respective mean ranks of the pheochromocytoma kindred, thyroid and control groups, were 90.6, 66.2, and 49.7, respectively. Using these values all three groups were found to differ from each other at the p < 0.05 level of significance (Dunn’s multiple comparison.)

The diagnoses of patients with thyroid disorders are summarized in table I. The endocrine indices in the kindred of the pheochromocytoma patient were all within normal range, except for elevated serum calcitonin in seven out of 12 subjects.

In the group of 49 patients with thyroid disorders, serum calcitonin levels were within normal range in 44 patients. In the remaining five, one was found to have medullary cancer of the thyroid, one had multiple endocrine neoplasia, one had acromegaly, and two remain undiagnosed.

Exclusive of the index case, calcitonin levels were distinctly increased in seven out of 12 kindred members of a patient with pheochromocytoma (figure 2). The endocrine indices were otherwise normal in all members of this group.

**Discussion**

Our data indicate that most patients under treatment for thyroid disorders had normal serum calcitonin levels as was the case with the majority of control subjects (figure 1). However, in a group of 49 such patients, five subjects were found to have above normal serum calcitonin concentrations. These data seem to suggest that thyroid abnormalities are not associated per se with the changes in serum calcitonin levels. However, patients with thyroid abnormalities and an elevated serum calcitonin probably are in a high risk group with respect to

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**TABLE I**

<table>
<thead>
<tr>
<th>Status of Patients with Thyroid Disorders* (Group 2)</th>
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</thead>
<tbody>
<tr>
<td>Multiple endocrine neoplasia</td>
</tr>
<tr>
<td>Acromegaly</td>
</tr>
<tr>
<td>Hyperthyroidism treated or untreated</td>
</tr>
<tr>
<td>Hypothyroidism or myxedema treated or untreated</td>
</tr>
<tr>
<td>Goiter - euthyroid</td>
</tr>
<tr>
<td>Non-toxic adenoma</td>
</tr>
<tr>
<td>Thyroiditis treated or untreated</td>
</tr>
<tr>
<td>Cancer of thyroid</td>
</tr>
<tr>
<td>Medullary cancer of thyroid</td>
</tr>
<tr>
<td>Hypercalcemia</td>
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<tr>
<td>Renal calculi</td>
</tr>
<tr>
<td>Miscellaneous</td>
</tr>
</tbody>
</table>

*Includes multiple disorders in some patients

**Figure 2.** Pedigree of the patient with pheochromocytoma. Shaded areas represent elevated serum hCT between 200 and 726 pg per ml. *Index Case. ( ) Age.
medullary carcinoma of the thyroid, multiple endocrine neoplasia, or other disorders attributable to calcitonin-producing C-cells. In general, patients with MTC had hCT levels twice above the normal level. Our findings pertaining to the relatives of the pheochromocytoma index subject seem to indicate that serum calcitonin may be increased in a majority of the kindred.

References