Determination of Intact Parathyrin by Immunoradiometric Assay Evaluated in Normal Children and in Patients with Various Disorders of Calcium Metabolism

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We report the reference values for intact parathyrin (PTH) measured by a two-site immunoradiometric assay (IRMA) during childhood. The study has been carried out in 215 healthy children and adolescents, ages 2.0 to 18.7 years. Some patients with altered mineral homeostasis were also studied to assess the sensitivity of the method in a clinical setting. Mean intact PTH concentrations were 30.8 (SD 9.6 ng/L; the median was 28.5 ng/L. Normal reference values were 16.0–59.0 ng/L (95% confidence interval). The distribution of intact PTH values was nongaussian. We found no significant variations between males and females and no age-related variations. The IRMA used was sufficiently sensitive to detect differences in PTH concentrations between healthy children and patients with hypocalcemia or hypercalcemia.

Additional Keyphrases: reference interval · pediatric chemistry

Intact parathyrin (PTH), after its release from the parathyroid gland, is cleaved, mainly in the liver, to yield carboxy- and amino-terminal fragments (I). The amino-terminal fragment, containing the biologically active fraction of PTH, disappears rapidly (2–3 min) from the circulation (I, 2). Larger circulating fragments of PTH, i.e., mid-molecule and carboxy-terminal sequences, persist in serum for several hours (3), their clearance being related to normal renal function (I, 4). PTH radioimmunoassays (RIAs) for biologically inactive mid-region and carboxy-terminal fragments of the hormone are the most widely used assays, because such fragments have the highest circulating concentrations in blood (5). However, the antibodies usually used in PTH RIAs are insufficiently sensitive to distinguish subnormal from normal values in various situations (4, 6).

The recent availability of an immunoradiometric assay (IRMA) of intact PTH provides many advantages—increased sensitivity, enhanced precision, and improved specificity—in comparison with the traditional PTH RIAs (6, 7); in fact, the IRMA method for intact PTH detects only biologically active intact molecules with no interference from circulating PTH fragments (6).

To obtain reference values for intact PTH during childhood, we measured with an IRMA serum hormones in a group of healthy children and adolescents. Some patients with altered mineral metabolism were also studied to evaluate whether such a method provided sufficient discrimination between normal values and sub- or supra-normal values.

Materials and Methods

Subjects. We measured serum concentrations of intact PTH in 215 healthy subjects (110 boys, 105 girls), ages 2.0 to 18.7 years. All subjects showed height and weight between the 3rd and 97th percentiles for Italian standards. They were healthy according to screening medical examinations and common blood and urine tests. None were taking drugs known to affect calcium metabolism or had signs and symptoms of metabolic or hormonal diseases. All had no personal or family history of illness related to an altered calcium and bone metabolism.

Serum concentrations of intact PTH were also determined in 22 untreated hypocalcemic patients (six with idiopathic hypoparathyroidism: three boys and three girls, ages 3.4–17.7 years; four with autoimmune hypoparathyroidism: two boys and two girls, ages 4.7–15.8 years; three with surgical hypoparathyroidism: one boy and two girls, ages 9.0–16.8 years; one four-month-old boy with Di George syndrome; and eight with pseudohypoparathyroidism: four boys and four girls, ages 1.6–12.1 years) and in 11 untreated hypercalcemic patients (two boys with primary hyperparathyroidism, ages 15.9 and 18.2 years; three with pulmonary tuberculosis: two boys and one girl, ages 5.6–10.5 years; three with Williams syndrome: one boy and two girls, ages seven months to 1.1 years; and three with various malignancies—cerebellar medulloblastoma, Hodgkin lymphoma,
and lymphosarcoma: two boys and one girl, ages 2.5-15.0 years).

Informed consent was obtained from the parents of each subject before the children were enrolled in the study. The study protocol was approved by the Ethical Committee for Investigation in Humans of our Department.

**Study protocol.** One-milliliter samples of venous blood were collected after an overnight fast at 0800 h without tourniquet into chilled polypropylene tubes for measurement of intact PTH and into anaerobic borosilicate glass tubes for measurement of ionized calcium. After clotting, the samples were centrifuged (1500 × g, 4 °C). Ionized calcium was determined in serum within 60 min from sample collection; serum aliquots for PTH evaluation were frozen in cryotubes at −20 °C until analyzed. All samples were measured in duplicate.

**Assays.** We detected ionized calcium values with an automated ion-selective analyzer (ICA 2; Radiometer A/S, Copenhagen, Denmark). The concentrations of ionized calcium were normalized to pH 7.4, and took into account CO₂ loss during sampling (8). Intact PTH values were measured with a two-site IRMA that detects the biologically intact 1-84 amino acid sequence of the hormone (Allegro Intact PTH; Nichols Institute, San Juan Capistrano, CA). The detection limit of the method is 1 ng/L (6).

**Statistical analysis.** The results are expressed as mean ± SD. Mean values were compared by using Student's t-test. Distribution of the concentrations of intact PTH in serum was analyzed by the method of Lilliefors (9). The 95% confidence limits of ionized calcium and intact PTH were calculated according to Rodbard and Hutt (10). For correlation coefficients, we used Pearson's formula. P < 0.05 was considered significant.

**Results**
The distribution of intact PTH values in the healthy children and adolescents was asymmetrical and non-gaussian. More than half of the subjects (58.8%) had intact PTH concentrations between 22.0 and 36.0 ng/L (Figure 1). The geometric mean of intact PTH from the 215 subjects was 30.8 (SD 9.6) ng/L; median 28.5 ng/L. The 95% confidence reference interval ranged from 16.0 to 59.0 ng/L. We found no significant difference by sex, age-related variations, and no correlation between intact PTH values and sex or age. The concentrations of ionized calcium were within the normal reference interval for our laboratory (1.17-1.31 mmol/L; n = 376) (11) in all healthy subjects (Figure 2).

In untreated hypercalcemic patients, intact PTH concentrations were 9.5 (SD 4.4) ng/L (range 5-17 ng/L, P < 0.001 vs results for healthy children) in those with hypoparathyroidism and 374.3 (SD 182.3) ng/L (range 150-720, P < 0.001 vs healthy children) in those with pseudohypoparathyroidism. Untreated hypercalcemic patients with primary hyperparathyroidism showed above-normal intact PTH values, but in subjects with pulmonary tuberculosis the intact PTH concentrations were in the low normal range in one case and suppressed in the other two (Figure 2).

**Discussion**
The IRMA we used for measuring intact PTH detects the biologically active intact molecule of the hormone with no interference from circulating inactive PTH fragments (6, 12, 19). This method for determining intact PTH values overcomes some of the limitations of traditional PTH RIAs (4, 6, 7).
Values for intact PTH assessed in our healthy subjects were remarkably similar to those reported for healthy adults (6, 14–17). This may suggest that intact PTH values do not significantly change with age; however, the earlier studies (6, 14–17) measured intact PTH in normal volunteers or donors with no well-defined age distribution, so no conclusive data are available for comparison.

Although the nongaussian distribution of intact PTH values during childhood is not well defined, such data agree with the results of Sokoll et al. for adults (17). However, the values are not related to PTH degradation during storage (13, 17), to age distribution, or to the distribution of the concentrations of ionized calcium. Although we studied only a few patients in each clinical group, the intact PTH IRMA we used provides an excellent discrimination between values of the major categories of children and adolescents with disturbances of calcium metabolism and those of healthy subjects, as has also been reported by Kruse et al. (18). In fact, this IRMA was able to distinguish low PTH values in patients with hypoparathyroidism from those of healthy children and adolescents. Moreover, it seems to be sufficiently sensitive to discriminate the patients with hypercalcemia due to primary hyperparathyroidism from those affected by hypercalcemia related to malignancy, pulmonary tuberculosis, or Williams syndrome, in which the high calcium concentrations are not due to an increased PTH secretion. The occurrence of intact PTH concentrations in the normal range (40 ng/L) in a child with hypercalcemia of malignancy due to cerebellar medulloblastoma is a very rare event, also reported by Blind et al. (19) in two of 40 patients with hypercalcemia of malignancy. Unfortunately, we are not able to define the cause(s) of the unexpected normal values of intact PTH in this patient because he died thereafter.

This IRMA for intact PTH was sufficiently sensitive to detect differences in PTH concentrations during childhood. Hence, we conclude that this method could be valuable in clinical investigations of disorders of calcium metabolism in children and adolescents.

References

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