Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Parathyroid Hormone

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Slide 1:
Hello, my name is Dr. Amy Pyle. I am Assistant Core Lab Director at Nationwide Children's Hospital in Columbus, OH. Welcome to this Pearl of Laboratory Medicine on "Parathyroid Hormone."

Slide 2:
Parathyroid hormone, or PTH, is an 84 amino acid-long protein that is made, stored, and released by the chief cells of the parathyroid glands, which are 4 small glands usually located on the dorsal aspect of the thyroid.

The concentration of PTH in circulation is regulated by its release from the parathyroids, its catabolism, and renal and hepatic clearance.

Slide 3:
PTH regulation is largely dictated by circulating calcium concentration: PTH is synthesized and secreted by the parathyroid glands in response to low free, or ionized, calcium in the blood and extracellular fluid, through activation of a calcium-sensing receptor in the membranes of parathyroid cells. Likewise, increased ionized calcium inhibits PTH synthesis and release, and increases PTH degradation and inactivation.

PTH release is also regulated, though to a lesser extent, by 1,25 dihydroxyvitamin D which suppresses PTH synthesis, phosphate, which promotes PTH synthesis and release, and to a very small degree, magnesium, which at high concentrations can suppress PTH synthesis through the calcium-sensing receptor.

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PTH secretion and free calcium exist in an inverse sigmoidal relationship, as PTH secretion is highest and lowest with hypo- and hypercalcemia, respectively. Only small changes are needed in the concentration of ionized calcium to change the amount of circulating PTH. In one study by Conlin et al, lowering the ionized calcium by only 0.05 mmol/L resulted in an increase in PTH of approximately 30 ng/L.

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Once in circulation, PTH has several mechanisms of action, culminating in an increase in circulating ionized calcium. First, PTH increases the activity and number of osteoclasts, though the exact mechanism by which this happens is not well understood. The outcome, however, is to promote bone resorption, and rapidly increase circulating calcium and phosphate. Second, PTH triggers increased reabsorption of calcium by the renal distal convoluted tubules, but decreasing reabsorption of phosphate at the renal proximal tubule, thereby increasing circulating calcium and decreasing phosphate. PTH also induces 25-hydroxyvitamin D-1 alpha-hydroxylase, which converts 25-OH Vitamin D to its active form, 1,25-dihydroxyvitamin D. This active vitamin D then triggers increased absorption of calcium and phosphate in the GI tract. The cumulative result of this action is increased free calcium in circulation, which feeds back to inhibit PTH synthesis and release.

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PTH determination is particularly useful in assessing hypercalcemia due to hyperparathyroidism. Hyperparathyroidism can be caused by a parathyroid gland disease, such as parathyroid adenoma or hyperplasia, causing primary hyperparathyroidism. Secondary hyperparathyroidism can arise in patients with severe renal disease in which the conversion of 25-OH Vitamin D to 1,25-di-OH Vitamin D is diminished. Most patients with hyperparathyroidism are asymptomatic at the time of diagnosis, and are identified during screening for low bone mineral density.

For symptomatic patients, classic symptoms which arise from both elevated PTH and calcium are often remembered with the mnemonic device “Bones, stones, abdominal groans, and psychiatric moans.” Bone and skeletal manifestations may occur, most commonly decreased bone mineral density, osteoporosis, and osteomalacia. And very rarely, osteitis fibrosa cystica, which is characterized by general bone demineralization and bone cysts.

Nephrolithiasis, or kidney stones, occur in 15-20% of patients with primary hyperparathyroidism, as the formation of calcium oxalate stones is promoted by hypercalciuria. Other renal manifestations include nephrocalcinosis, polyuria, and renal insufficiency.

Hypercalcemia can cause gastrointestinal symptoms, including constipation, nausea, and indigestion.

Finally, neuropsychiatric disturbances such as depression and lethargy may arise with mild hypercalcemia, and can escalate to psychosis, ataxia, and coma with more severe hypercalcemia.

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Measuring PTH is generally accomplished by immunoassay, however, doing so is confounded by the multiple forms of PTH present in circulation. Intact-PTH is comprised of a 34 amino-acid long N-terminal region which possesses most of the biological activity of the peptide, and a relatively inactive C-terminal portion. The N-terminal portion alone is known to act with similar potency to intact-PTH at the PTH receptor.

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Once in circulation, intact, bio-active PTH, with a half-life of less than 5 minutes, is readily fragmented into shorter, inactive fragments, primarily of the C-terminus, which have a much longer half-life.

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Due to its rapid degradation and clearance, intact bio-active, or 1-84, PTH is generally present as only 5-30% of the immunoreactive PTH in circulation; the remainder is primarily C-terminal fragments and some N-Truncated PTH, or non 1-84 PTH, in which the first 6 amino acids have been cleaved off.

In individuals with chronic kidney disease, decreased renal clearance may cause accumulation of PTH, especially the non-intact fragments. While the PTH fragments do not function the same as intact PTH, they are believed to have some distinct activity, possibly to antagonize the activity of intact PTH.

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With all the different lengths of PTH in circulation, immunoassays may measure non-active forms of PTH, depending on what portion of the molecule is recognized by the antibodies.

The first PTH assays were competitive radioactive immunoassays, which used a single antibody against the mid- or C-terminal portions of the molecule. These assays therefore detect not only bioactive, intact PTH, but also the inactive C-terminal fragments. This assay is no longer used in routine practice.

Second generation tests use sandwich immunoassays with antibodies against both the C- and N-termini and are sometimes referred to as “intact PTH” assays, since they were believed to measure the complete molecule. However, studies using HPLC indicate that these assays also detect an N-truncated, or non-1-84, PTH, which is not functionally equivalent to 1-84 PTH. These are currently the most commonly used PTH assays.

Finally, third generation assays were created with antibodies that specifically target amino acids 1-4, thereby detecting only full length PTH. Since N-truncated PTH comprises up to 30% of circulating PTH in normal individuals, and even more in those with chronic kidney disease, second and third generation assays may not agree, particularly in patients with kidney disease.

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When measuring PTH, preferred sample types are serum and plasma, either EDTA or Li-Heparin.

Reference ranges for PTH are generally around 10-65 pg/mL, depending on the assay and institution. One range is usually applied to all ages and genders.

Since PTH results are best interpreted in light of the serum calcium results, some labs may offer PTH bundled with a calcium test. If this is the case, the sample must not be in an EDTA tube, which is often preferred for PTH alone, as EDTA will chelate the calcium, producing a falsely low result.

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Parathyroidectomy may be performed as a treatment for primary hyperparathyroidism by surgically removing the hypersecreting tissue. However, identifying the parathyroids can be challenging. By following the concentration of PTH intra-operatively, surgeons get rapid and accurate feedback about the concentration of PTH before and after tissue excision. Generally, guidelines recommend that PTH should drop by at least 50% once the hypersecreting tissue has been removed.

This graph shows the results from a recent surgery in which tissues were excised three times before the parathyroid adenoma was removed. This is reflected in the concentration of PTH which did not fall significantly after the first or second excisions. However, within 10 minutes of the third excision, PTH fell from over 150 pg/mL to less than 50 pg/mL. The patient's calcium also dropped to a normal concentration following the surgery.

Since the intraoperative PTH assay is run while the patient is on the operating table, a rapid turnaround time is necessary to both save money and shorten the operating time. Several manufacturers offer intra-operative versions of their PTH assays, which are usually the same assay, but with a shorter incubation time. While this improves turnaround time—some assays have an on-board time of less than 10 minutes—the low-end sensitivity may decrease, but this isn't a major concern, as very low concentrations of PTH aren't seen in these patients.

Slide 13:
Hypercalcemia is a fairly common finding in patients with cancer, and is sometimes referred to as humoral hypercalcemia of malignancy. One cause of malignancy-associates hypercalcemia is the excretion of PTH-related protein, or PTHrP, from certain tumors. Malignancies associated with PTHrP include breast and ovarian cancer, squamous cell carcinomas, renal and bladder carcinomas, and certain leukemias and lymphomas.

PTHrP is unique from PTH, and is believed to play a role in normal physiology. However, due to a small N-terminal portion which is homologous to PTH, PTHrP can mimic the activity of PTH, thereby elevating calcium, and suppressing secretion of PTH. Therefore, PTHrP should be considered in the work-up for hypercalcemia, especially when PTH is low.

PTHrP is usually measured by immunoassay, and has a normal range of less than 1-2 pmol/L, depending on the assay.

PTHrP is unstable in serum and plasma and therefore requires special collection and handling procedures. Samples should be drawn into a chilled tube, transported on ice, centrifuged in a cold centrifuge, immediately removed from the cells or clot, and frozen. Ideally, protease inhibitors should be added to improve PTHrP stability.

Slide 14:
To review, the parathyroid glands make and release PTH in response to low ionized calcium. PTH then regulates bone and mineral metabolism, particularly calcium. Elevations in PTH in hyperparathyroidism cause hypercalcemia and low bone mineral density.

Full-length PTH is 84 amino acids long, but shorter lengths are generated via degradation and remain in circulation.

First, second, and third generation PTH immunoassays are available and differentially recognize the shorter forms of PTH, depending on where the antibodies bind PTH.

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PTH should be measured in serum or plasma, and may be reported along with calcium results.

PTH assays may be shortened and used to measure PTH intra-operatively. This aids surgeons in the accurate identification and removal of hyper-secreting parathyroid tissue.

Finally, PTH-related protein is a common cause of hypercalcemia of malignancy. PTHrP should be considered in any case of hypercalcemia with a suppressed PTH.

Slide 16: References

Slide 17:
Thank you for joining me on this Pearl of Laboratory Medicine on "Parathyroid Hormone." I am Amy Pyle.