Parathyroid hormone-related peptide and primary hyperparathyroidism

Mario Testini¹, Angela Gurrado¹, Germana Lissidini¹, Giuseppe Piccinini¹, Luigi Greco², Francesco Basile³, Antonio Biondi³

¹Section of General Surgery, Department of Applications in Surgery of Innovative Technologies, University Medical School of Bari, Italy, ²Section of General Surgery, Department of Emergency and Organ Transplantation, University Medical School of Bari, Italy, ³Section of General Surgery and Oncology, Department of General Surgery, University Medical School of Catania, Italy

TABLE CONTENTS

1. Abstract
2. Introduction
3. Hypercalcaemia
4. Primary hyperparathyroidism and humoral hypercalcaemia
5. Parathyroid hormone-related protein
6. Parathyroid hormone-related protein and parathyroid adenoma
7. Summary and perspective
8. References

1. ABSTRACT

The parathyroid hormone-related peptide (PTHrP) has been shown to be the major pathogenic factor to humoral hypercalcemia of malignancy (HHM). The presence of PTHrP in many normal tissues and in normal or abnormal parathyroids has been described in literature and its role has been investigated. PTHrP release from parathyroid cells into the extracellular space has been demonstrated to depend on the extracellular calcium concentration. The hormone binds to PTH type 1 Receptor (PTH1R) with a high affinity, as well as parathyroid hormone (PTH). These hormones’ amino-terminal (1-34) peptide fragments are considered sufficient to achieve efficient receptor activation and action on mineral ion homeostasis. Generally, diagnosis of primary hyperparathyroidism (PHPT) is based on hypercalcemia and elevated levels of PTH. The advent of intact-PTH immunoradiometric assay has further increased the reliability of results, enabling PHPT to be distinguished from non-parathyroid-dependent hypercalcemia. The presentation of a normal PTH level and hypercalcemia due to a parathyroid adenoma is unusual; however, its incidence has been estimated to be between 5% and 33% (2-4).

2. INTRODUCTION

The parathyroid hormone-related peptide (PTHrP) has been shown to be the major pathogenic factor to humoral hypercalcemia of malignancy (HHM). However, the presence of PTHrP in many normal tissues has been described in literature and a role in normal physiology has been assumed (1).

Diagnosis of primary hyperparathyroidism (PHPT) is generally based on hypercalcemia and high levels of parathyroid hormone (PTH); additional laboratory tests include hypophosphatemia and elevated urinary cyclic adenosine monophosphate (cAMP). The advent of the intact-PTH immunoradiometric assay has further increased the reliability of results, enabling PHPT to be distinguished from non-parathyroid-dependent hypercalcemia. The presentation of a normal PTH level and hypercalcemia due to a parathyroid adenoma is unusual; however, its incidence has been estimated to be between 5% and 33% (2-4).

PTHrP has been found in parathyroid tissue, but its role in causing hyperparathyroidism has not yet been defined (5).
Pthrp and Primary Hyperparathyroidism

The aim of the study is to identify the relationship between the production of PTHrP without malignancy and the diagnosis of PHPT by means of a systematic review according to recently presented guidelines on the argument. A comprehensive literature search was performed in December 2008 by consulting PubMed MEDLINE for publications and matching the terms of PTHrP and normal PTH level AND primary hyperparathyroidism/hypercalcaemia/parathyroid adenoma.

3. HYPERCALCAEMIA

Although the upper limit of normal can vary depending on the laboratory, hypercalcaemia is usually defined as a serum calcium level greater than 10.2 mg/dl, corrected for serum albumin concentration. Levels of higher than 14 mg/dl can be life threatening.

The clinical manifestations of hypercalcaemia will depend on the magnitude of this disorder. The most common symptoms are the nausea, vomiting, constipation and abdominal pain. Peptic ulcer disease and pancreatitis are rarely among the gastrointestinal manifestations. Furthermore, hypercalcaemia can determine difficulty in concentrating, corneal calcification, confusion and lethargy, fatigue and muscle weakness. Other effects are represented by vascular calcification, hypertension, shortening of the QT interval on the electrocardiogram and rare cardiac arrhythmias like digitalis toxicity. Significantly, hypercalcaemia can induce nephrolithiasis resulting from hypercalciuria, nephrogenic diabetes and, in the setting of volume depletion, acute renal failure.

However, diagnosis often is made incidentally in asymptomatic patients. The hypercalcaemia is one of the most common metabolic disorders and it could be generated by many different pathologic conditions. The most common categories are malignancy, PHPT and vitamin D-induced hypercalcaemia; the less frequent ones include thyrotoxicosis, drug-induced conditions (eg, thiazide diuretics, lithium, estrogens and antiestrogens, androgens, vitamin A), immobilization, tuberculosis, rhabdomyolysis, sarcoidosis, total parenteral nutrition, milk-alkali syndrome, kidney disease (acute and chronic, usually from medications) and familial hypocalciuric hypercalcaemia.

Although careful examination of personal and family history - focusing on clinical manifestations of hypercalcaemia and risk factors for malignancy and causative therapies - physical examination and laboratory investigations can differentiate the causes in most cases, hypercalcaemia often remains a challenging disease for clinicians.

4. PRIMARY HYPERPARATHYROIDISM AND HUMORAL HYPERCALCAEMIA

Identifying the aetiology of hypercalcaemia is very important, since subsequent management differs according to pathology. The main challenge in management is distinguishing the IPHP from conditions that will not respond to parathyroidectomy.

PTH level is the classic discriminator between parathyroid disease-dependent hypercalcaemia and others, whereas PTHrP - identified as tumour-associated factor - is the most useful analytical method in HHM.

The humoral hypercalcaemia of malignancy is one of the most frequent paraneoplastic syndromes and it is reported in up to 20 to 30% of patients with cancer (6-12).

In 1889, Stephen Paget stated that "in a cancer of the breast the bones suffer in a special way, which cannot be explained by any theory of embolism alone...the same thing is seen much clearly in those cases of cancer of the body where secondary deposition occurs in bones with astonishing frequency" and furthermore that "a general degradation of the bones sometimes occurs in carcinoma of the breast, yet without any distinct deposition of cancer in them" (13). Therefore, the current consideration that the cancer affects bone both by direct metastatic localization and through systemic humoral mechanism had been presciently recognized.

However, hypercalcaemia has only been associated with malignancy since 1920, when the serum calcium assay was introduced to clinical practice (6,14) and, in 1987, parathyroid-hormone-related peptide was isolated as a causative factor of HHM from human lung cancer (15) breast cancer (16) and renal cell carcinoma (17) and cloned shortly after its discovery (18).

5. PARATHYROID HORMONE-RELATED PROTEIN

This hormone is produced by a different gene - located on distinct chromosomes compared with PTH (mapped to the short arm of chromosome 12 and 11, respectively) - as a 141-amino acid protein or as a protein comprising either 139 or 173 amino acids, through different mRNA splicing (19).

Subsequently, PTHrP was detected in numerous tumour types like prostate cancer (20-22), epithelioid leiomyosarcoma (23), uterine carcinoma (24), cancer of the exocrine pancreas (10), pancreatic neuroendocrine tumour (25,26), squamous cell carcinoma (27-31), medulloblastoma (32), craniopharyngioma (33), rhabdomyosarcoma (34,35), haematological tumors (36-38), tumors of the neck and head (39-42), carcinoma of ovary (43,44), gallbladder carcinoma (45), cholangiocellular carcinoma (46,47), colorectal adenocarcinoma (48-52), carcinoma of the stomach (53), and melanoma (54).

PTHrP mediated hypercalcemia has already been reported in benignancy on rare occasions (55-58). Indeed, the presence of the hormone has been described in cases of gastrointestinal stromal tumours, leiomyoma and schwannoma (59), pheochromocytoma (60-62), mammary hyperplasia (63), uterine leiomyoma (64,65), as well as in other diseases, such as osteoporosis, sepsis, atherosclerosis,
hypertension and chronic inflammatory/autoimmune diseases (66-70).

Although PTHrP is undetectable in the circulating blood of normal subjects, this humoral factor is also produced by several normal tissues, suggesting that the hormone has a role in normal physiology as a local regulator in a paracrine/autocrine manner (6-71).

A role of PTHrP in the regulating cartilage differentiation and bone formation (72,73), in the growth and maturation of skin, mammary glands and teeth (74-76), and in lung development (77), has been demonstrated in recent studies.

Further interaction has been observed in the cardiovascular function (78,79), in the transepithelial calcium transport in mammary epithelia and placenta (80,81), in the relaxation of smooth muscle in uterus, bladder, vessels and ileum (82-85), and in the host immune function (86,87).

Other effects are the increase of beta cell mass and insulin secretion in the pancreas (88-91), and the involvement in the central nervous system function (92-96).

Further interaction has been observed in the cardiovascular function (78,79), in the transepithelial calcium transport in mammary epithelia and placenta (80,81), in the relaxation of smooth muscle in uterus, bladder, vessels and ileum (82-85), and in the host immune function (86,87).

Other effects are the increase of beta cell mass and insulin secretion in the pancreas (88-91), and the involvement in the central nervous system function (92-96).

However, the main effect of PTHrP is the interaction on bone growth and development and it is mediated by PTH type 1 receptor (PTH1R) binding, a G protein (98). The reaction with PTH1R in the bone and kidney results in the increase of the calcium serum level; therefore the biological responses elicited by PTH and PTHrP through this common PTH1R are largely indistinguishable, at least as regards mineral ion homeostasis (99-103). PTHrP stimulates bone resorption and mimics all PTH-like effects on tubules, including calcemic and phosphaturic effects.

The hormone binds to PTH1R with a high affinity, in the same way as PTH. These hormones’ amino-terminal (1-34) peptide fragments are considered sufficient to achieve efficient receptor activation and action on mineral ion homeostasis (99-102).

PTHrP actually shows significant sequence homology with PTH within the first 13 amino acid residues, and this fragment conservation explains the similar function of the amino-terminal residues in receptor signalling (101-106).

Indeed, the homology between PTHrP and PTH decreases markedly in the 14-34 sequence, because only three amino acids are identical, and these hormones are completely different beyond residue 34. Several evidence suggests that midregional and/or carboxy-terminal fragments of PTHrP and PTH also have biological activities (81,107,108), which are not likely to be related to mineral ion homeostasis and are probably mediated by other, not yet defined receptors (109-111).

However, the main PTH1R binding domain of PTHrP is localized in the 15-34 region peptide, and it is the same for PTH (112,113). Therefore, despite the absence of homology, the two different receptor-binding domains of PTHrP and PTH adopt a similar conformation. Indeed, the early N-terminal sequence of each hormone is required for biological activity, allowing the activation of the adenylcyclase/protein kinase A (AC/PKA) pathway.

This pathway and the phospholipase C/protein kinase C (PLC/PKC) pathway are at least the two second messenger signalling systems activated by PTHrP and PTH (107,114).

Currently the non-adenylyl-cyclase-mediated pathway is considered more complex than the AC/PKA system, because of the multiple phospholipase isoforms involvement, the sensitivity to variations in cell type and in receptor density (115,117).

The possibility of N-terminal PTHrP fragments activating a novel receptor in keratynocytes, insulinoma cells, lymphocytes and squamous carcinoma cells has been demonstrated; the activation determines the increase of intracellular free calcium, but not cAMP (90,118).

6. PARATHYROID HORMONE-RELATED PROTEIN AND PARATHYROID ADENOMA

Although the production of PTHrP secondary to parathyroid adenomas has been reported (5,119-122), this seems to be the first review in literature investigating the incidence of primary hyperparathyroidism with hypercalcemia and expression of PTHrP in association or not to the hypersecretion of PTH.

Primary hyperparathyroidism indicates the inappropriate or unregulated overproduction of PTH leading to abnormal calcium homeostasis. The effects of hypersecretion of PTH are the increase of renal resorption of calcium, bone resorption, synthesis of 1,24-dihydroxyvitamin D3 (1,24 (OH)2D3) and phosphaturia (123).

Generally, laboratory hallmarks are hypercalcemia, hypophosphataemia, hypercalciuria, elevated serum PTH levels and undetectable plasma levels of PTHrP. Most patients are asymptomatic or mildly symptomatic and the symptoms are correlated with hypercalcemia (124-127).

PHPT is secondary to an adenoma of the parathyroid gland accounting for 80% to 85%, a hyperplasia, for 10%-15%, and a carcinoma, for less than 1% (124,125,128,129).
Table 1. Primary hyperparathyroidism with hypercalcaemia and normal level of PTH: literature review

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhadada SK, 2008 (168)</td>
<td>2</td>
</tr>
<tr>
<td>Gurrado A, 2008 (169)</td>
<td>1</td>
</tr>
<tr>
<td>Khoo TK, 2007 (170)</td>
<td>1</td>
</tr>
<tr>
<td>Lafferty FW, 2006 (171)</td>
<td>1</td>
</tr>
<tr>
<td>Bergengfelz A, 2003 (2)</td>
<td>20</td>
</tr>
<tr>
<td>Perez JB, 2001 (119)</td>
<td>1</td>
</tr>
<tr>
<td>Bundgaard MJ, 2000 (172)</td>
<td>1</td>
</tr>
<tr>
<td>Marcinkowski W, 2000 (173)</td>
<td>22</td>
</tr>
<tr>
<td>Mischis-Troussard C, 2000 (174)</td>
<td>20</td>
</tr>
<tr>
<td>Baugmart DC, 1998 (175)</td>
<td>1</td>
</tr>
<tr>
<td>Glendning P, 1998 (4)</td>
<td>11</td>
</tr>
<tr>
<td>Haddock L, 1998 (176)</td>
<td>5</td>
</tr>
<tr>
<td>Okazaki R, 1992 (177)</td>
<td>3</td>
</tr>
<tr>
<td>Bergengfelz A, 1991 (178)</td>
<td>6</td>
</tr>
<tr>
<td>Hollenberg AN, 1991 (120)</td>
<td>1</td>
</tr>
<tr>
<td>Broughan TA, 1986 (179)</td>
<td>36</td>
</tr>
<tr>
<td>Coutrée J, 1980 (180)</td>
<td>1</td>
</tr>
<tr>
<td>Setton H V, 1979 (181)</td>
<td>1</td>
</tr>
<tr>
<td>Hammonds JC, 1976 (182)</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>157</strong></td>
</tr>
</tbody>
</table>

The parathyroid adenoma has been reported to be ectopic in 5 to 10% of cases (130), and this evidence is a frequent cause of surgical failure, requiring preoperative imaging to localise the condition in patients with PHPT before initiating surgery. Indeed, about 5% of patients who underwent parathyroidectomy present persistent or recurrent hyperparathyroidism (131).

The main cell types in parathyroid adenoma are chief cells, oxyphil and/or transitional oxyphil cells. Generally, PHPT is secondary to a solitary adenoma composed mainly of chief cells producing PTH. Indeed, the histology referring to an oxyphil adenoma is not commonly reported. This is an infrequent histological form considered exclusively non-functioning until 1970, consisting of cells with abundant eosinophilic cytoplasm that correlates ultrastructurally with numerous mitochondria (132).

In order to identify an oxyphil adenoma, the following histological criteria should be respected: 1) at least 90% of the cells should be oxyphil; 2) histologically normal excision or biopsy of a second gland should exclude the possibility of parathyroid hyperplasia; and 3) immediate postoperative normalization of hypercalcaemia should be reported (133).

At present, several Authors have reported cases of oxyphil parathyroid adenoma producing PTH (133-164).

Since the introduction of the intact PTH assay (immunoradiometric assay, IRMA) in 1984, the PTH level is high or included in the upper third of the normal range in order to diagnose PHPT (165-167).

The literature review regarding the reported cases of surgically proven PHPT with hypercalcaemia and normal levels of PTH has been reported (Table 1).

Several hypotheses have been performed in literature to explain the correlation between the atypical biochemical presentation and the histology of adenoma of parathyroid in these cases. There are quite a few pathological conditions, like coexistent sarcoidosis and/or vitamin D toxicity and/or hypomagnesaemia, which might suppress intact PTH level (183,184).

Another cause is related to the heat-lability/fragility of the hormone that degrades rapidly if the “cold chain” is not guaranteed during sample collection and transportation (185).

Moreover, an intact PTH level difference can be secondary to various methods, including the IRMA and immunochromiluminescent assay (ICMA) The IRMA is a “sandwich” assay formed by the solid-phase antibody, the antigen and the excess labelled antibody. In the event of an elevated concentration of antigen and insufficient solid-phase antibody, the unreacted antigen in solution competes with the antigen already extracted onto the solid-phase antibody for labelled antibody. Therefore, the count in the solid phase declines with the increase of the level of the antigen, inverting the dose-response curve, and determining the “hook effect” (186). However, this phenomenon might be avoided through serial dilution serum intact PTH assays (171), or through the ICMA method (168).

The intact PTH level should always be measured on more than two occasions with similar results in each patient.

In literature, the secretion of biologically active PTH fragment by adenoma has been considered, resulting from a post-translational change in the molecule that is not measurable by the current assay (171).

Hollenberg et al. (120) proposed different theories to explain the mechanism of the inappropriately low PTH level in the PHPT case observed: 1) the presence of a circulating PTH inhibitor; 2) the pulsatile secretion of the hormone; 3) an abnormal PTH molecule with increased biologic activity; 4) a rise of peripheral tissue sensitivity to normal PTH, 5) the presence of another mediator of hypercalcaemia (eg, PTHrP).

None of these hypotheses has been verified until 2008, when the association of immunohistochemical
Table 2. PHPT due to parathyroid adenoma producing PTHrP: literature review

<table>
<thead>
<tr>
<th>Author</th>
<th>n. of cases</th>
<th>hypercalcaemia</th>
<th>Serum PTH</th>
<th>Histology of adenoma</th>
<th>PTHrP immunoreactivity</th>
<th>PTHrP gene mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gurrado A, 2008</td>
<td>169</td>
<td>yes</td>
<td>n.r.</td>
<td>+</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td>Kitazawa R, 2002</td>
<td>36</td>
<td>yes</td>
<td>n.r.</td>
<td>o</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Matsushita H, 1997</td>
<td>187</td>
<td>yes</td>
<td>e</td>
<td>n.r.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Matsushita H, 1992</td>
<td>122</td>
<td>yes</td>
<td>e</td>
<td>1 o; 11 co; 1c</td>
<td>+</td>
<td>n.r.</td>
</tr>
<tr>
<td>Docherty, 1991</td>
<td>188</td>
<td>yes</td>
<td>n.r.</td>
<td>+ (+4/11)</td>
<td>+ (+7/11)</td>
<td></td>
</tr>
<tr>
<td>Danks JA, 1990</td>
<td>189</td>
<td>yes (12/14)</td>
<td>n.r.</td>
<td>+</td>
<td>n.r.</td>
<td></td>
</tr>
<tr>
<td>Ikeda, 1989</td>
<td>5</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>99</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: normal; e: elevated; n.r.: not reported; n.d.: not determined; c: chief cell type; o: oxyphil type; co: mixed type.

posivity for PTHrP-antigens, the immediate postoperative normalization of hypercalcaemia, and the histological features of oxyphil parathyroid adenoma were observed in a patient with hypercalcaemia and normal serum PTH level (169).

A literature review regarding the parathyroid adenomas positive for PTHrP immunoreactivity or overexpression of the messenger ribonucleic acid (mRNA) of the hormone gene was performed (Table 2).

PTHRP expression in normal or abnormal parathyroid tissue was analysed in some series and, so interestingly the positivity was most frequently related to adenoma with a dominance of oxyphil cells, and very uncommonly to chief cell type or mixed (5,36,121,122).

The age-related increase in oxyphil cells explains the frequency of immunohistochemical detection of PTHrP in normal parathyroid in adult population. Several studies have, indeed, defined the correlation of this hormone with the age-related metaplastic change of parathyroid cells into the oxyphil phenotype, through a paracrine/autocrine regulation mechanism (36,122).

Besides the same target, the PTH and PTHrP have been demonstrated to be secreted simultaneously by parathyroid adenoma cells and inversely related to the extracellular calcium ion concentration (97). Matsushita et al. 187 demonstrated furthermore that PTHrP could be co-secreted by the parathyroid gland together with PTH via a regulated pathway and that a constitutive pathway could barely operate in the secretory mechanism of PTHrP in parathyroid adenoma cells.

At present, the role of PTHrP secreted by reported cases of oxyphil parathyroid adenoma associated with hypercalcaemia has not been explicated (Table 2). In most series, the PTH level has not been reported (5,36,189), and in others, the hypercalcaemia depended on the simultaneous hypersecretion of PTH (122,187-189).

Although there are discordant opinions (137,190-192,194,195), several Authors observed a statistically significant rise of Tc-99m-sestamibi scan sensitivity of oxyphil adenoma, due to affinity of the mitochondrial-rich cell for the sestamibi uptake and retention (134,140,143,193-195). This should be considered an important factor in order to perform the diagnosis of adenoma in the unusual biochemical cases of PHPT.

To the best of our knowledge, one is the report regarding the association of immunohistochemical positivity for PTHrP-antigens with normal serum PTH level, the immediate postoperative normalization of hypercalcaemia, and the histological features of parathyroid adenoma (169).

7. SUMMARY AND PERSPECTIVE

Despite considerable advances in the understanding of the synthesis, secretion, molecular structure and target activation of PTH and PTHrP, hypercalcaemia can sometimes, but rarely, represent a diagnostic dilemma and a therapeutic challenge.

PTHRP could play a critical role in determining the pathogenesis of atypical biochemical presentation of hypercalcaemia due to parathyroid adenoma and the PTHrP measurement should be assessed not only in the presence of malignancy. Moreover, the diagnostic suspicion of PHPT should not be eliminated when serum PTH levels are in normal range.

Further research with larger populations is necessary to define a novel diagnostic flow-chart of hypercalcaemia considering the whole of the clinical and biochemical presentations of PHPT.

8. REFERENCES


Pthrp and Primary Hyperparathyroidism


67. J.L. Funk: A role for parathyroid hormone-related protein in the pathogenesis of inflammatory/autoimmune diseases. *Int Immunopharmacol* 1, 1101-21 (2001)


Pthrp and Primary Hyperparathyroidism


Pthrp and Primary Hyperparathyroidism


Pthrp and Primary Hyperparathyroidism


Pthrp and Primary Hyperparathyroidism


Pthrp and Primary Hyperparathyroidism


Pthrp and Primary Hyperparathyroidism


**Abbreviations:** PTHrP: parathyroid hormone-related peptide; HHM: humoral hypercalcemia of malignancy; PTH1R: PTH type 1 Receptor; PTH: parathyroid hormone; PHPT: primary hyperparathyroidism; cAMP: cyclic adenosine monophosphate; AC/PKA: adenylyl cyclase/protein kinase A; PLC/PKC: phospholipase C/protein kinase C; 1,24 (OH)2D3: 1,24-dihydroxyvitamin D3; IRMA: immunoradiometric assay; ICMA: immunochemiluminescent assay; mRNA: messenger ribonucleic acid; Tc-99m-sestamibi: Technetium-99m sestamibi.

**Key words:** PTHrP, PTH, Primary Hyperparathyroidism, Hypercalcaemia, Parathyroid Adenoma, Review

**Send correspondence to:** Mario Testini, Unit of Endocrine Surgery, Section of General and Thoracic Surgery, Department of Applications in Surgery of Innovative Technologies, University Medical School of Bari, Policlinico, P.zza G. Cesare 70124 BARI, Italy, Te.: 0039.080.5592882, Fax.: 0039.080.5592882 E-mail: mario.testini@chirgen2.uniba.it

http://www.bioscience.org/current/volS2.htm