Pancreatitis as the First Manifestation of Multiple Endocrine Neoplasia Type 2A

clinical case report

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ABSTRACT

Multiple endocrine neoplasia type 2A (MEN2A) is an autosomal dominant inherited condition that predisposes to the triad of medullary thyroid cancer (MTC), pheochromocytoma (Pheo), and primary hyperparathyroidism (PHT). Nearly 100% of MEN2A are associated with germ line mutation of the *RET* proto-oncogene (*RET*), and DNA-based *RET* genotype analysis is now considered essential for earlier diagnosis. The first manifestation of MEN2A is most often due to MTC, and less frequently to Pheo. Rarely, MEN2A is recognized during the search for PHT associated conditions. Most patients with primary hyperparathyroidism are asymptomatic, and the focus of the presentation may be the side effects of chronic hypercalcemia, osteoporosis, renal lithiasis, peptic ulcer disease, and hypertension. Hypercalcemic pancreatitis is rare, being an uncommon first manifestation of PHT. Here, we report on a patient who presented recurrent pancreatitis as the first manifestation of MEN2A. In the present case, prompt sequential dosage of calcium, diagnosis of PHT, and genetic analysis would have resulted in pancreatitis prevention and early MEN2A management. **(Arq Bras Endocrinol Metab 2008; 52/8:1332-1336)**

Keywords: Pancreatitis; Multiple endocrine neoplasia type 2; Primary hyperparathyroidism; Medullary thyroid carcinoma; Molecular diagnosis

RESUMO

Pancreatite como Primeira Manifestação de Neoplasia Endócrina Múltipla do Tipo 2.

Neoplasia endócrina múltipla do tipo 2 (NEM2A) é uma síndrome genética com herança autossômica dominante, que predispõe à tríade de carcinoma medular de tireóide (CMT), feocromocitoma (Feo) e hiperparatireoidismo primário (HPP). Aproximadamente 100% dos casos de NEM2A estão associados a mutações germinativas do protooncogene RET (*RET*), e a análise molecular do *RET* é atualmente considerada essencial para diagnóstico precoce. A primeira manifestação da NEM2A é geralmente em decorrência de CMT, e menos freqüentemente devido ao Feo. Raramente, a NEM2A é descoberta durante investigação para condições associadas ao HPP. A maioria dos pacientes com HPP é oligossintomática e a apresentação ocorre devido a sintomas relacionados à hipercalcemia, à osteoporose, à dispepsia, à hipertensão ou à litíase renal. A pancreatite hipercalcêmica é rara, sendo uma manifestação incomum do HPP. Este artigo relata um caso de paciente que apresentou pancreatite recorrente como primeira manifestação de NEM2A. Neste caso, abordagem seqüencial com determinação do cálcio sérico, diagnóstico de HPP e análise genética poderiam ter resultado prevenção de pancreatite e manejo precoce da NEM2A. **(Arq Bras Endocrinol Metab 2008; 52/8: 1332-1336)**

Descritores: Pancreatite; Neoplasia endócrina múltipla do tipo 2; Hiperparatiroidismo primário; Carcinoma medular de tireóide; Diagnóstico molecular

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INTRODUCTION

Multiple endocrine neoplasia type 2A (MEN2A) is a multi-glandular autosomal dominant inherited condition that affects tissues of neural crest origin. It is caused by germline mutations of the *RET* proto-oncogene (*RET*) located on cromossome 10q11.2 (1). The *RET* encodes a transmembrane tyrosine kinase receptor, expressed by cells of neural crest origin that regulates cell growth. The MEN2A gain-of-function mutations predispose to the triad of medullary thyroid cancer (MTC), pheochromocytoma (Pheo), and primary hyperparathyroidism (PHT) (1,2). The estimated prevalence of MEN2A is around 1 in 40,000 individuals. With a penetrance of ~100%, MTC is the hallmark of MEN2A. In patients with MEN2A, C-cell hyperplasia develops early in life and can be viewed as the precursor lesion for MTC, which often arises multifocally and bilaterally. Penetrance for Pheo and PHT is around ~40% and ~15%, respectively. Nearly 100% of MEN2A are associated with *RET* germ line mutation, and DNA-based *RET* genotype analysis is now considered essential for earlier diagnosis and treatment (3). The first manifestation of MEN2A is most often due to MTC, with symptoms of Pheo less frequently being the first sign of the disease. Rarely, recognition of MEN2A occurs during the search for PHT associated conditions (3). Symptomatic manifestations of PHT are most commonly due to hypercalcemia, osteoporotic fractures or nephrolithiasis. Hypercalcemic pancreatitis is rare and is an uncommon first manifestation of PHT (4). In this article we describe a patient with recurrent episodes of acute pancreatitis, a potentially lethal condition, as the first manifestation of MEN2A.

SUBJECTS AND METHODS

Case report

A 58-year-old male patient was referred to our institution (Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil) for genetic evaluation due to a diagnosis of MTC and PHT. He had begun a clinical investigation for diffuse bone pain and hypercalcemia about two years before. Laboratorial evaluation disclosed serum parathyroid hormone (PTH) levels of 1445 pg/ml (CLIA, normal range [NR] 7-53 pg/ml), serum calcium 11.7 mg/dl (NR 8.4-10.2 mg/dl), serum phosphorus 1.9 mg/dl (NR 2.5-4.5 mg/dl), serum creatinine 1.0 mg/dl and urinary calcium of 414 mg/24h (NR 100-300 mg/24h). Imaging with ^{99m}Tc-Methylene diphosphonate (^{99m}Tc-MDP) demonstrated alterations suggestive of metabolic bone disease. Bone density analysis showed osteoporosis of the lumbar spine and femoral neck (0.794 g/cm^2 [T score of -3.3] and 0.567 g/cm^2 [T score of -3.6], respectively; *General Electric GE Lunar DPX)*. Parathyroid ^{99m}Tc-2-Methoxy-isobutyl-isonitrile (^{99m}Tc-MIBI) scan showed two images suggestive of parathyroid hyperfunction. Cervical ultrasonography (US) described two augmented parathyroid glands measuring around 1.9 cm in diameter and two thyroid nodules measuring 1.6 cm and 1.8 cm in diameter. The two thyroid nodules were solid, irregular, heterogeneous, and had microcalcifications. Serum calcitonin was 1140 pg/ml (CLIA, NR < 12.0 pg/ml). A cervical surgical intervention comprising dual parathyroidectomy and total thyroidectomy were performed. The anatomopathological exam showed two hyperplastic parathyroid glands measuring 2.6 cm and 2.5 cm in diameter. The thyroid gland examination disclosed C-cell hyperplasia and two encapsulated nodules measuring 2.4 cm and 0.8 cm in diameter, with cells displaying amphophilic cytoplasm on hematoxylin and eosin stain, angiolymphatic and capsular invasion, accentuated mitotic activity and positive amyloid deposits, histological findings suggestive of MTC. Thyroid tissue immunohistochemistry (IMHC) confirmed the histological findings, displaying positivity for calcitonin, synaptophysin and chromogranin A and negativity for thyroglobulin. The patient was then referred to our center.

METHODS

Genetic screening

Genomic DNA was prepared from peripheral blood leukocytes by standard procedures and *RET* mutations were screened by restriction enzyme digestion and/or direct sequencing as previously described (5). Briefly, Oligonucleotide primers for amplification of different *RET* exons were designed on the intronic sequences flanking exons 10, 11, 13, 14, 15, and 16 (5). PCRs were run in a final volume of 50µl using 100 or 200ng genomic DNA, containing 20mM Tris HCl (pH 8.4), 50mM KCl, 1.5mM MgCl2, 0.2mM deoxynucleotide triphosphate, 1 U Taq polymerase, and 1µM of specific primer. Genomic DNA was denatured for 3 min at 94 C before 35 cycles at 94, 65, and 72 C for 1 min at each temperature, followed by a 5-min 72 C step in a pro-

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grammable thermal controller (MJ Research, Inc., Waltham, MA). Following PCR, the amplicon sizes were analyzed in 1.5% agarose gel and the products visualized by ethidium bromide staining. Whenever necessary, the presence of mutations is confirmed by direct sequencing of the PCR product using the Sanger method in an automated sequencer, according to the manufacturer's instructions (Alf Express, Pharmacia Biotech AB, Uppsala, Sweden). Before undergoing genetic testing, our patient gave his written informed consent, as required by the institution's Ethics Committee.

Laboratorial Investigation

Evaluation and follow up for patients with MEN2 at our center comprise a complete physical examination, basal plasma calcitonin (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA; NR. Male < 12.0 pg/ml and female < 6.0 pg/ml]), plasma calcium and PTH levels (Immulite 2000 Intact PTH, Diagnostic Products, Los Angeles, CA, USA). Further, urinary fractionated and total metanephrines, and catecholamines are measured by high-performance liquid chromatography to rule out pheochromocytoma, besides an extensive diagnostic imaging investigation, which includes cervical US, cervical, thorax, and abdominal computed tomography (CT), and whole-body metaiodobenzylguanidine (^{131}I-MIBG) scintigraphy on selected patients.

RESULTS

Molecular analysis disclosed a C634Y *RET* proto-oncogene mutation, thus confirming the clinical hypotheses of MEN2A. Laboratorial investigation showed persistent hyperparathyroidism (PTH 122 pg/ml and serum calcium 10.9 mg/dl), a serum calcitonin of 1841 pg/ml, and cervical US demonstrating enlargement of lymph nodes. Thorax and abdominal CT were normal, and the complementary evaluation for Pheo was negative.

Physical examination was unremarkable except for a fibrous scar on the neck due to thyroidectomy. The patient has undergone surgical intervention for resection of lymph nodes in the central zone of the neck combined with lymph node dissection of both cervicolateral compartments, and complement of total parathyroidectomy, with implantation of slices of parathyroid tissue in the arm. Five out of 14 lymph nodes removed were positive for MTC and thymic metastasis was found. In the second year of follow-up at our institution, urinary metanephrines increased and the abdominal CT showed a 1cm diameter nodule of in the left adrenal gland. The patient was submitted to a videolaparoscopic adrenalectomy and the anatomopathological exam confirmed Pheo. Currently the patient is being followed, with persistent elevated calcitonin of 1841 pg/ml, and no detectable metastatic MTC on imaging studies.

Interestingly, a review of medical records revealed that until starting investigation for bone pain, the patient medical history was unremarkable, except for two prior episodes of acute pancreatitis two years and six months previously. On both occasions, evaluation for pancreatitis etiology disclosed no anatomical anomaly or evidence of gallstones. Prior to the episodes, the patient was using no drugs, and had no history of alcohol ingestion, trauma, bug bites or infection. The patient had no evidence of autoimmunity, renal function was normal and serum triglycerides were within the normal range. Except for elevated serum calcium of 11.2 mg/dl, all the investigation to determine pancreatitis etiology was unremarkable. Despite lack of evidence for biliary pancreatitis, the patient had undergone videolaparoscopic cholecystectomy after the first episode of pancreatitis. After the second acute pancreatitis episode the patient was discharged home from his hometown hospital with a presumptive diagnosis of hypercalcemic pancreatitis.

DISCUSSION AND CONCLUSIONS

In the present study, we describe a patient harboring a 634-RET mutation presenting recurrent episodes of acute pancreatitis as the first manifestation of MEN2A. Pancreatitis is a severe condition, with mortality and morbidity rates near 4% and 20% respectively (6). Prompt identification of the cause of pancreatitis may help reduce cost and morbidity. When extensive investigation with no cause is found, the term idiopathic pancreatitis is applied. The incidence of idiopathic pancreatitis varies across different studies, according to definition criteria and population studied, accounting for ~15-25% of the acute cases (7). Hypercalcemic pancreatitis is rare, accounting for ~1% of the acute pancreatitis episodes and is an uncommon first manifestation of PHT (4,7-9). As serum calcium lessens in the acute pancreatitis inflammatory process, it is possible that some hypercalcemic pancreatitis cases may be missed, and mislabeled as idiopathic. Because acute pancreatitis is a potentially lethal condition, dosage of serum calcium during initial evaluation

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and in the follow-up is warranted as a means of stratifying risk though the application of the Ranson's Criteria and for etiologic investigation.

Our patient underwent extensive investigation in search for the cause of pancreatitis in the two episodes reported. Patient history and laboratorial investigation do not support other etiologic diagnosis than hypercalcemia. It is well known that, even when investigation discloses no evidence of biliary obstruction or gallstones, biliary pancreatitis is far more prevalent than hypercalcemic pancreatitis (7). Indeed, based on this assumption, the patient was submitted to cholecystectomy after the first pancreatitis episode, but a recurrence of pancreatitis after cholecystectomy had occurred. Pancreatitis is associated with PHT, whereas hypercalcemia seems to be a major factor in the development of this condition. Some studies have described severe acute pancreatitis as a first manifestation of PHT (4,8,9). It is interesting however, that no association with MEN2A has been reported so far. This probably can be partially explained by the fact that PHT in MEN2 is usually mild, develops slowly and in most cases have no symptoms, with diagnostic been performed by screening.

The *RET* proto-oncogene is expressed in cells of neuronal and neuroepithelial origin and encodes a receptor tyrosine kinase (10). Mutations on the highly conserved extracellular cysteine ligand-binding domain encoded by exons 10 and 11 induce constitutive tyrosine kinase activity due to aberrant homodimerization (10,11). The transforming capacity of the c-*RET* examined in transfected NIH-3T3 cells has been shown to be dependent on specific mutated codons with the C634R mutant showing a 3-to 5-fold higher transforming activity compared with any exon 10 Cys mutants (11). Although the three-dimensional structure of the *RET* extracellular domain is still unknown, it is likely that these cysteines likely form intramolecular disulfide bonds in the wild-type receptor, and the mutation results in an unpaired cysteine, which forms an activating intermolecular bridge (10,11).

Differences in dimerization induction intensities are a reasonable explanation for the phenotypes resulting from mutations of the different cysteines. In fact, the international *RET* mutation consortium analysis studied 477 MEN2 families from 18 tertiary referral centers and demonstrated that specifically mutated *RET* codons correlate with MEN2 variants (3,6,12,13). In agreement with the results of the consortium analysis, in our series, the most frequent phenotype was the MEN2A syndrome with codon 634 mutation (5). The presence of *RET* 634 mutations predicts the development of MTC, Pheo and HPT, such as the case of the patient described here. However, there is a general consensus that MTC occurs before or concurrently with the development of Pheo in almost all cases of MEN2, and that the overwhelming cause of death in MEN2 is metastatic MTC. PHT is rarely the first manifestation of MEN2A (14). Our patient, not only presented PHT as the first manifestation, but also presented recurrent episodes of acute pancreatitis before being diagnosed with PHT. The diagnosis of PHT associated to MTC warranted prompt genetic screening for *RET* proto-onocogene mutation, that confirmed the clinical hypothesis of MEN2A. Although uncommon, the diagnosis of inherited forms of primary hyperparathyroidism are clinically important since the management of the parathyroid tumors in familial parathyroid syndromes often differs from that of sporadic primary hyperparathyroidism. Moreover, extraparathyroidal manifestations of hereditary syndromes may need treatment, and awareness of familial clustering should prompt systematic family screening. Of note, the molecular diagnosis preformed in this case was crucial for the close surveillance of Pheo that has allowed early diagnosis and management of this potential fatal disorder. HPT is also one of the major components of the multiple neoplasia type 1 (MEN1) (15).

Our Division is a reference center for molecular screening of germ-line *RET* mutation in Brazil. In the cohort of 94 patients with MEN2A evaluated at our institution, MTC was already present in all but 1 individual (29/30) identified by genetic screening and in 96.8% (62/64) who manifested clinical disease at diagnosis (Table 1). Of the two patients with no-MTC as the first manifestation at diagnosis, one presented with Pheo and the other (present case description) with PHT. In our cohort, PHT developed in only 3.3% (1/30) patients diagnosed by genetic screening and in 25% (16/64) with manifested clinical disease, a finding that is in accordance with data showing that PHT is a late manifestation in MEN2A (14). Despite the low prevalence of Pheo and PHT as the first manifestation of disease, our cohort data highlight the importance of close follow-up of these patients. Surveillance for theses associated neoplasias is warranted as a means of reducing morbidity and mortality in affected individuals. One should note that Pheo and PHT develop in nearly 40% and 25% of MEN2A patients during long term follow-up (Table 1).

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Table 1. Phenotype of the patients with Multiple Endocrine Neoplasia Type 2A (MEN2A) followed at our institution (n=94).

	Total	MTC	Pheo	PHT
Asymptomatic Carriers - n (%)	30 (32)			
Clinical disease - n (%)	64 (68)			
First manifestation of disease - n (%)		62 (96.8)	1 (1.6)	1 (1.6)
Developed at follow-up – n (%)		64 (100.0)	26 (40.6)	16 (25.0)

MTC = medullary thyroid carcinoma; Pheo = pheocromocytoma; PHT = primary hyperparathyroidism.

To our knowledge this is the first report of acute pancreatitis as the first manifestation of MEN2A. In this case, prompt sequential dosage of serum calcium and PTH, diagnosis of PHT and genetic analysis would have resulted in pancreatitis prevention and early MEN2A management.

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