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Caso Clínico

Multiple Endocrine Neoplasia Type 2B (MEN2B) delayed diagnosis: importance of opportune recognition of MEN2 Syndromes in pediatric thyroid cancer.

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A B S T R A C T

Background: *RET* proto-oncogene mutations are responsible for familial thyroid medullary carcinoma and multiple endocrine neoplasia (MEN) type 2A and 2B. These syndromes develop specific biomarkers and, in the case of MEN2B, clinically observable stigmas. However, the diagnosis of patients with MEN2B is usually delayed. Because of the close genotype-phenotype correlation, molecular testing is the final approach for the diagnosis to establish preventive care and therapeutic behaviors.

Case report: We present the case of a woman diagnosed with a thyroid nodule at the age of nine. She underwent a total thyroidectomy plus radical cervical lymph node dissection, with a diagnosis and initial management of papillary thyroid carcinoma. During the evolution of the disease, she developed pulmonary metastases. At the age of 24, after her first endocrinological evaluation, typical physical manifestations of MEN2B were observed. A re-evaluation of the original thyroidectomy revealed a medullary carcinoma, with positive manifestation CEA and calcitonin. The analysis of *RET* proto-oncogene identified a *de novo* mutation in exon 16 (pM918T).

Discussion: pM918T is classified as "highest risk" for medullary carcinoma with a 50% of lifetime risk for developing pheochromocytoma. Most cases of MEN2B are due to a *de novo* mutation. Even with the increased risk of developing pheochromocytoma, our 24-year-old patient does not yet present one. Other factors may be involved in the modulation of the phenotype in different populations.

Conclusion: The timely diagnosis of MEN2B offers opportunities to make appropriate preventive and therapeutic decisions that may change the natural evolution of the disease and its complications.

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INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN2) is a relatively rare pathology that results from an autosomal dominant inheritance caused by specific *RET* (REarranged during Transfection) proto-oncogene mutations. MEN2 encompasses two syndromes, MEN type 2A, with familial medullary thyroid cancer included (OMIM # 171400) and MEN type 2B (OMIM # 162300)[1].

The occurrence of medullary thyroid carcinoma (MTC) in patients with MEN2 is nearly 100% and can occur at an early age. In MEN2B patients, the MTC often manifests during infancy and is highly aggressive, metastasizing to regional lymph nodes and beyond [2]. Approximately 75% of MEN2B cases are due to *de novo RET* mutations, while 25% of cases occur in families with manifestations of MEN2B[3]. About 95% of patients with MEN2B present exon 16 (codon pM918T) *RET* proto-oncogene germline mutations and less than 5% have *RET* exon 15 (codon pA883F) germline mutations[4,5].

The American Thyroid Association (ATA) identifies three levels of risk for hereditary MTC: highest risk, high risk, and moderate risk. "Highest risk"(HST) includes patients with MEN2B with the *RET* codon M918T mutation. “High risk"(H) includes patients with the *RET* codon C634 and the codon A883F mutation. "Moderate risk" (MOD) includes patients with hereditary MTC and other *RET* mutations [3].

MEN2 patients have an increased lifetime risk for pheochromocytoma (PHEO) with a prevalence rate of about 50%[6]. Different degrees of penetrance are observed depending on the RET proto-oncogene mutations, with those in 634 (MEN2A) and 918 (MEN2B) being the most frequently associated with this phenotype [7,8].

Patients with MEN2B present universal extra-endocrine features, mainly bowel deficiency due to diffuse intestinal ganglioneuromatosis, in addition to mucosal neuromas and marfanoid body habitus characterized by narrow long facies, pes cavus, pectus excavatum, high-arched palate, scoliosis, slipped capital femoral epiphyses, and arachnodactyly with some muscle wasting and possibly, weakness, which may not be become clinically apparent until several years of age [6,9].

The RET proto-oncogene, with 21 exons located on chromosome 10 (10q11.2), encodes for a transmembrane receptor tyrosine kinase for members of the glial cell line–derived neurotrophic factor family (GDNF) and associated ligands (artemin, neuturin, persephin), is involved in proliferation and cell survival, and is significantly expressed in thyroid para-follicular C-cells [10,11]. Hyperactivation of the receptor leads to induction of downstream signals responsible for oncogenesis[12].

Because RET proto-oncogene analysis is the final approach for an accurate diagnosis of MEN2, in developing countries like Ecuador, the limited availability of molecular testing has been a restriction. Understanding the fact that diagnosis may be a challenge, reporting these types of pathologies will bring new insights and possibilities for physicians, patients, and their families to experience the best possible management of the disease. To our knowledge, this publication is one of a few reports about a complete clinical, histological and molecular characterization of a patient with MEN2B in South America and the first one in Ecuador.

CASE REPORT

The patient is a 24-year-old woman with no family history of thyroid cancer who was diagnosed with a thyroid nodule at the age of nine. She underwent a total thyroidectomy and a complete cervical lymph node dissection (in three different surgical procedures). The first histological evaluation revealed a papillary thyroid carcinoma. No prior review of the histological material was performed during the subsequent 15 years of follow-up. She was treated with radioactive iodine (350 milicuries in cumulative dosage). Thyroglobulin and anti-thyroglobulin antibodies were consistently negative, with a null uptake of ^{131}I. During this primary follow-up, the case was interpreted as aggressive papillary carcinoma, refractory to radioactive iodine treatment with a progressive dedifferentiation.

After several years of ambulatory treatment, at the age of 22, the patient was diagnosed with pulmonary metastases. She was admitted for the first time by the Oncology Department of the Eugenio Espejo Hospital, with a compromised general condition. Due to the critical situation of the patient and the first referred clinical information, she received a therapeutic regimen of sorafenib, with a notable improvement of the respiratory symptomatology.

She received multidisciplinary care to treat the different symptomatologies she exhibited. After the cardiologist observed acromegaly-like signs, the case was referred to the Endocrinology Department. During the first clinical evaluation, marfanoid biotype with clear arachnodactyly were observed. Thickened lips, high arched palate and multiple yellowish papules with well-defined borders and arboriform vessels were observed across the tongue, vermilion, oral mucosa, and palpebral edge with eversion of the upper eyelids (Figure 1).

Considering the typical stigmas of MEN2B, a histological re-evaluation of the histological material and cervical lymph nodes was performed with a conclusion of medullary thyroid carcinoma. Immunohistochemistry showed a positive thyroid transcription factor 1 (TTF-1) as well as calcitonin. Chromogranin, cytokeratin 7 (CK7) and synaptophysin were also positive, while thyroglobulin, CK20 and S-100 were negative, supporting the diagnosis of MTC (Figure 2).

Blood tests revealed elevated calcitonin 892 (<11 pg / ml); carcinoembryonic antigen (CEA) 13.5 (<3.8 mg / ml) and chromogranin A 164.62 (<100 ug / l). Free plasmatic metanephrines were negative (metanephrine result: 0.23 nmol/L, reference value: <0.50 nmol/L; normetanephrine result: 0.70 nmol/L, reference value: <0.90 nmol/L) measured

by high performance liquid chromatography-HPLC (13). Serum calcium, phosphorus and parathormone (PTH) levels were compatible with hypoparathyroidism, probably as a consequence of the numerous surgical procedures. CT scan presented diffuse bilateral micronodular pulmonary metastases. No liver or bone metastases were demonstrated.

Due to the presence of mucosal lesions, she was referred to a dermatologist for clinical evaluation and biopsy. Histological results from the tongue showed neural bundles hyperplasia, compatible with mucosal neuromas (Figure 2).

Patient's family history did not show familial genetic contributions compatible with MTC or other MEN2B associated characteristics presuming a *de novo* mutation. Genomic DNA from peripheral blood was analyzed for *RET* mutations in exons 10, 11, 13, 14, 15 and 16. A heterozygous pM918T missense mutation was identified in exon 16 (rs74799832, codon 918, ATG>ACG, Met>Thr). In addition to referred mutation, two polymorphic homozygous genetic variants were found; pG691S (rs1799939, GGT>AGT, Gly>Ser), establishing a missense in exon 11; and another synonym change in exon 15, pS904S (rs1800863, TCC>TCG, Ser>Ser) (Figure 3).

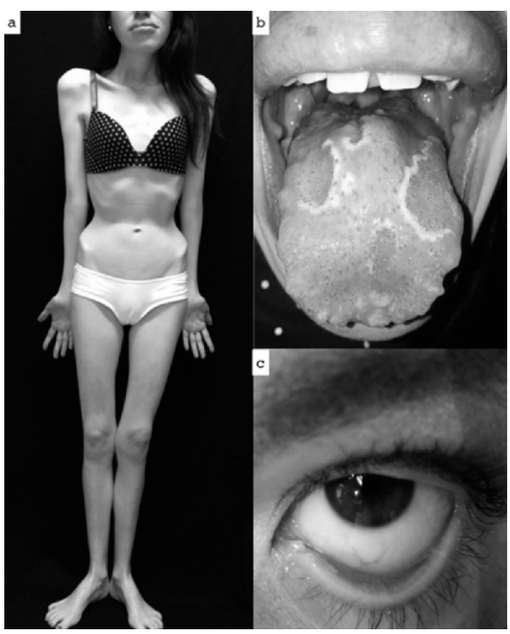

Figure 1: Patients phenotype with marfanoid habitus and mucosal neuromas.

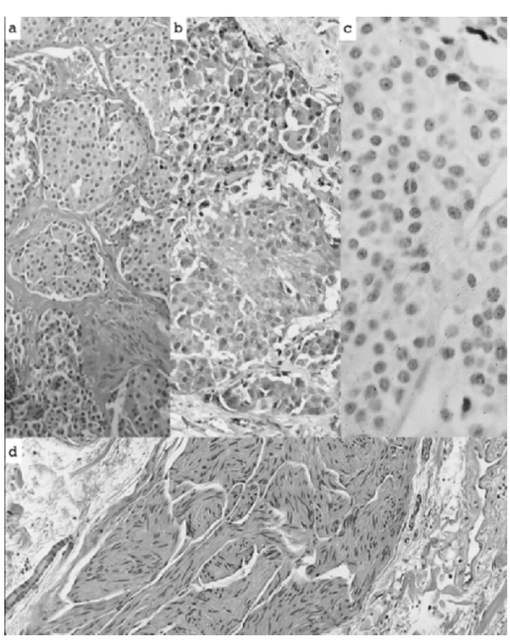

Figure 2: a. Medullary carcinoma (H&E staining), b. Positive Calcitonin, c. Negative Tyroglobulin, d. mucosal neuromas of the tongue H&E staining).

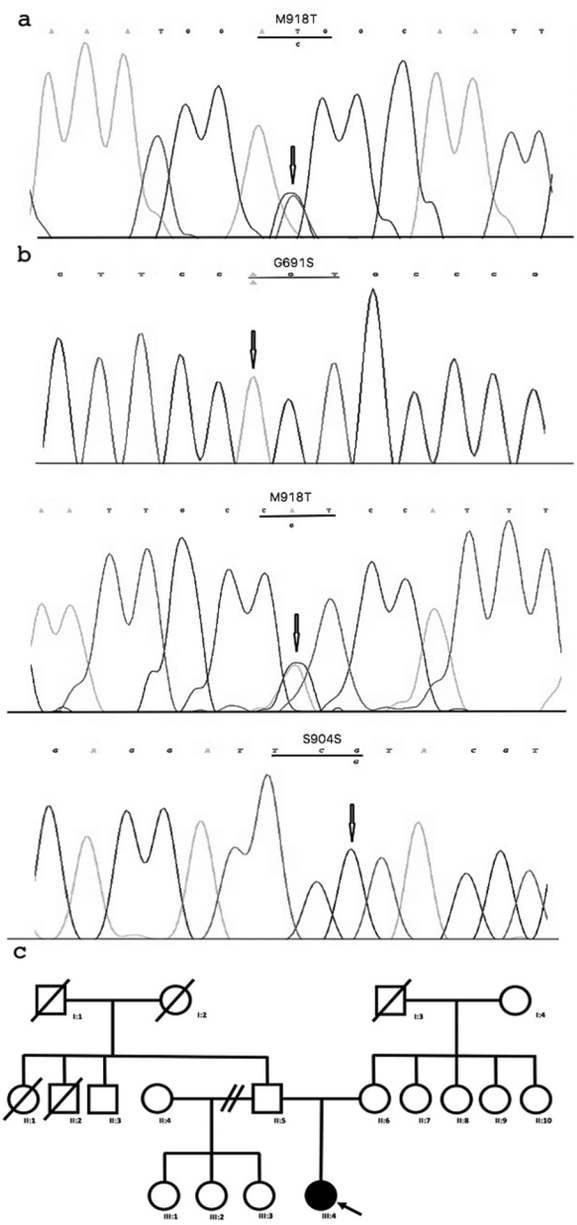

Figure 3: a. Heterozygous point mutation pM918T in exon 16 (right and left panel show the sense and antisense sequence respectively), b. Polymorphic homozygous genetic variants (right panel, pG961S in exon 11 and left panel, pS904S in exon 15), c. Family tree with de novo mutation

DISCUSSION

We present the case of a young patient with a pM918T mutation of the *RET* proto-oncogene, causing an aggressive MTC accompanied by typical phenotypic characteristics of MEN2B. Nevertheless, it took 15 years from cancer occurrence to the endocrinology evaluation and definitive diagnosis that included a molecular description. To our knowledge, this is the first diagnosis and report of a MEN2B case in Ecuador.

The analysis of the *RET* proto-oncogene, since its identification in 1993, has changed the history of MEN2 syndromes. The characterization of specific genetic mutations leads to preventive and therapeutic decisions that will change the course of the disease[11,14,15].

Another distinctive feature in MEN2B is the presence of multiples mucosal neuromas; these are usually benign tumors, involving hyperplastic nerves that might be located in the tongue, lips and conjunctiva. The presence of these lesions can produce an appearance of thickened lips and eversion of the eyelids [12]. Less frequently, there have been reports of associations between this syndrome with cutaneous melanoma [14], macular amyloidosis [15], lichen nitidus and cutaneous metastases of primary endocrine tumors like pheochromocytoma [16]; none of these are evident in this case. Our patient presents a unique combination of multiple neuromas and a geographic tongue that have not been described before. Although PHEO is not yet evident, it is expected. We are, therefore, conducting close follow-up because of the mutation evident in the patient [8,17]. Regarding MTC, international guidelines recommend a prophylactic thyroidectomy during the first year of life [3] and even before six months for the highest risk group [18,19]. Since the availability of genetic testing in Ecuador is relatively new, it is important to identify patients and families at risk in order to apply preventive measures, similar to other countries of the region [20,21].

The family history and pedigree suggested a *de novo* mutation. More than half of all new cases result from sporadic mutations, this may be associated with paternal origin and advanced age of the parent, suggesting a difference in susceptibility depending on whether the *RET* onco-gene mutation is derived from the paternal or maternal line. 95% of this *de novo* variations arise in the male germline [22,23]. In the case of our patient, the paternal age at the time of conception was 40 years. The overwhelming majority (95%) of new MEN2B mutations occur at the same nucleotide site (rs74799832) resulting in the pM918T substitution[24].

Although the diagnosis was confirmed, an homozygous nonpathogenic mutation (pG691S rs1799939, GGT>AGT, Gly>Ser) in exon 11 and another one in exon 15 (pS904S rs1800863, TCC>TCG, Ser>Ser) are present [11,25]. It has been suggested that RET polymorphisms (pL769L, pS836S, and pG691S/S904S) might modify the natural history of MEN2 PHEO; patients with two of these polymorphisms indeed presented a younger age at diagnosis [10]. Although information about this mutation is scarce, some studies suggest that they have a role of modifying the prognosis and clinical presentation of the disease due to changes in the level of expression of the mRNA and the activation of the cascade by tyrosine kinase activity [10,26,27]. Nevertheless, our patient has not developed PHEO to this date. Ancestry-specific protective alleles could also explain the difference in the clinical manifestations[28].

CONCLUSION

Notwithstanding the distinctive characteristics present in people affected by MEN 2B, its diagnosis may be delayed. Timely diagnosis offers opportunities to make appropriate preventive and therapeutic decisions changing the natural evolution of the disease and its complications. We emphasize the importance of a high suspicion index for MEN2B when young thyroid nodule or cancer plus marfanoid biotype and mucosal neuromas are altogether observed. In view of this diagnostic impression, an endocrinological and genetic-molecular evaluation is necessary to determine the best course of treatment or management for the benefit of the patient.

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ETHICAL DISCLOSURES

Protection of human and animal subjects.

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data.

The authors declare that they have followed the protocols of their work center on the publication of
patient data.

Right to privacy and informed consent.

The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors declare that they have no conflict of interest.

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