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CASE REPORT

Disparity between tissue and serum calcitonin and carcinoembryonic antigen in a patient with medullary thyroid carcinoma

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Abstract Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor of parafollicular or C-cells of thyroid that comprises 5–10% of all thyroid cancers [1, 2]. The neoplastic cells secrete calcitonin, carcinoembryonic antigen (CEA), and chromogranin A. Typically, increased serum levels of these tumor markers permit them to be used for initial diagnosis and long-term disease status surveillance. This article reports a case of sporadic MTC (pT2N0M0) in a young patient with normal serum tumor markers. A 16-year-old woman presented with MTC without evidence for this to be a familial case due to the absence of germline mutations in the RET proto-oncogene and negative family history. Surprisingly, there were normal preoperative serum levels of calcitonin, CEA, and chromogranin A, despite the immunohistochemistry showing strong and diffuse positive staining for these markers. This disparity between serum levels and tumor expression of calcitonin and CEA in MTC is quite rare. The relevant features of this case are discussed in consideration of the published experiences. This case may represent an unique subgroup of MTC with abnormal secretory capacity that requires reliance upon radiological evaluation for evidence of recurrent or disseminated disease, without the diagnostic benefit of serum tumor markers.

Keywords Medullary thyroid carcinoma · Calcitonin · CEA · Chromogranin A

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Introduction

Medullary thyroid carcinoma (MTC) arises from parafollicular C-cells of the thyroid gland that typically produce calcitonin, carcinoembryonic antigen (CEA), and chromogranin A, among other proteins. The CEA and chromogranin A production and immunoreactivity is seen in about 80–100% of all MTC [3, 4], however, most of these tumors typically express calcitonin. These tumors arise as sporadic disease in just under 80% of cases predominantly affecting adults (ages 40–60 years) and presenting as a solitary thyroid nodule. The inherited forms of MTC are less frequent (20–30%) and occur in a younger population, as a part of multiple endocrine neoplasia syndromes (MEN 2A, MEN 2B) or isolated familial medullary thyroid carcinoma (FMTC) [1]. Familial cases arise from activating germline mutations in the RET proto-oncogene and are detected in more than 95% of the patients with hereditary MTC, whereas somatic RET mutations are detected in 40–70% of sporadic MTC [2]. Just as with their progenitor

C-cells, MTC cells are able to synthesize and secrete calcitonin, CEA, and chromogranin A, providing utility as clinical markers for this malignancy. Calcitonin has been suggested as a preoperative serum marker to diagnose MTC, as well as for postoperative monitoring to detect residual or recurrent disease [5, 6]. It is distinctly unusual for the patients with unresected tumor masses to have normal serum levels of calcitonin. To the best of our knowledge, there have been few reported cases of sporadic MTC without elevation of serum or plasma tumor markers [4, 7–12]. These cases were generally considered sporadic MTC after failing to find germline RET proto-oncogene mutations [7, 9, 11]. In addition, most cases were associated with weak or negligible immunoreactivity of the tumor cells for calcitonin. [4, 7, 11–13]. We present another case of sporadic MTC occurring in a 16-year-old patient without germline proto-oncogene mutations and with normal serum calcitonin and CEA levels before resection of the tumor, while expressing strong and diffuse immunoreactivity for these markers.

Patient history

The patient is a 16-year-old woman who presented with a 3-year history of a slowly growing left neck mass causing pressure symptoms and shortness of breath. There was no history of any previous malignancy. There was a history of a thyroidectomy for her paternal great aunt with a thyroid cancer of unknown type and thyroidectomy in her paternal great grandmother for unknown reason, but no history of any previous exposure to radiation. A fine needle aspiration (FNA) biopsy of the thyroid mass revealed evidence of MTC based on cytomorphology and immunocytochemistry. Preoperatively, the basal serum calcitonin level was 4.0 pg/ml (normal range <4.6 pg/ml), and the CEA level was less than 1.0 ng/ml (normal range <3 ng/ml). The calcitonin test was performed at Mayo Clinic using Siemens Immulite 2000 method. The patient did not undergo calcium stimulation testing for calcitonin measurements preoperatively. Pentagastrin stimulation test was also not performed (test not available in the United States for at least a decade). Plasma catecholamine levels and fractionated metanephrine levels were within normal limits. Standard patient identification verification procedures were followed for this patient's samples in the clinical laboratory. Preoperative computer tomography (CT) scan of the body were negative for other extrathyroidal lesions. Intraoperatively, a 3-cm hard mass was identified in the midportion of the left thyroid lobe. A total thyroidectomy with complete central compartment nodal dissection and left modified radical neck dissection was performed. After 3 months postoperatively, the serum calcitonin level was 0.6 pg/ml (normal range <4.6 pg/ml) and the serum CEA level was less than 2.0 ng/ml (normal range <3 ng/ml). While the calcitonin levels have decreased postoperatively compared to preoperative levels, both results were within the range of normal values, thus the difference cannot be considered statistically significant. Over the course of 20 months, multiple computerized tomography scans of the neck, chest, abdomen, and pelvis have been negative for residual or recurrent disease.

Cytomorphology

The FNA of the thyroid mass revealed neoplastic single cells showing abundant granular cytoplasm, and eccentric uniform round to oval nuclei with punctuate chromatin. The tumor cells were strongly positive for calcitonin immunostaining, supporting the diagnosis of MTC (Fig. 1a).

Histopathologic and immunohistochemical findings

Gross examination of the total thyroidectomy specimen revealed a single, unilateral, firm, white-tan, well-circumscribed, nonencapsulated mass in the mid-portion of the left lobe, measuring 3.0 × 2.5 × 2.3 cm. The diagnosis of MTC (pT2, N0, M0) was confirmed histologically. Hematoxylin and eosin (H&E)-stained slides showed nests, cords, follicles, and solid sheets of tumor cells with spindle to round to polygonal morphology separated by thick fibrous stroma (Fig. 1b). The tumor cells demonstrated slightly basophilic abundant finely granular cytoplasm with predominantly uniform nuclei and rare mitotic figures. However, there was no evidence of C-cell hyperplasia in the surrounding non-neoplastic thyroid tissue examined. No lymphovascular invasion or extension beyond the thyroid capsule was identified. All 5 perithyroidal and 39 cervical lymph nodes were negative for metastatic MTC. The tumor cells demonstrated strong and diffuse immunoreactivity for calcitonin, CEA, and chromogranin A (Fig. 1c, d, e), as well as strong and diffuse positivity for thyroid transcription factor (TTF-1) and complete absence of staining for thyroglobulin (Fig. 1f).

The immunohistochemical stains were performed using antibodies against calcitonin, CEA, chromogranin A, TTF-1, and thyroglobulin (all antibodies from DAKO, Inc., Carpinteria, CA) using an indirect immunoperoxidase technique. The Congo red stain was negative for amyloid deposits in three tumor blocks showing extensive homogenous fibrous stroma. The non-neoplastic thyroid tissue stained for calcitonin failed to demonstrate any evidence of C-cell hyperplasia.

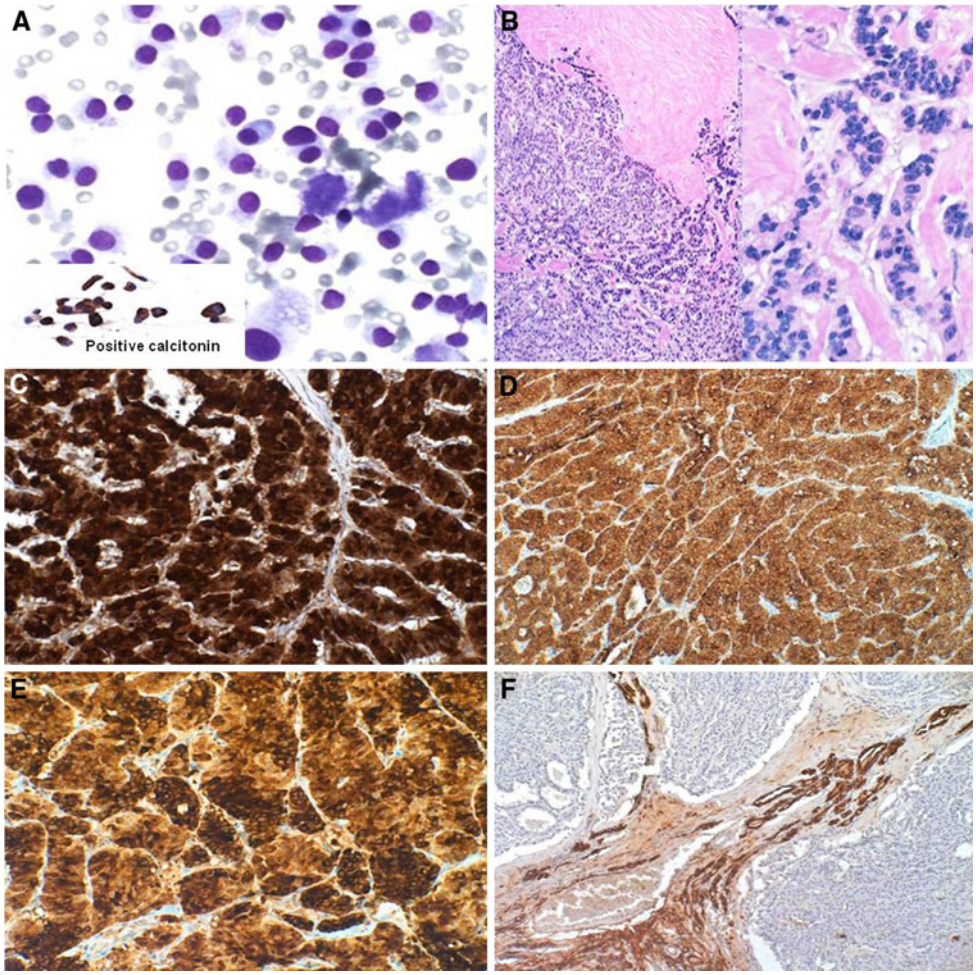

Fig. 1 **a** Fine needle aspirate (FNA) of the thyroid mass (*inset* demonstrates calcitonin immunostaining). **b** H&E stained tissue showing tumor cells in nests, cords and follicles with fibrous band outline. **c** Calcitonin immunohistochemical staining with diffusely positive immunoreactivity. **d** Diffusely positive immunostaining for CEA. **e** Diffusely positive immunostaining for chromogranin A. **f** Negative immunostaining for thyroglobulin

Germline mutation analysis of the RET proto-oncogene

Bi-directional sequencing of RET proto-oncogene exons 5, 8, 10, 11, 13, 14, 15, and 16 (including flanking intronic sequences) was performed using dye-terminator chemistry with analysis on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Exons 10, 11, and 13–16 were analyzed using DNA extracted from two distinct samples of this patient's blood. Exons 5–8 were analyzed using bi-directional sequencing from only one blood specimen. Results did not reveal any deleterious mutation. Two sequence variants were detected, both in homozygous form: Leu769 CTT>CTG (a known non-pathological silent polymorphism in exon 13) and IVS 8 + 84 G>A (intron 8). The latter sequence change was also found in homozygous form in normal control DNA and therefore likely is a common non-pathological polymorphism.

Discussion

The sporadic form of MTC usually occurs in adults and presents as a slow growing thyroid mass, sometimes with clinical symptoms of thoracic inlet obstruction [14]. This report depicts a very unusual case of a sporadic MTC (pT2, N0, M0) occurring in a young, 16-year-old patient. The chromogranin A production and immunoreactivity is seen in about 80–100% and increased serum levels in about 50% of all MTC [3, 4, 15]. To exclude the possibility of rare metastases from extra-thyroidal neuroendocrine tumors to the thyroid that may have a similar histological appearance as MTC and immunohistochemical expression of chromogranin A [16–20], we performed immunostaining for TTF-1. The primary thyroid origin of this tumor was supported, in part, by positive immunoreactivity of tumor cells for TTF-1, along with calcitonin, as well as by extensive imaging studies excluding evidence of any other extra-thyroidal source of tumor, including the lungs.

The sporadic (non-familial) presentation is evidenced by the absence of associated clinical features of MEN syndromes and the absence of germline mutations in clinically relevant regions of the RET proto-oncogene. Moreover, no evidence of C-cell hyperplasia, typically present in hereditary forms of MTC, was identified on histological evaluation of the surrounding normal thyroid tissue. This would generally be found as a precursor lesion for hereditary

MTC [14, 21]. Such hereditary forms of MTC are usually bilateral, multicentric, and affect younger age groups. Among them, MTC in MEN 2B is more aggressive than MEN 2A, FMTC, and sporadic forms [14]. Poor prognostic factors include age over 50 years, male gender, metastases, and the MEN 2B form. In the familial settings, MTC is a potentially lethal tumor having an autosomal dominant inheritance with high penetrance and variable expressivity for the other neoplastic features of the syndromes [1]. The patients may present with associated tumors other than MTC demonstrating neuroendocrine syndromic features and leading to the evaluation of hereditary forms like MEN 2A, MEN 2B, and FMTC [1, 14, 22].

Medullary thyroid carcinoma usually presents as an ill-defined, non-encapsulated, hard, invasive mass that most commonly originates in the upper central lobes of the thyroid. It is composed of columns of epithelial cells in a dense stroma that usually contains amyloid deposits and collagen. The tumor cells are immunoreactive for calcitonin, CEA, and chromogranin A, negative for thyroglobulin, and usually exhibit positive staining for amyloid deposits. Regional metastases occur early in the disease, while the distant metastases to the liver, lung, bone, brain, and adrenal glands occur later in the course of the disease [5, 22].

It has been shown that calcitonin and CEA are present in normal, hyperplastic, and malignant C-cells. These molecules serve as tumor markers for MTC and are used to support the diagnosis of MTC as well as assist in screening for residual or recurrent disease. Calcitonin and CEA can also be elevated in numerous other malignancies such as lung, breast, and pancreas, and in some benign conditions, such as renal failure and inflammatory bowel disease, or in heavy smokers [14]. A significant increase of baseline or stimulated (with calcium or pentagastrin infusion) calcitonin levels above 1000 pg/ml confirms the presence of MTC. Immunostaining for calcitonin can also be used to detect C-cell hyperplasia, a precursor to clinical MTC [21, 23]. Several studies demonstrate a correlation between tumor size and the basal calcitonin levels, although it can be difficult to distinguish benign processes from MTC based on a distinct calcitonin level threshold [24, 25]. Although some case reports note that a few patients with MTC had normal basal serum calcitonin levels, in most of these cases the tumor cells show very weak or no calcitonin expression by immunohistochemistry, suggesting a loss of tumor expression of calcitonin [4, 7, 11–13]. Also, while CEA and chromogranin are nonspecific and insensitive markers for detection of early MTC, the tumor in this case measured 3 cm in greatest dimension which corresponds to advanced stage pT2 disease. Discordance between calcitonin and tumor size can also be seen during pharmacotherapy of the patients with metastatic medullary thyroid cancer [9], however, no evidence of metastatic disease was present in this case. Our patient with MTC was distinct, considering her young age and the presence of large primary thyroid tumor that stained strongly and diffusely for calcitonin, CEA, and chromogranin A, despite the absence of elevated corresponding tumor markers in the circulation. This phenomenon might perhaps be explained by a failure of extracellular calcitonin secretion by these MTC cells.

In our patient, the serum CEA levels, along with other serum markers, were also normal preoperatively despite a strong immunoreactivity of tumor cells for CEA. This gives support for a potential secretory defect in these tumor cells affecting both CEA and calcitonin. Published studies on electron microscopy of normal human parafollicullar C-cells demonstrate that calcitonin and related peptide deposits are usually found in the cytosol as large secretory granules, but not in the cisterna of the endoplasmic reticulum, golgi apparatus, or mitochondrial matrix. The ultrastructural studies of MTC cells show cytoplasmic inclusions composed of particulate and fibrillar structures that stain positively for calcitonin [26, 27]. Disintegration of these inclusions is likely needed for the discharge of calcitonin into the cellular matrix and for extracellular secretion. Unfortunately, we did not have the opportunity to perform electron microscopy on this patient's tumor.

There are two distinct localization patterns for calcitonin and CEA. This is interpreted to mean that these two markers are secreted via two different intracellular secretory pathways, a regulated pathway for calcitonin and a constitutive pathway for CEA. Abnormalities of these pathways in tumor cells may restrict the secretion of these tumor markers, perhaps leading to lack of elevated serum levels of calcitonin and CEA in the patient's circulation [28].

Although there is a small number of cases of MTC reported with low preoperative basal calcitonin levels, only two of such cases reported this finding in association with a strong immunohistochemical staining of the primary tumor for calcitonin [9, 10]. Our case, along with these two other cases, may constitute an unique subgroup of MTC with abnormal secretory capacity. The clinical consequence of this feature is the need to rely upon radiological evaluation for recurrent or disseminated disease without the benefit of serum tumor markers.

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