Case Report

Detection of early stage medullary thyroid carcinoma by measuring serum calcitonin using an electrochemiluminescence immuno-assay: A case report of a young Japanese woman with a high-risk \textit{RET} mutation

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Abstract. Medullary thyroid carcinoma (MTC) in multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant disorder caused by the mutation of the \textit{RET} proto-oncogene, that shows nearly complete penetration. The American Thyroid Association guidelines recommend prophylactic thyroidectomy for patients with high-risk \textit{RET} mutations. However, in Japan, ethical and medical issues may preclude prophylactic treatment in young patients. Hence, an early diagnosis of MTC is necessary to ensure a favorable outcome. Here, we report the case of a young Japanese girl with a high-risk \textit{RET} mutation, diagnosed with very early stage MTC using serum calcitonin (Ctn) values measured using an electrochemiluminescence immuno-assay (ECLIA). The Japanese girl with a family history of MEN2 had been followed at our hospital since she was 5-mo-old. \textit{RET} analysis revealed that she displayed a Cys634Gly mutation. The patient underwent annual neck ultrasonography and calcium infusion testing. When she was 8-yr-old, the Ctn level, measured using ECLIA, dramatically increased with calcium stimulation, from a baseline of 11.3 pg/mL to 333 pg/mL. She subsequently underwent total thyroidectomy and was diagnosed with stage I MTC. Detecting early stage MTC by monitoring serum Ctn measured by ECLIA, may represent a useful strategy for patients with high-risk \textit{RET} mutations.

Key words: medullary thyroid carcinoma, multiple endocrine neoplasia, \textit{RET} mutation, calcitonin, electrochemiluminescence immuno-assay

Introduction

Hereditary medullary thyroid carcinoma (MTC) is one of the primary tumors found in patients with multiple endocrine neoplasia type 2 (MEN2). MTC in MEN2 shows nearly complete penetration, and its prognosis highly depends on the mutation of the \textit{RET} proto-oncogene. Based on this fact, the American Thyroid Association (ATA) guidelines recommend \textit{RET} analysis in patients with both hereditary and sporadic
Abe and Tsukano

Furthermore, for patients with high-risk RET mutations, the guidelines recommend prophylactic thyroidectomy, which is defined as the removal of the thyroid before MTC develops or while it is still clinically unapparent and confined to the gland (1). According to the guidelines, these patients should undergo total thyroidectomy before the age of five. However, such radical prophylactic treatment may raise ethical concerns. Additionally, health insurance coverage for prophylactic thyroidectomy is not always equally available to all patients (2).

Here, we report the case of a young Japanese girl with a high-risk RET mutation, who was diagnosed with stage I MTC by monitoring serum calcitonin (Ctn) levels. Total thyroidectomy was performed before metastasis occurred.

Case Presentation

A Japanese girl with a family history of MEN2A, a subtype of MEN2 characterized by MTC, pheochromocytoma, and primary parathyroid hyperplasia, had been followed at our hospital since she was 5-mo-old. Her mother had been previously diagnosed with MEN2A and underwent thyroidectomy at the age of 32. The girl was born healthy and presented no medical problems. Her parents were non-consanguineous Japanese individuals with a strong family history of MTC in the maternal lineage (Fig. 1). There were no abnormalities in her clinical features or laboratory findings at her initial presentation. The parents wished to determine whether their daughter was at risk for developing MEN2A. After obtaining written informed consent from her parents, exons 10, 11, and 16 in RET were analyzed (SRL, Inc., Tokyo, Japan). The patient’s mother declined a personal genetic analysis. A heterozygous missense mutation, p. Cys634Gly, was found in codon 634 of exon 11. According to the ATA guidelines, patients with mutations in codon 634 have a high risk of developing MTC, and prophylactic thyroidectomy before 5-yr of age is recommended. In this case, however, both ethical and medical issues precluded prophylactic treatment. Additionally, the patient did not have insurance coverage for preventative surgery. For these reasons, prophylactic thyroidectomy was deferred. In an effort to detect the development of
MTC as early as possible, the patient subsequently underwent annual neck ultrasonography and calcium infusion testing to monitor serum Ctn levels. When she was 8-yr-old, the laboratory procedure for measuring Ctn levels was switched from a solid two-site immunoradiometric assay (RIA; LSI Medience Co., Tokyo, Japan) to an electro chemiluminescence immuno-assay (ECLIA; Roche Diagnostics GmbH, Manheim, Germany). Until the age of seven, the patient’s Ctn levels, as measured by RIA, were normal both before and after a 2.5 mg/kg calcium gluconate infusion. When she reached 8-yr-old, the basal Ctn level (b-Ctn) was mildly elevated and the stimulated peak Ctn level (s-Ctn) was elevated to 30 × the baseline level (ECLIA), while the neck ultrasonography remained negative (Table 1). Computed tomography imaging revealed no distant metastasis. RET sequences of the patient and her mother were reanalyzed to confirm that they had the same mutation in codon 634 (Fig. 2). Two months later, when the patient was 9-yr-old, a Ctn provocation test was performed on the same blood sample using both ECLIA and

**Table 1** Calcium infusion testing

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Calcitonin (pg/mL)</th>
<th>Laboratory method</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>2 min</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>44</td>
</tr>
<tr>
<td>8</td>
<td>11.3</td>
<td>333</td>
</tr>
<tr>
<td>9</td>
<td>13.7</td>
<td>332</td>
</tr>
<tr>
<td>55</td>
<td>55</td>
<td>456</td>
</tr>
</tbody>
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Reference values for females. ECLIA: ≤ 6.40 pg/mL, RIA: 29.8–68.6 pg/mL. ECLIA: electro chemiluminescence immuno-assay, RIA: solid two-site immunoradiometric assay. The tests in the 9-yr-old (* and **) were performed using the same blood sample.

![Fig. 2. Direct sequencing of RET. The patient and her mother were found to have the same heterozygous mutation, C634G. Mutations in codon 634 of RET are classified as high-risk mutations by the American Thyroid Association guidelines; prophylactic thyroidectomy before 5-yr of age is recommended.](image-url)
The b-Ctn and s-Ctn values, as measured by ECLIA, were almost identical to the previous results. The s-Ctn value, as measured by RIA, was significantly elevated, but the b-Ctn level remained normal (Table 1). Although the patient presented no clinical symptoms or abnormal imaging findings, the elevated Ctn levels suggested that she had developed very early stage MTC. Surgical intervention was approved by the ethics board of Niigata City General Hospital. Four months after the first positive provocation test, she underwent total thyroidectomy and central lymph node dissection. The pathological analysis demonstrated features of stage I MTC (Fig. 3). One week after surgery, the serum Ctn level, as measured by ECLIA, decreased from a preoperative value of 10.8 pg/mL to < 0.50 pg/mL. No difference in pre- versus post-operative Ctn levels were observed using RIA. The Ctn level, as measured by ECLIA, remained < 0.50 pg/mL 1 yr after the thyroidectomy (Table 2).

**Fig. 3.** Histopathological manifestations. The pathological investigation demonstrates the presence of medullary thyroid carcinoma (MTC) in the left upper pole of the resected thyroid. The tumor measures 0.2 cm at its largest diameter, and contains several C-cell hyperplastic lesions. No lymph node metastasis or extra thyroidal expansion are detected, resulting in a final diagnosis of stage I MTC. A) Hematoxylin-Eosin stain × 400: Parafollicular cells causing partial destruction of follicles. B) Calcitonin immunostaining × 400: Tumor cells strongly reactive for calcitonin invade a thyroid follicle.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Calcitonin values before and after thyroidectomy</th>
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<tbody>
<tr>
<td></td>
<td>Calcitonin (pg/mL)</td>
</tr>
<tr>
<td></td>
<td>ECLIA</td>
</tr>
<tr>
<td>Before thyroidectomy</td>
<td>10.8</td>
</tr>
<tr>
<td>POD 7</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>POD 14</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>6-mo after</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>1-yr after</td>
<td>&lt; 0.5</td>
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</tbody>
</table>

Reference values can be found below Table 1. POD: postoperative day, other abbreviations are the same as in Table 1.
Discussion

Our case demonstrated two major problems in the management of hereditary MTC. One is the timing of thyroidectomy in the regions where the medical environment is similar to the one in Japan. The other is the Ctn measurement method, which in Japan was recently switched from RIA to ECLIA. To our knowledge, no Japanese child case has been reported describing these problems in detail.

The ATA guidelines recommend total prophylactic thyroidectomy before 5-yr of age for patients with high-risk \textit{RET} mutations, including mutations in codon 634. Although prophylactic therapy can provide a complete cure, it is not without barriers. For example, the procedure on very young children is associated with longer hospitalization times (3). Additionally, prophylactic therapy is not always covered by health insurance. Finally, ethical concerns regarding potentially unnecessary surgery may represent an obstacle to preventive treatment. Heterogeneity is also the reason that makes prophylactic therapy difficult. As exemplified by our patient’s family, the phenotype of hereditary MTC, including the age of onset, is highly variable even among family members with the same \textit{RET} mutation. It has been reported that almost all patients with mutations in codon 634 of \textit{RET} develop MTC before the age of 20 (4). However, the patient’s mother developed the disease at the age of 32. Furthermore, the patient’s grandfather and his elderly brother were not affected by MTC. We thus propose that the timing of thyroidectomy should be decided on an individual basis, considering various situations. If this is the case, it is essential that the patient should be diagnosed with MTC early enough to receive radical thyroidectomy.

However, the current situation in Japan is rather inadequate. According to the Japanese Multiple Endocrine Neoplasia Research Consortium, thyroidectomy is often performed too late in Japan. Although 50 out of all 51 children with MTC presented the hereditary disease, 33 were diagnosed after the clinical symptoms or the positive imaging findings had developed. As a result, 18 of 46 (39%) patients who underwent thyroidectomy showed relapse. This relapse rate was similar to that in adults, which included many non-hereditary patients (5). Despite the fact that the development of MTC can be predicted by genetic analysis, these outcomes were less hopeful than anticipated, and suggest that a thyroidectomy-induced cure is not guaranteed, particularly after the clinical or imaging signs of MTC have developed. The current management of MTC in Japan may be influenced by the fact that, in general, thyroid cancer has a more favorable life expectancy than other malignancies. However, MTC tends to metastasize early to the paratracheal and lateral cervical lymph nodes (6), and no conclusive medical interventions for advanced MTC currently exist. The 10-yr survival rates for patients with stages I, II, III, and IV MTC are 100, 92.6, 70.9, and 20.7%, respectively (7), and the N0 status in the tumor, node, metastases (TNM) classification scheme was reported to be associated with disease-free survival (8). Therefore, in order to obtain a radical cure, we must detect MTC and perform thyroidectomy before nodal involvement occurs (stage I).

To ensure an early diagnosis and a radical cure, our case had been monitored using serum Ctn measurements, and was detected at stage I MTC, that could be radically resected. Serum Ctn is a sensitive marker for detecting micro MTC (9). Based on Elisei’s data of 53 patients with \textit{RET} mutations, stage I MTC is found in patients with a mildly elevated b-Ctn level, and in those with a normal b-Ctn but an elevated s-Ctn level (10). Our patient presented a mild elevation in the b-Ctn level, as measured by ECLIA (11.3 pg/mL) and was thus likely to present early stage MTC. According to their data, an evaluation of the s-Ctn value might not be necessary, because the patient demonstrated an elevated b-Ctn level. However, we must note the following important facts.
Since 2015, the procedure for measuring Ctn in Japan has been changed from RIA to ECLIA, that is an immune chemiluminometric assay (ICMA) and is more sensitive. Although there are many studies on serum Ctn reference values as measured by ICMA, reports describing Ctn values measured by ECLIA are rare. Moreover, their reference values are not completely the same (11). This fact indicates that it might be difficult to determine whether a mild elevation of b-Ctn levels measured by ECLIA, as in the case of our patient, is significant. In our case, both ECLIA and RIA-measured s-Ctn values showed significant elevation during the provocation test using the same blood sample. However, only ECLIA could detect a mild elevation in the b-Ctn value during the test (Table 1). These facts indicate the possibility that we can detect early stage MTC by using ECLIA-measured Ctn values. Furthermore, ECLIA might allow us to avoid using the stimulating test to detect MTC, when we could determine an appropriate cut-off value for the b-Ctn level. Concerning post-operative management, we could not evaluate the effect of thyroidectomy based on the Ctn values, as measured by RIA. These showed no change in contrast to the rapid decrease of the ECLIA-measured Ctn values (Table 2). It has been reported that the Ctn value measured using ICMA is more sensitive and more specific than the value measured using RIA. Hence it has advantages in the post-operative monitoring of MTC (12). Our case also showed that ECLIA was more advantageous than RIA. Taken together, the Ctn values measured using RIA, that have been used in Japan for a long time, lack sufficient sensitivity and specificity for the early detection and for the postoperative management of MTC. To establish a favorable management of MTC, more studies are needed to determine the most appropriate reference values for both b-Ctn and s-Ctn levels, as measured by ECLIA.

In conclusion, we believe that monitoring the serum Ctn level, as measured by ECLIA, may make it possible to detect extremely early stage MTC and to avoid the progression to nodal involvement. Moreover, the b-Ctn level, as measured by ECLIA, may be comparable with the s-Ctn value, regarding its sensitivity. By deciding on an appropriate cut-off value for the b-Ctn level, we may be able to avoid using the stimulating test in the future.

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