Mild Persistent Hypercalcitoninemia after Total Thyroidectomy in Patients with Papillary Thyroid Carcinoma

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Abstract. Total thyroidectomy was performed in 455 patients with differentiated thyroid carcinoma between 1978 and 1999. Serum calcitonin (CT) was determined preoperatively in all patients using polyclonal antibodies. Among the subjects, 25 patients showed elevated serum calcitonin levels preoperatively. Pathological diagnoses of 18 patients were confirmed as medullary thyroid carcinoma (MTC) postoperatively. Eight patients were diagnosed as papillary thyroid carcinoma (PTC) in the final pathological diagnosis without evidence of minimal foci of MTC or C cell hyperplasia, and they showed elevated CT levels preoperatively. Hypercalcitoninemia in 8 patients with PTC continued throughout the 24 follow-up months with normal CEA levels. Extrathyroidal CT-producing diseases were all neglected, and precise pathological examination showed negative evidence of minute MTC or C cell hyperplasia in these 8 patients. Serum CT levels were simultaneously determined by a different CT assay kit using the same blood samples in 7 of 8 patients. Serum CT levels were all within normal values in another CT kit applying a different polyclonal antibody, although elevated CT values continued in the routine CT kit. The recognition of polymeric or fragmented CT by polyclonal antibody was thought to be the causative factor for the hypercalcitoninemia after total thyroidectomy in the patients with PTC. Knowledge of the false positive CT determination makes it important to employ different CT assay kits, especially the new generation of two-site immunoassays using two monoclonal antibodies against distinct epitopes of human CT, although the new generation kits are not clinically available in Japan.

Key words: Hypercalcitoninemia, Papillary thyroid carcinoma, Total thyroidectomy, Immunoassay, Calcitonin

(SERUM calcitonin (CT) is a sensitive and accurate marker of medullary thyroid carcinoma (MTC). MTC occurs in both familial and nonfamilial (sporadic) forms. Familial forms can be suspected by family history, screened by basal and pentagastrin-stimulated serum CT measurement, and treated by total thyroidectomy. Sporadic forms, on the other hand, are seldom suspected, and are usually diagnosed after surgery when serum CT is not determined preoperatively. Fine needle aspiration cytology (FNAC) is a very sensitive and specific procedure for the diagnosis of malignant thyroid nodules, but cytological typing of MTC may be difficult with routine staining, especially when the pathologist is not alerted by a suspicion of MTC. The determination of serum CT in all patients with thyroid nodules as a screening procedure for the early diagnosis of MTC has been recently proposed [1–5]. Conversely, the limited specificity of mildly elevated serum CT may create diagnostic problems in some patients. After total thyroidectomy, detectable serum CT levels are unequivocal evidence of locally persistent or metastatic disease in patients with MTC. Mild hypercalcitoninemia persists in some patients with papillary thyroid carcinoma (PTC) who have received total thyroidectomy, and this persistence worries surgeons who may miss the occult MTC. The aim of this study is to assess the specificity of the routine measurement method of serum CT in thyroid cancer patients and to clarify the clinical management of a

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mild elevation of serum CT unrelated to MTC. The statement of CT assays in Japan and other countries are also discussed.

Patients and Methods

Subjects

Total thyroidectomy was performed in 455 patients with differentiated thyroid carcinoma between 1978 and 1999 at the Department of Surgery II, Nagoya University School of Medicine. Serum CT was determined preoperatively in all the patients. Among the subjects, 25 patients showed elevated serum calcitonin levels preoperatively. Pathological diagnoses of 18 patients were postoperatively confirmed as MTC, although the preoperative serum CT level was within the normal range in 1 patient [6]. Eight patients were diagnosed as PTC in the final pathological diagnosis without evidence of minimal foci of MTC or C cell hyperplasia, and their serum CT levels were elevated preoperatively (Table 1). The completeness of thyroidectomy was checked by a postoperative radioiodine scan, or by measuring the thyroglobulin concentration.

Profiles of 8 PTC patients with elevated CT

Seven of 8 patients were females aged 13 to 61 (average 39.3 ± 6.8) yr. Preoperative CT and CEA levels were 181.6–340.0 pg/ml (average 261.0 ± 20.8) and 0.2–2.7 ng/ml (average 1.3 ± 0.4), respectively. There were no clinical signs of renal insufficiency, acute pancreatitis, or hypergastrinemia. Normal serum calcium (s-Ca) levels were noticed in all cases (4.4–4.9 mEq/l, average 4.6 ± 0.3). All patients were in a euthyroid state, and TSH levels were 0.99–5.92 µU/ml (average 2.85 ± 0.72). No ectopic CT-producing tumors were found in any patients, i.e., there was no evidence of small cell lung cancer, pheochromocytoma, melanoma, breast cancer, colorectal cancer, or bronchial carcinoma. The median follow-up was 108 ± 18 months (37–192). Seven patients showed low serum thyroglobulin levels (<1.5 ng/ml), and there were no signs or symptoms of recurrences from PTC in any of the patients. Serum CT levels were determined before surgery, and at 1, 3, 6, 12, and 24 months after total thyroidectomy. CT levels at 24 postoperative months were still high at 229.0–403.2 pg/ml (average 317.5 ± 50.3); however, CEA levels were normal (0.2–3.0 ng/ml, average 1.2 ± 0.5) (Table 2).

Serum CT levels were determined with immunometric assays (Calcitonin kit Daiichi; Daiichi Radioisotope Lab. Co., Kyobashi, Tokyo) using rabbit antihuman calcitonin antibodies (normal range 0–100.0 pg/ml). Values above 100 pg/ml were considered to be abnormal and highly suspicious for the presence of MTC. The anti-calcitonin antibody is polyclonal and does not cross-react with other species of CT, but does cross-react with human CT fragments.

Plasma carcinoembryonic antigen (CEA) levels

Table 1. Preoperative serum CT in 455 patients with differentiated thyroid carcinoma

<table>
<thead>
<tr>
<th>CT (pg/ml)</th>
<th>Number of patients</th>
<th>Sporadic MTC</th>
<th>Familial MTC</th>
<th>PTC</th>
<th>FTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>100&gt;</td>
<td>430</td>
<td>0</td>
<td>1</td>
<td>413</td>
<td>16</td>
</tr>
<tr>
<td>100–500</td>
<td>13</td>
<td>0</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>&gt;500</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

FTC: Follicular thyroid carcinoma

Table 2. Profiles of 8 PTC patients with elevated CT

<table>
<thead>
<tr>
<th>case</th>
<th>sex</th>
<th>age (y.o.)</th>
<th>CT (pg/ml)</th>
<th>CEA (ng/ml)</th>
<th>TSH (µU/ml)</th>
<th>TG (ng/ml)</th>
<th>follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>39</td>
<td>288.0</td>
<td>3.2</td>
<td>1.0</td>
<td>2.6</td>
<td>16.7</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>41</td>
<td>260.0</td>
<td>2.7</td>
<td>3.0</td>
<td>2.7</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>38</td>
<td>340.0</td>
<td>0.8</td>
<td>1.0</td>
<td>0.8</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>61</td>
<td>220.0</td>
<td>0.8</td>
<td>0.3</td>
<td>0.8</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>22</td>
<td>197.3</td>
<td>0.2</td>
<td>0.2</td>
<td>1.4</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>13</td>
<td>181.6</td>
<td>0.2</td>
<td>0.2</td>
<td>2.25</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>61</td>
<td>333.7</td>
<td>0.7</td>
<td>0.7</td>
<td>3.44</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>27</td>
<td>275.1</td>
<td>2.5</td>
<td>2.5</td>
<td>0.99</td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

TG: thyroglobulin, pre: preoperative determination, 24 m: 24 months after thyroidectomy
were also determined using a CEA kit (Wako Co., Osaka, Japan) containing rabbit anti-human CEA antibodies.

In 2 patients, a gastrin stimulation test was performed with informed consent. CT levels were determined before administration, and at 1, 3, 5, 10, and 15 min after 0.5 μg/kg of pentagastrin (Cambridge Lab. NE2 1GX, England).

CT immunohistochemistry was determined on formalin-fixed, paraffin-embedded tissue using monoclonal mouse anti-human calcitonin antibody (DAKO Co., Carpinteria, CA, USA) at a dilution of 1:50.

At the latest examination, CT levels were determined with another calcitonin kit (Mitsubishi Yuka, Mitsubishi Kagaku BCL Co., Tokyo, Japan), used at the same time as the Calcitonin kit Daiichi. Seven of 8 patients’ samples were available for the simultaneous assay. The antibody in the Mitsubishi Yuka kit was recognized with both the carboxyl terminal and the midportion of human CT (normal range 0–100 pg/ml). The assay antibody did not cross-react with other species or human CT fragment 1–18, but did cross-react with human CT fragment 19–32.

Results

Preoperative serum CT levels in 455 patients with differentiated thyroid carcinoma

Serum calcitonin levels were greater than 500 pg/ml in 12 patients, and MTC was confirmed pathologically in all 12 patients. Among these 12 patients, serum CT levels remained high in 4 patients postoperatively. The metastatic lesions were detected in 3 patients, but the origin of high serum CT was undetermined in one patient. Serum calcitonin levels were between 100 and 500 pg/ml in 13 patients. Pathological diagnosis revealed that 5 patients had MTC postoperatively. The other 8 patients had PTC, and the foci of MTC or C cell hyperplasia were not found pathologically. One patient was diagnosed with MTC among patients with preoperative serum calcitonin below 100 pg/ml; however, the patient had multiple endocrine neoplasia type 2A (Table 1).

Changes in postoperative CT levels of 8 patients with hypercalcitoninemic PTC

Serum CT was mildly and continuously elevated in all 8 patients with PTC throughout the 24-month follow-up period (Fig. 1).

Response to pentagastrin stimulation test in 2 patients with hypercalcitoninemic PTC

Serum CT did not increase after pentagastrin stimulus (Fig. 2).

Fig. 1. Postoperative persistent elevation of CT levels in patients with PTC.

Fig. 2. Pentagastrin provocative test showed negative in two patients with PTC preoperatively.
lation in the 2 patients with PTC (Fig. 2).

**Profiles of CT immunostaining**

All cases showed negative staining. There was no evidence of small foci of MTC or C cell hyperplasia in 8 patients (data not shown).

**Simultaneous CT determination of Daiichi and Mitsubishi Yuka**

Serum CT levels were normal in all 7 patients using the Calcitonin kit Mitsubishi Yuka, although the levels were elevated in all 7 patients using the Calcitonin kit Daiichi at the simultaneous examination (Fig. 3).

**Discussion**

Measurement of the serum CT level is an essential tool for the diagnosis of MTC [7–9]. Although a prominent elevation of serum CT allows one to readily determine an MTC diagnosis, a mildly elevated serum CT makes it sometimes difficult to determine the true significance. The preoperative serum CT levels were reviewed in patients with differentiated thyroid carcinoma who received total thyroidectomy during the past 22 years, and 8 patients with PTC showed a mild, persistent elevation of serum CT when using the Daiichi CT kit. Precise pathological examination, pentagastrin provocative test, and long-term follow-up results suggested the nonexistence of MTC in these 8 patients with PTC. Seven of the 8 patients had normal serum CT levels using the Mitsubishi Yuka CT assay system, indicating the presence of hypercalcitoninemia unrelated to MTC.

In the IMMUNOASSAY inspection control survey (2001.10), three kinds of samples were adjusted to low level (A-1), median level (A-2), and high level (A-3) of CT, respectively, and the CT concentration of each sample was determined in 14 laboratories in Japan. The CT concentration in A-1 was 120.5 measured by the Daiichi kit, and 40.5 pg/ml by the Mitsubishi Yuka kit. CT in A-2 was 345.2 by Daiichi kit, and 468.9 pg/ml by Mitsubishi Yuka kit. CT in A-3 was 652.2 by the Daiichi kit, and 899.6 pg/ml by the Mitsubishi Yuka kit. The CT concentration was three times higher in the Daiichi kit compared to Mitsubishi Yuka kit in low-level samples, although lower by the Daiichi kit compared to the Mitsubishi Yuka kit in the median and high-level samples.

Immunoreactive CT, which can be measured by the RIA method, consists of: 1) monomer CT, 2) high molecular CT (precursory body), 3) polymer CT, 4) low molecular CT (fragmentation), etc. [10]. Since the antibodies of each kit differ slightly, data may differ depending on which of the antibodies reacts. Since immunoreactive CT consists almost solely of monomer CT in the high level sample, the correlation is comparatively good. Although one immunoreactive CT does not obtain the main peak in the median and low-level samples, there is a great difference. In current CT measurement, dissociation of data may occur since there are no uniform standard articles or standardization.

When a patient with a nodular goiter is diagnosed as PTC as the result of FNAC, and serum CT is mildly elevated to, for example, 300 pg/ml, what procedure to use next becomes an issue. The pentagastrin provocative test is the most sensitive and reliable method to determine the presence of MTC. However, the reagents, pentagastrin or its derivatives, are not clinically available now in 2002 [11]. Alternative provocative tests, such as calcium or omeprazole, have been reported, but the sensitivity and accuracy of those tests are
not as good as pentagastrin [12, 13]. Analysis of the germ line mutation of RET proto-oncogene is another alternative only applicable to hereditary MTC patients [14, 15]. Re-examination of FNAC to inform the pathologist of the possibility of MTC is also worthwhile [16]. In this report, the preoperative pentagastrin provocative test was studied for only 2 among 8 patients with elevated serum CT. The provocative test was not performed more frequently because total thyroidectomy with bilateral lymph node dissection is employed as a standard operative procedure for PTC [17, 18]. The operative procedures are the same for PTC and MTC. Precise postoperative pathological examination revealed the final diagnosis as to whether or not the patients had PTC alone, PTC with minute foci of MTC or C cell hyperplasia, or MTC alone. All patients with a preoperative mild increase of serum CT level had PTC alone.

Cohen et al. reported that preoperative CT levels appeared to be predictive of postoperative CT normalization in patients with MTC, insofar as the CT level is not significantly high [19]. From a clinical point of view, the simultaneous evaluation of serum levels of CT and CEA may be useful in the postoperative follow-up of patients with elevated serum CT [20].

In order to investigate the possibility of ectopic CT production, there was a need for patients in whom C cells from a residual thyroid or an incomplete MTC extirpation could be excluded as a possible source of detectable CT values [7, 21]. C cells remaining in the residual thyroid may be responsible for the verifiable CT levels. Patients who had undergone total thyroidectomy because of a non-medullary differentiated thyroid carcinoma were ideally suited for the examination of this problem. Patients with a total thyroidectomy have detectable immunoreactive CT in their serum and urine, suggesting that human CT may be secreted by extrathyroidal tissues. Significant amounts of CT were found in many human extrathyroidal tissues [22–25]. Furthermore, extrathyroidal tissue contains CT fractions of the same molecular size and charge characteristics as do the serum and thyroid.

The usefulness of routine measurement of preoperative CT levels in every case with thyroid nodules to predict minute foci of medullary thyroid carcinoma has been reported [1–5]. These studies employed a new generation of two-site immunoassays using two monoclonal antibodies against distinct epitopes of human CT [26–28]. These new assay systems have been reported to have greatly improved both the sensitivity and the specificity of CT determination. However, clinically available routine CT measurements in Japan recognize multiple heterogeneous forms of circulating immunoreactive CT, which are variously detected by polyclonal antiserum, and mainly account for the wide range of reported reference values. The new two-site immunoassays using two monoclonal antibodies against distinct epitopes of human CT are only available for research use in Japan at the present time, whereas the new assays are routinely used in Europe and the United States.

It is important to keep in mind the possibility of false positive CT determination, and to order the determination of the serum CT in patients with mild hypercalcitoninemia using different CT assay kits providing different characterization of polyclonal antibody for human CT.

References


