Study of Calcitonin and Calcitonin Gene-related Peptide in Patients with Medullary Thyroid Carcinoma

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OBJECTIVE To provide a basis for treatment and prognosis for MTC patients by investigating CT and CGRP.

METHODS Fifty-eight cases of MTC were selected and the relationship between the CT levels and metastasis was investigated. An immunohistochemical method was used to detect the expression of CT and CGRP in the 58 samples of MTC tissues. The CT and CGRP of new MTC inpatients were measured before operation and in the first few days after operation using a radioimmunoassy.

RESULTS 1) The metastatic rate of cervical lymph nodes was a significantly different between the normal serum CT group and the elevated group ($P<0.01$). 2) The rate of residual tumor was significantly different between the normal serum CT group one month after operation and the elevated group at the same period ($P<0.01$). 3) In the immunohistochemical study we found the positive rate of CT was about 98%, and the CGRP was 87.8%. 4) Part of the patients had an elevated CGRP level while CT levels were normal. 5) The serum CT levels dropped to a stable range one week after operation.

CONCLUSION The CT levels are a useful index to evaluate the probability of cervical lymph node metastasis and the efficacy of surgical treatment. Measurement of the serum CGRP is an aid for the diagnosis of MTC, especially for those patients whose preoperative CT levels are normal.

KEYWORDS: calcitonin, calcitonin gene-related peptide, medullary thyroid carcinoma.

Medullary thyroid carcinoma (MTC) is a more aggressive disease of thyroid cancer and has a poorer prognosis compared to other types of thyroid carcinoma. Calcitonin (CT) remains the cornerstone of clinical management as a serum tumor marker for MTC, but the nature of the changes of CT after operation are not clear. Also in some patients with MTC, they have a normal preoperative CT level. Calcitonin gene-related peptide (CGRP) is an alternate product of the CT gene, so it is a possible tumor marker for MTC.[1,2]

In this study, in order to offer a basis for the diagnosis, treatment and prognosis for MTC patients, an immunohistochemical investigation for CT and CGRP was performed on tumor tissues from 58 patients with MTC. A radioimmunoassy was used to investigate the pattern of changes of serum CT and CGRP before and after operation.

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MATERIALS AND METHODS

Materials

The MTC tumor tissues were obtained from patients (19 men and 39 women; ages 23~72 years, median 50) who had undergone surgery in Tianjin Medical University Cancer Hospital from May 1992 to May 2002. Serum CT and CGRP levels of 15 patients who were admitted since May 2002, (6 men and 9 women; ages 23~71 years, median 48) were measured using a radioimmunoassy before and at the 1st, 2nd, 3rd and 7th day after operation.

Methods

The expression of CT and CGRP were detected by immunohistochemical staining. Mouse monoclonal antibody anti-CT, anti-CGRP and a immunohistochemical kit were purchased from the Beijing Zhongshan Biotechnology Co.. Ltd. Main steps: Sections were incubated with the primary antibody for 24 hr at 37℃ and then incubated with biotinylated secondary antibody for 30 min at room temperature, followed by peroxidase-conjugated streptavidin. Staining was visualized using DAB, washing in water, and counterstaining with haematoxylin. Normal thyroid tissues were used as a positive control and PBS replaced the primary antibody for a negative control. The CT and CGRP radioimmunoassy kits were provided by the Tianjin Jiuding Co. Ltd.

Counting methods

Five typical microphotographs were observed on each slide and on every microphotograph 100 tumor cells were counted. The staining index was obtained as the ratio of CT-positive cells to the total number of cells counted. Slight staining: the expression was in the cytoplasm and the number of positive cells was less than 25% . Moderate staining: 25% ~75% ; Heavy staining: >75% .[4] The normal CT level: 95.9 ± 26 pg/ml. The normal CGRP level: 50 ± 8 pg/ml.

Statistical methods

A SPSS for Windows 10.0 statistical software package was used to conduct χ^2 test and Radit analysis. A P <0.05 was considered significant.

Follow-up results

In all patients the follow-up was 1~10 years (median 5.4 years). Twenty of the cases recurred.

RESULTS

Serum study

In this study, 41 of 58 patients had a primary tumor focus. Of these patients 17 had normal serum CT levels (group A) and 24 had elevated CT levels (group B). Also there were 17 patients who had no primary tumor focus because of a prior thyroidectomy. Of these 17 patients, 7 had normal CT levels (group C) and 10 had elevated CT levels (group D). In group C one patient was found with cervical lymph node metastases, a rate of 14.3% (1 of 7). In group B, the positive rate was 87.5%. In groups A plus C (who had normal preoperative CT levels), the metastatic rate of cervical lymph nodes was 20.8% which showed a significant difference compared with groups B plus D (P <0.01).The four groups also showed a significant differences (Table 1).

Table 1. The relationship between the preoperative serum CT level and cervical lymph node metastasis (% cases)

Group	Cases	Positive	Negative	%
A	17	4	13	23.5
B	24	21	3	87.5
C	7	1	6	14.3
D	10	8	2	80.0
Total	58	34	24	58.6

Positive rate of groups A + C *vs* B + D P<0.01.

All patients had their serum CT levels remeasured one month after operation, and have been followed-up until the present time. Of 23 patients who had elevated CT levels at one month after operation 17 had a recurrence, and 3 of 35 who had normal CT levels at that time recurred. The difference between the two groups had a statistical significance (Table 2). Of 58 patients 19 had preoperative CT levels up to 1000

pg/ml, and to the present 14 have developed distant metastases. The metastatic rate was 73.7%.

Table 2. The relationship between the serum CT level one month after operation and tumor recurrence (% cases)

Group	Cases	Positive	Negative	%
Normal	35	3	32	8.6
Elevated	23	17	6	73.9
Total	58	20	38	34.5

$P<0.01$

Immunochemistry study

Forty-one of 58 patients had a primary tumor focus. Of these 41 patients 24 had elevated CT levels. Positive CT staining was detected in 94.1% of the 41 patients, mainly the slight-positive type (Fig.1,2). The staining reached 64.7%. Of the patients who showed positive CT staining 100% had elevated serum CT levels, mainly the heavy-positive type. It reached 70.8%. These values showed a significant difference (Table 3).

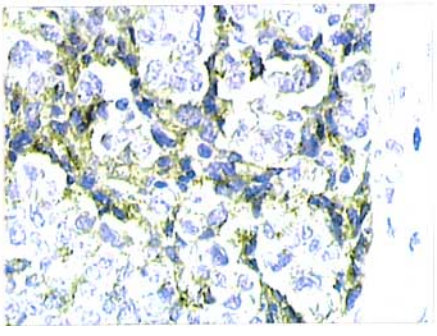

Fig.1. MTC is CT-positive × 400.

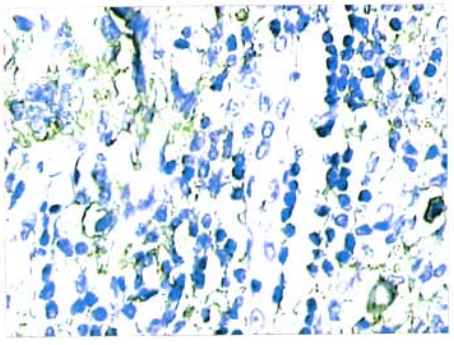

Fig.2. The metastatic lymph node of MTC is CT-positive × 400.

Table 3. The expression of CT in cases of MTC (% cases)

		Immunohistochemical staining				
Group	Cases	Negative	Slight	Moderate	Heavy	%
Normal	17	1	11	3	2	94.1
Elevated	24	0	3	4	17	100.0
Total	41	1	14	7	19	97.6

$P<0.01$

The result of CGRP was similar to CT staining, but its density dropped somewhat. Two patients had heavy-positive CGRP staining whose CT staining was the slight-positive type (Table 4).

Table 4. The expression of CGRP in cases of MTC (% cases)

		Immunohistochemical staining				
Group	Cases	Negative	Slight	Moderate	Heavy	%
Normal	17	2	8	3	4	82.4
Elevated	24	2	2	7	13	91.7
Total	41	4	10	10	17	87.8

$P<0.05$

Radioimmunoassy study

Ten of 15 MTC patients had elevated CT levels who were admitted to the hospital since May 2002. Eight of the 15 patients had elevated CGRP levels. One patient was noted to have an elevated CGRP level of the 5 patients who had normal CT levels. Based on the post-operation assays, we found the CT serum levels dropped over one or two days. In the first three days it showed a continuous course. By the 7th day the CT level was similar to the 3rd day (Fig.3). The serum CGRP levels also dropped after operation, but the characteristics of its change should be studied further.

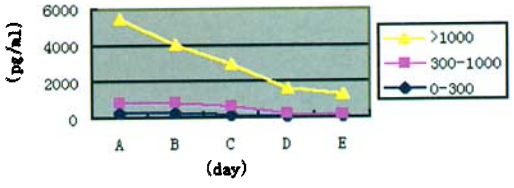

Fig.3. The pattern of serum CT changes. A: before operation B: 1st day after operation C: 2nd day D:3rd day E :7th day

DISCUSSION

Serum study

MTC is a relatively highly malignant tumor compared to other thyroid cancers, Early identification of patients with MTC results in a much better prognosis, with a high cure rate, so the test for tumor markers before and after operation is very important.

Former studies have shown that metastases to lymph nodes were found in 60%~70% of MTC patients, so thyroidectomy and neck dissection are requreded. [2,3] We found 90% of our patients had lymph node metastases whose preoperative CT levels were elevated, and the number and district of lymph node metastases were also higher in these patients. For these patients whose preoperative CT levels were normal, especially for those patients who had undergone thyroidectomy, most of them had no lymph node metastases. Whether those patients should be followed-up closely and neck dissection postponed until an elevated CT level is detected should be considered further. Only 8.6% of the patients recurred who had normal CT levels one month after operation. If CT levels were elevated at one month there was more recurrence. So the postoperative CT level was a good index for the assessment of surgical treatment.

Immunohistochemistry study

Through our immunohistochemical study of the tumor tissues we found that a higher positive cell rate correlated with a higher serum CT level. We believe the serum CT levels very well reflect the synthesis and secretion of CT and also the tumor burden. Part of the MTC patients had normal preoperative CT levels, perhaps because at this time the tumor was in an early stage, the tumor burden was lower and the CT secretion was lower. In this condition the patients had no or less lymph node metastases and the surgical treatment had more effective results. If the primary tumor focus can be resected completely, the rate of recurrence is also lower. If the CT level was much higher before operation, especially for those whose preoperative CT level was up to 1000 pg/ml, the disease was in a late stage. More care would be needed after operation and new treatment ways should be adopted in addition to the surgery in order to avoid a tumor recurrence. [4-6]

Radioimmunoassy study

The two precursor proteins of CT and CGRP are both encoded by the CT/CGRP gene. [7-10] In those 15 patients in which serum CT and CGRP were measured, 5 had normal preoperative CT levels, and one of the 5 patients had an elevated CGRP level. Also in the retrospective study we found 2 patients who had heavy-positive CGRP staining whose CT staining was slight-positive. This indicated that the 2 patients may have elevated CGRP levels before operation. So we think the measurement of preoperative CGRP can improve the diagnosis of MTC. We believe the test point should be at about the 7th day after operation. Thus we can alter the treatment measures according to the postoperative CT levels when the patients are still in the hospital.

In summary, the CT levels are a useful index to evaluate cervical lymph node metastasis and the efficacy of surgical treatment. The metastatic rate of cervical lymph nodes had a significant difference between the normal serum CT group and the elevated group. The preoperative CT levels up to 1000 pg/ml meant there was a higher tumor burden and more opportunity for distant metastases. The rate of residual tumor had a significant difference between the normal serum CT group one month after operation and the elevated group at the same time. The serum CT levels dropped to within the stable range one week after operation. This is the best time to assay CT levels. The serum CGRP measurement is an aid in the diagnosis of MTC especially for those patients whose preoperative CT levels are normal, but changes in its level should be studied further.

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