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Clinical and pathological analysis of 19 cases of medullary thyroid carcinoma without an increase in calcitonin

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ABSTRACT

Background: Medullary thyroid carcinoma (MTC), defined as a malignant tumour with C-cell differentiation, is of neuroendocrine origin and is characterized by the synthesis and secretion of calcitonin (CT). MTC without CT secretion has been reported on rare occasions. The purpose of this study was to evaluate the histological, immunohistochemical, and molecular pathologic features as well as the clinical significance of non-secretory MTC (NCR-MTC).

Methods: A retrospective analysis of patients with NCR-MTC was performed. The clinical features of NCR-MTC, including age, gender, tumour size and number, clinical signs of hypocalcaemia and diarrhoea, and the presence of lymph node metastasis, as well as the pathologic features of the disease, including tumour morphology, presence of neuroendocrine structures, capsular invasion, and immunohistochemical expression and presence of mutations in the *RET* gene, were evaluated.

Results: Nineteen patients with NCR-MTC were identified among 158 patients with MTC, resulting in a prevalence rate of 12.02%. Patients with NCR-MTC typically had masses less than 1 cm in size (73.7%, 14/19). Hypocalcaemia was not present in 94.7% (18/19) of patients. While 42.1% (8/19) of patients with NCR-MTC did not have amyloid deposits, only 18% (25/139) of patients with secretory MTC did not have such deposits. While 95.7% (133/139) of the control group of patients with secretory MTC had neuroendocrine tumour structure, only 84.2% (16/19) of the patients with NCR-MTC had this type of tumour structure. Patients with NCR-MTC were also less likely to have vascular tumour thrombus, lymph node metastasis or thyroid capsular invasion. With regard to immunohistochemistry, CT expression was mostly negative, and carcinoembryonic antigen (CEA) expression was positive in 21.1% (4/19) of patients with NCR-MTC, while only 5.8% (8/139) of patients in the control group had positive CEA expression.

Conclusions: The prevalence of NCR-MTC was low (12.02%). This type of tumour was smaller in size and more differentiated. Compared with the control group, relatively few patients had obvious symptoms, hypocalcaemia, lymph node metastasis, thyroid capsular or vascular invasion, or tumours with amyloid or neuroendocrine tumour structure.

1. Introduction

Medullary thyroid carcinoma (MTC) originates from the neural crest ectoderm and is a very rare neuroendocrine tumour, accounting for approximately 5% of thyroid cancer (Cooper, 2009). Basal or stimulated serum calcitonin (CT) levels are highly sensitive and specific markers (Kloos et al., 2009) for MTC, and more than 80% of patients with MTC have elevated serum CT levels. Patients with MTC who do not have elevated serum CT levels are said to have NCR-MTC. As early as the 1970s and 1980s, some reports found that approximately 30% of patients with MTC had normal CT levels (Saad et al., 1984; Samaan et al., 1973). However, the lower limit of the reference range used for CT was 293 pmol/L (1000 pg/mL), which is quite insensitive for detection of altered CT levels, compared to today's reference ranges. NCR-MTC was first proposed as a subtype of MTC in 1999, and the first official report of NCR-MTC was in 2000. However, the first prevalence data were only reported in 2013. So far, MTC that does not secrete CT into the serum has been rarely reported (Diez and Iglesias, 2004; Redding et al., 2000a; Alapat et al., 2011; Frank-Raue et al., 2013). The clinical, histological, immunohistochemical and molecular features of

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19 cases of NCR-MTC were evaluated in this study, and possible clinical treatments and prognosis are also discussed.

2. Materials and methods

2.1. Patients

A total of 158 cases of medullary thyroid carcinoma treated at Zhejiang Cancer Hospital between January 1, 2008 and April 30, 2016 were identified for retrospective statistical analysis. All cases had confirmed MTC by surgery and had recorded calcitonin levels. CT levels were increased in 139 cases (139/158, 88%), and this group was defined as the control group. The experimental group consisted of 19 cases of NCR-MTC, in which there was no preoperative elevation in CT levels (19/158, 12%). Clinical and pathological parameters including patient age and gender, mass size, morphological and immunohistochemical characteristics, bloodwork parameters, and surgical approach were all recorded.

2.2. Tumour markers

Serum calcitonin was measured by electrochemiluminescence, using a Cobas e602 instrument; the normal reference ranges were as follows: 0–9.52 pg/mL for males and 0–6.4 pg/mL for females.

2.3. Immunohistochemistry

The immunohistochemical stains were performed using antibodies against chromogranin A (CgA), synaptophysin (Syn), thyroglobulin (TG), and CEA using an indirect immunoperoxidase technique. All antibodies were from DAKO (Denmark).

The primary antibody for calcitonin is a rabbit monoclonal antibody (clone number: EPR68 (2)) with a dilution ratio of 1:100 using a dyeing platform (BOND Detection Systems (Leica)). The second antibody was from Polymer Refine Detection. The method of antigen repair used was Novocastra Epitope Solution, pH 9, for 20 min. The immunohistochemical method was an indirect method for peroxidase labelling. The positive staining of CT, Syn, TG was localized in the cytoplasm, and CEA positive staining was located in the capsule. Brown was positive, and no staining was negative.

2.4. Mutation analysis of the rearranged during transfection (RET) proto-oncogene

The *RET* gene detection method was via PCR gene sequencing using the following primers: 13F5, '-GTGCTGCATTTCAGAGAACGC-3′, and 13R5, '-AGAACAGGGCTGTATGGAGC-3′. A TaKaRa ExTaq PCR reaction kit with Taq enzyme was used for PCR. The automatic sequence analyser used was an ABI Prism 3100 Genetic Analyzer.

2.5. Statistical methods

The statistical software used was SPSS 17.0, and the statistical method used was the Chi square test. Statistical significance was defined as $p < 0.05$.

3. Results

A total of 158 patients were included, including 84 males and 74 females. Sixteen patients were 30 years old or older, and the remaining 142 cases were younger than 30 years old. Forty-one cases had a tumour diameter of less than 1 cm, and 117 cases had a tumour diameter of greater than 1 cm.

In terms of clinical manifestations, out of 158 total cases of MTC, 19 cases were NCR-MTC (12.02%). In patients with NCR-MTC, 73% (14/19) had a mass diameter less than 1 cm, while in the control group with secretory MTC, only 19.4% of patients had similar mass dimensions ($P < 0.05$). Of patients with NCR-MTC, 94.7% did not have hypocalcaemia, whereas in the control group 73.4% of patients did not have hypocalcaemia, which was a statistically significant difference between groups ($p = 0.041$). There was no significant difference between the sex, age, site of operation or occurrence of diarrhoea between the NCR-MTC group and the control group (Table 1).

Table 1
Comparison of Clinical features of NCR-MTC and Secretory MTC.

	Medullary carcinoma with elevated calcitonin n = 139 n	Medullary carcinoma without elevated calcitonin n = 19 n	$\chi 2$	*P*
Sex				
M	73	11	0.194	0.660
F	66	8		
Age				
≧30	13	3	0.761	0.383
< 30	126	16		
Tumour size				
≦1cm	27	14	25.610	0.000
> 1cm	112	5		
Hypocalcaemia				
Yes	37	1	4.173	0.041
No	102	18		
Diarrhoea				
Yes	34	1	3.572	0.059
No	105	18		
Genetic				
Familial	4	0	0.561	0.454
Sporadic	135	19		
Mass number				
Single	120	18	1.068	0.301
Multiple	19	1		

With regard to histomorphology, compared with other thyroid neoplasms, amyloid- and neuroendocrine-type tumours are more characteristic of medullary carcinoma. In this study, only 57.9% of NCR-MTC cases had tumours with amyloid deposits, compared to 82% in the control group ($p = 0.015$). In NCR-MTC, a neuroendocrine tumour structure is relatively less common; however, 84.2% (16/19) of patients with NCR-MTC had neuroendocrine tumour structure in this study. This result was only a slightly lower percentage of neuroendocrine-type tumours than the control group (95.7%, 133/139) ($p = 0.043$). In addition, the number of patients with lymph node metastasis, thyroid capsular invasion and vascular tumour thrombus in the NCR-MTC group was significantly lower than the control group. There were no significant differences in the two groups in terms of the differentiation of papillary carcinoma or follicular carcinoma, the number of tumours, genetics, or tumour cell morphology (Table 2).

Immunohistochemically, compared with the control group, the positive rate of CT in NCR-MTC was significantly lower, while the positive rate of CEA was significantly higher. Other immunohistochemical markers such as CgA, Syn, TG and *RET* gene mutations were not significantly different between the NCR-MTC group and the control group.

In addition, during follow-up of the 19 cases of NCR-MTC, after 6 years, there were 2 cases of local recurrence, 1 case of lymph node metastasis, and 1 case of lymph node metastasis and local recurrence. In all 4 of these cases, the serum calcitonin levels had increased again prior to treatment (Table 3).

4. Discussion

Medullary thyroid cancer is a neuroendocrine tumour deriving from the thyroid follicular cells, of which approximately 80% of cases are