

ORIGINAL ARTICLE

Diagnostic Value of Serum Cytokeratin 18, Carcinoembryonic Antigen, and Thyroglobulin in Patients with Papillary Thyroid Carcinoma

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SUMMARY

Background: The current study aims to explore the expression and diagnostic value of cytokeratin 18 (CK) in patients with papillary thyroid carcinoma (PTC).

Methods: Patients diagnosed with thyroid tumor, including 127 cases of PTC and 60 cases of benign tumors and 50 healthy controls were included. Serum CK 18, carcinoembryonic antigen (CEA) and thyroglobulin (Tg) were detected using enzyme-linked immunosorbent assay (ELISA). The diagnostic value of serum CK 18, CEA, and Tg for PTC was analyzed by receiver-operating characteristic (ROC) curve.

Results: We showed novel data that the level of serum CK 18 was higher in PTC patients than that in controls. Upregulation of serum CK 18 correlated with aggressive clinicopathologic characteristics of PTC, including lymph node metastasis, high ASA risk classification, advanced TNM stage, and larger tumor size. Furthermore, serum CEA and Tg levels of thyroid cancer patients were significantly higher than those of the benign group. More importantly, the AUC-ROC of combined serum CEA, Tg, and CK 18 was higher than that of CEA, Tg, and CK 18 alone, indicating the combination of serum CEA, Tg, and CK 18 significantly improved the diagnostic efficiency in PTC patients.

Conclusions: Altogether, combined use of serum CK 18, Tg, and CEA may be a promising biomarker in screening benign and malignant thyroid tumors.

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KEY WORDS

papillary thyroid carcinoma, cytokeratin 18, carcinoembryonic antigen, thyroglobulin, diagnosis

INTRODUCTION

Thyroid tumor is one of the most common endocrine tumors, which can be divided into benign and malignant cancer [1]. Papillary thyroid carcinoma (PTC) comprises the vast majority (80%) of total thyroid cancer cases [2]. The prognosis of most PTC patients is good, but there are still some patients with postoperative recurrence and distant metastasis [3]. At present, fine-needle biopsy (FNA) is the gold standard for preoperative diagnosis of PTC [4]. However, due to the influence of inadequate sampling and indeterminate results, about 30% of the samples cannot be diagnosed [5]. Al-

though the ultrasound examination is noninvasive, it is largely challenged because of the techniques and clinical experience of the operators [5]. Therefore, it is of great significance to identify noninvasive biomarkers for PTC diagnosis, preoperative risk assessment and recurrence monitoring.

Cytokeratin (CK) 18 is widely expressed in multiple organs and participants in different cellular processes, such as apoptosis, mitosis, proliferation, and cell cycle progression [6,7]. During apoptosis and necrosis processes, elevated levels of caspase-cleaved (M30) and uncleaved (M65) CK 18 fragments can be detected in blood serum [8]. Hence, serum levels of these fragments may be a promising marker for diagnosis of tumors. A previous study has shown that CK 18 was extensively expressed in thyroid cancer tissues [9]. However, the expression and diagnostic value of serum CK 18 in PTC patients have not been studied.

The present study evaluated the level of serum CK 18 in patients with PTC and further explored the diagnostic value of CK 18, by which we raise the possibility that measurement of CK 18 combined other existing indicators may provide useful diagnostic information in the evaluation of thyroid nodules.

MATERIALS AND METHODS

Patient samples

From June 2018 to June 2019, patients diagnosed with thyroid tumor according to American Thyroid Association Guidelines [10] were selected in the Affiliated Jianhu Hospital of Nantong University, including 127 cases of PTC and 60 cases of benign tumors (including 32 follicular adenoma samples and 28 multinodular goiter samples). The details of PTC patients were shown in Table 1. In the benign group, there were 14 males and 46 females, aged 32 - 72 years, with an average age of 53.0 ± 16.8 years. There was no significant difference in age and gender between the two groups. Exclusion criteria: 1. Preoperative radiotherapy and chemotherapy history; 2. Combined with other malignant tumor; 3. Long-term use of immunomodulatory drugs; 4. Combined with rheumatic disease and connective tissue disease.

Meanwhile, 50 healthy controls were selected from the Physical Examination Center in the Affiliated Jianhu Hospital of Nantong University. Among them, there were 24 males and 26 females, aged 29 - 69 years, with an average age of 51.8 ± 17.2 years.

This study was approved by the ethics committee of the Affiliated Jianhu Hospital of Nantong University, and all patients signed an informed consent.

Enzyme-linked immunosorbent assay (ELISA)

Venous blood samples of 5 mL were collected after fasting from all participants. The samples were centrifuged at 2,500 g for 15 minutes immediately after sampling and then stored at -20°C until the test of CK18

level. An M65 EpiDeath[®] ELISA kit (DiaPharma[®], USA) was used to determine the level of serum CK18 in all participants. Serum carcinoembryonic antigen (CEA) and thyroglobulin (Tg) levels were determined using a Human CEA ELISA Kit (RAB0411-1KT, Sigma, USA) and a Human Thyroglobulin ELISA Kit (RAB0458-1KT) according to the instructions.

Statistical analysis

All statistical analysis was performed on GraphPad Prism (version 7.0; GraphPad Software, San Diego, CA, USA). The data were represented as the mean \pm standard deviation (SD). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 20.0) followed by Tukey's post hoc test were used for comparisons of two more groups. A receiver-operating characteristic (ROC) curve was constructed for serum CK18 to establish the cutoff value. Statistical significance was considered at $p < 0.05$.

RESULTS

Elevated serum CK 18 in PTC patients

The levels of serum CK 18 in PTC patients, benign tumor group, and healthy controls were 321.67 ± 48.79 U/L, 54.62 ± 18.26 U/L, 47.98 ± 18.57 U/L, respectively (Figure 1). Obviously, serum CK 18 was significantly increased in PTC patients compared with that of the benign tumor group and healthy controls.

The relationship between serum CK 18 and the clinicopathological parameters in PTC patients

Furthermore, we analyzed the level of serum CK 18 based on different clinicopathological parameters in PTC patients. As shown in Figure 2A, the level of serum CK 18 in PTC patients with tumor diameter > 2 cm was higher than that in PTC patients with tumor diameter ≤ 2 cm (387.61 ± 49.56 U/L vs. 289.23 ± 35.87 U/L). Increased serum CK 18 was found in the PTC patients with lymph node metastasis compared with those without lymph node metastasis (379.16 ± 42.98 U/L vs. 301.25 ± 32.16 U/L, Figure 2B). In addition, the relative expression of serum CK 18 in PTC patients with TNM stage II - IV was higher than that in PTC patients with stage I (385.97 ± 45.16 U/L vs. 296.75 ± 32.86 U/L, Figure 2C). Besides, elevated expression of serum CK 18 was also found in PTC patients in the medium and high risk groups compared with those in the low risk group (401.23 ± 43.27 U/L vs. 268.78 ± 35.18 U/L, Figure 2D).

Enhanced CEA and Tg levels in PTC patients

Subsequently, we detected the levels of other existing indicators for PTC patients, including CEA, a common tumor marker that is also increased in thyroid cancer [11], and Tg, a thyroid-specific protein produced by thyrocytes [12]. As shown in Figure 3A and 3B, the lev-

Table 1. The clinicopathological parameters in PTC patients.

	n
Age (years)	
≥ 45	67
< 45	60
Gender	
Female	69
Male	58
Tumor diameter (cm)	
> 2	73
≤ 2	54
Lesion number	
Single	63
Multiple	64
Lymph node metastasis	
Yes	47
No	80
ASA risk classification	
Low risk	69
Medium and high risk	58
TNM staging	
I	62
II - IV	65
Tg level (ng/mL)	
≤ 77	64
> 77	63
CEA level (ng/mL)	
≤ 5	23
> 5	104

els of CEA (17.34 ± 8.23 ng/mL vs. 2.21 ± 1.64 ng/mL) and Tg (98.67 ± 35.23 ng/mL vs. 35.32 ± 18.53 ng/mL) were significantly increased in PTC patients compared to those of the benign tumor group.

Diagnosis of thyroid cancer by serum CEA, Tg, and CK 18

Then, we compared the diagnostic efficiency of CEA, Tg, and CK 18 between PTC patients and benign controls. As shown in Figure 4, the AUC-ROC of CK 18 was 0.761, with the sensitivity and specificity of 72.85% and 85.64% (cutoff value: 315.43 U/L). In comparison, the AUCs-ROC of CEA and Tg were 0.608 (sensitivity: 26.98%; specificity: 30.17%; cutoff value: 5.01 ng/mL) and 0.629 (sensitivity: 30.17%; specificity: 89.21%; cutoff value: 65.4 ng/mL), respectively. Obviously, the sensitivity and AUC value of CK 18 were higher than those of CEA and Tg in the differ-

ential diagnosis of thyroid cancer. More importantly, the AUC-ROC of combined serum CEA, Tg and CK 18 was 0.916, with the sensitivity and specificity of 87.92% and 94.18%, respectively. Obviously, combination of serum CEA, Tg, and CK 18 significantly improved the diagnostic efficiency in PTC patients.

DISCUSSION

PTC is a malignant tumor originating from thyroid follicular cells, and its incidence has increased significantly in recent years [13]. Although PTC has a good prognosis, there are still a few patients with local recurrence or distant metastasis [14]. At present, ultrasound, CT and FDG-PET/CT can be used to detect metastasis or recurrence, but these methods are expensive and time-consuming [15]. Hence, it would be helpful to identify the serum markers of PTC to solve this problem.

Abnormal expression of CK 18 has been found in different tumors [16,17]. According to Lam KY et al., CK 18 is widely expressed in thyroid cancer [9]. However, whether serum CK 18 could be used for the diagnosis of PTC has not been explored. In the present study, we showed novel data that the level of serum CK 18 was higher in PTC patients than in controls. Moreover, up-regulation of serum CK 18 correlated with aggressive clinicopathological characteristics of PTC, including lymph node metastasis, high ASA risk classification, advanced TNM stage, and larger tumor size. These data indicate that the high expression of serum CK 18 may be involved in the pathogenesis of PTC and related to the progress of PTC.

Previous studies have also suggested CEA as a tumor marker for thyroid cancer [11,18]. However, it is commonly accepted that CEA is a specific biomarker for medullary thyroid carcinoma (MTC) [19,20], but sensitivity of CEA for PTC is low [21,22]. Besides, elevated level of serum Tg is a risk factor for PTC and may be useful to predict lymph node metastasis (LNM), but it is also challenged by a lack of specificity [23,24]. For instance, serum Tg may be significantly increased in thyroid nodules or thyroid autoimmune diseases [25]. Clinically, for patients with suspected thyroid cancer, an increase in serum Tg and other reference indicators should be comprehensively evaluated to reduce the risk of misdiagnosis [26]. Here, we found that the serum CEA and Tg levels of PTC patients were significantly higher than those of the benign group. ROC analysis showed that the sensitivity and specificity of CK 18 were higher than Tg and CEA alone. More importantly, the diagnostic value of the combined three serological indicators was ideal, and the specificity of the diagnosis can reach more than 90%. Therefore, serum CK 18 can be used to assist in the diagnosis of PTC.

In conclusion, for the first time, we demonstrated that combined use of serum CK 18, Tg, and CEA may be a promising biomarker in screening benign and malignant thyroid tumors. However, the cases of PTC patients are

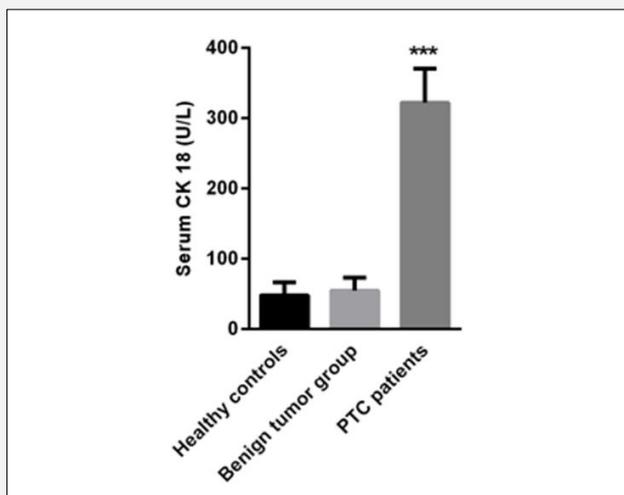


Figure 1. ELISA assay showed that serum CK 18 was significantly increased in PTC patients compared with that of the benign tumor group and healthy controls.

*** $p < 0.001$ vs. control.

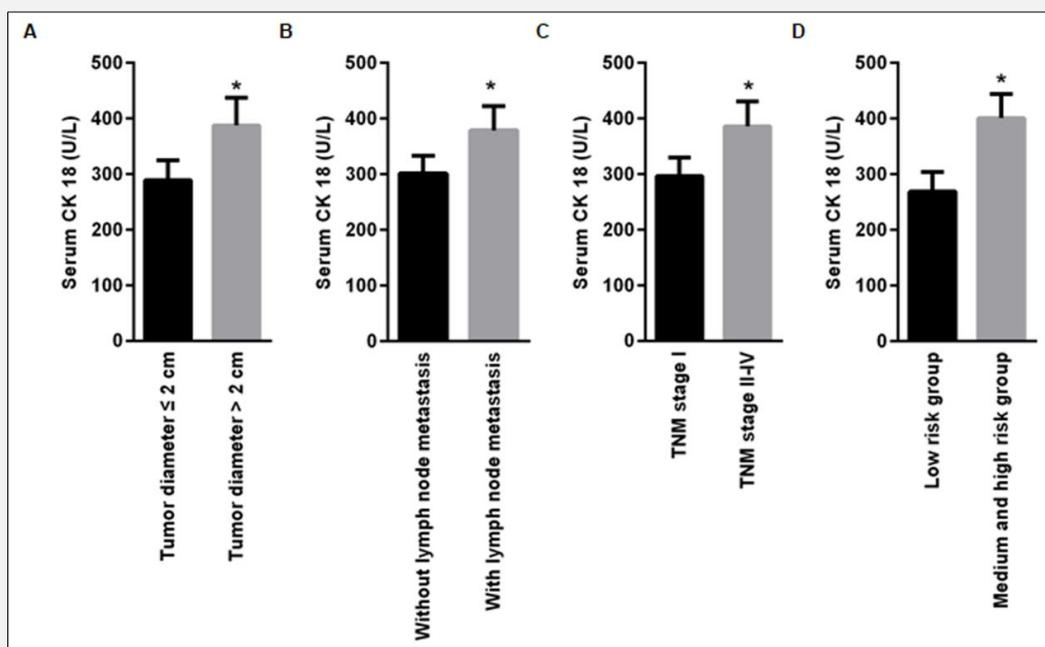


Figure 2. Serum CK 18 was analyzed based on the clinicopathological parameters in PTC patients.

(A) The level of serum CK 18 in PTC patients with tumor diameter > 2 cm was higher than that in PTC patients with tumor diameter ≤ 2 cm. (B) Increased serum CK 18 was found in the PTC patients with lymph node metastasis compared with those without lymph node metastasis. (C) The relative expression of serum CK 18 in PTC patients with TNM stage II ~ IV was higher than that in PTC patients with stage I. (D) Elevated expression of serum CK 18 was also found to be increased in PTC patients in medium and high-risk groups compared with those in the low risk group. * $p < 0.05$ vs. control.

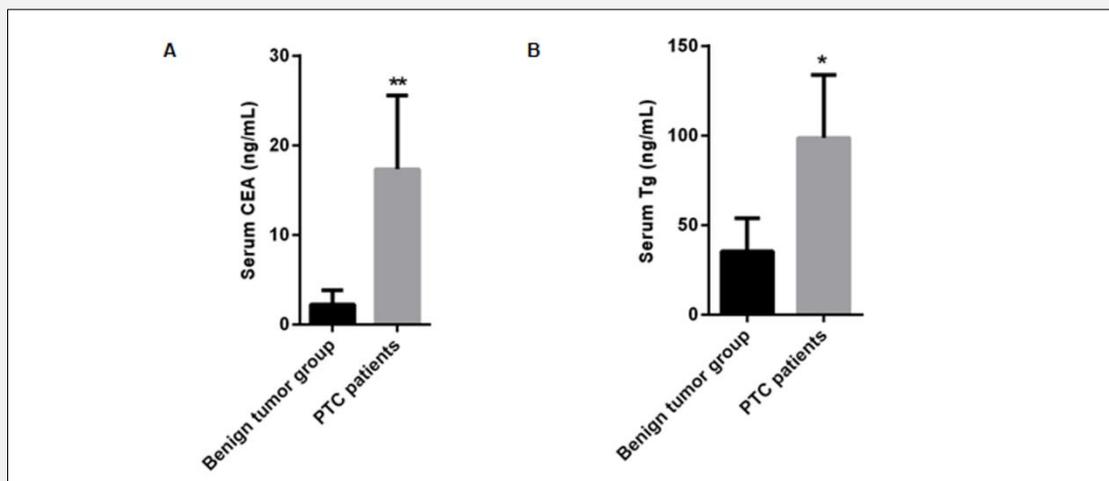


Figure 3. The levels of CEA and Tg were evaluated in PTC patients and in the benign tumor group. Enhanced CEA (A) and Tg (B) levels were found in PTC patients compared to that of controls.

* p < 0.05, ** p < 0.01 vs. controls.

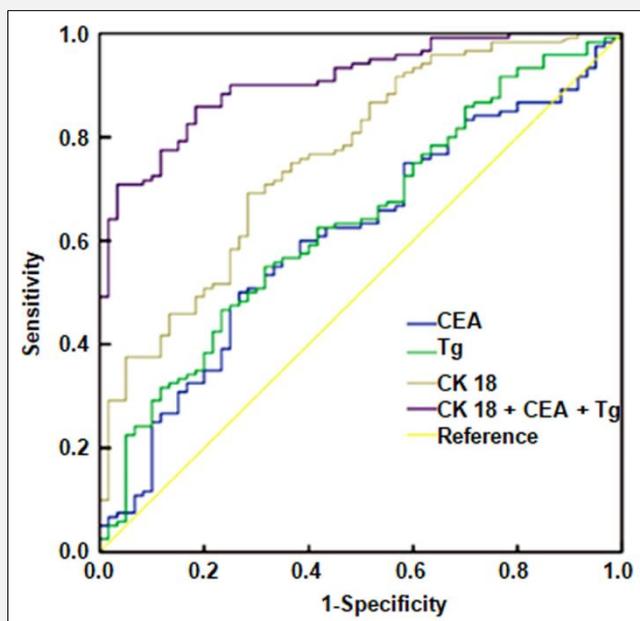


Figure 4. ROC analysis was carried out to explore the diagnostic efficiency of serum CEA, Tg, and CK 18 in screening PTC from benign tumor.

relatively small and large samples are necessary to strengthen the proof of serum CK 18 as a new biomarker to distinguish between PTC and benign thyroid tumor with papillary hyperplasia and other clinical diagnoses.

Declaration of Interest:

We declare no conflicts of interest.

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