THE EFFICACY OF PLASMA CIRCULATING TUMOR DNA CONTENT COMBINED WITH BRAF~(V600E) GENE DETECTION IN THE DIAGNOSIS OF THYROID CANCER

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ABSTRACT

Objective*: To study the efficacy of plasma circulating tumor DNA (ctDNA) content combined with BRAF~(V600E) gene detection in the diagnosis of thyroid cancer.*

Methods: *From July 2018 to July 2019, 42 thyroid cancer patients admitted to our hospital for thyroid surgery were selected as the thyroid cancer group, and 60 patients with benign thyroid nodules were selected as the benign thyroid nodule group. The ctDNA and BRAF~(V600E) gene levels of the 2 groups of patients were detected and compared to explore the efficacy of ctDNA content combined with BRAF~(V600E) gene detection in the diagnosis of thyroid cancer.*

Results: *The content of ctDNA in patients with thyroid cancer was higher than that of patients with benign thyroid nodules, and the difference was statistically significant (P<0.01). There were 9 BRAF~(V600E) gene mutations in the group of patients with thyroid cancer. The BRAF~(V600E) gene mutation rate was 40.91%. The patients with benign thyroid nodules had no BRAF~(V600E) gene mutations. The difference between the 2 groups was statistically significant (P<0.01). The BRAF~(V600E) gene mutations in patients with thyroid cancer had no statistical significance with the patients' age, gender, and lymph node metastasis (P>0.05). The ROC curve analysis showed that the AUC for the ctDNA diagnosis of thyroid cancer was 0.892, the optimal cutoff was 71.12 ng/mL, the sensitivity was 88.36%, and the specificity was 81.12%. The sensitivity of the parallel combined detection of thyroid cancer (90.91%) was higher than that of the system combined detection (63.64%), and the difference was statistically significant (P<0.05). The specificity of the systemic detection of thyroid cancer (100.00%) and the specificity of the systemic parallel detection (91.67%) showed no significant difference (P>0.05).*

Conclusion: *Plasma ctDNA content combined with BRAF~(V600E) gene detection has a certain value in the diagnosis of thyroid cancer and can be widely used in clinical settings.*

Keywords: *Plasma circulating tumor DNA, combination, BRAF~(V600E) gene, thyroid cancer.*

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Introduction

Thyroid cancer is a common malignant tumor, accounting for approximately 1% of systemic malignancies. There are 4 pathological types: undifferentiated cancer, myeloid cancer, follicular cancer, and papillary cancer. Among them, papillary cancer has the highest incidence[(1)]. Iodine deficiency, X-ray irradiation, chronic TSH stimulation, sex hormone effects, and family factors can all lead to the occurrence of thyroid cancer[(2)]. There are no obvious symptoms in the early stage of thyroid cancer. Generally, there are symptoms such as hard, fixed, and uneven surface thyroid masses, and the glands will reduce the range of up-and-down movement when swallowing. In the later stage, hoarseness, dyspnea, Horner syndrome, occipital shoulder pain, and lymph node metastasis[(3-5)].

ctDNA is a characteristic tumor biomarker that is released from tumor cell somatic cells or released into the circulatory system after apoptosis[(6)]. The BRAF~(V600E) gene is the most common mutant gene of thyroid cancer, and its mutation rate in papillary thyroid cancer is as high as 75%. In particular, studies have shown that mutations in the BRAF~(V600E) gene are important for the early di-

agnosis of thyroid cancer[7]. The hospital conducted this test to investigate the efficacy of plasma ctDNA content combined with BRAF~(V600E) gene detection in the diagnosis of thyroid cancer.

Materials and methods

General information

From July 2018 to July 2019, 82 thyroid patients who were admitted to our hospital for thyroid surgery were selected and divided into a thyroid cancer group and a benign thyroid nodule group, according to the patient's disease type.

The inclusion criteria for thyroid cancer were as follows:

• The symptoms of all patients with thyroid cancer met the criteria of the "Diagnosis and Treatment Guidelines for Adult Thyroid and Differential Thyroid Cancer" issued by the American Thyroid Association in 2015;

• An ultrasound and fine-needle aspiration biopsy confirmed thyroid cancer;

• There was normal liver and kidney function; (4) the patient was informed and signed the informed consent document;

• No iodine contrast agent was used within one month prior;

• The patient had no chronic hypertension, diabetes, endocrine disease, liver or kidney disease, or other surgical diseases;

• And the patient had received no high-dose radiation or X-ray treatment for the head and neck.

The exclusion criteria were as follows:

• The patient was younger than 18 years old;

• Combined with other malignant tumors;

• Combined with thyroid tuberculosis;

• Combined with autoimmune diseases;

• And the patient had severe liver and kidney dysfunction.

The inclusion criteria for benign thyroid nodule group were as follows:

• The patient had single or multiple solid nodules confirmed by a B-ultrasound;

• And the patient underwent a pathological examination for benign lesions.

The exclusion criteria were as follows:

• The patient was younger than 18 years old;

• Combined with other malignant tumors;

• Combined with acute coronary syndrome;

• The patient had an unstable pituitary function;

• And the patient had severe liver and kidney dysfunction.

There were 22 patients in the thyroid cancer group, including 10 males and 12 females. The average age was 42.00±8.95 years, and the average BMI was 20.15±1.25. There were 60 patients in the benign thyroid nodule group, including 24 males and 36 females. The average age was 41.75±7.85 years, and the average BMI was 20.21±0.89. There was no statistically significant difference in general information, such as age, gender, and BMI, between the 2 groups of patients (P>0.05). See Table 1.

Group	Age	Gender		BMI Value
		Male	Female	
Thyroid cancer (n = 22)	42.00±8.95	10	12	20.15±1.25
Benign thyroid nodule (n = 60)	41.75±7.85	24	36	20.21±0.89
t/χ^2	0.197	0.197		0.241
P	>0.05	>0.05		>0.05

Table 1: A comparison of general information between the 2 groups of patients (cases, $\bar{x}\pm s$).

Observation indicators

Take 5 mL of fasting venous blood from 2 groups of subjects in the morning, centrifuge at 2000 r/min for 15 minutes, carefully take the upper serum and refrigerate it at -80oC. The plasma ctDNA content of all subjects was detected by fluorescence quantitative PCR, and the mutation of the BRAF~(V600E) gene was detected by fluorescence quantitative PCR and sequencing.

• Compared with the ctDNA content and BRAF~(V600E) gene mutation in the 2 groups of patients.

• The relationship between BRAF~(V600E) gene mutation and age, sex, and lymph node metastasis.

• The ROC curve was used to explore the efficacy of ctDNA in the diagnosis of thyroid cancer.

• The value of ctDNA and BRAF~(V600E) gene mutation in the diagnosis of thyroid cancer was analyzed through a parallel combined test (One of the 2 methods is positive, that is, the parallel combination test is positive.) and a systematic combined test. (If both tests are positive, then the system test is positive).

Statistical methods

The SPSS 20.0 software package was used for the statistical data analysis. Measurement data were compared using a one-way analysis of variance and an LSD-t test. The count data were compared using a χ^2 test. The ROC curve was used to explore the efficacy of ctDNA in the diagnosis of thyroid cancer.

The parallel and systematic detections of ctDNA and BRAF~(V600E) gene mutations were used to diagnose thyroid cancer. Statistical results were statistically significant, with P<0.05.

Results

A comparison of ctDNA content and BRAF~(V600E) gene mutation between the 2 groups

The ctDNA content in patients with thyroid cancer was higher than that of patients with benign thyroid nodules, and the difference was statistically significant (P<0.01). There were 9 patients with BRAF~(V600E) gene mutations in the thyroid cancer group, and the BRAF~(V600E) gene mutation rate was 40.91%. There was no BRAF~(V600E) gene mutation in the benign thyroid nodule group, and the difference between the 2 groups was statistically significant (P<0.01). See Table 2.

Group	ctDNA (ng/mL)	BRAF~(V600E) gene mutation (Cases, %)
Thyroid cancer (n = 22)	81.36±25.26	9 (40.91%)
Benign thyroid nodule (n = 60)	49.36±20.19a	0 (0.00%)a
t/χ^2	6.491	8.963
P	<0.01	<0.01

Table 2: A comparison of ctDNA content and BRAF~(V600E) gene mutation between the 2 groups ($\bar{x}\pm s$).
Note: a means that it was compared with the thyroid cancer group, $^aP<0.05$.

The relationship between BRAF~(V600E) gene mutation and age, sex, and lymph node metastasis

The BRAF~(V600E) gene mutations in patients with thyroid cancer had no statistical significance with the patients' age, gender, and lymph node metastasis (P>0.05). See Table 3.

Index	Age		Gender		lymph node metastasis	
	<42	≥42	Male	Female	Yes	No
BRAF~(V600E) Gene mutation	4/11 (36.36%)	5/11 (45.45%)	3/10 (30.00%)	6/12 (50.00%)	5/12 (41.67%)	4/10 (40.00%)
χ^2	0.188		0.903		0.006	
P	>0.05		> 0.05		> 0.05	

Table 3: The relationship between BRAF~(V600E) gene mutation and age, sex, and lymph node metastasis (Cases, %).

ROC curve analysis: The value of the diagnosis of thyroid cancer with ctDNA

The ROC curve analysis showed that the AUC of ctDNA for thyroid cancer diagnosis was 0.892, the optimal critical value was 68.12 ng/mL, the sensitivity was 88.36%, and the specificity was 81.12%. See Table 4 and Figure 1.

Index	AUC	95%CI	Optimum critical value (ng/ml)	Sensitivity (%)	Specificity (%)
ctDNA	0.892	0.846–0.956	68.12	88.36	81.12

Table 4: ROC curve analysis: The value of the diagnosis of thyroid cancer with ctDNA.

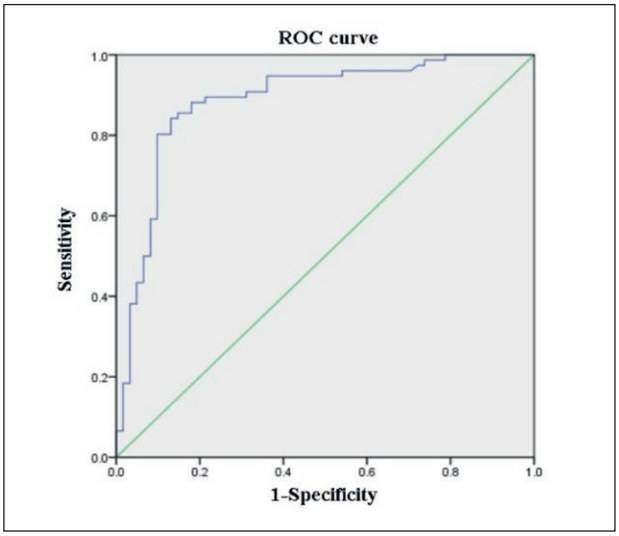

Figure 1: ROC curve analysis: The value of the diagnosis of thyroid cancer with ctDNA.

The value of ctDNA content combined with BRAF~(V600E) gene detection in the diagnosis of thyroid cancer

The optimal critical value for the ctDNA diagnosis of thyroid cancer was 68.12 ng/mL; the positive was greater than 68.12 ng/mL, and the negatives were less than 68.12 ng/mL. Patients with the BRAF~(V600E) gene mutation were positive, and patients with no BRAF~(V600E) gene mutation were negative. The sensitivity of the parallel combined detection of thyroid cancer (90.91%) was higher than that of the system combined detection (63.64%), and the difference was statistically different (P<0.05). The specificity of the system combined detection of thyroid cancer (100.00%) was higher than that of the system parallel detection (91.67%), and the difference was not statistically significant (P>0.05). See Table 5.

Group	Cases	Parallel combined detection		System combined detection	
		Positive	Negative	Positive	Negative
Thyroid cancer	22	20	2	14	8
Benign thyroid nodule	60	5	55	0	60

Table 5: The value of ctDNA content combined with BRAF~(V600E) gene detection in the diagnosis of thyroid cancer (Cases).

Conclusions

Thyroid cancer is the most common malignant tumor of the endocrine system in China, and its incidence has increased significantly in recent years (8). At present, the main methods for diagnosing thyroid cancer include a B-ultrasound and fine-needle biopsy, etc., but some cases still cannot be diagnosed clearly[9]. Therefore, the early diagnosis of thyroid cancer and the timely monitoring of thyroid disease are of great significance.

ctDNA is a kind of extracellular DNA in a cell-free state. It is mainly composed of single-stranded or double-stranded DNA and a mixture of single-stranded and double-stranded DNA. It exists in the form of a DNA protein complex or free DNA in the blood, synovial fluid, cerebrospinal fluid, and other bodily fluids[10, 11]. As a tumor marker for the genomic mutation of tumor cells, ctDNA rarely detects false positives, and the ctDNA half-life is short, which can accurately reflect the current condition of the tumor. ctDNA is widely used in the diagnosis, treatment, and prognostic detection of tumors due to its advantages, such as non-invasive technology, simple acquisition, high specificity, and strong sensitivity[12]. The ctDNA content of thyroid cancer patients in this study was higher than that of the benign thyroid nodule patients, and the difference was statistically significant ($P<0.01$). This showed that the increase in ctDNA content has a certain relationship with thyroid disease, and the more serious the disease, the higher the ctDNA content. The ROC curve analysis showed that the AUC for the ctDNA diagnosis of thyroid cancer was 0.892, the optimal critical value was 68.12 ng/mL, the sensitivity was 91.36%, and the specificity was 88.12%. It was suggested that ctDNA content has a certain value in the diagnosis of thyroid cancer and can be used as an effective method for early diagnosis.

The BRAF gene is located on human chromosome 7 and encodes a serine/threonine-protein kinase of the RAF family[13]. It can regulate the MAPK/ERK signaling pathway and affect cell division, differentiation, and secretion. The most common carcinogenic mechanism of thyroid cancer is the activation of the MAPK signaling pathway, and BRAF is a key gene in this pathway. BRAF mutations can promote the activation of downstream signaling pathways to a certain extent and then induce the production of thyroid cancer[14]. The BRAF~(V600E) gene is the most common mutation type of the BRAF gene. It is most frequently mutated in malignant tumors such as thyroid cancer, melanoma, colorectal cancer, hairy cell leukemia, non-small cell lung cancer, and adenocarcinoma (15). In this study, there were 9 cases of BRAF~(V600E) gene mutation in the thyroid cancer group, and the BRAF~(V600E) gene mutation rate was 40.91%. There were no BRAF~(V600E) gene mutations in the patients with benign thyroid nodules. The differences between the 2 groups were statistically significant ($P<0.01$). It was suggested that the mutation of the BRAF~(V600E) gene induces the generation of thyroid cancer to a certain extent, and this mutation has nothing to do with the patient's age, gender, and lymph node metastasis.

In this study, the parallel and combined detections of ctDNA content and BRAF~(V600E) gene mutations were used to detect thyroid cancer. The sensitivity of the parallel combined detection of thyroid cancer (90.91%) was higher than the system combined detection's sensitivity (63.64%), and the difference was statistically significant ($P<0.05$). The specificity of the systemic combined detection of thyroid cancer was 100.00%, the specificity of the systematic parallel detection was 91.67%, and there was no significant difference ($P>0.05$). It was suggested that the content of ctDNA combined with the BRAF~(V600E) gene mutation has a certain significance in the diagnosis of thyroid cancer and can help in its diagnosis.

In summary, patients with thyroid cancer have a certain increase in plasma ctDNA content, and the BRAF~(V600E) gene mutation rate increases. The combination of plasma ctDNA content and BRAF~(V600E) gene mutation is helpful for the diagnosis of thyroid cancer, which can be widely used in clinical settings.

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