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Original Article

Diagnostic value of high-frequency ultrasound combined with fine needle aspiration cytology and BRAF gene for papillary thyroid microcarcinoma

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Abstract

We attempted to evaluate clinical application value of high-frequency ultrasound (HFUS), fine needle aspiration cytology (FNAC), BRAF gene, and combination of HFUS, FNAC, and BRAF gene in diagnosing papillary thyroid microcarcinoma (PTMC). The 150 patients with thyroid minimal lesions who underwent HFUS, FNAC and BRAF gene testing before surgery in our hospital from June 2020 to December 2021 were selected as research subjects. Patients were divided into two groups based on postoperative pathological results. The consistency of diagnostic results of HFUS, FNAC, and BRAF gene and their combination with those of pathological examination, diagnostic efficacy of HFUS, FNAC and BRAF gene combined detection and individual detection for PTMC lymph node metastasis, and diagnostic value of HFUS, FNAC and BRAF gene combined detection and individual detection for PTMC lymph node metastasis received analysis and comparison. The consistency of diagnostic results of combined detection with pathological examination exhibited elevation relative to that of HFUS, FNAC and BRAF gene detection alone ($P < 0.05$). The negative predictive value, sensitivity and accuracy of combined detection exhibited elevation relative to individual detection ($P < 0.05$). The AUC of combined detection in diagnosing PTMC lymph node metastasis exhibited elevation relative to that of HFUS and BRAF gene alone ($P < 0.05$). HFUS combined with FNAC and BRAF genes possesses high diagnostic value, with high diagnostic sensitivity, specificity, and accuracy. Thus, combined detection for PTMC before surgery can accurately determine whether lymph node metastasis occurs, reduce occurrence of missed diagnosis and misdiagnosis, and thus improve diagnostic precision.

Keywords: Papillary thyroid microcarcinoma, High-frequency ultrasound, Fine needle aspiration cytology, BRAF gene, Diagnostic value.

1. Introduction

Papillary thyroid microcarcinoma (PTMC) refers to thyroid papillary carcinoma with a maximum diameter of less than 1 cm, which is a malignancy with a high incidence in endocrine system. Patients often have hoarse voice, dysphagia and other symptoms, seriously affecting their quality of life [1-3]. Cervical lymph node metastasis in PTMC often occurs on the same side. It has been demonstrated that thyroidectomy and lymph node dissection can achieve good treatment outcomes for patients with early PTMC lymph node metastasis. Nevertheless, misdiagnosis and missed diagnosis are prone to occur when utilizing palpation to diagnose small-volume lymph node metastasis, which often delays optimal time for therapy [4]. Thus, elevating accuracy of early diagnosis in PTMC is crucial.

Imaging examinations are often applied in clinical practice based on palpation [5, 6]. High-frequency ultrasound (HFUS) is a commonly applied imaging examination method with advantages of non-invasion, high repeatability, direct display of lesion status, etc. [7]. The popularization of HFUS screening has elevated detection rate of thyroid nodules less than 1 cm. Color Doppler ultrasound, a type of HFUS ultrasound, majorly judges benign and malignant thyroid nodules based on their echo, boundary, aspect ratio, calcification, blood flow and other characteristics [8, 9], providing vital reference for clinical diagnosis and therapy; nevertheless, its value in diagnosing PTMC remains controversial. Fine needle aspiration cytology (FNAC) is currently a diagnostic method for thyroid nodules recommended by guidelines [10, 11]; though it has advantages of simplicity, safety, and high repeatability, it is still limited by quality of samples and personal experience of pathologic physicians. The V600E mutation in BRAF gene locus is the most common gene mutation site in PTMC. It has been reported that 52%-87% of PTMC can experience mutations in BRAF gene V600E locus [12].

Our research evaluated clinical application value of HFUS, FNAC, BRAF gene, and combination of HFUS, FNAC, and BRAF genes in diagnosing PTMC. The current report is as follows.

2. Materials and methods

2.1. General data

The 150 patients with thyroid minimal lesions who

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underwent HFUS, FNAC and BRAF gene testing before surgery in our hospital from June 2020 to December 2021 received selection as research subjects, including 54 males and 96 females; mean age was (38.45 ± 3.13) years old; mean body mass index (BMI) was (24.36 ± 1.06) kg/m^2. Inclusive criteria: (1) Thyroid masses can be touched during physical examination; (2) all agreed to undergo pathological examination and have an ideal cooperation level; (3) all voluntarily signed informed consent. Exclusion criteria: (1) With previous history of thyroid surgery; (2) with acute thyroiditis within one month; (3) accompanied by other malignancies; (4) severe atherosclerosis; (5) complicated with other endocrine system diseases; (6) with mental disorders or intellectual disabilities. This research received approval by the Medical Ethics Committee of our hospital.

2.2. Methods

HFUS examination. (1) Instrument: The Vivid E9 color Doppler ultrasound instrument (GE, USA) received utilization, and probe frequency received was set to 7-12 MHz. (2) Examination method: The patients were in a supine position, fully exposing the skin of neck. Firstly, bilateral lobes and isthmus of thyroid gland received conventional scanning, and morphology and internal echo status of thyroid gland received recording. When lesions were found during examination, they received continuous scanning from multiple perspectives and angles. The imaging features and internal blood flow distribution of the nodules received careful observation and detailed recording, longitudinal and transverse diameters at the maximum section of lesion received measurement, and aspect ratio received calculation. Simultaneously, two-dimensional ultrasound and color Doppler ultrasound received application for scanning bilateral cervical lymph nodes. After examination, two experienced imaging physicians jointly observed ultrasound images; joint opinions of two physicians were regarded as diagnostic results, and selection of physicians strictly followed "double blind" principle.

FNAC examination. (1) Fine needle puncture: The patients were in a supine position, skin in the anterior cervical area received disinfection, and sterile sheet was paved. SEQOUTA ultrasound (Siemens) received application for detecting thyroid nodules, and a 25G needle entered suspected nodule under ultrasound guidance, which was repeatedly inserted 10-15 times without negative pressure. The syringe aspired 4-5 mL of air and pushed samples inside the needle onto a slide. The slide received fixation with 95% ethanol and staining with HE. Another needle of cell sample was taken and placed in a centrifuge tube containing 2 mL of physiological saline and stored in a refrigerator at 4℃. (2) Cytological diagnosis: Cell smears were interpreted by the same cytopathologist. According to the Bethesda classification criteria, specimens received division into 6 categories: undetermined or unsatisfactory specimens, benign follicular nodules, atypical cellular lesions with unclear significance, follicular tumors, suspected malignant tumors, and malignant tumors. This research classified suspected malignant tumors and malignant tumors as cytologically positive, while the remaining four were defined as cytologically negative.

BRAF gene mutation detection. (1) DNA extraction: The cytological specimens received centrifugation at 3000 g/min for 10 min, and supernatant received removal. (2) BRAF gene testing: The amplification refractory mutation system (ARMS) method received application for detection through the human BRAF gene V600E mutation detection kit (Amoy Diagnostics Co., Ltd.). The primers received designing and conventional reverse transcription to generate cDNA. The mixture received thawing and mixing with Taq enzyme and DNA specimens to be tested in a ratio of 20:0.25:5, followed by amplification in a PCR instrument. The FAM and HEX signals received collection at 60℃. If sample Ct ≥ 28, it was negative, and if sample Ct < 28, it was positive.

2.3. Observation indicators

(1) The consistency of diagnostic results of HFUS, FNAC, and BRAF gene and their combination with those of pathological examination received analysis. (2) The diagnostic efficacy of HFUS, FNAC and BRAF gene combined detection and individual detection for PTMC lymph node metastasis received comparison based on pathological examination results as golden standard. (3) The diagnostic value of HFUS, FNAC and BRAF gene combined detection and individual detection for PTMC lymph node metastasis received analysis through receiver operating characteristic (ROC).

2.4. Statistical analysis

The data obtained from research received processing using SPSS 27.0 software. Counting data received expression in ratios, and differences between groups received comparison through χ^2 test. Measuring data received expression in ($\chi \pm s$), and differences between groups received comparison using t-test. The diagnostic value of HFUS, FNAC and BRAF gene combined detection and individual detection for PTMC lymph node metastasis received analysis through receiver operating characteristic (ROC). $P < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Comparison of general data between both groups

Patients were divided into two groups based on postoperative pathological results. Negative group: 36 cases in total; 16 males and 20 females; mean age was (45.1 ± 10.3) years old. Positive group: 114 cases in total; 38 males and 76 females; mean age was (45.7 ± 13.1) years old. No statistical significance was exhibited in general data between both groups ($P > 0.05$; Table 1).

Table 1. General data in both groups.

Groups	N	Gender (male/female)	Age
Negative group	36	16/20	45.1 ± 10.3
Positive group	114	38/76	45.7 ± 13.1
χ^2/t		1.466	0.47
P		0.226	0.642

3.2. Analysis of results of HFUS, FNAC and BRAF gene individual detection and combined detection in diagnosing lymph node metastasis in PTMC

Postoperative pathology demonstrated that among 150 patients, there were 114 cases of PTMC, and 36 cases of non-PTMC, including 21 cases of nodular goiter, 14 cases of follicular adenoma, and 1 case of chronic lymphocytic thyroiditis. The consistency of diagnostic results of combined detection with pathological examination exhibited elevation relative to that of HFUS, FNAC and BRAF gene detection alone, indicating statistical significance ($P < 0.05$; Tables 2 and 3).

3.3. Comparison of diagnostic efficacy of HFUS, FNAC, BRAF gene individual detection and combined detection for lymph node metastasis in PTMC

The negative predictive value, sensitivity and accuracy of combined detection exhibited elevation relative to individual detection, indicating statistical significance ($P < 0.05$; Table 4).

3.4. The diagnostic value of HFUS, FNAC and BRAF gene individual detection and combined detection for lymph node metastasis in PTMC

ROC demonstrated diagnostic value of HFUS, FNAC and BRAF gene detection alone and combined detection in PTMC lymph node metastasis (Figures 1 and 2.)

3.5. Comparison of diagnostic value of HFUS, FNAC, BRAF gene individual detection and combined detection for lymph node metastasis in PTMC

The AUC of combined detection in diagnosing PTMC

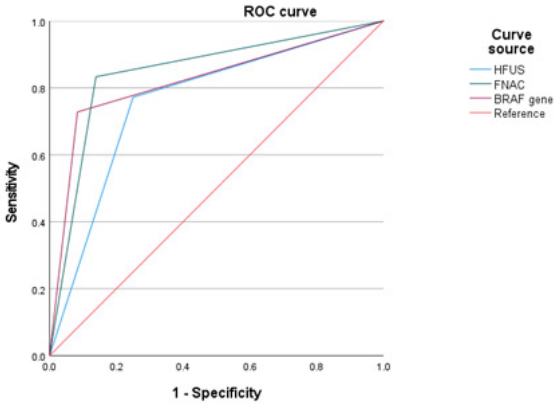

Fig. 1. Diagnostic value of HFUS, FNAC and BRAF gene detection alone in PTMC lymph node metastasis.

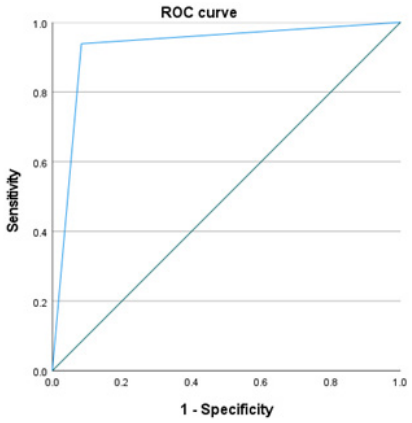

Fig. 2. Diagnostic value of combined detection in PTMC lymph node metastasis.

Table 2. Diagnostic results of combined detection and HFUS, FNAC and BRAF gene detection alone.

Diagnostic methods		Pathological examination results		Total	Kappa
		Positive (n=114)	Negative (n=36)		
HFUS	Present	88	9	97	0.449
	Absent	26	27	53	
FNAC	Present	95	5	100	0.613
	Absent	19	31	50	
BRAF gene	Present	83	3	86	0.509
	Absent	31	33	64	
Combined detection	Present	107	3	110	0.824
	Absent	7	33	40	

Table 3. Consistency of combined detection and HFUS, FNAC and BRAF gene detection alone with pathological examination.

Groups		Combined detection	χ^2	P
HFUS	0.449		29.533	< 0.001
FNAC	0.613	0.824	10.821	0.001
BRAF gene	0.509		21.569	< 0.001

Table 4. Diagnostic efficacy of HFUS, FNAC, BRAF gene individual detection and combined detection for lymph node metastasis in PTMC.

Groups	N	Positive predictive value	Negative predictive value	Sensitivity	Specificity	Accuracy
HFUS	150	90.72 (88/97)#	50.94 (27/53)*	77.19 (88/114)*	75 (27/36)*	76.67 (115/150)*
FNAC	150	95 (95/100)#	62 (31/50)*	83.33 (95/114)*	86.11 (31/36)#	84 (126/150)*
BRAF gene	150	96.51 (83/86)#	51.56 (33/64)*	72.81 (83/114)*	91.67 (33/36)#	77.33 (116/150)*
Combined detection	150	97.27 (107/110)	82.5 (33/40)	93.86 (107/114)	91.67 (33/36)	93.33 (140/150)

Note: Versus combined detection, *$P < 0.05$ and #$P > 0.05$.

Table 5. Diagnostic value of HFUS, FNAC, BRAF gene individual detection and combined detection for lymph node metastasis in PTMC.

Groups		Combined detection AUC	χ^2	P
HFUS AUC	0.761		11.033	< 0.001
FNAC AUC	0.847	0.928	3.269	0.071
BRAF gene AUC	0.822		5.531	0.019

lymph node metastasis exhibited elevation relative to that of HFUS and BRAF gene alone, indicating statistical significance ($P < 0.05$; Table 5).

4. Discussion

The malignancy of PTMC is low, whereas as it progresses, risk of lymph node metastasis remarkably increases, which will elevate mortality to some extent [13]. For lymph node metastasis with small volume and soft texture, it is difficult to make accurate judgments, and clinical staging often differs from actual pathological staging, which may lead to changes in treatment methods and be unfavorable for prognosis [14]. Pathological examination is "golden standard" for diagnosing PTMC, with extremely high accuracy [15]; nevertheless, it is an invasive operation that can cause certain trauma to patients' bodies, and examination time is long, with high expense, thus some patients are unwilling to accept it, which cannot be promoted and applied in clinical practice [16]. Thus, finding a non-invasive, short time-consuming, and low-cost examination method for early diagnosis of PTMC is of great significance for prognosis of PTMC.

It has been demonstrated that preoperative imaging examination for patients can help physicians make judgments on lymph nodes with typical sonographic features, and perform biopsy during surgery, which can lead to early diagnosis and timely intervention, thereby improving prognosis [17]. HFUS possesses advantages of non-invasion, easy operation, high repeatability, etc., making it the preferred examination method for multiple tissue and organ lesions. Nevertheless, when PTMC lymph node metastasis lesions have a small volume, regular shape, clear boundaries, and limited blood flow, it is easy to miss diagnosis; moreover, ultrasound has marked disadvantages in examining lymph nodes in posterior pharyngeal, mediastinal, and some lower regions [18]. FNAC is a quite high-sensitive and specific diagnostic method for thyroid cancer, and it diagnoses thyroid cancer via observing cellular morphological changes under guidance of color Doppler ultrasound [19]. Research has illustrated that accuracy of FNAC in nodules less than 1 cm is 89.1%, while accuracy in nodules over 1 cm is 94.3%, indicating that FNAC has certain limitations in diagnosis of small thyroid nodules [20]. Some misdiagnosed cases may cause confusion for physicians, and radical diagnostic surgery may cause surgical complications and lifelong replacement of thyroid hormones caused by thyroid deficiency; conservative observation may lead to progression and distant metastasis of malignancies. The BRAF gene, a member of the RAF-MEK-ERK signaling pathway, exerts a vital role in tumor cell proliferation, differentiation, and apoptosis [21]. BRAF gene receives classification into wild type and mutation, and the most common site of BRAF gene mutation is V600E [22]. When a mutation occurs at V600E site, BRAF kinase can be activated, further activating related pathways and leading to cancerization. The detection rate of BRAF V600E gene mutation in papillary thyroid cancer is high, which possesses direct relation to classic type and adverse prognosis [23]. Thus, perhaps combination of HFUS, FNAC and BRAF genes can complement each other in diagnosing PTMC.

Herein, we combined HFUS with FNAC, and BRAF genes in diagnosing PTMC, and we discovered that consistency of diagnostic results of combined detection with pathological examination exhibited elevation relative to that of HFUS, FNAC and BRAF gene detection alone, and negative predictive value, sensitivity and accuracy of combined detection exhibited elevation relative to individual detection, suggesting that combined detection in diagnosing PTMC lymph node metastasis presents a marked superiority. This is major because HFUS is a continuous examination that can effectively compensate for shortcomings of FNAC and BRAF genes, while FNAC and BRAF genes are less affected by surrounding tissue and blood flow status, which can reduce occurrence of missed diagnosis due to ultrasound being affected by surrounding tissue. Herein, the AUC of combined detection in diagnosing PTMC lymph node metastasis exhibited elevation relative to that of HFUS and BRAF gene alone, suggesting that combined detection presents high diagnostic value for PTMC lymph node metastasis. Thus, application of combined detection before surgery can determine whether PTMC patients have lymph node metastasis, helping them choose appropriate treatment methods and provide reasonable therapy, thereby improving prognosis.

In conclusion, HFUS combined with FNAC and BRAF genes possesses high diagnostic value, with high diagnostic sensitivity, specificity, and accuracy. Thus, combined detection for PTMC before surgery can accurately determine whether lymph node metastasis occurs, reduce the occurrence of missed diagnosis and misdiagnosis, and thus improve diagnostic precision.

Informed consent

The authors report no conflict of interest.

Availability of data and material

We declared that we embedded all data in the manuscript.

Authors' contributions

LC conducted the experiments and wrote the paper; ZX, ZJG, ZJ and AL analyzed and organized the data; WG conceived, designed the study and revised the manuscript.

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