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Original Article

Ultrasound features suspicious for malignancy predict the risk of BRAF mutation in papillary thyroid carcinoma

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Abstract: Purpose: This study aimed to evaluate the BRAF mutation status in papillary thyroid carcinoma (PTC) on the basis of ultrasound (US) features suspicious for malignancy. Methods: The clinical, pathologic, and radiologic characteristics of patients who were diagnosed with PTC from August 2014 to August 2015 were reviewed. A radiologist who was blinded to the BRAF status, independently reviewed all existing preoperative US examinations and recorded the sonographic characteristics for each PTC case. The US features included echogenicity, composition, height and width, margin, shape, capsule, vascularity, calcification, and number. Univariate and multivariate analyses were performed to determine sonographic predictors. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for each US characteristic that was suspicious for malignancy were calculated. The diagnostic accuracies for predictors of BRAF mutation were calculated via receiver operating characteristic analysis. Results: There was a significant association between echogenicity, composition, height, width, margin, shape, and calcification and BRAF mutation status. However, no association was observed between capsule, vascularity, and number and BRAF mutation status. Poorly defined margins and microcalcification were independent predictors of BRAF mutation via multivariate analysis. PTC with poorly defined margins predicted BRAF mutations with 86.4% sensitivity, 55.0% specificity, and 76.6% accuracy, while microcalcification predicted BRAF mutation with 84.1% sensitivity, 50.0% specificity, and 73.4% accuracy. Conclusions: Poorly defined margins and preoperative microcalcification could effectively predict the BRAF mutation status and provide a valuable guideline for aggressive treatment in PTC. Further investigations should be performed to understand the association between US features and BRAF mutation.

Keywords: Ultrasound features, BRAF mutation, papillary thyroid carcinoma

Introduction

Papillary thyroid carcinoma (PTC) is the most common malignant thyroid neoplasm and accounts for 80% of all thyroid cancer cases. The incidence of PTC has increased in recent decades [1-3].

BRAF is the strongest activator of downstream MEK signaling among the 3 Raf isoforms. Downstream of MEK, ERK phosphorylation activates substrates located in the cytoplasm and nucleus [1]. The $BRAF^{V600E}$ mutation commonly occurs in PTC, with a reported frequency ranging from 25% to 82.3%. Many authors have demonstrated that the $BRAF^{V600E}$ mutation is associated with aggressive clinical features and poor prognosis [4].

Ultrasound (US) has been a valuable tool for characterizing and diagnosing thyroid pathology for many years since its invention. Currently, the rapid technological evolution of US, including color Doppler and three-dimensional imaging, could provide much more precise evaluation of thyroid nodules. For example, US characteristics, such as taller-than-wide shape, microcalcifications, intratumoral hypervascularity, and irregular margins, often predict malignancy [5]. However, whether or not the

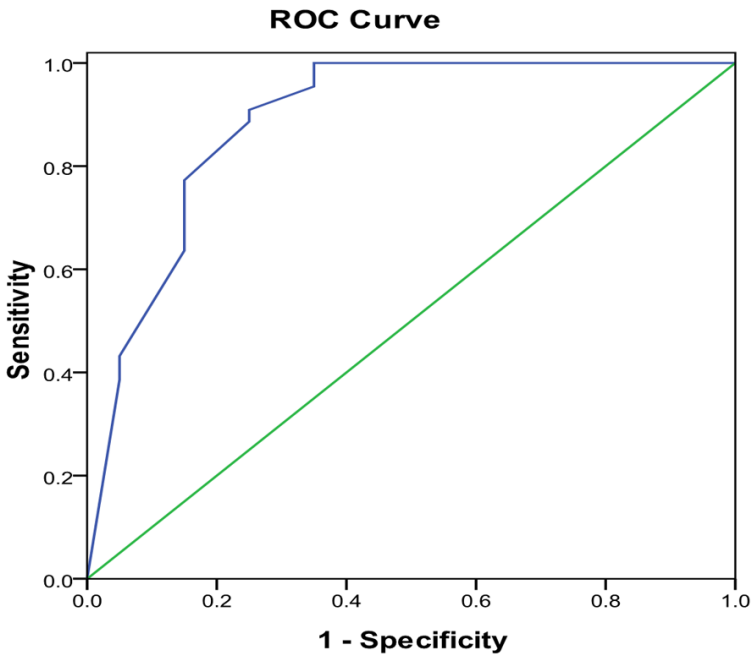

Figure 1. Receiver operating characteristic (ROC) curve of the relationship between malignant ultrasound features and BRAF-positive papillary thyroid carcinoma.

suspicious US features predict the risk of BRAF mutation in PTC remains unknown [6]. In this study, we examined the associations between suspicious US features and BRAF mutation and provide a valuable reference for clinical decisions.

Materials and methods

Clinicopathological patient information

The clinical, pathologic, and radiologic characteristics of patients who were diagnosed with PTC from August 2014 to August 2015 were reviewed. All patients with PTC who underwent total thyroidectomies plus central lymph node dissection, had complete clinical parameters, and underwent molecular testing for $BRAF^{V600E}$ using either the fine needle aspiration or pathology specimen were included. A series of 64 cases that met the inclusion criteria were further studied. This retrospective study was approved by the ethics committees of Union Hospital.

BRAF testing

DNA was extracted from each sample using the commercial kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. A portion of *BRAF* exon 15 that encompassed codon 600 was amplified using polymerase chain reaction (PCR) with specific primers (Forward (wild): 50-AGGTGATTTTGGTCTAGCTAC-AGT (70124-70101); Forward (mutant): 50AGGTGATTTTGG-TCTAGCTACAGA (70124-701-01); Reverse: 50TAGTAACTC-AGCAGCATCTCAGGGC (699-76-70000)), and the amplified product was analyzed with fluorescence-labeled hybridization probes in a melting curve assay on the real-time LightCycler 480 PCR system (Applied Biosystems, Basel, Switzerland). A melting temperature of approximately 65°C corresponded with the wild-type sequence, while melting at approximately 60°C is seen in transcripts expressing the T to A transversion at nucleotide 1799 that results in the V600E mutation. This assay was validated for sensitivity to the V600E mutation detection down to a minimum of at least 25% of the tumor cells in the specimen.

Ultrasonography evaluation

In this study, a single radiologist (Y.W.), who was blinded to BRAF status, independently reviewed all existing preoperative US examinations and recorded the sonographic characteristics for each PTC. Each US examination under review was performed using the Acuson S2000 diagnostic ultrasound system (Siemens Medical Solutions, Berlin, Germany). Patients were positioned in the supine position with a fully exposed neck. A standardized data collection tool was used by the reviewer for the following US features: echogenicity, composition, height and width, margin, calcification, shape, capsule, and number (**Figure 2**).

Statistical analysis

Initial clinical and pathological data were collected using EpiData Software v3.1 (EpiData Association, Odense, Denmark). Comparisons of frequency distributions were performed via a

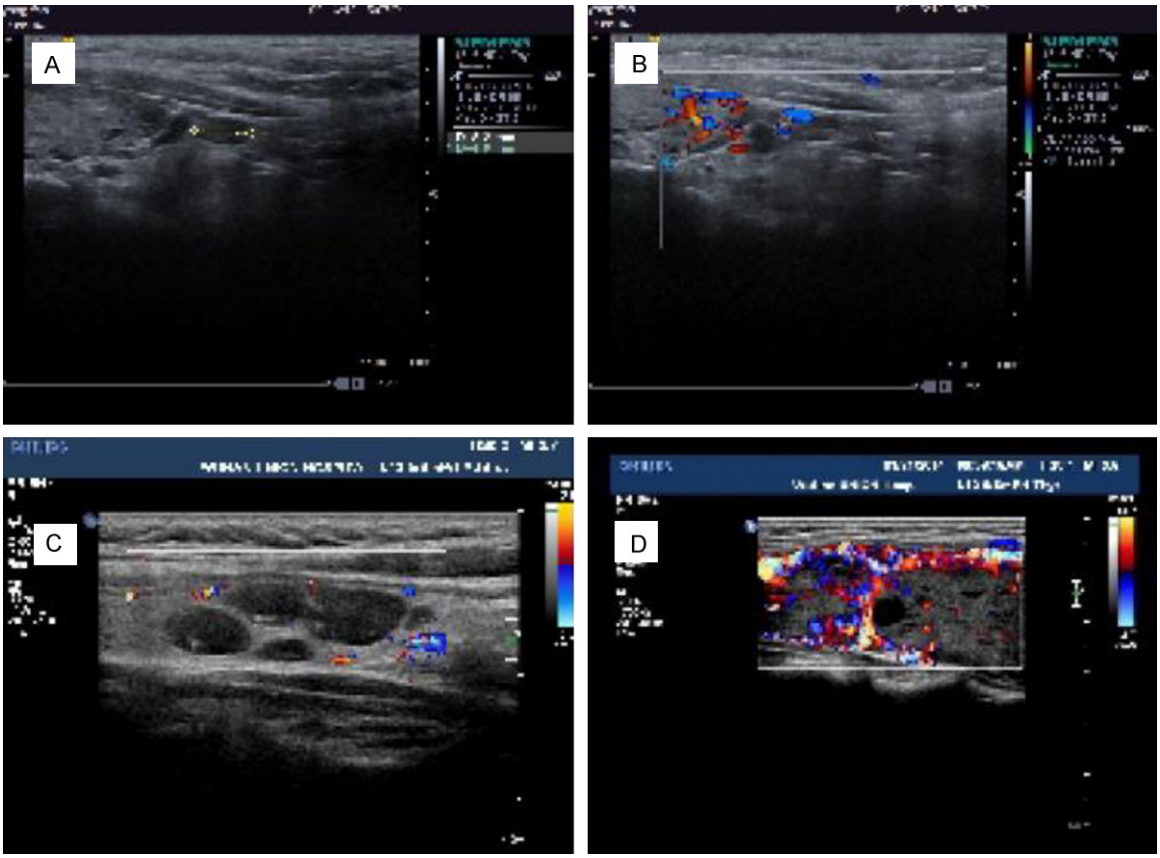

Figure 2. Representative Ultrasonography features. A. Hypoechoic lymph node; B. Taller than wide, Poorly defined, Irregular shape, Incomplete capsule lymph node; C. Microcalcication lymph node; D. Cystic >50%, Abundant vascularity lymph node.

χ^2 test. Multivariate logistic regression analysis was performed to determine independent sonographic predictors. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for each US characteristic suspicious for malignancy were calculated. The diagnostic accuracy of predictions of BRAF mutation was calculated with receiver operating characteristic (ROC) analysis. All statistical analyses were performed by SPSS software, version 13.0 (SPSS, Chicago, IL), and a two-tailed *P* value of less than .05 was considered as statistical significance.

Results

Sixty-four patients were included in our analysis, of whom 44 (68.8%) had BRAF-positive PTC and 20 (31.2%) had BRAF-negative PTC. All correlations between suspicious US features and BRAF mutation are shown in **Table 1**. According to our results, there were significant correlations between echogenicity, composition, height, width, margins, shape, and calcification and BRAF mutation. However, there was no association between capsule, vascularity, or number and BRAF mutation. Compared with patients with PTC that did not express BRAF mutations, PTCs with BRAF mutations were more likely to present as hypoechoic (P< 0.001), solid masses (P=0.001), taller than wide (P=0.021), with poorly defined margins or boundaries (P=0.001), irregular shape (P< 0.001), and microcalcifications (P=0.004) on US. However, capsule (P=0.537), vascularity (P=0.289), and tumor number (P=0.061) did not correlate with BRAF status.

After evaluating the five US features associated with BRAF mutation by chi-squared analysis, we further evaluated independent predictive factors for BRAF mutation by multivariate analysis. Poorly defined margins and microcalcification were independent predictors of BRAF mutation in PTC (**Table 2**).

Table 1. Basic ultrasound features, and BRAF mutation status in PTC

Features	BRAF- (n=20)	BRAF+ (n=44)	P
Echogenicity			<0.001
Hypoechoic	12	42	
Isoechoic or Hyperechoic	8	2	
Composition			0.001
Cystic >50%	7	1	
Predominantly solid or solid	13	43	
Taller and wide			0.021
Oval to round	12	13	
Taller than wide	8	31	
Boundary or margin			0.001
Well defined	11	6	
Poorly defined	9	38	
Shape			<0.001
Regular	9	2	
Irregular	11	42	
Capsule			0.537
Complete	13	25	
Incomplete	7	19	
Vascularity			0.289
Negative or peripheral	6	8	
Central	14	36	
Calcification			0.004
No calcification or Macrocalcification	10	7	
Microcalcication	10	37	
Number			0.061
Solitary	15	22	
Multifocality	5	22	

Table 2. Multivariate analysis of the features that predict BRAF mutation in PTC

US features	B	Sig.	Exp (B)	95% confidence interval for Exp (B)	
				Lower Bound	Upper Bound
Hypoechoic	1.384	0.407	3.991	0.152	104.955
Solid Composition	3.073	0.082	21.617	0.676	690.868
Taller than wide	1.488	0.100	4.429	0.750	26.152
Poorly defined margin	1.819	0.050	6.166	0.979	38.822
Microcalcification	2.169	0.018	8.753	1.449	52.863
Irregular shape	1.905	0.144	6.717	0.521	86.585

PTC with poorly defined margins predicted BRAF positivity with 86.4% sensitivity, 55.0% specificity, and 76.6% accuracy. In addition, PTC with microcalcification predicted BRAF positivity with 84.1% sensitivity, 50.0% specificity, and 73.4% accuracy (**Table 3**). A ROC curve was created to verify the relationship between the number of malignant US features and BRAF positivity (**Figure 1**). The area under the curve was 0.889, indicating that the test was very accurate, as a value of 1.0 represents a perfect test.

Discussion

The BRAF-V600E mutation causes constitutive serine/threonine kinase activation. BRAF mutation correlates with many aggressive clinicopathological characteristics and poor prognosis in PTC, according to many studies [4, 6-10]. Therefore, BRAF mutation is used to guide preoperative and postoperative treatment, including radioactive iodine therapy and targeted therapy. BRAF mutation status may help determine the necessary extent of thyroidectomy as well as the need for and extent of cervical lymphadenectomy. BRAF mutation status can be detected via fine needle aspiration biopsy (FNAB) preoperatively. However, FNAB is invasive and unreliable for thyroid nodules <5 mm in size [11]. Therefore, a safe and feasible approach to detect BRAF status, which contributes to the risk stratification of patients with PTC, would be helpful.

US is an accurate and noninvasive modality used to characterize thyroid nodules and follow patients with PTC. A number of US features are reportedly associated with malignancy, such as height vs. width, poorly defined margins, microcalcifications, and central vascularity [12-16]. Furthermore, lymph node characteristics on US provide valuable information concerning the need for FNAB and the extent of surgical approach, i.e., thyroidectomy or the need for and extent of cervical lymphadenectomy [6, 17]. Total thyroidectomy should be considered for patients with ≥2 US features,

Table 3. Predictive values of US features for BRAF mutation status in PTC

US features	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
Poorly defined margin	86.4	55.0	80.9	64.7	76.6
Microcalcification	84.1	50.0	78.7	58.8	73.4

including microcalcifications, irregular borders, hypoechogenicity, and more height than width [17]. The ability to predict the BRAF mutation status using US characteristics would be very valuable for clinicians, as it would require adopting appropriate treatment measures with a less invasive assessment technique [6].

The significance of BRAF mutation and US features in the management of PTC remains unclear and controversial [6, 18]. Recently, Park et al. suggested that there was no significant correlation between BRAF mutation and US features [18]. However, Kabaker et al. [6] reported that US characteristics, such as larger height than width, poorly defined margins, hypoechogenicity, microcalcification, and missing halo, correlated with BRAF mutation significantly, and the risk of positive BRAF mutation was high (91%) when >4 US features were present in PTC. As a result of these ambiguous results, further research on the correlation between US features and BRAF mutation in PTC is necessary.

In our study, there was a significant correlation between echogenicity, composition, height, width, margins, shape, and calcification and BRAF mutation, and poorly defined margins and microcalcifications were found to be independent predictors of BRAF mutation in PTC. Accurate preoperative risk assessment of BRAF mutation can help identify those PTCs that are more likely to be associated with aggressive histopathology or prognosis in order to inform the extent of initial surgery.

Our study has some limitations. The sample size was not large enough, our study was retrospective, and all the US images were reviewed by a single, experienced radiologist. Additionally, our current study contained only a qualitative analysis, whereas quantitative analysis should be used in future studies. The mechanism that underlies BRAF-mutant tumors presenting with these US features should also be further analyzed.

Conclusion

Poorly defined margins and microcalcifications could effectively predict the BRAF mutation status preoperatively and provide a valuable screening tool for patients who may benefit from surgical adjuncts, such as prophylactic central compartment lymph node dissection. Further investigation should be performed to assess the association between US features and BRAF mutation.

Disclosure of conflict of interest

None.

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