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대한영상의학회지

Prevalence of the B Type Raf Kinase V600E Mutation in Cytologically Indeterminate Thyroid Nodules: Correlation with Ultrasonographic and Pathologic Features[1]

세침흡인술로 확인된 비결정 갑상샘 결절에서의 B Type Raf Kinase V600E Mutation의 유병률 및 영상의학적 소견[1]

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Purpose: To study the prevalence of B type Raf kinase (BRAF) mutations, and to evaluate the ultrasonographic and clinicopathological features associated with thyroid cytology of indeterminate nodules.

Materials and Methods: We assessed the presence or absence of BRAF mutation in 44 specimens from patients with cytologically indeterminate thyroid nodules according to two consecutive preoperative fine needle aspiration cytology procedures. In 9 specimens, the test for BRAF mutation was not possible due to scant cellularity. DNA was extracted from the atypical cells and then analyzed for the BRAF V600E mutation by pyrosequencing. The ultrasonographic and clinicopathological features of the patients were characterized according to their mutation status.

Results: The BRAF V600E mutation was present in 17 (48.6%) of 35 patients with indeterminate cytology results and in 17 (54.8%) of the 31 patients with papillary thyroid cancer (PTC). Twenty two of 35 cytologically indeterminate nodules had calcifications, and among them 14 cases were proven to be positive for BRAF V600E mutations. Extrathyroid extension was significantly more frequent in the presence of the BRAF V600E mutation (p = 0.027), while tumor size, lympho-vascular invasion, or lymph node metastasis were not associated with the mutation.

Conclusion: Screening for BRAF V600E mutations in conjunction with cytology may increase the diagnostic accuracy for PTC with indeterminate cytology results.

Index terms
Atypia
Fine-Needle Aspiration
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INTRODUCTION

Ultrasonography-guided fine needle aspiration (US-FNA) is the most accurate method for distinguishing malignant thyroid nodules from benign nodules. It is, however, an operator-dependent method that is limited by indeterminate cytological results in 22-42% of cases (1, 2). Persistent indeterminate or non-diagnostic cytology results with no clear distinction between benign and malignant cells can be problematic. It has been reported that 10-52% of thyroid nodules with indeterminate cytological findings are ultimately diagnosed as malignant on post-surgical pathological reports (3, 4). Previously, to avoid missed malignancies, surgical excisions were done or intraoperative frozen sections were acquired in the treatment of thyroid nodules with indeterminate cytology (5, 6). In recent efforts to avoid unnecessary surgical procedures in cases of indeterminate cytology, many investigators have sought molecular markers that will improve the reliability of cytology-based diagnostics. The B type Raf kinase (BRAF) gene is one of the most studied markers because of its high reliability

and testing feasibility in the pre-operative stage (7-10). A cytology-based BRAF mutation test can give certain clinical information, but not all papillary thyroid cancer (PTC) cases carry BRAF mutations. Thus, the mutation alone is not sufficient to diagnose PTC. Since the prevalence of BRAF V600E is reportedly much higher (73-84%) in Korean PTC patients (11, 12) compared to PTC patients in western countries (29-69%) (13), we hypothesized that a BRAF mutation test in conjunction with a cytology exam would help identify the true status of thyroid nodules diagnosed as indeterminate on fine needle aspiration cytology (FNAC). Therefore, we assessed the prevalence of the BRAF mutation and ultrasonographic and clinicopathologic features of thyroid nodules with indeterminate cytology.

MATERIALS AND METHODS

Patient Selection

This retrospective study was approved by the institutional review board at our institution. The patient dataset was reviewed to specifically identify individuals with preoperative FNA demonstrating cytologic findings that were indeterminate for malignancy. We studied patients who presented between January 2008 and December 2009 at our institution. Patients who underwent US-FNA of a thyroid nodule with suspicious malignant ultrasonographic features and indeterminate cytology results all underwent a second FNA performed by the same radiologist to exclude the possibility of technical problems. Despite a minimum of two repeat samples with ultrasonographic features of at least one malignant finding, cases with indeterminate cytology results underwent surgical procedures. Of these cases, 44 were selected for the BRAF mutation study (Fig. 1).

Definition of Indeterminate Cytology Cases

Indeterminate cytology was defined as atypia of undetermined significance; these specimens contained cells (follicular, lymphoid, or other) with insufficient architectural and/or nuclear atypia to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignant according to the Bethesda system for reporting thyroid cytopathology (14). Most cases included focal features suggestive of papillary carcinoma including nuclear grooves, enlarged nuclei with pale chromatin, and alterations in the nuclear contour and shape in an otherwise predominantly benign-appearing sample. Some samples with preparation artifacts such as air-drying artifacts or clotting artifacts were included when enough follicular cells were present. The diagnostic correlation was

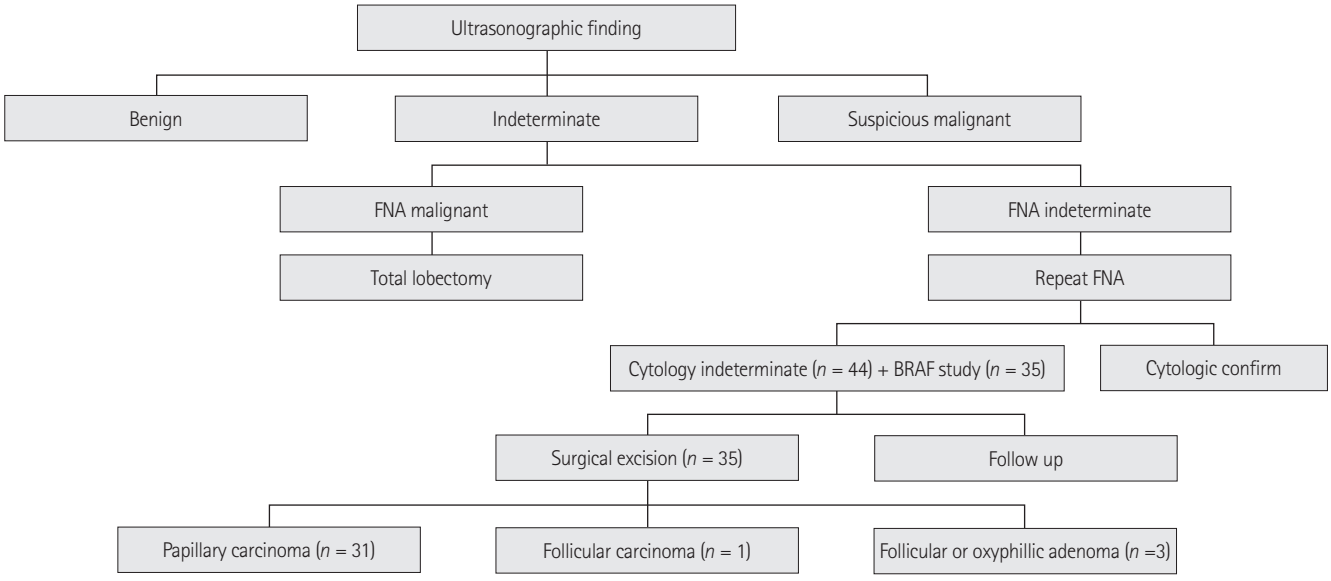

Fig. 1. Flowchart of the strategy for follow-up US and US-guided fine needle aspiration (US-FNA) biopsy according to US findings and cytology results for thyroid nodules.
Note.—BRAF = B type Raf kinase, US = ultrasonography

restricted to cases in which the cytology was reported as indeterminate within a six-month period preceding the histology report. We reviewed and analyzed the features of specimens diagnosed as indeterminate on preoperative FNAC and correlated the results with a cytology-based BRAF mutation analysis and with a final histologic diagnosis.

US-Guided FNA

For all patients examined, US images were obtained with a LOGIQ 9 ultrasound scanner (GE Medical Systems, Milwaukee, WI, USA), which was used for guidance with broad band high-frequency (7-15 MHz) linear transducers. US-FNA was performed on solitary or multiple thyroid nodules by a single board-certified radiologist using a 22-gauge needle attached to a 10-mL syringe with 2-4 passes through the target lesion. The specimens were smeared on slides, fixed immediately in 95% ethanol, and stained using the Papanicolaou method. All the nodules selected for FNA, regardless of size, had at least one malignant ultrasonographic finding such as marked hypoechogenicity, microlobulated or irregular margins, microcalcifications, or a nonparallel shape. Repeated FNA was performed at two-month intervals when the cytology results were indeterminate or non-diagnostic due to scanty cellularity.

DNA Isolation from FNA Samples

A pathologist reviewed all the slides, chose one for each case that contained the most atypical cells, and extracted DNA using the slide scrape lysate method and a single-edged razor blade. Following manual microdissection, a 50.0 μL aliquot of Proteinase K (3 mg/mL) digestion solution (50 mM Tris, 1 mM EDTA, pH 8.0, 1% Tween 20) was pipetted onto the scraped area of the slide to pick up any remaining cells. Using the same pipette tip, the digestion solution was then pipetted from the slide and used to rinse the scalpel blade that was positioned inside the labeled microcentrifuge tube.

DNA Extraction

DNA was extracted from five 10-μm thick paraffin sections containing a representative portion of tumor tissue. DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissue was performed using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Amplification of the B-Raf Gene

Fifty nanograms of DNA were amplified in a 20 μL reaction solution containing 10 μL of 2 X concentrated HotStarTaq Master Mix (Qiagen, Hilden, Germany), including polymerase chain reaction (PCR) buffer with 3 mM $MgCl_2$, 400 μM of each dNTP, and 0.3 μM of each of the primer pairs (exon 15, F: 5'-ATGCTTGCTCTGATAGGAAAATGA, R: 5'-AGCAGCATCTCAGGGCCA). Amplifications were performed using a 15-minute initial denaturation time at 95°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C, 45 seconds at 72°C, and a 10-minute final extension at 72°C. PCR products were then 2% gel-purified with a QIAgen Gel Extraction Kit (Qiagen, Hilden, Germany).

Direct Sequencing

DNA templates were processed for the DNA sequencing reaction using the ABI-PRISM BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA) with both forward and reverse sequence-specific primers. Twenty nanograms of purified PCR products were used in a 10 μL sequencing reaction solution containing 1 μL of BigDye Terminator v3.1 and 0.1 μM of the same PCR primer. Sequencing reactions were done using 25 cycles of 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C. Sequence data were generated with the ABI PRISM 3100 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence variations were compared using Sequencing Analysis 5.1.1. software (Applied Biosystems, Foster City, CA, USA).

Data and Statistical Analysis

Statistical comparisons were performed using a *t*-test for parametric variables and the chi-square test and Fisher's exact test for nonparametric variables. A p-value < 0.05 was considered statistically significant. All statistical tests were performed using the personal computer Statistical Package for the Social Sciences for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Analysis of Data for Patients Who Underwent a BRAF Mutation Test

Among the 44 patients, only 35 patient slides were available for the BRAF mutation test because we excluded 9 slides that

had insufficient levels of tumor DNA after nucleic acid preparation. We identified 35 consecutive patients (29 women and 6 men), who subsequently underwent a hemi (n = 17) or total (n = 18) thyroidectomy. The mean age of the patients at the time of the operation was 46.4 years (range, 25-61 years). The mean nodule size was 0.81 cm and ranged from 0.20 cm to 2.80 cm. On surgical pathology, there were 31 papillary cancers (89%), 1 follicular carcinoma, and 3 follicular or oxyphillic adenomas.

Prevalence of the BRAF V600E Mutation in Indeterminate Thyroid Fine-Needle Aspirates

We identified 17 (48.6%) BRAF transversion mutations in 35 thyroid aspirates with indeterminate results by the fine needle aspiration biopsy (FNAB) method. Additionally, 17 nodules proved to be papillary thyroid cancer on histopathology.

Clinicopathologic Features of the Indeterminate Cytology Cases

No significant statistical differences were found for sex, patient age, or lesion size between the group with the mutation and the group without the BRAF V600E mutation. Lymphovascular invasion or lymph node metastasis accompanying PTC was independent of whether the PTC was positive or negative for the BRAF V600E mutation, but a significantly higher frequency of extrathyroidal extension was noted in PTCs positive for the BRAF mutation (Table 1).

The clinicopathological features of the nodules with a corresponding BRAF V600E mutation test and final histology are listed in Table 2. PTC with the BRAF V600E mutation was more frequently found in women than in men (82.4% vs. 17.6%, respectively). Of the 17 patients with the BRAF V600E mutation, 12 had papillary microcarcinoma (PMC), which is de-

Table 1. Clinicopathological Features of Indeterminate FNA Nodules (n = 35)

Clinicopathological Features	BRAF Mutation (-) (n = 18)	BRAF Mutation (+) (n = 17)	p-value
LVI (n = 1)	1 (5.6%)	0 (0%)	1.000
ETE (n = 10)	2 (11.1%)	8 (47.1%)	0.027
LNM (n = 7)	4 (22.2%)	3 (17.6%)	1.000

Note.—BRAF = B type Raf kinase, ETE = extrathyroidal extension, FNA = fine needle aspiration, LNM = lymph node metastasis, LVI = lymphovascular invasion

Table 2. Clinicopathological Features of BRAF V600 Mutation-Positive Patients (n = 17) among 35 Indeterminate FNA Cases

Sex	Age (y)	Pathology	Size (cm)	ETE	LNM	Treatment
F	58	PTC	1.2	Yes	No	Total
F	28	PMC	0.6	No	No	Total
F	37	PMC	0.8	No	No	Lobectomy
F	51	PTC	1.1	Yes	No	Total
M	30	PTC	1.3	No	No	Lobectomy
F	58	PTC	1.2	No	No	Total
F	47	PMC	0.8	No	No	Total
M	61	PMC	0.6	Yes	No	Total
F	52	PMC	0.5	Yes	No	Total
F	41	PMC	0.6	No	No	Lobectomy
F	49	PMC	0.7	Yes	Yes	Total + RND
F	48	PMC	0.5	Yes	No	Total
F	34	PTC	1.5	No	No	Total
F	61	PMC	0.4	No	Yes	Total + RND
F	46	PMC	0.7	Yes	Yes	Total + RND
M	39	PMC	0.9	Yes	No	Total
F	49	PMC	0.5	No	No	Lobectomy

Note.—BRAF = B type Raf kinase, ETE = extrathyroidal extension, F = female, FNA = fine needle aspiration, LNM = lymph node metastasis, M = male, PMC = papillary microcarcinoma, PTC = papillary thyroid carcinoma larger than 1 cm, RND = radical neck dissection

fined as being equal to or less than 1.0 cm, while only 5 cases had a PTC larger than 1.0 cm. Extrathyroidal extension was identified in 8 patients (47%) - two PTCs and six PMCs. All cases (n = 3) of lymph node metastasis were present with PMC.

Ultrasonographic Features of Nodules with Persistent Indeterminate Cytology

The ultrasonographic features of nodules with a corresponding BRAF mutation test and final histology are summarized in Table 3. All cases of indeterminate cytology that proved to be malignant at final histopathology were spongiform in appearance with an irregular margin (n = 6), peripheral rim calcifications (n = 8) (Figs. 2-4), coarse internal calcifications (n = 4), taller than wide with hypoechogenicity (n = 7) (Figs. 5-7), and hypoechoic, irregularly marginated with internal microcalcifications (n = 10). Twenty two of 35 indeterminate specimens showed calcifications including peripheral rim calcifications, coarse internal calcifications, or internal microcalcifications. Among them, 14 nodules were proven to be positive for the BRAF V600E mutation.

DISCUSSION

Diagnostic Relevance of the BRAF Mutation in a Thyroid Lesion Work-up

The clinical implications of "non-diagnostic" FNAB have yet to be defined. The number of consecutive FNABs needed in cytologically indeterminate cases and the duration of follow-up has not achieved world-wide consensus. Also, there are issues surrounding the justification of indicating surgical treatment for persistent "non-diagnostic" FNAB. We aimed to determine if the expression of a BRAF mutation in non-diagnostic cytology is a useful indicator of patient selection for surgery. In our study, we found that BRAF mutation analysis

Table 3. Predominant Ultrasonographic Features of Cytologically Indeterminate Nodules Positive for the BRAF Mutation

Predominant Ultrasonographic Feature	No. of Nodules (n = 35)	No. of BRAF Mutation-Positive
Spongiform nodule	6	1/6 (16.7%)
Peripheral rim calcifications	8	4/8 (50.0%)
Coarse internal calcifications	4	2/4 (50.0%)
Taller than wide	7	2/7 (28.6%)
Irregular marginated hypoechoic nodule with internal microcalcifications	10	8/10 (80.0%)

Note.—BRAF = B type Raf kinase

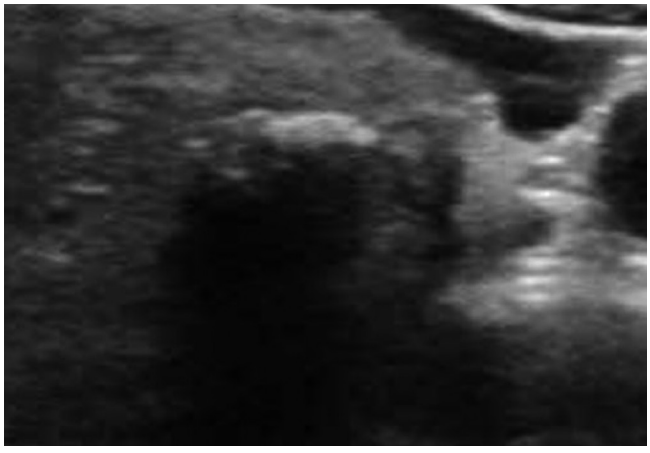

Fig. 2. A 58-year-old woman with a papillary thyroid carcinoma positive for the BRAF V600E mutation. Note the thyroid nodule has an irregular margin with hypoechoic lesion through the disrupted peripheral rim calcification, suggestive of a malignant nodule. However, most of the lesion cannot be characterized, including the internal echogenicity and presence of internal microcalcification, because of the strong posterior shadow accompanying the dense rim calcification.
Note.—BRAF = B type Raf kinase

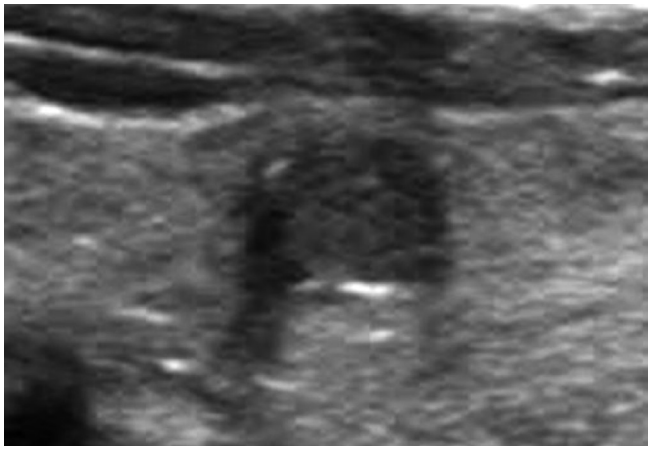

Fig. 3. A 50-year-old woman with a BRAF V600E mutation positive papillary thyroid carcinoma. The nodule has marked hypoechoic internal echogenicity and peripheral rim calcification, as well as a spiculated margin, particularly at the right aspect, indicating malignant ultrasonographic features.
Note.—BRAF = B type Raf kinase

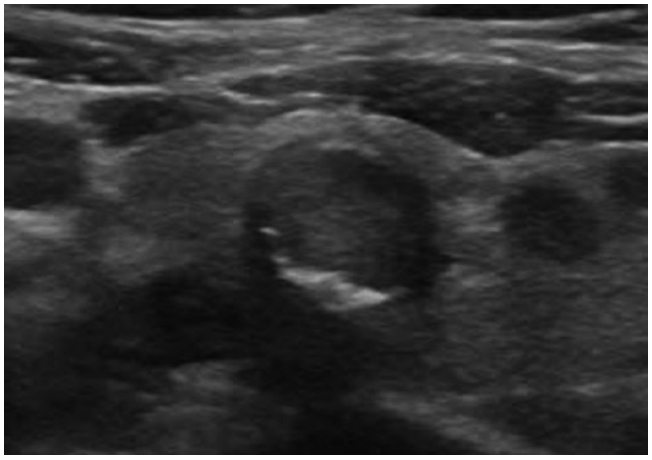

Fig. 4. A 48-year-old woman with a papillary thyroid carcinoma and negative for the BRAF V600E mutation. The thyroid nodule with peripheral rim calcification does not have any ultrasonographic features suggesting malignancy (unlike Fig. 2 and Fig. 3) and it was regarded as a benign nodule on preoperative ultrasonography.
Note.—BRAF = B type Raf kinase

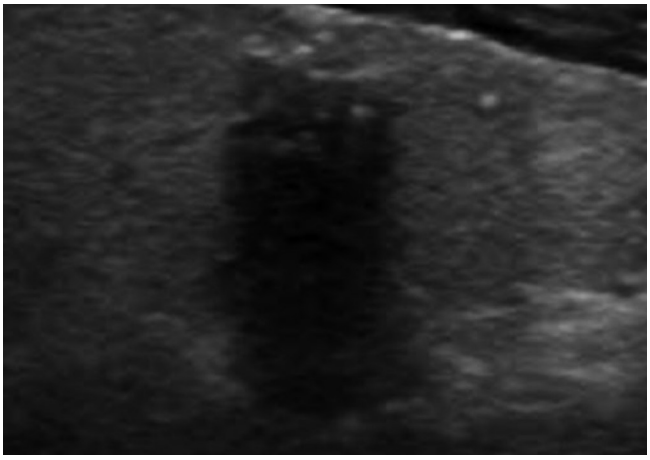

Fig. 6. A 50-year-old woman with a papillary thyroid carcinoma that was negative for BRAF V600E mutation. The taller-than-wide nodule has an irregular margin and marked low internal echogenicity, as well as internal microcalcifications. The preoperative ultrasonographic diagnoses was malignancy.
Note.—BRAF = B type Raf kinase

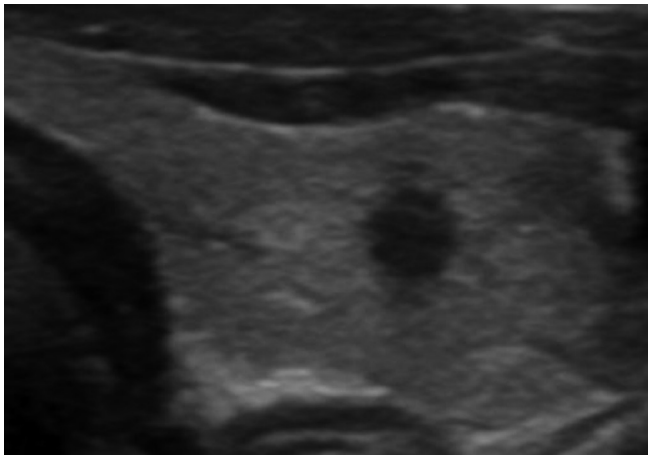

Fig. 5. A 51-year-old woman with a BRAF V600E mutation negative papillary thyroid carcinoma in the left thyroid lobe. Transverse gray-scale US image shows a marked hypoechoic, irregular, and taller-than-wide nodule. It was considered to be a suspicious malignant thyroid nodule.
Note.—BRAF = B type Raf kinase

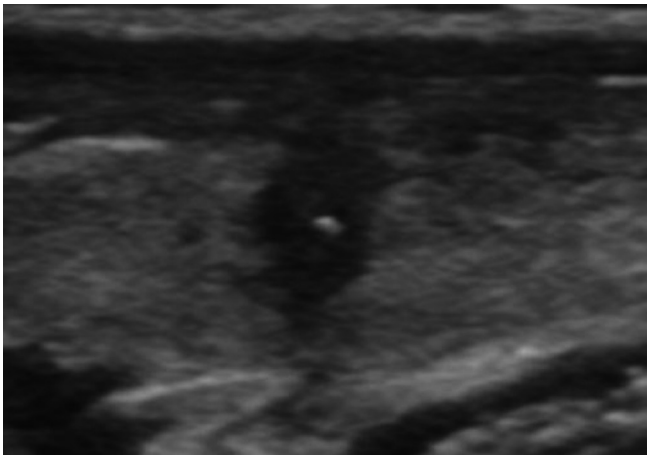

Fig. 7. A 49-year-old woman with a BRAF V600E mutation positive papillary thyroid carcinoma. A marked hypoechoic taller-than-wide nodule with an irregular margin and internal microcalcification in the left thyroid was considered malignancy on ultrasound.
Note.—BRAF = B type Raf kinase

of indeterminate specimens aided in the decision between surgery and follow-up in half of the cases when the nodule was less than 1 cm. According to Salvatore et al. (8), BRAF mutations are rarely identified in non-diagnostic FNAs due to the lack of DNA. In our study, we excluded all the slides that showed inadequate cytology due to scanty cellularity (probably due to insufficient levels of tumor DNA in nucleic acid preparations). We were able to test for BRAF mutations using a direct pyrosequencing method in 35 of 44 (80%) paraffin-embedded FNA tissue samples.

The prevalence of the BRAF mutation in thyroid nodules with indeterminate cytological diagnoses is reported to range from 8% to 16% of cases (7-10, 15-18). In our study, we noted BRAF mutations in more than 40% of cases and we presume that this is due to nodules we selected having more than one ultrasonographic feature suspicious for malignancy. Of the nodules we encountered that were less than 1 cm in size (n = 20), 12 (60%) were positive for the BRAF mutation. The presence of this mutation may be useful for determining the need for a surgical lobectomy. For patients that were negative for

the BRAF mutation (8/20), the surgeon proceeded to perform a frozen biopsy and wait for the results, only performing total thyroidectomy when a malignancy was confirmed.

The limitation of the BRAF mutation test in preoperative diagnosis is in its sensitivity for diagnosing malignancy, but as our study shows, about 60% of PMC cases carried the BRAF mutation. There is a further need for the development of adjunctive diagnostic assays that would help diagnose nodules with indeterminate cytology. We think that the BRAF mutation could serve as an adjunctive diagnostic tool to overcome the limitations of FNAB because the BRAF V600E mutation is highly specific for PTC. The BRAF V600E mutation analysis increased the accuracy of papillary thyroid carcinoma diagnosis by fine-needle aspiration biopsy, which can be limited by the presence of atypical cytologic patterns where a treatment concept is not established. Considering our analysis of the BRAF mutation in preoperative FNA smears, the diagnosis of PTC would have been affirmed in 48.6% (17/35) of indeterminate or suspicious FNA smears. Of the 35 indeterminate FNAB specimens, 17 (48.6%) BRAF mutations were found and 17 of the nodules proved to be papillary thyroid cancer on histopathology. DNA sequencing analysis for detecting a BRAF V600E mutation had a positive predictive value of 100%.

The size of the PTCs that had a pre-operative indeterminate cytology result was relatively small, ranging from 0.20 cm to 2.80 cm, with a mean size of 0.81 cm (standard deviation, 0.32). It is controversial whether PMC holds a significant risk of local or distant metastasis or recurrence. In our study, however, PMCs, as well as larger PTCs, demonstrated aggressive clinicopathological features, and these findings were consistent with those from a previous report by Park et al. (19). Therefore, PMC should not be underestimated and lesions that appear as suspicious malignant features on ultrasonography, even if smaller than 10 mm, should be evaluated using FNAB and molecular analysis. However, a large-scale prospective study to determine the acceptable criteria for identifying proper candidates for molecular investigation is needed, and this may increase the value of the BRAF mutation analysis as an adjunctive diagnostic tool.

We classified the ultrasonographic findings of enrolled persistent cytologically indeterminate nodules, and noted the common presence of calcifications in 22 of 35 cases, including 8 peripheral rim calcifications, 4 internal coarse calcifications, and 10 microcalcifications. In any shape or form, the existence of calcifications seems to make the decision difficult for the radiologist in rendering a diagnosis of malignant or benign. Although microcalcifications are a characteristic ultrasonographic feature of malignant nodules (20-22), they often appear in benign thyroid nodules as well. Furthermore, there is no established consensus about the predictability of benignity or malignancy of internal coarse macrocalcification or peripheral rim calcification of thyroid nodules. The strong posterior shadow accompanying the macrocalcification or rim calcification can hinder the precise characterization of the calcified nodule. In the same context, whether or not to regard the 12 nodules with macrocalcifications or peripheral rim calcifications in our study as suspicious malignant nodules could be a matter of debate. The BRAF V600E mutation was confirmed in more than half of the thyroid nodules exhibiting calcifications. In cases with indeterminate cytology results, identifying the presence or absence of the BRAF V600E mutation can facilitate the decision-making process because of its relatively high sensitivity and reliable specificity.

Recently, there have been divergent opinions regarding the relationship between the BRAF mutation and aggressive clinicopathologic features that may have an influence on the worst case prognosis in a patient. Many investigations have revealed that mutated PTCs exhibit more aggressive features such as lymph node metastasis, extrathyroidal extension and distant metastasis (23-27). Conversely, some studies failed to show this correlation between the occurrence of the BRAF mutation and its prognostic factors (28). In our study, extrathyroidal extension was significantly more frequent in the presence of the BRAF V600E mutation ($p = 0.027$), while tumor size, lympho-vascular invasion, and lymph node metastasis were not associated with the mutation. However, it is difficult to investigate the exact effect of BRAF mutation on the high risk clinicopathologic characteristics of PTC due to the relatively small number of patients included in this study.

The limitations to this study include potential interobserver variability in that we only included patients who had two consecutive incidences of indeterminate cytology results under the somewhat strict definitions defined in the Materials and Methods section. Further, a selection bias was introduced when we selected the slides to perform the BRAF mutation test from

among the patients with indeterminate cytology results. In addition, this was a retrospective study and among 44 patients with two consecutive US-FNABs, only 35 patients were able to be tested for BRAF V600E mutation status. A high degree of selection bias was introduced when analyzing the image features of selected thyroid nodules because only patients with ultrasonographic features suspicious of malignancy underwent consecutive FNAs leading to a surgical procedure, despite the fact that the cytology results really gave no specific information in terms of whether the nodule was suspected to be malignant or benign. Also, thyroid malignancies with the BRAF V600E mutation had higher rates of extrathyroidal extension and a higher stage than those without the mutation; however, the number of selected patients was too small to prove the relationship between BRAF V600E mutation and clinicopathologic features. Furthermore, routine lateral neck dissections were not performed in all cases, and follow-up exams were not performed.

Because the prevalence of the BRAF V600E mutation was very high for PTC cases, the results of a previous report (29) indicate that pre-operative molecular detection of the BRAF mutation would have helped to establish the diagnosis and make therapeutic decisions. In our study, DNA sequencing analysis to detect the BRAF V600E mutation had a positive predictive value of 100%, thus ensuring the diagnosis of PTC when the BRAF V600E mutation was detected on the pre-operative examination of the FNAB thyroid nodule specimens.

Preoperative knowledge of the BRAF mutation status of indeterminate cytology results obtained through US-FNA could be valuable in guiding the management of PTC, as well as determining the extent of initial surgical treatment in countries with a high prevalence of BRAF in papillary thyroid cancer. Detection of the BRAF mutation in FNA specimens is feasible and could be used as an adjunct tool for preoperative diagnosis of PTC cases classified as indeterminate or suspicious with conventional cytology.

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세침흡인술로 확인된 비결정 갑상샘 결절에서의 B Type Raf Kinase V600E Mutation의 유병률 및 영상의학적 소견[1]

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목적: 세포병리검사상 비결정 갑상샘 결절을 갖는 환자의 B type Raf kinase (이하 BRAF) 유전자변이의 유병률과 결절의 초음파 소견 및 임상병리적 특성에 대해 알아봄으로써 BRAF 유전자변이 검사의 진단적 유용성을 알아보고자 한다.

대상과 방법: 수술 전 세침흡인술에서 2회 연속 비결정 갑상샘 결절로 확인된 44명 중 세포가 불충분하여 유전자검사를 할 수 없었던 9명을 제외한 35명의 비정형 세포의 DNA를 추출하여 pyrosequencing 기법으로 BRAF 유전자변이 여부를 분석하고, 유전자변이 여부에 따른 초음파 소견과 임상병리적 특성에 대해 알아보았다.

결과: 35명의 세포병리검사상 비결정 갑상샘 결절 환자 중 17명(48.6%)에서 BRAF 유전자변이가 있었으며, 이는 31명의 갑상샘유두암종 환자의 54.8%를 차지하였다. 35개의 세포병리검사상 비결정 결절 중 22개는 석회화를 포함하였고, 이 중 60% 이상(14/22)에서 BRAF 유전자변이가 확인되었다. BRAF 유전자변이군은 그렇지 않은 환자군에 비해 갑상샘외침범이 더 자주 동반되었으나(p = 0.027), 종양의 크기, 림프-혈관계 침범, 그리고 림프절 전이는 두 환자군 간에 유의한 차이를 보이지 않았다.

결론: 세포병리검사상 비결정 갑상샘 결절에서 BRAF 유전자변이검사를 함께 시행함으로써 갑상샘유두암종에 대한 진단적 정확성을 높일 수 있다.

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