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$BRAF^{V600E}$ Mutation is a Strong Preoperative Indicator for Predicting Malignancy in Thyroid Nodule Patients with Atypia of Undetermined Significance Identified by Fine Needle Aspiration

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세침흡인검사 결과 Atypia of Undetermined Significance로 진단된 갑상선 결절에서 악성을 예측할 수 있는 위험인자

최혜랑 · 최보윤 · 조재훈 · 임영창

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Background and Objectives This study aimed to identify a reliable preoperative predictive factor for the development of thyroid cancer in patients with atypia of undetermined significance (AUS) identified by fine needle aspiration biopsy (FNAB).

Subjects and Method This was a retrospective cohort study. Two hundred and ninety-nine patients diagnosed with AUS by preoperative FNAB who underwent curative thyroid surgery at our institution between September 2005 and February 2014 were analyzed. Clinical, radiological and molecular features were investigated as preoperative predictors for postoperative permanent malignant pathology.

Results The final pathologic results revealed 36 benign tumors including nodular hyperplasia, follicular adenoma, adenomatous goiter, nontoxic goiter, and lymphocytic thyroiditis, as well as 263 malignant tumors including 1 follicular carcinoma and 1 invasive follicular carcinoma; the rest were papillary thyroid carcinomas. The malignancy rate was 87.9%. The following were identified as risk factors for malignancy by univariate analysis: $BRAF^{V600E}$ gene mutation, specific ultrasonographic findings including smaller nodule size, low echogenicity of the nodule, and irregular or spiculated margin ($p<0.05$). Multivariate analysis revealed that only $BRAF^{V600E}$ mutation was a statistically significant risk factor for malignancy ($p<0.05$). When $BRAF^{V600E}$ mutation was positive, 98.5% of enrolled patients developed malignant tumors. In addition, the diagnostic rate of malignancy in these cases was approximately 16-fold higher than BRAF-negative cases.

Conclusion Patients with AUS thyroid nodules should undergo $BRAF^{V600E}$ gene mutation analysis to improve diagnostic accuracy and if the mutation is confirmed, surgery is recommended due to the high risk of malignancy.

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Key Words Atypia of undetermined significance · $BRAF^{V600E}$ · Fine needle aspiration biopsy · Thyroid cancer · Thyroid nodule.

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Introduction

Thyroid nodules are commonly detected by diagnostic imaging techniques such as ultrasonography (US) that are now widely used.[1,2] As a result, the prevalence of unexpected thyroid nodules has increased by up to 67% with the improved resolution capacity of US.[3] Fine needle aspiration biopsy (FNAB) is a useful technique that is used worldwide to evaluate thyroid nodules and is a simple, cost-effective and relatively accurate test for differentiating benign from malignant nodules.[4-6] The results of thyroid cytopathology following FNAB are categorized using the Bethesda System for Reporting Thyroid Cytopathology (BSRTC), which is now recognized as a standardized reporting system.[7-9] Despite this, the diagnostic value of atypia of undetermined significance (AUS) as defined by Bethesda criteria is still vague.[10,11]

According to the 2017 BSRTC edition, the risk of malignancy of AUS increases by 10–30% when noninvasive follicular thyroid neoplasm with papillary-like nuclear features are included.[12] Due to the ambiguous interpretation of indeterminate nodules, the optimal management of AUS is still unresolved.[2] The usual management options for nodules in this category are repeated FNAB, additional molecular testing or diagnostic thyroid lobectomy, which highlights the lack of standardization among treatment guidelines for patients with AUS.[12] Therefore, the identification of a preoperative predictive factor for the development of pathologically confirmed thyroid cancer will be extremely valuable in deciding whether a surgical procedure is necessary or if observation will suffice. To date, there are no definitive predictors to identify postoperative confirmed thyroid cancer in AUS cases. Although some previous reports suggested possible predictive factors, these studies enrolled very small patient numbers.[13,14] A large cohort study of AUS nodules followed by surgical pathologic confirmation providing statistically significant predictors for malignancy has not yet been reported.

In this study, we investigated preoperative factors including demographical factors, US features, and molecular findings to predict pathologically confirmed thyroid cancer in patients with AUS thyroid nodules. The present study provides a valuable guideline to aid decision making with regard to further treatment in these patients.

Subjects and Method

Two hundred and ninety-nine patients who underwent thyroid surgery following diagnosis of AUS by preoperative FNAB at Konkuk University Hospital (Seoul, Korea) between September 2005 and February 2014 were retrospectively reviewed. Patients were aged between 23 and 82 years, with a mean age of 64.25 years.

All subjects underwent at least one US-guided FNA prior to surgery. FNAB results were analyzed according to the Bethesda classification; category III AUS was diagnosed when cytologic findings were not definitively benign but cytologic or architectural atypia was not sufficient for diagnosis of follicular neoplasm or suspicious malignancy. $BRAF^{V600E}$ gene mutational analysis was performed as previously reported by Kim, et al.[15] Briefly, extracted DNA from atypical cells obtained by FNA were amplified and pyrosequencing mutation analysis was carried out in order to detect $BRAF^{V600E}$ mutation. A total of 252 patients (84.3%) underwent preoperative $BRAF^{V600E}$ examination and 240 patients had positive $BRAF^{V600E}$ mutation. All surgery was performed under general anesthesia and lobectomy or total thyroidectomy was performed on a case-by-case basis. The subjects were divided into two groups, malignant or benign, according to the final pathologic result.

Retrospectively, we collected data on age, gender, number and maximum size of thyroid nodules, bilaterality, ultrasound features, $BRAF^{V600E}$ gene mutation, and final pathologic results. Other US features were assessed including echogenicity, margin, shape, presence of calcification, and enlargement of cervical lymph nodes.

Statistical analyses were performed with SPSS version 24.0 (IBM Corp., Armonk, NY, USA). To compare variables between the malignant and benign thyroid nodules, we used the chi-square test for categorical variables and t-test for continuous variables. To identify risk factors for malignancy, we used logistic regression analysis. Statistical significance was assumed when the p value was <0.05.

Results

During the study period, 36/299 patients (12.1%) with AUS thyroid nodules had benign nodules and 263/299 patients (87.9%) had malignant nodules following the final pathology examination. Benign nodules included nodular hyperplasia (n=25), multinodular goiter (n=1), nontoxic goiter (n=2), adenomatous goiter (n=3), follicular adenoma (n=3), and lymphocytic thyroiditis (n=2). Malignant nodules were papillary thyroid carcinoma (n=264), follicular carcinoma (n=2), and

invasive follicular carcinoma (n=1).

We found no statistically significant difference in age, gender, bilaterality, calcification, or positive lymph node enlargement between patients with benign nodules and those with malignant nodules. However, the maximum size of a nodule was statistically smaller in patients with malignant nodules compared with benign nodules (mean size: 1.51±11.32 vs. 2.45±1.51, $p<0.001$). Malignant nodules were also significantly related to other US features including irregular or spiculated margin ($p<0.001$), hypo-echogenicity ($p=0.006$) and taller than wide shape ($p=0.014$). More importantly, 131/133 patients (98.5%) with AUS and simultaneous positive $BRAF^{V600E}$ mutation were reported to have thyroid cancer in the final pathologic report ($p<0.001$) (Table 1).

In the univariate analysis, $BRAF^{V600E}$ mutation, smaller size, hypo-echogenicity, and irregular margin significantly increased the risk of malignancy (Table 2). However, in multivariate analysis, positive $BRAF^{V600E}$ mutation was the only significant critical factor associated with increased risk of malignancy (odds ratio=16.195) (Table 3).

Discussion

Although the risk of malignancy in AUS thyroid nodule cases has been reported to be up to 30%, the treatment guideline for AUS nodules are still controversial.[12] As such, the identification of preoperative predicting factors for malignancy may help to inform treatment decisions. In this study, the malignancy rate of AUS nodules after surgery was 87.9%, which is much higher than the estimated malignancy risk pre-

Table 1. Clinicopathologic characteristics in patients with atypia of undetermined significance nodule

Characteristic	Benign (n=36)	Malignancy (n=263)	*p*-value
Age (year)	56.94±12.08	54.73±11.14	0.268
Gender			0.153
Male	8	35	
Female	28	228	
Ultrasonography features			
Bilateral	15	98	0.864
Maximum nodule size (cm)	2.45±1.51	1.51±11.32	0.001*
Margin (irregular or spiculated)	4	59	0.001*
Echogenicity (hypoechoic)	12	119	0.006*
Shape (taller than wide)	2	53	0.014*
Calcification (+)	10	112	0.069
Lymph node enlargement (+)	4	28	0.121
$BRAF^{V600E}$ mutation			<0.001*
Wild type	21	86	
Mutant type	2	131	

*$p<0.05$

Table 2. Univariate analysis of variables predicting malignancy in atypia of undetermined significance nodules

Variable	Odds ratio	Lower limit (95% CI)	Upper limit (95% CI)	*p*-value
$BRAF^{V600E}$	15.994	3.657	69.959	<0.001*
Size of nodule	0.691	0.559	0.855	0.001*
Echogenicity	0.377	0.195	0.750	0.006*
Margin	5.784	1.855	18.033	0.001*

*$p<0.05$. CI: confidence interval

Table 3. Multivariate analysis of variables predicting the malignancy of atypia of undetermined significance

Variable	Odds ratio	Lower limit (95% CI)	Upper limit (95% CI)	*p*-value
$BRAF^{V600E}$	16.195	1.907	137.568	0.011*
Size of nodule	1.136	0.752	1.716	0.544
Echogenicity	0.371	0.115	1.195	0.097
Margin	4.207	0.925	19.145	0.063

*$p<0.05$. CI: confidence interval

sented by the Bethesda system for AUS nodules. This could be explained by inevitable bias because some patients did not receive any treatment as their risk was defined as low based on US findings.

Demographically, we found no differences in age or gender distribution in patients with malignant or benign nodules. Therefore, age and gender should not be neglected when dealing with AUS thyroid nodules. The size of thyroid nodules is another helpful factor that can be used to predict malignancy. Nodule size has been positively correlated with malignancy risk in some studies. Seo, et al.[13] reported that the malignancy rate was higher in the group with nodules <1.5 cm compared with patients who had nodules >1.5 cm. In contrast, Kiernan and Solórzano[7] reported that nodule size alone could not predict malignancy in patients with AUS. In our study, nodule size alone was not found to be a significant predictor of malignancy based on multivariate analysis.

In most studies, US malignant features include hypoechogenicity, spiculated margin, microcalcifications, and a taller than wide shape. US is useful and shows very good diagnostic accuracy for the differentiation of Bethesda class III AUS nodules.[5] Lee, et al.[6] reported that US features are particularly useful for stratifying the level of malignant risk; however, a large proportion of nodules without any suspicious US features were eventually found to be malignant (6.25−32.4%). Therefore, repeated FNAB has been recommended for diagnostic accuracy; however, AUS nodules with low suspicious patterns as defined by the 2015 American Thyroid Association guidelines might be followed up with US instead of repeat FNA, because of low malignancy in that group. In our study, US features such as hypo-echogenicity and spiculated margin increased the risk of malignancy in univariate analysis (p=0.005 and p=0.002, respectively). However, after adjustment by multivariate analysis, these features were no longer significant in defining the risk of malignancy.

Molecular diagnostics has the potential to overcome the limitations of indeterminate cytology using FNA.[16,17] Among the genetic mutations in thyroid cancer patients, the $BRAF^{V600E}$ mutation is the most common and is found in 45% of papillary thyroid cancer cases. Seo, et al.[13] performed BRAF gene analysis in 38 AUS nodules and found that 13 patients had a BRAF mutation. All of them were diagnosed with thyroid carcinoma, although it was not statistically significant due to the small sample size. Decaussin-Petrucci, et al.[14] reported that neuroblastoma rat sarcoma (NRAS), harvey rat sarcoma (HRAS), and telomerase reverse transcriptase (TERT) gene mutations are strong predictors of malignancy regardless of the cytological classification according to the Bethesda system. According to the study in 61 AUS nodules, NRAS/HRAS mutations were significantly more frequent in malignant nodules and $BRAF^{V600E}$ mutation was not. Valderrabano and McIver[18] stated that the $BRAF^{V600E}$ cancer-specific mutation is typically associated with Bethesda category V or VI nodules but rarely category III or IV specimens. Kim, et al.[19] suggest a triage scheme for AUS nodules based not only on $BRAF^{V600E}$ mutation but also other US findings; repeat biopsy results due to the use of single gene mutations shows low sensitivity for the detection of malignancy. Therefore, to our knowledge, this is the first report in a large study population to show that BRAF mutation is a statistically significant risk factor for predicting malignancy in patients with AUS nodules.

The present study showed that 98.5% patients with AUS and simultaneous positive $BRAF^{V600E}$ mutation were found to have thyroid cancer following surgery. Also, positive $BRAF^{V600E}$ mutation led to a statistically significant increased risk of malignancy in patients with AUS, which was identified by both univariate and multivariate analyses. As such, it is a valuable predictive indicator of cancer in thyroid nodule patients with AUS.

In conclusion, our study shows that $BRAF^{V600E}$ gene mutation should be evaluated when dealing with AUS thyroid nodules detected by FNA. If the mutation is confirmed, there is a strong risk of malignancy; therefore, surgery or close observation with very short-term intervals is recommended.

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