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Cytologic Features and *BRAF* Mutation of Hyalinizing Trabecular Adenoma of the Thyroid – A Case Report with Review of the Literature –

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A hyalinizing trabecular adenoma (HTA) is a rare benign thyroid tumor of follicular epithelial cell origin with a trabecular-alveolar growth pattern and marked intratrabecular hyalinization. The cytological and histological features of HTA are very similar to those of papillary and medullary carcinomas of the thyroid. Therefore, an accurate diagnosis of HTA is important to avoid unnecessary and potentially harmful management of patients. However, the results of *BRAF* gene mutation analysis shown by many studies are distinctly different between HTAs and papillary thyroid carcinomas. Herein, we describe a rare case of HTA of the thyroid in a 49-year-old female and consider its characteristic cytological features and *BRAF* gene mutation analysis results with a brief review of the literature.

Key Words: Adenoma; Cytology; *BRAF*; Thyroid

Hyalinizing trabecular adenoma (HTA) of the thyroid, characterized by the presence of a trabecular-alveolar growth pattern and marked intratrabecular hyalinization, was initially described 100 years ago.[1] In 1987, Carney *et al*.[2] introduced the term HTA to describe this rare tumor of the thyroid composed of polygonal, oval, and spindle cells arranged in a trabecular pattern and separated by a hyalinized stroma. The most important aspect of HTA is that it can resemble other thyroid malignancies both cytologically and histopathologically, specifically, papillary and medullary carcinomas.[3] The nuclear characteristics of HTA result in confusion with papillary thyroid carcinoma (PTC), and hyaline material does with that of medullary thyroid carcinoma (MTC).[4] These misinterpretations may result in unnecessary or aggressive surgical management. In practice, fine needle aspiration cytology (FNAC) is the best tool for preoperatively evaluating the thyroid nodules.[3] Cytological features of HTA have been reported, and Casey *et al.*[4] described a detailed cytological profile of HTA compared with that of PTC and MTC. However, HTA is frequently misinterpreted by FNAC as PTC and sometimes diagnosed as MTC or atypical cells.[4] In contrast to the cytological similarities between HTA and PTC, there are distinct differences among many studies detailing the prevalence of the *BRAF* gene.[5]

Recently, we experienced a case of HTA in a 49-year-old woman. Here, we report the cytological features of HTA correlated with the histopathological findings and a briefly review of the Korean literature. Additionally, we present results of a *BRAF* gene mutation analysis for three HTA cases, including another two tumors from a different institution.

CASE REPORT

A 49-year-old woman was admitted to our hospital to evaluate a palpable small mass on the anterior neck of 2 years duration. On physical examination, the mass was movable, with no accompanying pain or tenderness. Neck ultrasonography revealed a hypoechoic nodule, measuring 1.6×1.2×0.7 cm in the mid portion of the right lobe of thyroid, and the mass displayed indistinct, lobulated margins with heterogeneous echogenecity and microcalcification (Fig. 1A). No preoperative diagnostic procedures were performed. The patient underwent a lobectomy

of the right lobe of thyroid. The cut surface revealed a well-circumscribed, lobulated, solid mass with a pink-grayish appearance in the mid portion, measuring 1.6 cm in maximum diameter (Fig. 1B). The mass was confined to the thyroid without evidence of invasion into adjacent tissues. Frozen sectioning and a touch imprint preparation were performed for cytology.

The touch imprint cytological preparation revealed moderate cellularity with a partly bloody background (Fig. 2A). The preparation exhibited radially oriented cohesive aggregates of atypical follicular epithelial cells with indistinct cell borders, surrounding central hyaline materials, and scattered single cells (Fig. 2B). The tumor cells showed a polygonal or elongated shape with abundant cytoplasm and a low nuclear to cytoplasmic ratio. The nuclei were uniformly ovoid or elongated and slightly eccentrically located. Nuclei also showed finely granular chromatin, occasional intranuclear pseudoinclusions, nuclear grooves, a vague nuclear palisading pattern, and a perinucleolar halo (Fig. 2C, D). Nucleoli were small or inconspicuous. A papillary architecture or sheet-like arrangement was not present. The frozen section diagnosis with a cytological evaluation was misinterpreted as PTC.

On the histopathological evaluation, the tumor was well-defined and composed of medium to large-sized polygonal and spindle cells arranged in a trabecular-alveolar growth pattern with varying amounts of hyaline material between the tumor cell nests, mimicking amyloid-like material (Fig. 3A). The tumor cells exhibited abundant amounts of finely granular, acidophilic, amphophilic, or clear cytoplasm and occasional nuclear grooves and intranuclear pseudoinclusions. The hyaline material was negative for Congo red staining. Immunohistochemically, the tumor cells were all strongly positive for MIB-1 with a characteristic cytoplasmic or membranous staining pattern but negative for HBME-1 and cytokeratin 19 (CK19), which are known to be positive in PTC (Fig. 3B).

Additionally, we performed a *BRAF* mutation analysis by directly sequencing the tumor. For the analysis of the *BRAF* gene mutation, genomic DNA was isolated from the hematoxylin and eosin-stained slides of three HTAs; the present case (case 1) and two additional HTA cases (cases 2 and 3) from another hospital (Hanyang University Seoul Hospital). The samples were deparaffinized with xylene/ethanol. DNA was extracted using the QIAamp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Exon 15 of the *BRAF* gene was amplified by the polymerase chain reaction (PCR) using the following primers: forward, 5′-GCTTGCTCTGATAGGAAAATGAG-3′; reverse, 5′-GTAACTCAGCAGCATCTCAGG-3′. Each PCR product was purified using the QIAGEN-QIAquick PCR purification kit, and then subjected to bi-directional direct DNA sequencing analysis using an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). No *BRAF* mutation was detected in cases 1 or 3. However, a *BRAF* mutation was identified in case 2 at the V600E site (Fig. 4). The mutation was present at nucleotide position 1,799, where thymine was replaced by adenine, resulting in the substitution of valine for glutamic acid at position 600. The tumor demonstrating the *BRAF* mutation showed typical histopathological features of HTA with no immunoreactivity for CK19, HBME-1, or galectin-3. The clinicopathological data of the three HTA cases with the results of the *BRAF* gene mutation analysis are summarized in Table 1.

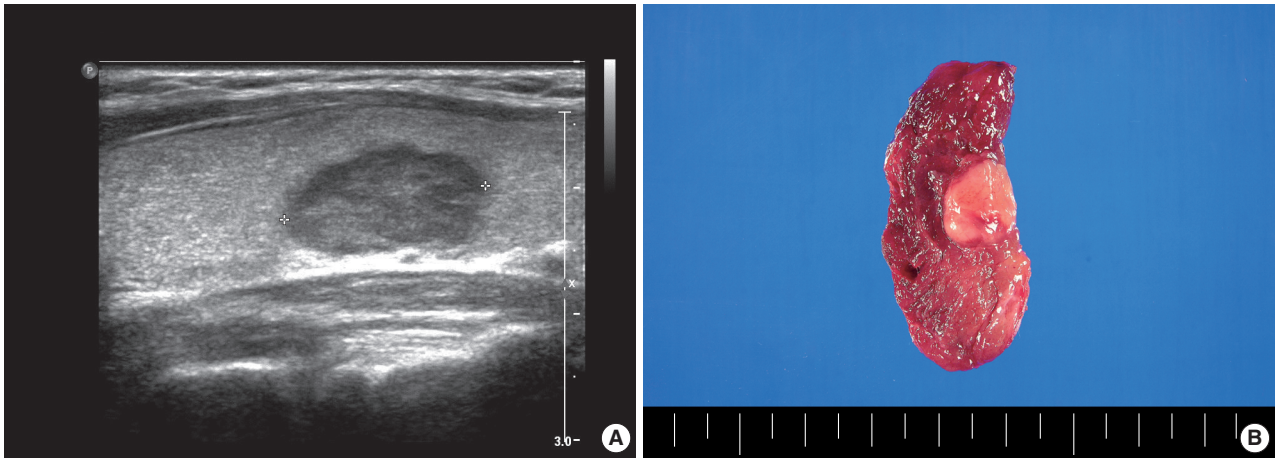

Fig. 1. Ultrasonography shows a well circumscribed, hypoechoic, hypervascular mass with heterogeneous echogenecity in the right lobe of the thyroid (A). Cut surface reveals a well defined, pinkish-gray, solid mass, measuring 1.6×1.2×0.7 cm (B).

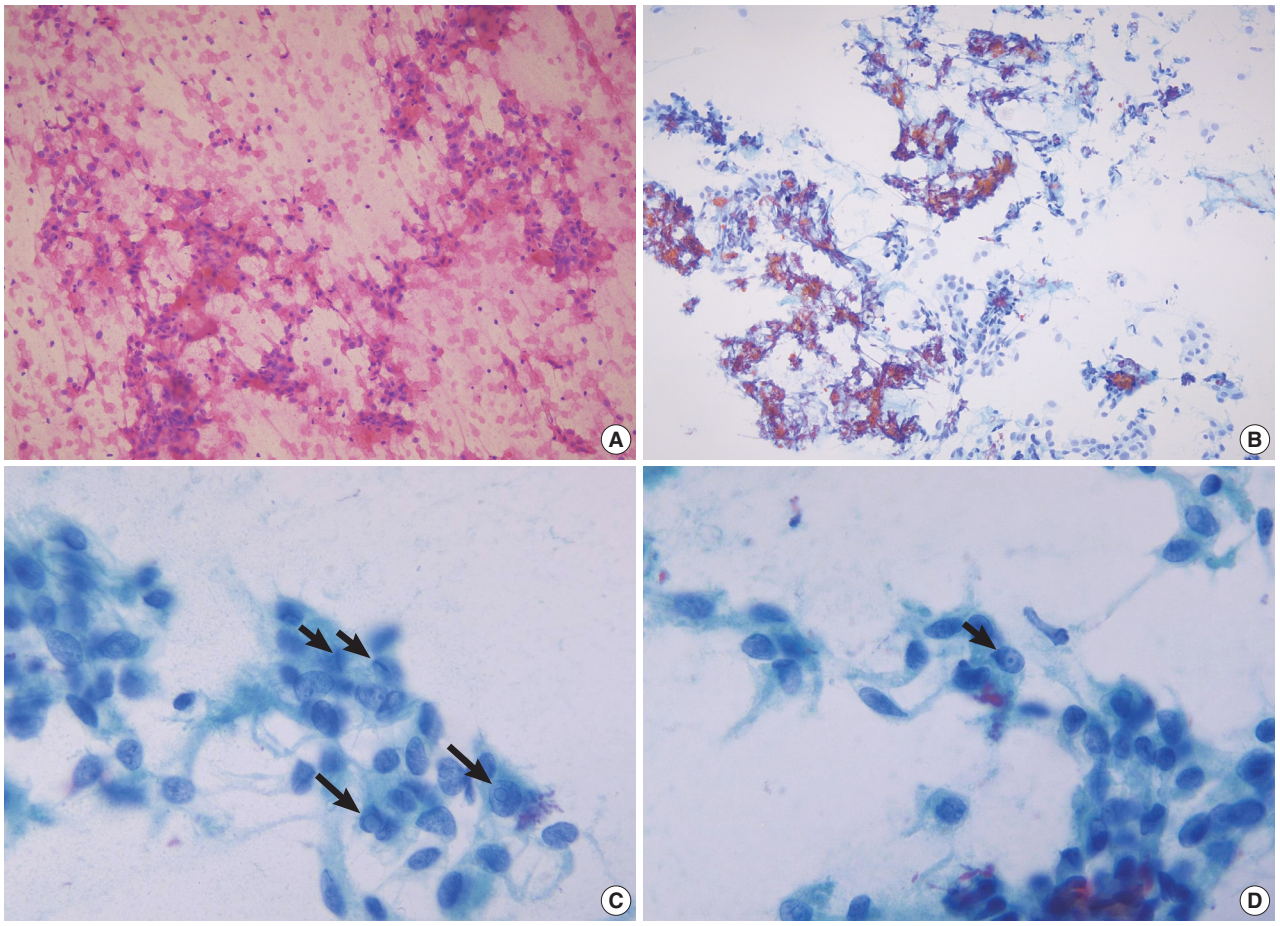

Fig. 2. A touch imprint preparation shows moderate cellularity in a bloody background (A). The smear pattern shows syncytial tissue fragments of atypical follicular epithelial cells and, less frequently, single, isolated tumor cells. The tumor cells have a polygonal or elongated shape with abundant cytoplasm and a low nuclear to cytoplasmic ratio. The tumor cells appear to radiate from acellular hyaline material (B) (Papanicolaou method). Higher magnification reveals the rather eccentrically located nuclei with intranuclear pseudoinclusions (long arrows), nuclear grooves (short arrows) (C), and perinucleolar halo (arrow) (D) (Papanicolaou method).

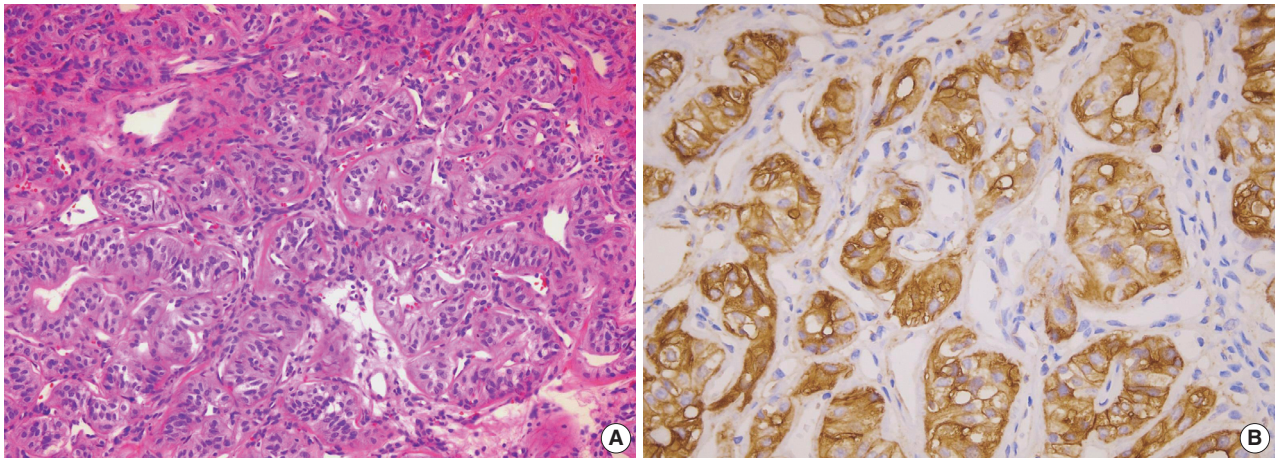

Fig. 3. The tumor has a polygonal and spindle cell shape in a trabecular-alveolar growth pattern with a variable amount of hyalinized material in and between the tumor cell nests. The amorphous, lumpy, hyaline substance mimics amyloid, raising the possibility of a medullary thyroid carcinoma (A). The tumor shows strong MIB-1 immunoreactivity with a characteristic membranous and cytoplasmic pattern (B).

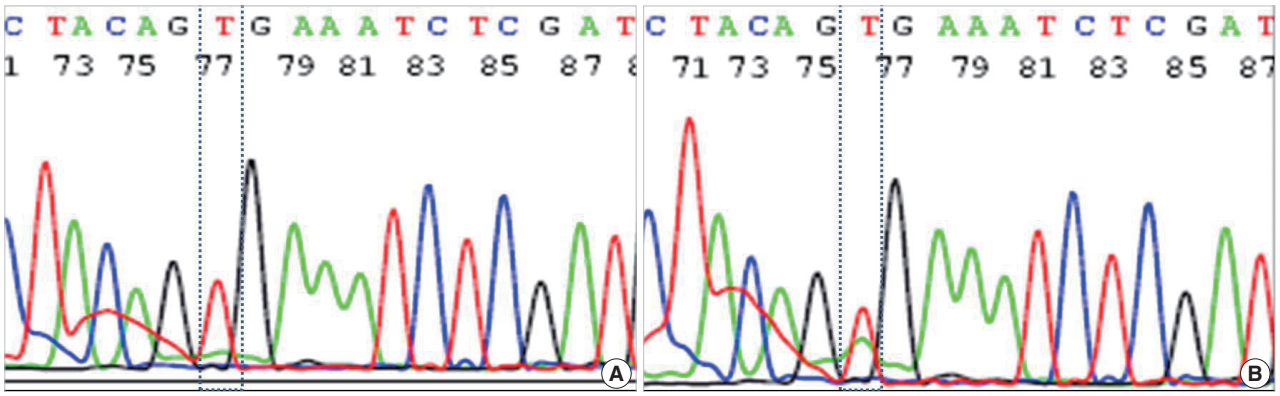

Fig. 4. Polymerase chain reaction (PCR) amplification followed by direct nucleotide sequencing of the PCR products to identify the *BRAF*^{V600E} mutation. The present hyalinizing trabecular adenoma (case 1) shows a wild-type peak (A), whereas the tumor from case 2 displays a *BRAF*^{V600E} mutation. T → A transversion at nucleotide 1,799 (B).

Table 1. Analysis of the *BRAF*^{V600E} mutation in the three cases of hyalinizing trabecular adenoma (HTA)

Characteristics	Case 1	Case 2	Case 3
Age (yr)/Sex	49/Female	53/Female	60/Female
Duration	2 yr	7 mo	7 mo
Past history	None	None	None
Gross finding (cm)	Solitary/1.6	Solitary/1.5	Solitary/3
Initial cytopathologic diagnosis			
FNAC	ND	PTC	ID
FS with TIC or SSC	PTC	PTC	PTC
Associated lesion	None	None	None
Follow-up	21 mo/NED	67 mo/NED	12 mo/NED
BRAF mutation	No	Yes	No

FNAC, fine needle aspiration cytology; ND, not done, PTC, papillary thyroid carcinoma; ID, insufficiency for diagnosis; FS with TIC or SSC, frozen section with touch imprint cytology or scrape smear cytology; NED, No evidence of disease.

DISCUSSION

HTA is a rare benign thyroid tumor of follicular cell origin with a trabecular-alveolar pattern of growth and marked intratrabecular hyalinization. It can be easily misdiagnosed as PTC mainly due to a significant overlap of nuclear features observed in both HTA and PTC.[3,6] Cytological findings of HTA are characterized by similar features that are more frequently seen on FNAC than in PTC, including hypercellularity, nuclear grooves, intranuclear pseudoinclusions, and a powdery nuclear chromatin pattern.[3] In the present case, the cytological touch imprint and scrape smear findings indicated a PTC, leading to the misinterpretation on the frozen section.

Casey *et al.*[4] studied cytological material from 29 cases of HTA to establish the cytological profile of this neoplasm and compared it with that of PTC and MTC. They emphasized that the important cytological features of HTA that differ from PTC and MTC are a bloody background, cells with ample cytoplasm arranged in cohesive aggregates or single polygonal nuclei with frequent nuclear grooves and intranuclear pseudoinclusions and hyaline material. They also suggested that perinucleolar clearing, which is not present in either PTC or MTC, may be the most reliable feature for a cytological diagnosis. The present case also revealed similar cytological findings to HTA including perinucleolar clearing cited by Casey *et al.*[4]

Galgano *et al.*[6] observed a consistent lack of HBME-1 and CK19 immunoreactivity in HTA, which are sensitive markers for PTC. More interestingly, membranous staining with the antibody to MIB-1 is characteristic of HTA but absent in PTC.[7] In this case, the tumor was non-reactive to HBME-1 and CK19, but showed characteristic membranous and cytoplasmic staining for MIB-1 on immunohistochemistry. As described before, these immunohistochemical markers are helpful to distinguish HTA from PTC.

BRAF is one of three isoforms of the serine-threonine RAF kinase family in mammalian cells.[8] It is frequently mutated in a wide range of human cancers.[9] Many studies have reported an association between a *BRAF* gene mutation and aggressive tu-

Table 2. Clinicopathological features of hyalinizing trabecular adenomas reported in Korean patients

Characteristics	Yim *et al.* (1998)[13]	Kim *et al.* (1999)[14]	Choi *et al.* (2003)[15]	Kang *et al.* (2008)[12]	Present case
Age (yr)/Sex	53/Female	40/Female	55/Female	47/Female	49/Female
Duration	Unknown	14 yr	6 mo	5 yr	2 yr
Past history	None	None	None	Minocyclin, 9 years	None
Gross finding (cm)	Solitary/2	Solitary/4	Solitary/3.4	Solitary/2	Solitary/1.6
Initial cytopathologic diagnosis					
FNAC	ND	PTC	PTC	PTC	ND
FS with TIC or SSC	MTC	PTC	ND	HTA[a]	PTC
Associated lesion	None	None	PTC, occult[b]	Black pigmentation[c]	None
Follow-up	Unknown	Unknown	Unknown	Unknown	21 mo/NED

[a]Frozen section diagnosis was reported as "suspicious for hyalinizing trabecular tumor, benign vs. malignant"; [b]An occult PTC was found on opposite lobe of the thyroid; [c]Black pigmentation in the thyroid parenchyma and the oral cavity associated with long term use of minocyclin was present.
FNAC, fine needle aspiration cytology; ND, not done; PTC, papillary thyroid carcinoma; FS with TIC or SSC, frozen section with touch imprint cytology or scrape smear cytology; MTC, medullary thyroid carcinoma; HTA, hyalinizing trabecular adenoma; NED, no evidence of disease.

mor behavior. *BRAF* mutations, particularly a valine-to-glutamate substitution at nucleotide 1,799 (V600E), are the most common genetic abnormalities in PTCs.[8] In PTCs and its variants, $BRAF^{V600E}$ mutations have been reported in 36-69% of studied cases, but other benign or malignant thyroid tumors do not reveal a *BRAF* mutation.[5] Recently, Sheu *et al.*[10] evaluated *BRAF* gene mutations in 18 HTAs, and they observed no *BRAF* mutations in any case. Furthermore, no *BRAF* mutations were seen in 23 cases of HTA studied by Nakamura *et al.*[11] The current HTA cases (cases 1 and 3) also did not include a *BRAF* gene mutation. However, case 2 displayed a *BRAF* gene mutation, although histopathological and immunohistochemical findings were entirely compatible with HTA. A similar case demonstrating discrepant results was previously documented by Kang *et al.*[12] They reported a case of black thyroid associated with HTA. In their case, the thyroid tumor showed typical histopathological features of HTA and characteristic membranous MIB-1 staining, whereas a *BRAF* mutation, which is specific to PTCs, was also present. Taken together, these results suggest that *BRAF* mutations may play a role in the pathogenesis of HTA and PTC.

In Korea, two cases of HTA have been reported using cytological findings, whereas two other cases have been reported without cytological findings.[12-15] The clinical and cytohistological findings of all reported Korean cases are summarized in Table 2. All patients, including the present case, were females ranging from 40 to 55 years old. They had no remarkable history such as irradiation, except for the long-term use of minocyclin in one patient, which led to black discoloration in the oral cavity and thyroid parenchyma, possibly related to the minocyclin. The pathogenetic mechanism of minocyclin associated with HTA has not been described.[12] As for the HTA itself, they were all solitary masses ranging from 1.6 to 4 cm. According to those studies, all masses were well circumscribed or encapsulated and homogeneously solid and devoid of hemorrhage or necrosis, as in the present case. Preoperative FNAC was performed in three cases, and a frozen section with touch imprint or scrape smear cytology was performed in four, including the present case. All FNACs were misinterpreted as PTC. In the frozen sections subjected to touch imprint or scrape smear cytology, two of the four cases were initially reported as PTC and one as MTC but the remaining one was "suspicious of a hyalinizing trabecular tumor, adenoma vs carcinoma."[12] These results may be rare for this tumor and are not typical cytological features of HTA. One patient also had an occult papillary carcinoma in the opposite lobe of the thyroid, measuring 0.3 cm.[15] Some reports have indicated that HTA is a variant of PTC based on the occasional coexistence of HTA and PTC, similar microscopic features including intranuclear inclusions and grooves, and the presence of a *RET*/*PTC* gene rearrangement in both HTA and PTC.[6,15,16] In contrast, other studies have supported that HTA is a peculiar entity that differs from PTC because of different immunohistochemical staining patterns, as described above.[7] Additional study will be necessary to distinguish HTA and PTC.

Currently, HTA is referred to as hyalinizing trabecular tumor, which signifies the unknown malignant potential of this tumor and a possible relationship to PTC, because the *RET*/*PTC* gene rearrangement, which is specific to PTC, was detected in six HTA cases.[6,16] However, some thyroid experts claim that true HTAs are almost always benign.[17,18] The present case also showed benign features with no evidence of aggressive behavior, such as capsular, vascular, or parenchymal invasion, and no clinical evidence of local recurrence or distant metastasis during a 21-month follow-up period. Additionally, distinguishing HTA and carcinoma using cytology is extremely difficult, similar to cases of thyroid follicular neoplasms. However, it can be distinguished from PTC or MTC as long as it is recognized that this is a rare

entity.

Herein, we have briefly described the cytological and histopathological findings of a HTA in a 49-year-old woman and a separate case with a *BRAF* mutation.

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