

Original Article

Hyalinizing trabecular tumor of the thyroid: a clinicopathological analysis of four cases and review of the literature

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Abstract: Objective: To explore the clinicopathological features, diagnosis and differential diagnosis of hyalinizing trabecular tumor (HTT) of the thyroid. Methods: The four HTT specimens were collected including demographics, clinical information, relevant images, the extent of thyroidectomy, the follow-up and representative pathological data of tumors were available for analysis. In addition, the immunohistochemical staining related to the tumor as well as the BRAF and N-ras mutation analysis were analysed. Results: The mean age of four patients was 47 years old and the mean size of the tumor was 2.8 cm. Most of the patients were asymptomatic, while detecting incidentally by using neck ultrasound test. Ultrasound imaging of all cases showed demarcated substantial hypoechoic nodules in ipsilateral thyroid lobe. Computed Tomography (CT) showed a clear low density shadow in the affected thyroid lobe. Tumors of three cases were located at the left, but the other one was located at the right thyroid gland with a complete fibrous capsule. The cytological features resembled papillary thyroid carcinoma (PTC). The histological test indicated that the tumors had characteristic of trabecular growth pattern with hyalinizing material. The tumor cells were in shape of polygonal, oval or high columnar with an acidophilic or clear cytoplasm. The nuclei were oval with inconspicuous small nucleoli, prominent grooves and pseudoinclusion body in cell nucleus. Mitosis and psammoma bodies were rare to be observed. Cytoplasmic "yellow bodies" were frequently observed. The hyaline material was prominent, with positive periodic acid-Schiff (PAS) and negative Congo red staining. Immunohistochemically, tumor cells were positive for thyroglobulin (Tg), thyroid transcription factor-1 (TTF-1), CD56 and negative for calcitonin, cytokeratin 19 (CK19), HBME-1, S-100 and synaptophysin (Syn). Chromogranin A (CgA) and galectin-3 were expressed weakly in some cases. Staining with the MIB-1 antibody showed membranous/cytoplasmic immunoreactivity. Whereas, another clone of Ki-67 (SP6) showed a common nuclear pattern with an index of <1%. None of the four cases exhibited the BRAF V600E protein reactivity. Gene mutation analysis demonstrated no BRAF and N-ras mutation. There was no evidence of local recurrence or metastasis after 6 to 36 months of follow-up. Conclusions: HTT is an uncommon thyroid tumor with very low malignant potential. It has no particular clinical features, so it's often misdiagnosed in fine needle aspiration cytology (FNAC)/Ultrasonography-guided fine needle aspiration cytology (US-FNAC) and frozen section (FS). Its final diagnosis mainly relies on typical histopathological features and characteristic expression pattern of MIB-1 immunohistochemical staining.

Keywords: Hyalinizing trabecular tumor, thyroid tumor, immunohistochemistry, BRAF mutation, N-ras mutation

Introduction

Hyalinizing trabecular tumor (HTT) is an uncommon neoplasm of the thyroid follicular derivation, with morphology that is similar with papillary thyroid carcinoma (PTC). The tumor contains hyaline material that often confuses it

for medullary thyroid carcinoma (MTC), because it mimics amyloid. So it's often misdiagnosed as solid variant papillary thyroid carcinoma (SPTC), hyalinizing papillary thyroid carcinoma (HPTC) and hyalinising trabecular adenoma-like variant of medullary thyroid carcinoma (HTALMTC), because of its cytological features

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Table 1. Summary of clinical and pathological data in patients with surgically diagnosed with hyalinizing trabecular tumor (HTT)

Patient	Sex	Age (yr)	Location	Size (cm)	Symptom	Multiplicity	CT	FNAC	FS	Operation	Associated findings	Follow-up (mo)
1	F	46	Left thyroid gland	3.5 cm	Solid hypoechoic mass	Multiple nodule b) FA, Right	A clear low density shadow in the left thyroid lobe, with multiple plaque high density shadows in the nodule and displayed displacement of trachea due to compression of a large mass.	NA	NA	Hemitotal	Lymphocytic thyroiditis	30
2	F	51	Left thyroid gland	5.0 cm	Solid hypoechoic mass	Solitary nodule	A clear low density shadow in the left thyroid lobe	Suspicious for PTC	NA	Hemitotal	Lymphocytic thyroiditis	36
3	F	46	Left thyroid gland	0.4 cm	Solid hypoechoic mass	Multiple nodular goiter a)	A clear low density shadow in the left thyroid lobe	AUS/FLUS, Suspicious for PTC	Defer, HTT vs. PTC	Subtotal	Lymphocytic thyroiditis	6
4	M	45	Right thyroid gland	2.3 cm	Solid hypoechoic mass	Solitary nodule	NA	Suspicious for PTC	Defer, HTT vs. PTC	Hemitotal	Lymphocytic thyroiditis	10

FNAC, fine needle aspiration cytology; FS, frozen section; IHC, immunohistochemical staining; F, female; FA, follicular adenoma; PTC, papillary thyroid carcinoma; CK19, cytokeratin 19; M, male; MTC, medullary thyroid carcinoma. a) Probably benign nodules on US. b) Incidentally detected HTT after surgery for FA in the contralateral lobe. NA, Not available; US, ultrasonography; US-FNAC, Ultrasonography-guided fine needle aspiration cytology.

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Table 2. Summary of antibodies and conditions used for immunohistochemical staining

Antibody	Clone	Source	Dilution	Antigen retrieval	IHC system	Antibody incubation
RAF V600E	VE1	Spring Bioscience	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
CD56	56C04	Thermo Scientific	1:100	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
Chromogranin A	SP12	Thermo Scientific	1:100	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
CK19	A53-B/A2.26/Ks19.1	Thermo Scientific	1:100	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
Calcitonin	SP17	Thermo Scientific	1:100	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
Galectin-3	9C4	Thermo Scientific	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
HBME-1	HBME-1	Maixin Bioscience	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
Ki67	MIB-1	Maixin Bioscience	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
Ki67	SP6	Thermo Scientific	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
S-100	4C4.9	Maixin Bioscience	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
SyN	SP11	Thermo Scientific	1:100	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
Thyroglobulin	2H11/6E1	Thermo Scientific	1:150	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min
TTF-1	8G7G3/1	Thermo Scientific	1:50	EDTA (pH 9.0), Autoclave HIER, 3 min	DAKO, EnVision, Manual	RT, 30 min

CK19, cytokeratin 19; CgA, Chromogranin A; HIER, heat induced epitope retrieval; IHC, immunohistochemical staining; RT, room temperature; Synaptophysin, SyN; TTF-1, thyroid transcription factor 1.

resemble PTC and its hyaline material resemble amyloid [1, 2]. This make it difficult to differentiate it from PTC and MTC, especially by FNAC/US-FNAC and FS [3-7]. The misdiagnosis rate derived from FNAC/US-FNAC is even up to 100% [5, 6] and the correct rate is only 53% in FS [7]. But the correct rate is higher when the core-needle biopsy is used [5]. Herein, we present the clinicopathological features, diagnosis and management of four typical HTT cases and general information related to the HTT are also presented to raise awareness of this tumor and to prevent misdiagnosis and subsequent surgical treatment under the misimpression of PTC or MTC.

Materials and methods

Clinical data of patients

All the procedures were approved by our Institutional Review Board with agreement of the patients. We identified four patients with HTT (as confirmed by permanent histopathology) who had undergone thyroidectomy and diagnosed from February 2013 to October 2016 at the department of pathology of Yichang Central People's Hospital, Hubei Province, China. We analyzed the data including demographics, clinical information, relevant imaging, the extent of thyroidectomy and the follow-up (**Table 1**).

Pathological data of patients

The pathological data including FNAC/US-FNAC, the gross appearance, FS results, permanent histopathology, PAS, Congo red and immunohistochemical (IHC) staining results

were analyzed. There were two patients underwent FNAC and one patient underwent US-FNAC prior to their operations and two patients underwent FS analysis during the operations (**Table 1**). The cytological smears were stained with Diff-Quik Staining. The tissue samples from the resected thyroid nodule and/or the adjacent thyroid parenchyma were obtained and processed for FS analysis. Frozen samples were cut into 6- μ m-thick sections and stained with hematoxylin and eosin (HE) for histological analysis, and the surgical team in the operating room was notified with the results. FS results were further classified into benign (including HTT), malignant, and deferred diagnosis. All tumor specimens were surgically resected and fixed in 10% buffered formalin and embedded in paraffin, and then cut into 4- μ m-thick sections and stained with HE, PAS and Congo red. Immunohistochemical staining (EnVision method) was performed using commercially available antibodies to the following antigens: Tg, TTF-1, CD56, calcitonin, CK19, HBME-1, S-100, SyN, CgA, galectin-3, Ki-67 (Clone:MIB-1), Ki-67 (Clone:SP6) and BRAF V600E. All protocols were performed according to the manufacturers' instructions (**Table 2**).

BRAF and N-ras gene mutation detection

All the samples embedded in paraffin were analyzed in exon 2, exon 3 and exon 4 of N-ras gene and in exon 15 of the BRAF gene mutation detection, respectively. Briefly, (1) Nucleic acid was extracted from 4 paraffin embedded

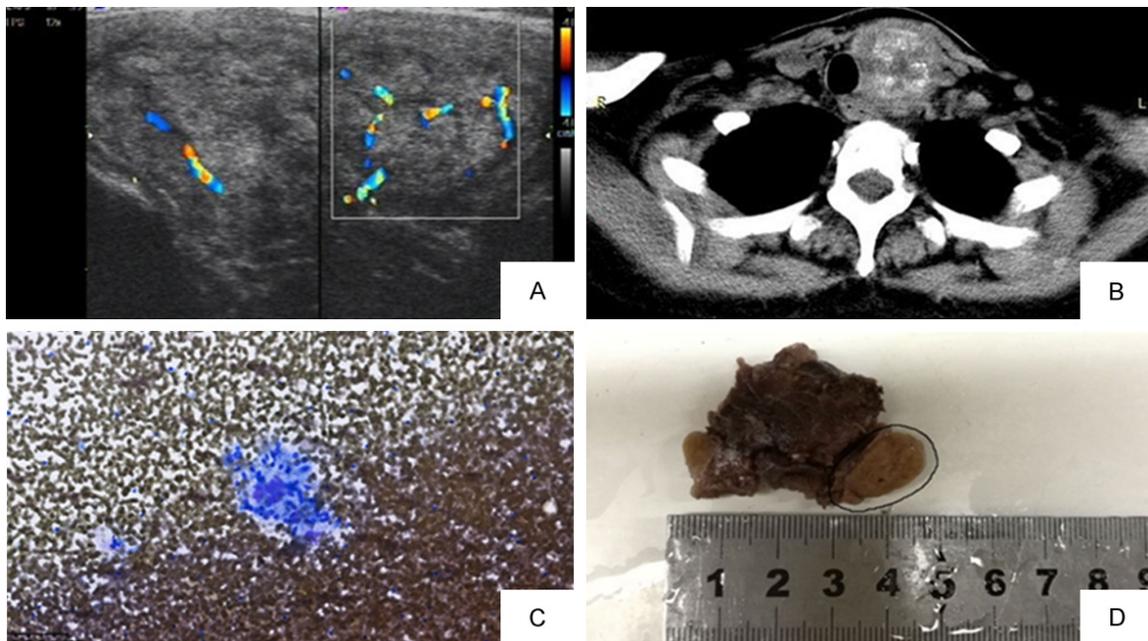


Figure 1. A. Ultrasound showed a demarcated solitary hypoechoic nodule with abundant blood flow signals in ipsilateral thyroid lobe. B. CT scanning showed that there was a clear low density shadow in the affected thyroid lobe. C. Variably cellular with cohesive clusters of tumor cells aggregates around a red hyaline stromal core in a bloody background in FANC. Diff-Quik staining (magnification $\times 20$). D. Gross investigation showed an encapsulated mass in the thyroid gland lobe, the cut surface was homogeneously pale and rigid.

tissues using QIAamp DNA Tissue Kit (QIAGEN, America), then nucleic acids extract were measured by Thermo Nanodrop 2000 (Thermo, America); (2) The exon 15 of BRAF gene and the exon 2, 3, 4 of N-ras gene were amplified by using Lifepro PCR (HANGZHOU BIOER Technology Co., China), according to the BRAF and N-ras gene sequencing Kit instructions (Shanghai YUANQI Biological Medicine Technology Co., China); (3) PCR products after denaturation were analyzed in ABI 3500 Dx gene analyzer (ABI, America) for Sanger sequencing. All protocols were performed according to the manufacturers' instructions.

Results

Clinical features of HTT patients

The demographics, clinical presentation, relevant images, the extent of thyroidectomy and the follow-up from our hospital study group are summarized in **Table 1**. All the patients were female except one male patient, with age of 46, 51, 46 and 45 years old, respectively. The mean age was 47 years (range 45 to 51 years). The tumors of three cases were located at the left thyroid gland, the other one was at the right

thyroid gland, and the mean size of the thyroid nodules was 2.8 cm (range 0.4 to 5 cm). All the patients were in good physical condition, except one patient claimed with symptoms of pharynx discomfort and was detected bilateral thyroid masses on neck US imaging. One patient was noticed with a palpable mass for ten years, while the other 2 patients were asymptomatic, with incidentally detected thyroid masses on neck US imaging for routine physical examination. Ultrasound showed demarcated substantial hypoechoic nodules in ipsilateral thyroid lobe of all cases. The US imaging of left thyroid mass and right thyroid mass was similar of the case with bilateral thyroid masses. All of them showed solitary hypoechoic nodules with abundant blood flow signals (**Figure 1A**). Permanent histopathology confirmed the nodules on both sides of the thyroid gland were different, the left side was a HTT, and the right side was a follicular adenoma (FA). Among all the HTT nodules, three cases of nodules were not uniformly distributed, so that the abundant branches of the color flow signal could be observed. In our study, three cases were examined by CT, and the results showed that there was a clear low density shadow in the affected thyroid lobe (**Figure 1B**), one case showed multiple plaque

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with high density shadows in the nodule and displayed displacement of trachea due to compression of a large mass. Of all the patients, two patients were underwent FNAC and one patient was underwent US-FNAC prior to their operations and two patients were underwent FS analysis during the operations. The decision to perform total thyroidectomy or hemithyroidectomy was based on the results of the FNAC/US-FNAC and FS analysis. Of all four patients, one underwent total thyroidectomy, the other three cases were underwent hemithyroidectomy. There was no local recurrence or metastasis during the follow-up (mean follow-up period 20.5 months, range 6 to 36 months) in all cases.

Pathological features of HTT

FNAC/US-FNAC characteristics: As is shown in **Figure 1C**, the cytological images were similar of the patients undergoing FNAC/US-FNAC. The scattered tumor cells in a bloody background exhibited with elongated nuclei, a well-formed nuclear pseudoinclusion and nuclear grooves. In local areas, variably cellular with cohesive clusters of cells aggregated around a red hyaline stromal core can be observed. Based on these cytological features, the cytology was suspicious for PTC.

Gross investigation: Gross investigation of all cases showed an encapsulated mass in the thyroid gland lobe, and the tumor volume was measured with 3.5 cm × 3.0 cm × 2.2 cm, 5.0 cm × 3.0 cm × 3.0 cm, 0.4 cm × 0.4 cm × 0.3 cm, and 2.3 cm × 2 cm × 1 cm, respectively. The cut surface was homogeneously pale and rigid (**Figure 1D**).

FS characteristics: The FS showed follicles with varied size and were characterized of trabecular structures and nuclei with prominent grooves and cytoplasmic inclusions (**Figure 2A**). The intratrabecular hyalin and colloid was prominent, it mimicked amyloid. Moreover, the cytological features also mimicked PTC. This makes it difficult to differentiate it from PTC and MTC by FS. The lumps were surrounded by a thin capsule. And mitosis and psammoma bodies were rare or absent. In one case, the yellow peculiar intracytoplasmic inclusion bodies called “yellow bodies” could be observed in FS.

Histologic appearance: All the tumors were well encapsulated without any evidence of capsular or vascular invasions, and the thyroid tissues adjacent to the tumor showed lymphocytic thyroiditis (**Figure 2B**). The tumors had polygonal to elongated cells arranged in a trabecular or alveolar pattern. Hyaline material was present extensively in both intracellular and extracellular locations. The cell nuclei often exhibited perinucleolar clearing, nuclear grooves, and nuclear inclusions similar to PTC. “Yellow bodies” can be observed in all cases (**Figure 2C**). Calcifications and psammoma bodies could be seen interspersed throughout the stroma in one case.

PAS and Congo red stain: The hyaline material of all the tumors were PAS positive (**Figure 2D**) and Congo red negative.

IHC phenotype

Immunohistochemically, tumor cells were positive for Tg, TTF-1, CD56 and negative for calcitonin, CK19, HBME-1, S-100 and SyN. CgA was expressed in one case (25%). Staining with the MIB-1 antibody showed membranous/cytoplasmic immunoreactivity (**Figure 2E**), whereas another clone of Ki-67, SP6, showed a common nuclear pattern, with an index of <1%. Galectin-3 was expressed weakly in two of four cases (**Figure 2F**). None of the four cases possessed the BRAF V600E protein reactivity.

BRAF gene and N-ras gene mutation

Gene mutation analysis demonstrated none of the four cases possessed BRAF (**Figure 3A**) and N-ras gene mutations (**Figure 3B**).

Diagnosis

All the cases were confirmed as HTT by pathological analysis.

Discussion

HTT is a rare and unique neoplasm derived from thyroid follicular epithelium. It is thought to be an exclusive tumor of thyroid gland with characteristic of trabecular structures and prominent intratrabecular hyalin. Since 1987, when it had been the first time described as “Hyalinizing trabecular adenoma” [8]. Hyalinizing trabecular adenoma was replaced by Hyalinizing trabecular neoplasm in the recent

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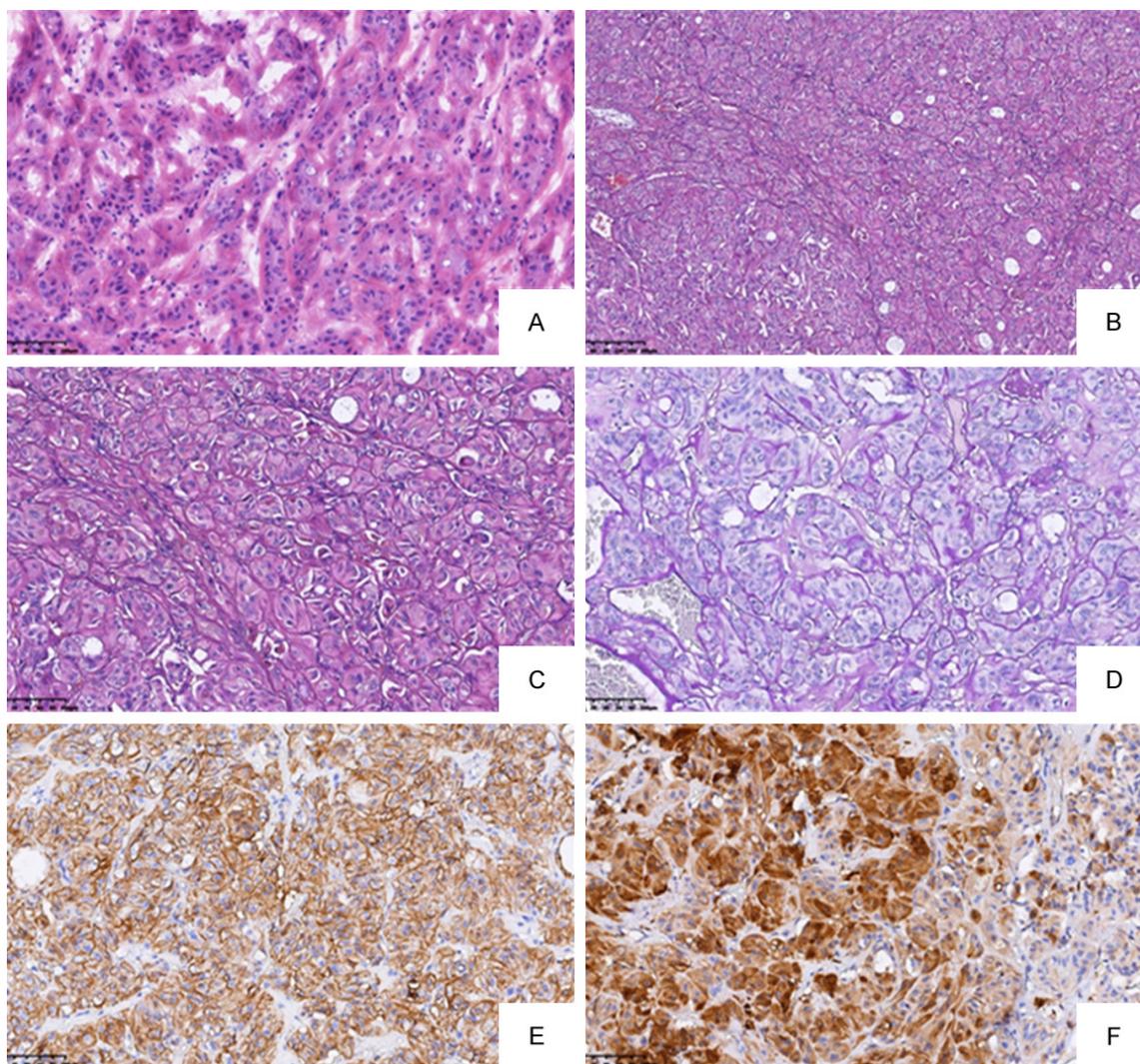


Figure 2. A. The FS showed follicles of varying sizes with focal characterized by trabecular structures and nuclei with prominent grooves and cytoplasmic inclusions, the intratrabecular hyalin was prominent. In some areas, the yellow peculiar intracytoplasmic inclusion bodies called “yellow bodies” can be observed. HE staining (magnification $\times 20$). B. The tumor was well encapsulated with characterized by trabecular structures and the thyroid tissues adjacent to the tumor showed lymphocytic thyroiditis. HE staining (magnification $\times 10$). C. The tumor presenting characteristics consisting of a trabecular growth pattern and hyalinizing material deposited intratrabecularly, the tumor cells were polygonal, oval or high columnar with an acidophilic or clear cytoplasm. The nuclei were oval with inconspicuous small nucleoli, prominent grooves and pseudoinclusions, the mitosis was rare and cytoplasmic “yellow bodies” were frequently found. HE staining (magnification $\times 20$). D. The hyaline material of the tumor was PAS positive. PAS staining (magnification $\times 20$). E. Staining with the MIB-1 antibody showed membranous/cytoplasmic immunoreactivity. EnVision method (magnification $\times 20$). F. Galectin-3 weakly expressed in some cases of HTT. EnVision method (magnification $\times 20$).

World Health Organization (WHO) classification of tumors of endocrine organs. This classification has defined this terminology as “a rare tumor of follicular cell origin with a trabecular pattern of growth and marked intratrabecular hyalinization” [9]. WHO classification has assigned it the term, i.e., Hyalinizing trabecular tumor (HTT) [10]. With the increasing number of

reported cases, the tumor has attracted more and more attention. Most of the published literature is case reports, and the largest case study has been also reported by Carney [11]. As HTT and PTC share significantly overlapping nuclear features and a high prevalence of RET/PTC translocations [12, 13], some authors consider HTT as a morphologic variant of PTC or a

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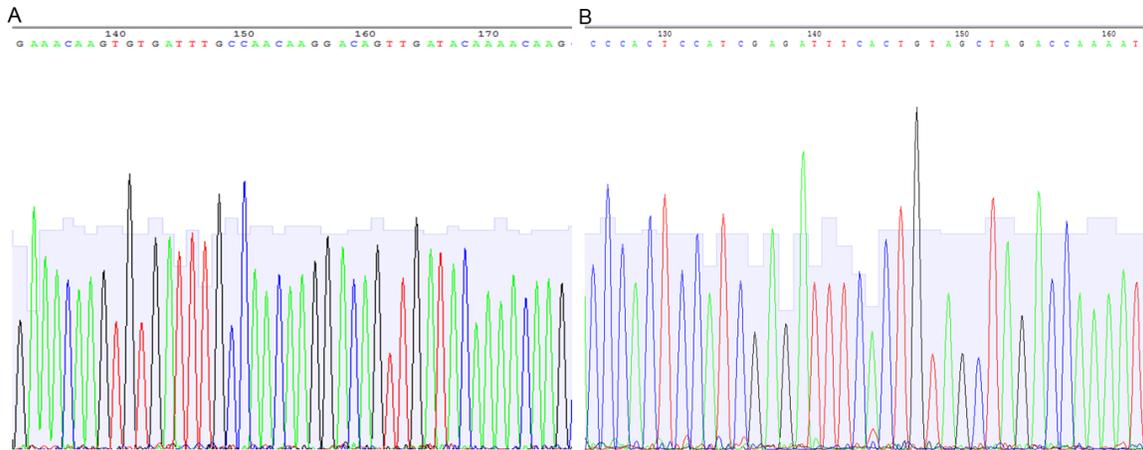


Figure 3. Gene mutation analysis demonstrated no mutation of the BRAF gene (A) and the N-ras gene (B).

precancerous lesion. HTT usually follows an indolent clinical course. The majority of the reported cases are benign, their biology remains controversial, whereas a few isolated cases, which are accompanied by metastasis in the lymph nodes or the lung, which named as hyalinizing trabecular carcinoma (HTC) [11-15]. Due to the uncertain malignant potential and property of this tumor, a more general term, HTT, has been adopted by most pathologists and the WHO classification, reflecting the controversy.

Clinical features of HTT

HTT often occurs more in female than in male, between the fourth and seventh decade of age, as presented our cases. Carney had reported 119 cases of HTT, which were the biggest series to date, only 18 cases were male, the others were female, and the mean age of the patients was 50 years (range 21 to 79 years) [11]. It is unusual to be diagnosed less than 20 years old and over 80 years old.

Clinically, most patients with HTT have no obvious symptoms and are found with neck mass during the physical examination. HTT is usually shown as a single clear, hypoechoic nodule on the ultrasound. A small number of cases showed abundant color flow signals in the nodules [16]. However, some patients may be accompanied with nodular goiter, FA, PTC and other thyroid diseases, so there are numerous nodules images presented on the ultrasound. Of all our cases, except one case with bilateral thyroid solitary nodules, all the other three cases are unilateral solitary nodules. In that

case, the nodules on both sides of the thyroid gland are different. The left side is a HTT, and the right side is a FA.

To date, the etiology of HTT remains to be fully clarified. It has been reported that the previous history of radiation exposure may play an important role in the development of HTT. This tumor may arise in the background of chronic lymphocytic thyroiditis and multinodular goiter, or in association with PTC [17]. The link between HTT and lymphocytic thyroiditis may be significant due to similarities in molecular genetics and age or gender distribution. The thyroid tissues adjacent to tumor are lymphocytic thyroiditis in all of our cases. But none of the patients had a history of accidental or therapeutic radiation exposure. It is suggested that lymphocyte thyroiditis may be associated with HTT. However, this remains to be addressed in future studies.

Pathological features of HTT

Preoperative cytology using FANC or US-FANC of thyroid nodules is effective, reliable, and widely used to distinguish the nature of these lesions. However, the features of hypercellularity and grooves, pseudoinclusions and hyperchromaticity of the nuclei, which are the main diagnostic clues for PTC, are also observed in HTT frequently. The cytological diagnosis of HTT can be challenging as these neoplasms share cytomorphological features with PTC. In most reported cases, these tumors are diagnosed as 'suspicious or even diagnostic for PTC' in cytological evaluations, and few tumors

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are diagnostic or suspicious for HTT, it is possible that cytologists are not familiar with HTT. Another characteristic of HTT in cytological smears is the presence of a fibrotic/hyaline matrix and dense clumps of vaguely myxoid material resembling colloid. Particularly in air-dried smears, this matrix can also be confused with amyloid, which is also possible to be found in smears of MTC. Findings on FNAC/US-FNAC that should raise the suspicion of HTT include a bloody background, cells with a low nuclear-to-cytoplasmic ratio, cellular aggregates around the hyaline material, fine chromatin (rather than the optically clear chromatin of PTC), and numerous nuclear inclusions and grooves. Almost all tumors were circumscribed or encapsulated with intact membrane, firm to soft, and spheroidal, oval, or slightly irregular in shape. According to literature report, one-half measured mass was less than 3 cm in diameter and 40% measured mass was 2 cm in diameter or less, and the fresh cut surface showed a solid, slightly bulging, delicately lobulated tumor with a yellow, pink, orange, or white color [11]. Among our patients, the size of tumors is ranged from 0.4 cm to 5.5 cm in diameter, and the mean size of the tumor is 2.8 cm. The results are similar to those reported in the literature.

Microscopically, the tumor cells are polygonal or fusiform, arranged in a trabecular or alveolar pattern in FS and conventional HE staining. The red hyaline material is present extensively in both intracellular and extracellular locations. Calcifications and psammoma bodies can be seen interspersed throughout the stroma in a small proportion of cases. Follicular structures within the tumor are often dysplastic or absent, and the long axis of tumor cells and the basement membrane are arranged vertically. The tumor cells are rich in cytoplasm, and the characteristic “yellow bodies” in cytoplasm can be visible [18]. It has been observed by electron microscopy that the “yellow bodies” are giant secondary lysosomes of multivesicular body subtype [19]. The nuclei of tumor cells can show different degrees of polymorphism, and the intensity of staining is different. The chromatin of the cell is fine, and it is easy to see the intranuclear inclusion bodies, and the nuclear groove is visible similar to PTC. However, the nuclear overlap and crowding is scarce and mitotic figures are rare.

Special staining

The hyalinizing material of HTT is PAS-positive and Congo Red-negative in special staining.

IHC

As HTT is of the thyroid follicular derivation, so it usually stains positive for thyroid follicular epithelial markers, such as Tg and TTF-1, and negative for CK19, calcitonin, Carcinoembryonic antigen (CEA), NSE, SyN and S-100. CgA is mostly expressed negatively in literature [4], but a recent study has shown that HTT has immunoreactivity for CgA, although the staining intensity is different according to the cases [20]. Our data also showed that CgA is expressed weakly in one case. Therefore, more samples are needed to further evaluate the expression of CgA in HTT. The expression of galectin-3 and HBME-1 are uncertain [21, 22]. Galectin-3 often displays strong positive staining in PTC, whereas this marker is weakly expressed in some cases of HTT. Lenggenhager investigated the immunophenotype of eight cases of HTT, found that all cases are CK19 negative and galectin-3 is expressed weakly in four of eight cases (50%) [21]. In addition, three of eight cases (37.5%) are HBME-1 positive, with staining of tumor cells as well as of intratrabeular hyaline matrix material and five of eight cases (62.5%) showed weak-to-moderate cytoplasmic Ki-67 positivity [21]. Gaffney [22] detected galectin-3 expression in a number of different thyroid neoplasms including 58 HTTs, 60 PTCs, 21 follicular carcinomas (FCs), and 14 FAs, found that 60% of the HTTs are negative or have weak staining and 40% have strong staining of galectin-3, however, fifty of the 60 (83%) PTCs showed strong immunostaining results. Our results supported the findings of Lenggenhager. We speculate the different biological behaviors between the HTT and PTC may lead to the difference of galectin-3 expression. In addition, the hyalinizing material of HTT is positive for collagen type IV and laminin immunostaining, which has been demonstrated for basal lamina-like substance ultrastructurally [23]. MIB-1 staining pattern is useful as the membrane/cytoplasm positive reactivity is a distinctive feature of HTT, however, no such pattern in PTC and other thyroid neoplasms are found yet. Notably, it has been reported that the membrane/cytoplasm positive pattern is

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observed for the MIB-1 clone only but not other clones of Ki-67 [21, 24-26], which is also confirmed in our present study. So the characteristic aberrant cell membranous and peripheral cytoplasmic staining of MIB-1 may be a specific marker of HTT. Remarkably, because membranous staining of Ki-67 might be influenced by the modification of antigen spatial conformation caused by the different immunostaining methods, and the antigen retrieval procedure could significantly affect Ki-67 immunostaining [20]. Therefore, the IHC staining of Ki-67 varies according to different IHC systems.

Molecular genetics features

HTT and PTC share significantly RET/PTC translocations. Also HTT has a high prevalence of RET/PTC translocations, but no BRAF or N-ras mutations [1, 6, 27]. However, BRAF or N-ras mutations are common in PTC [28, 29]. Thus, the mutation of BRAF and N-ras reflects the difference between the two diseases at the molecular level [1, 27]. It can be used for the differential diagnosis of the two diseases.

Differential diagnosis

The differentiation of HTT from other thyroid tumors with similar images, such as SPTC, HPTC and HTALMTC, can be achieved by histochemistry and immunohistochemistry besides morphology. SPTC and HPTC are rare subtypes of PTC, showing the typical nuclear cytological characteristics of PTC including complex branching papillae, nuclear grooves, inclusions and ground glass nuclei, without complete capsules. Moreover, vascular invasion and extra-thyroidal extension are observed in some cases. HTALMTC is a rare subtype of MTC. The typical nuclear cytological characteristics and intracytoplasmic “yellow bodies” of HTT often do not appear. HTALMTC often expresses calcitonin and neuroendocrine markers and exhibits positive staining for Congo Red, but is negative for Tg. On the contrary, HTT usually stains positive for thyroid follicular epithelial markers, such as Tg and TTF-1, and positive for collagen type IV in immunostaining, but negative for calcitonin and SyN. The hyalinizing material of HTT is PAS-positive and Congo Red-negative in special staining.

The referential experience to diagnose HTT can be used as follows: ① Variably cellular with cohesive clusters of cells which radiate from a

hyaline stromal core in a bloody background and the cytological features resemble those of PTC, with nuclei that have frequent intranuclear inclusions and grooves in FANC/US-FANC [4, 6]; ② “Yellow bodies” are a distinctive feature unique from PTC; ③ Immunohistochemically, the tumor cells demonstrate aberrant membranous and peripheral cytoplasmic staining for MIB-1; ④ Mutation detection of BRAF and N-ras can be used for differential diagnosis of HTT and PTC.

Treatment and prognosis

Recently, Carney investigated 119 cases of HTT for invasion, recurrence and metastasis, and obtained follow-up for 96% cases. In his findings, only one case showed vascular and capsular invasion and pulmonary metastasis [11]. Thus, it has confirmed that the behaviors of most HTTs exhibited as benign neoplasms. In addition, since 1994, a number of cases of hyalinizing trabecular carcinoma (HTC) have been reported. Summary of all cases reported, all of the 9 cases show capsular invasion, 78% vascular invasion are occurred (7/9) and 33.3% pulmonary metastasis are observed (3/9) [11, 13-15]. Therefore, the presence of capsule or vascular invasion in HTT is malignant, which is thought to be unfavorable prognostic factor [11, 14, 15]. In our cases, there is no capsule and vascular invasion, and no recurrence or metastasis is observed from a follow-up of 6-36 months.

In clinical practice, sufficient dissection and careful observation of vascular and capsular invasion is critical to predict whether the neoplasm is malignant or not. Once the diagnosis of HTC is established, it will be treated as malignant tumor. HTT are not usually performed by total thyroidectomy because of the relative benign clinical course. The recommended surgical policy tends to be conservative according to the majority of reports in the literature. However, long-term follow-up is necessary for HTT patients after surgery.

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Disclosure of conflict of interest

None.

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