

Hyalinizing Trabecular Adenoma of the Thyroid Gland

Identification Through MIB-1 Staining of Fine-Needle Aspiration Biopsy Smears

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Key Words: Fine-needle aspiration biopsy; Hyalinizing trabecular adenoma; Medullary thyroid carcinoma; MIB-1 immunostaining; Papillary thyroid carcinoma

DOI: 10.1309/5KJU9LV8LLA6CL76

Abstract

Smears of fine-needle aspiration (FNA) biopsy specimens of 24 histologically proven and MIB-1–positive hyalinizing trabecular adenomas stained with Papanicolaou or Diff-Quik were stripped from the original slides, transferred to charged slides, destained, and restained with MIB-1. Positive immunoreactivity with MIB-1 in a peripheral cytoplasmic pattern was present in 17 specimens, no immunoreactivity was seen in 4 specimens, and equivocal staining was seen in 3 specimens. Papanicolaou-stained smears of papillary thyroid carcinoma, medullary thyroid carcinoma, thyroid follicular neoplasm, and Hürthle cell adenoma (5 specimens each) were treated similarly. None of the control cases stained with MIB-1 had the peripheral cytoplasmic pattern. Hyalinizing trabecular adenoma can be distinguished reliably from other thyroid neoplasms by MIB-1 staining of destained cells from FNA biopsy specimens.

Hyalinizing trabecular adenoma (HTA) of the thyroid gland is a rare benign neoplasm described by Carney et al in 1987.¹ It is characterized by circumscription or encapsulation, trabecular growth pattern, polygonal and elongated cells, nuclear cytoplasmic inclusions and grooves, hyaline material, dilated sinusoids, laminated calcospherites, and cytoplasmic yellow bodies.^{1–3} Molecular genetic studies have allied the tumor with papillary thyroid carcinoma (PTC), but other findings, in particular the absence of immunostaining for cytokeratin 19 and high-molecular-weight keratin, do not support this relationship.⁴ For this reason, it has been suggested that the lesion be referred to as *hyalinizing trabecular tumor*.^{5–9} Of great interest is the remarkable appearance of the HTA tumor cells with MIB-1 immunostaining—heavy staining of a narrow peripheral rim of the tumor cell cytoplasm^{10,11}—an appearance not shared with other thyroid tumors. MIB-1 is a monoclonal antibody raised against the recombinant part of the Ki-67 antigen and ordinarily is expressed in the nuclei during active parts of the cell cycle.^{12,13}

HTA is misdiagnosed almost uniformly in fine-needle aspiration (FNA) biopsy specimens, because of the confusing similarity of its nuclear features to those of PTC and the presence of a misleading hyaline material in the tumor that mimics amyloid, and often is diagnosed as medullary thyroid carcinoma (MTC).^{14–23}

We described the cytologic findings in specimens from 29 patients with HTA stained with the Papanicolaou and Diff-Quik methods.¹⁷ Important features of the neoplasm included a bloody background; cells with ample cytoplasm arranged in cohesive aggregates, often radially oriented around hyaline material; and polygonal nuclei with abundant grooves and cytoplasmic invaginations. These and other features

permitted diagnosis or suspicion of HTA in the majority of the 29 cases. In the remainder, the cytologic diagnosis was not possible, usually because the smears were poorly preserved or hypocellular, lacked hyaline material, or showed combinations of these findings.

We investigated whether the unique MIB-1 cytoplasmic staining characteristic of HTA seen in tissue sections could be seen in FNA biopsy samples suggestive of the neoplasm. Unstained cytologic slides of the neoplasm were not available, so previously stained FNA biopsy samples were used from patients with HTA proven by MIB-1 staining of tissue sections. The stained cells were transferred to charged slides, destained, and restained with MIB-1. The restained cells showed the typical peripheral cytoplasmic positivity of the neoplasm in 17 (71%) of 24 cases. The results indicate that this method is practical and should prove useful in the cytodagnosis of suspected cases of HTA.

Materials and Methods

This study was approved by the Mayo Foundation Institutional Review Board. We retrieved 29 specimens from HTA cases from the surgical pathology collection of Mayo Clinic, Rochester, MN, and our own collection. FNA biopsy specimens were present from each patient, in addition to tissue sections. Six biopsy samples were excluded, 2 because of insufficient cells and 4 because permission to reprocess the original slides was denied. The remaining 23 cases and an additional recent case formed the study group. H&E-stained slides of the tumor sections were reviewed, and the diagnosis of HTA was confirmed histologically in all **Image 1A**. Twenty-three samples had diffuse, typical MIB-1 positivity **Image 1B**; in the remaining case, the MIB-1 staining was focal (<5% of tumor cells showed staining). The original FNA biopsy cytologic diagnoses in the 24 cases had been PTC in 11 cases, “suspicious for” PTC in 8 cases, follicular neoplasm in 4 cases, and indeterminate in 1 case.

The cytologic material available comprised Papanicolaou-stained (19 cases) or Diff-Quik–stained (5 cases) FNA biopsy smears. The smears featured cohesive aggregates of cells, often radially oriented around hyaline material, and found singly less frequently. Cytoplasm was abundant. Intranuclear cytoplasmic inclusions, nuclear grooves, and nuclear overlapping were very common and best seen with Papanicolaou stain. Diff-Quik–stained smears highlighted the metachromatic hyaline material, the perinucleolar clearing, and the cytoplasmic bodies.

Cells from the previously stained smears were stripped from the original slides, using the “peel and stick” technique.²⁴ This method was used instead of direct

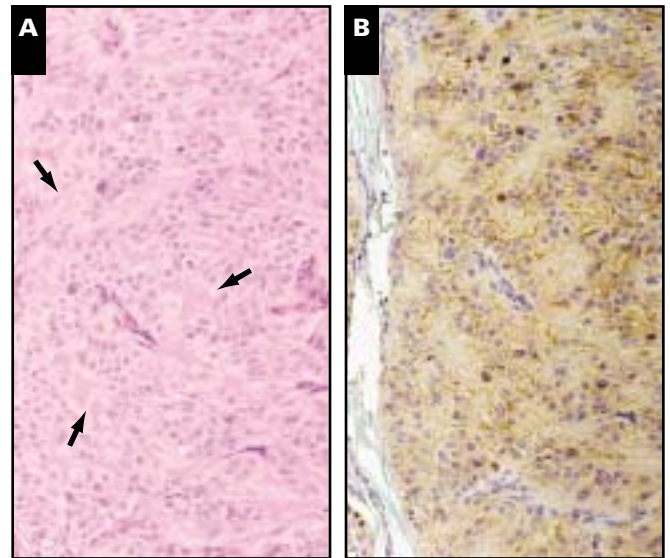


Image 1 Histology of hyalinizing trabecular adenoma.

A, Section showing a delicately encapsulated tumor with trabecular growth pattern, oval nuclei, and acellular hyaline material (arrows) (H&E, original magnification $\times 400$).

B, Tissue section from Image 1A (after destaining) showing peripheral cytoplasmic staining of the tumor cells. Rare diffuse nuclear staining is present (MIB-1, original magnification $\times 200$).

destaining and restaining of the original smear because it permitted the use of multiple stains in addition to MIB-1. The original peeled smear was cut into several pieces, and only 1 was used for MIB-1 staining. The stained slides were placed in xylene to loosen the coverslip so that it could be removed. The exposed cells were covered with organic solvent–based mounting media (Krystalon, EM Science, Gibbstown, NJ), placed in an 80°C to 90°C oven for 6 hours, permitted to cool to room temperature, and placed in 90°C distilled water for 20 to 30 seconds. The hardened mountant with the cells attached to it was undermined and separated from the slide, using a single-edged razor blade held at a 45° angle. Heated (90°C) distilled water was flooded onto charged glass slides, and the removed mountant with attached cells was placed on a new slide.

The new slides then were placed in an 80°C to 90°C oven for 30 minutes. They were removed, permitted to return to room temperature, and placed in xylene to remove the mountant. The slides were rehydrated in 70% alcohol and were decolorized in 0.3% acid alcohol, washed in running tap water for 5 minutes, and rinsed in distilled water. The samples then were restained with MIB-1 antibody (clone MIB-1, DAKO, Carpinteria, CA). Immunostaining was performed at a 1:800 dilution, using avidin-biotin complex detection chemistry and 3,3'-diaminobenzidine as the chromogen.

Papanicolaou-stained FNA smears of PTC, MTC, thyroid follicular neoplasm, and Hürthle cell adenoma (5 cases each) were treated similarly.

Results

The patients whose biopsy specimens were used were 24 women with a median age of 43 years (range, 25-84 years). Cytoplasmic MIB-1 staining showed that 17 smears (71%) (16 initially stained by the Papanicolaou method and 1 by the Diff-Quik method) had the typical peripheral cytoplasmic positivity seen in histologic sections of the tumor. Three smears (13%) showed equivocal staining, and 4 smears (17%) did not stain. Occasional nuclei were stained in 8 cases (33%). Amorphous material in the cell aggregates, interpreted as hyaline, was not stained.

Cytoplasmic Staining

The abundant cytoplasm of most individual tumor cells was stained in the 17 positive cases. The staining was light to heavy and most prominent in a narrow rim at the periphery of the cells, consistent with staining of the cell membrane or a restricted zone of the cytoplasm immediately interior to it. This staining usually had a solid linear appearance but occasionally had an interrupted beaded form, appearing as a series of stained granules or, less commonly, vacuoles, both forms just resolvable at high-power magnification **Image**

2A. The remainder of the cytoplasm also was stained but to a lesser degree than the narrow peripheral band.

Groups of cells had a variable appearance depending on their flatness, thickness, and complexity of folding. Staining of flat groups had a honeycomb pattern (**Image 2A**). With increasing complexity of cell arrangement, the clusters had a more disorderly “crazy paving” appearance **Image 2B** and sometimes a jumbled array of crisscrossing folds. Rarely, a linear unstained zone between 2 flanking stained zones confirmed that the stained material was peripheral intracellular and not extracellular (basement membrane). There were occasional aggregates of small cells in 2 specimens that did not stain or stained only lightly. Structures that might correspond to the cytoplasmic bodies seen in histologic sections and FNA smears of HTA were not seen in any specimens.

Amorphous Material

The amorphous hyaline material seen did not stain with MIB-1 **Image 2C**.

Nuclear Staining

In 4 specimens, some nuclei were diffusely stained **Image 3A**. The appearance of the stained nuclei was similar to that seen during DNA synthesis. In an additional 4 samples, a few nuclei had a different staining pattern, a distinct, heavy, irregularly shaped, or spherical stained area partially occupying the nucleus **Image 3B**. This zone in some cases appeared to be a portion of an intranuclear cytoplasmic inclusion.

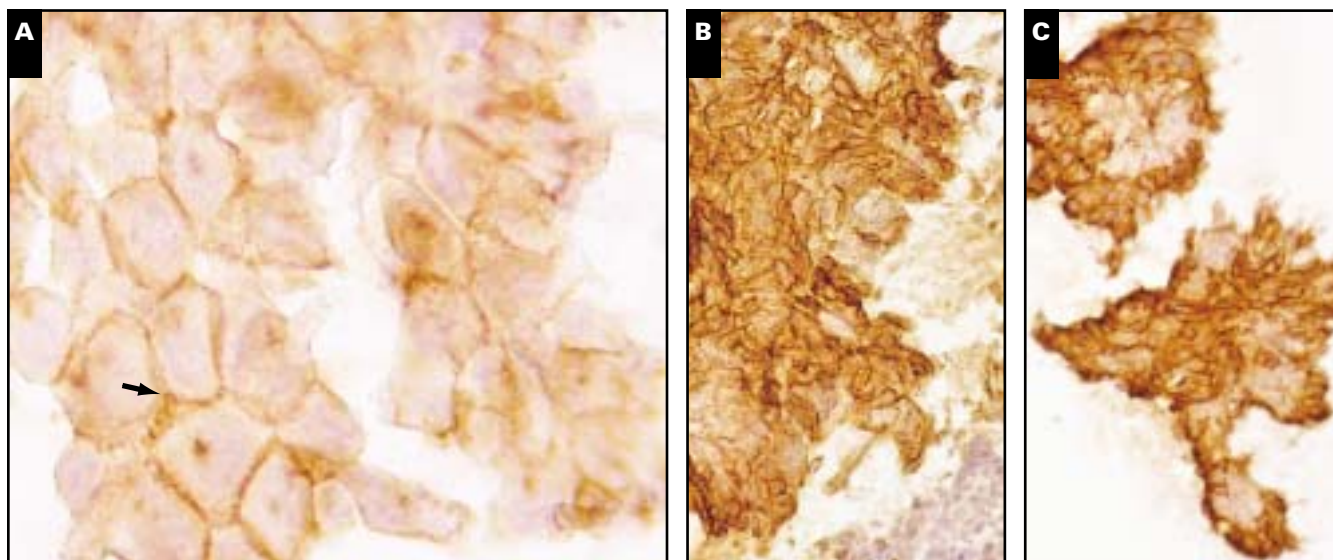


Image 2 MIB-1 stain of hyalinizing trabecular adenoma. **A**, Flat group of cells shows a honeycomb pattern. Peripheral cytoplasmic staining of the tumor cells is present. Staining is light to heavy and has a predominantly solid linear pattern but focally a vacuolar pattern (arrow) (original magnification $\times 600$). **B**, Complex cell arrangement with disorderly “crazy paving” appearance. Strong diffuse peripheral cytoplasmic staining of tumor cells is present. There is less prominent staining of the remainder of the cytoplasm. A group of smaller cells (bottom right) is not stained (original magnification, $\times 400$). **C**, Two aggregates of cells, one with branching architecture. Unstained areas in both are consistent with hyaline (original magnification, $\times 200$).

All of the control cases (5 cases each) of PTC, MTC, thyroid follicular neoplasm, and Hürthle cell adenoma were negative for the peripheral cytoplasmic staining pattern with MIB-1. Scattered positive diffuse nuclear staining was identified in each subset of the control cases.

Discussion

HTA of the thyroid is a neoplasm with distinctive morphologic features described by Carney et al in 1987.¹ It had been confused histologically in the Mayo Clinic practice with PTC and MTC. The nuclear features of the tumor, the grooves and cytoplasmic inclusions (“holes”), led to its confusion with PTC, and the homogeneous acidophilic hyaline material (that mimicked amyloid) led to mistaken diagnoses of MTC.

Subsequent investigative reports have produced new information about the neoplasm. It is important to note that HTA has been found to exhibit an unusual peripheral cytoplasmic MIB-1 staining pattern that does not occur in other thyroid neoplasms—a few nonthyroid epithelial neoplasms reportedly have the same cell membrane–associated staining pattern.^{25,26} In addition, *RET/PTC* mutations have been found in some HTAs, which suggests that HTA is a peculiar form of PTC and that it should be referred to as *hyalinizing trabecular tumor* because of uncertainty about the importance of the molecular changes.

At present, the problem posed by HTA is its distinction from PTC in FNA biopsy specimens. Recently, we studied the cytologic findings in a Papanicolaou- and Diff-Quik–stained series of HTA biopsy specimens that should facilitate suspicion and diagnosis of the neoplasm.¹⁷ In 8 cases (28%), however, the diagnosis remained difficult for a variety of reasons.

To address this problem and to confirm the diagnosis in cases in which the lesion was suspected cytologically but the diagnosis was uncertain, we wondered whether the unusual MIB-1 staining pattern seen in histologic sections of the neoplasm could be detected in FNA biopsy smears. The unique MIB-1 pattern indeed was seen in 17 FNA samples (71%). Equivocal staining was seen in 3 samples, and no staining was seen in 4. Histologic sections in 3 of the 4 nonstaining cases had the typical diffuse cytoplasmic MIB-1 staining characteristic; an explanation for failure of MIB-1 staining of the FNA specimens is lacking. The remaining case had staining of less than 5% of the tumor cells in the histologic sections, which likely explains the negative result with the biopsy sample. Negative results were more common in specimens initially stained by the Diff-Quik method.

The method we describe appears useful, and it would seem ideal if it could be applied to all FNA biopsy specimens in which the nuclear features suggested a diagnosis of PTC. The majority of cases of HTA thus would likely

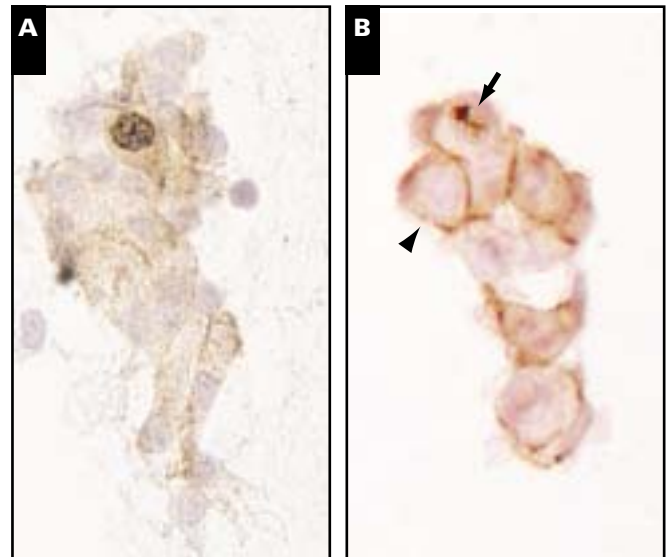


Image 3 MIB-1 nuclear staining. **A**, One nucleus shows strong diffuse staining. Variable linear peripheral cytoplasmic staining is evident (original magnification, $\times 400$). **B**, An irregularly shaped zone of partial nuclear staining is seen in a nucleus that also shows multiple inclusions (arrow). Peripheral cytoplasmic staining of variable thickness and with a barely visible focal vacuolar appearance (arrowhead) is seen (original magnification $\times 400$).

be identified because of diagnostic cytoplasmic staining, and, as a result, patients would be spared unnecessary anxiety and unnecessary surgery. General use of the procedure, however, would be expensive, probably prohibitively so, because PTC is a relatively common neoplasm and HTA is rare. It would be necessary to carefully select cases in which to apply the technique we describe. As an initial guideline, we suggest that it be reserved for cases in which there is suspicion of HTA but with inconclusive findings. Such cases would include those in which the smears were not bloody, were hypocellular, or both; those in which the nuclei showed an unexpectedly large number of nuclear grooves or cytoplasmic inclusions or both; and cases in which material consistent with hyaline was absent or sparse.

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