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Decalcification does not Affect Immunohistochemical Stain of HBME-1 on Papillary Thyroid Carcinoma

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Introduction

Thyroid carcinoma is the most common endocrine malignancy and 80% of all thyroid malignancies are papillary thyroid carcinoma [1]. The diagnosis of papillary thyroid carcinoma is based on architectural features combined with nuclear features, including nuclear clearing, overlapping, grooves and pseudoinclusions [2,3]. It is always challenging in our everyday practice to distinguish folicualr variant papillary thyroid carcinoma (FVPTC), one of the subtypes of PTC, from cellular adenomatous nodules. Nuclear clearing in benign thyroid lesion, such as in Hashimoto's disease may lead pathologists to misdiagnose papillary thyroid carcinoma. In an unusual condition, it may also be difficult for differentiating papillary hyperplastic nodules from papillary thyroid carcinoma.

HBME1 is a monoclonal antibody which was originally developed as a mesothelioma marker and directed against the microvillous surface of mesothelial cells [4]. Subsequently, it was applied to the diagnosis of malignant thyroid conditions. Several studies demonstrated that HBME-1 is a sensitive marker for papillary thyroid carcinoma [5-9].

Calcification of thyroid carcinoma and benign thyroid lesions are common [10-15]. In practice, decalcification process must be performed for surgical pathological slides formation to evaluate the nature of the calcified lesion. Decalcification has been demonstrated to affect the identification of markers by immunohistochemistry [16-18]. More importantly, nuclei of thyroid follicular cells close to calcified areas can appear to have (?) nuclear clearing or even nuclear grooves. As an important marker for diagnosing PTC, the effect of decalcification in HBME-1 staining for papillary thyroid carcinoma has not been elucidated. For this purpose, we performed immnuhistochemical stains for HBME-1 in calcified PTCs by using calcified benign thyroid lesions as controls and concluded that decalcification does not affect HBME-1 stain for PTCs.

Methods

The surgical pathology files from two participating institutions (Tisch Hopsital, New York University Langone Medical Center and the Hospital of University of Pennsylvania) were searched for cases on thyroid resection specimens that contained in the gross description and/or diagnosis with key word "decalcification" from 2008 to 2012. Twenty-six cases were recruited, including seventeen cases of papillary thyroid carcinoma, eight cases of nodular hyperplasia, and one case of follicular carcinoma. Hematoxylin and eosin slides from all cases were reviewed to confirm the diagnosis. Clinical information was obtained by reviewing the charts.

Immunohistochemical stain for HBME-1 was performed for all the cases:

Immunohistochemistry was performed on 4 micron formalin fixed, paraffin embedded papillary thyroid carcinomas using mouse anti-human mesothelial cell, tissue culture supernatant clone HBME-1 (Dako Carpentaria, CA USA). In brief, sections were deparaffinized in xylene (3 changes), rehydrated through graded alcohols (3 changes 100% ethanol, 3 changes 95% ethanol) and rinsed in distilled water. Heat induced epitope retrieval was performed in a 1200-Watt microwave oven at 100% power in 10 mM sodium citrate buffer, pH 6.0 for 10 minutes. Sections were allowed to cool for 30 minutes and then rinsed in distilled water. Antibody incubation and detection were carried out at 40°C on a NexES instrument (Ventana Medical Systems Tucson, Arizona USA) using Ventana's reagent buffer and detection kits unless otherwise noted. Endogenous peroxidase activity was blocked with hydrogen peroxide. Anti-HBME-1 was diluted 1:100 in Dulbecco's Phosphate Buffered Saline, (Life Technologies Grand Island, New York USA) and incubated overnight at room temperature. Primary antibody was detected with iView biotinylated goat anti-mouse followed by application of streptavidin-horseradish-peroxidase conjugate. The complex was visualized with 3,3-diaminobenzidene and enhanced with copper sulfate. Slides were washed in distilled water, counterstained with hematoxylin, dehydrated and mounted with permanent media. Appropriate positive and negative controls were included with the study sections.

Results

Most papillary thyroid carcinomas with calcification stain for HBME-1:

With appropriate controls seventeen papillary thyroid carcinoma with calcification were stained with HBME-1. Sixteen cases were positive for membraneous staining of the tumor cells with this antibody except for one case that was negative. Interestingly, noncalcified carcinoma blocks were available for six PTC cases. Five positive cases showed positive labeling for HBME-1 in the noncalcified carcinoma. The noncalcified carcinoma from the negative case was negative.

All non-papillary thyroid carcinoma cases are negative for HBME-1 staining:

Eight cases of calcified nodular goitre and one case of follicular carcinoma were stained with HBME-1 by using the same condition of PTC in this study. All these non-PTC cases were negative for HBME-1 staining (Figure 1).

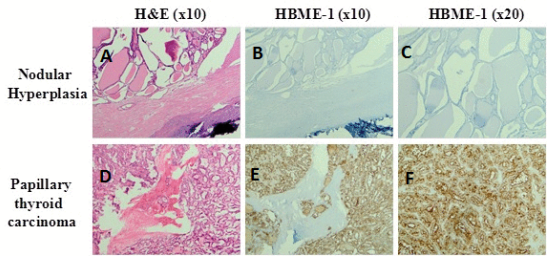

Figure 1: HBME-1 expression pattern in calcified thyroid lesions after decalcification process. The HBME-1 stain is negative in calcified benign thyroid lesion (Panel A-C). HBME-1 is identified in calcified papillary thyroid carcinoma (Panel D-F).

Discussion

Papillary thyroid carcinoma is the most common thyroid malignant tumors [1]. Calcification within the thyroid gland is a common finding both on thyroid imaging and thyroid histopathologic findings. Several reports in the literature have noted that calcification seems to be more common in malignant thyroid nodules than benign thyroid nodules [12-14]. Most of the calcified benign thyroid lesion was in multinodular goiters [14].

The use of immunohistochemical markers, such as HBME-1, CK19, CD15, and Galectin 3, has been proven in many histological studies to distinguish PTC from its mimics [5,7,19-21]. HBME-1 was noted as one of the most sensitive and specific markers for PTCs [7,8].

For severely calcified thyroid lesions, decalcification has to be performed to evaluate the nature of the lesion. Decalcification of the tissue has variable effects on immunohistochemical stains [19-21]. To elucidate the effect of decalcification on HBME-1 staining on PTCs and its mimics, we searched surgical pathological cases in two institutions and identified sixteen calcified PTCs, one calcified follicular carcinoma, and eight cases of thyroid multinodular hyperplasia.

All cases were stained for HBME-1 immunohistochemically on decalcified samples. All PTC cases were positive for HBME-1 labeling except one case. Interestingly, noncalcified carcinoma samples were available for six PTC cases. Five positive cases showed positive labeling for HBME-1 in the noncalcified carcinoma. The noncalcified carcinoma from the negative case was negative. All benign thyroid lesions and the case of follicular carcinoma were negative for HBME-1 staining. These results indicate decalcification does not have significant effect on immunohistochemical staining of HBME-1 for thyroid lesions. HBME-1 stain can be used in practice to distinguish difficult calcified cases between papillary thyroid carcinoma and benign thyroid lesions.

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