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아급성 육아종성 갑상샘염의 세침흡인 세포소견
-아급성 육아종성 갑상샘염 10예의 임상-세포소견 및 면역세포염색 소견 분석-

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Fine Needle Aspiration Cytology of Subacute Granulomatous Thyroiditis
-A Clinico-Cytological Review of 10 Cases with Immunocytochemical Analysis-

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Although subacute granulomatous thyroiditis(SGT) is usually diagnosed clinically, papillary carcinoma or other thyroid conditions must be considered in the differential diagnosis. We retrospectively reviewed the clinical and fine-needle aspiration(FNA) cytologic findings seen in 10 SGT cases to decide what are the most reliable cytologic findings and the most helpful molecular tools for reaching a confident cytologic diagnosis. The most representative smear slides were retrieved to perform immunocytochemistry for cytokeratin19(CK19) and Ret protein. Five papillary carcinomas(PTCs) were included as controls. The constant and typical cytologic findings of SGT were multinucleated giant cells(MGCs) (100%), epithelioid granulomas(90%), an inflammatory dirty background(90%) and plump transformed follicular cells(80%) without fire-flare cells, oncocytic cells or transformed lymphocytes. The immunoreactivities for CK19(37.5%) and Ret(10%) of the follicular cells of SGT were less than those(CK19 and Ret:100%) of PTC. CK19 immunoreactivity of the MGCs was seen in only one case of PTC. There was no significant difference between CK19 and Ret immunocytochemical staining for the MGCs of both SGT and PTC. The results of this study demonstrate that the cytological diagnosis of SGT can be improved by employing a combination of the typical and constant diagnostic cytological features and immunocytochemical results.

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Key Words : Fine needle, Biopsy, Thyroiditis, Subacute, Immuno histochemistry

INTRODUCTION

Subacute granulomatous thyroiditis(SGT) is also known as de Quervain' s thyroiditis, and this malady has an unknown cause, but is thought to be a postviral syndrome.[1,2] It typically presents in middle aged women who complain of pain and tenderness in the area of the thyroid gland, along with malaise and fever. The erythrocyte sedimentation rate(ESR) of SGT may be higher than that in any other form of thyroiditis.[3] The thyroid generally shows symmetrical or asymmetrical diffuse enlargement without well-defined nodules. SGT is usually diagnosed clinically without performing fine-needle aspiration(FNA). Consequently, any cyto-

logic literature on this condition is relatively scarce.[1,4-6] In the early phase of SGT, the typical cytologic findings may be lacking and atypical follicular cells may suggest malignancy.[7] Malignant thyroid lesions can recently be easily diagnosed by immunohistochemical staining in conjunction with using the known molecular markers.[8] To the best of our knowledge, there have been no studies that have examined the expression of molecular markers or immunohistochemical staining of atypical cells for making the differential diagnosis of SGT from malignancy.[9] This study focused on demonstrating the cytologic features and molecular findings of SGT to discriminate it from thyroid malignancy.

MATERIALS AND METHODS

All the cases of SGT that underwent FNA for thyroid nodules over a 2-yr period(2002-2004) at Severance Hospital were used as the subjects of this study. The patients' clinical records were reviewed and the relevant data was recorded. To be included in the study, a diagnosis of SGT was established by the appropriate clinical, serologic and follow up findings. These included pain and tenderness in the neck or thyroid area that was accompanied by fever and an elevated ESR, and the FNA cytologic features were compatible with the diagnosis of SGT.

The FNA was obtained to confirm whether the nodules in question were due to SGT or to other conditions, particularly neoplasm. Other entities that may show multinucleated giant cells(MGC) and granuloma on FNA were ruled out by a review of the patients' charts. These included previous surgery to the thyroid/neck area, treatment with phenytoins, prior radionuclide scanning and a history of sarcoidosis, tuberculosis or fungal infections. Palpation thyroiditis was not considered because there was no palpation history in the records.

The fine-needle aspiration biopsies were performed by a radiologist. 23-gauge needles, 20-ml syringes and a syringe holder were used. An average of two passes per thyroid nodule was performed. The majority of the material was placed on frosted slides, fixed in 95% alcohol and then stained using the Papanicolaou technique. The remainder of the aspirated material was rinsed in saline and centrifuged for preparation of a paraffin cell block. We reviewed all the cytology slides, including slides made from the cell blocks. The cytology slides were studied for the following parameters: 1) plump transformed follicular cells; 2) epithelioid granulomas, 3) MGCs, 4) an acute or chronic inflammatory dirty background, and 5) the presence of the fire-flare cells, hypertrophic follicular cells, oncocytic cells and transformed lymphocytes.[10] The presence of a given cytologic criterion was scored semi-quantitatively on a scale of 0 to 2+ (absent: 0, scarce: +, numerous: ++).

Immunocytochemical stains for cytokeratin 19 and Ret were performed. The archival cytologic slides were retrieved from the cytopathology laboratory slide archives. Two representative slides of each subject were chosen and these were processed using the labeled streptavidin-biotin-peroxidase method. Briefly, the slides were subsequently incubated in 10% normal blocking serum for 30 min. They were then incubated overnight at 4℃ in the appropriately diluted primary antibody. Rabbit polyclonal anti-Ret(1:200; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) antibody and mouse monoclonal anti-CK19(1:75; Biomeda, Hayward, CA, U.S.A.) antibody were used. After washing with Tris buffer, the sections were incubated with biotin-labeled secondary antibodies and then with streptavidin-horseradish peroxidase with using the DAKO LSAB kit(DAKO, Carpinteria, CA, U.S.A.) at room temperature for 30 min for each step. Nova red (Vector Laboratory, Burlingame, CA, U.S.A.) was used as the chromogen and hematoxylin was used for the nuclear staining. This procedure was performed for all the antibodies in the study. Diffuse cytoplasmic staining of the follicular cells or the MGCs was defined as positive immunoreactivity for Ret and CK19. The presence of the immunoreactivity was scored semi-quantitatively

on a scale of 0 to 2+ (absent: 0, less than 10% immunoreactivity of the corresponding cells: +, more than 10 % immunoreactivity of the corresponding cells: ++).

Statistical analysis

Differences in the expression of Ret proteins and CK19 in the follicular cells and MGCs between the groups were evaluated using Fisher exact test. A P value < 0.05 was considered significant. The clinical records, serological findings and follow up findings are presented as percentages.

RESULTS

1. Clinical Findings

Seven patients showed mild to severe painful neck swelling and two of them revealed fever. The history of upper respiratory tract infection before the onset of subacute granulomatous thyroiditis was not recorded.

The initial laboratory data at diagnosis demonstrated that the ESR of 4 out of 5 patients who underwent this test was increased(range: 15~55 mm/hr, mean: 37 mm/hr(NL. 0.0~20.0)).

On retrospective gray-scale ultrasound(US), only three patients revealed enlargements of the glands (more than 1.8 cm in the AP diameter). All of the patients had focal ill-defined hypo echoic areas and three of them were suspicious for malignancy. The flow on color Doppler US was not increased. Eight patients were treated with steroid and then one of them experienced disappearance of the focal lesion and the focal lesions of the other five patients were improved(Table 1).

Table 1. The Clinical Findings of Subacute Granulomatous Thyroiditis

Clinical History	Present	Absent
Mild to severe painful neck swelling	7	3
accompanying fever	2	8
History of upper respiratory tract infection before the onset of subacute granulomatous thyroiditis	not recorded	
Initial laboratory data at diagnosis (n=5)		
Increase of ESR (15~55 mm/hr, mean 37 mm/hr (NL. 0.0~20.0))	4	1
On retrospective gray-scale US,		
Enlargements of the glands (more than 1.8 cm in AP diameter)	3	7
Focal ill-defined hypo echoic areas	10	0
Suspicious thyroid carcinoma	3	7
Increase of flow on color Doppler US	0	10
After the steroid therapy, follow-up US(n=8)		
Disappearance or	1	
Improvement of the lesions	5	2

US: ultrasound
AP: anterior-posterior

Table 2. Cytologic Findings of Subacute Granulomatous Thyroiditis

Cytologic findings	++	+	0
Pump transformed follicular cells	5	3	2
Epithelioid granulomas	8	1	1
Multinucleated giant cells	10	0	0
Inflammatory dirty background	9	0	1
Fire-flare cells	1	0	9
Hypertrophic follicular cells	2	3	5
Oncocytic cells	0	1	9
Transformed lymphocytes	2	1	7

2. Cytologic Findings(Table 2)

Eighty percent of the subjects revealed plump transformed follicular cells. Ninety percent of the cases showed epithelioid granulomas and an inflammatory dirty background. All the subjects had MGCs(Table 2). Therefore, the most constant cytologic features of SGT were epithelioid granulomas, MGCs and an inflammatory dirty background with plump transformed follicular cells(Fig. 1).

There were hyertrophic follicular cells in 5 cases (50%) out of 10 SGTs, transformed lymphocytes in 3 cases(30%) out of 10 SGTs, fire-flare cells in 1 case (10%) out of 10 SGTs and oncocytic cells in 1 (10%) out

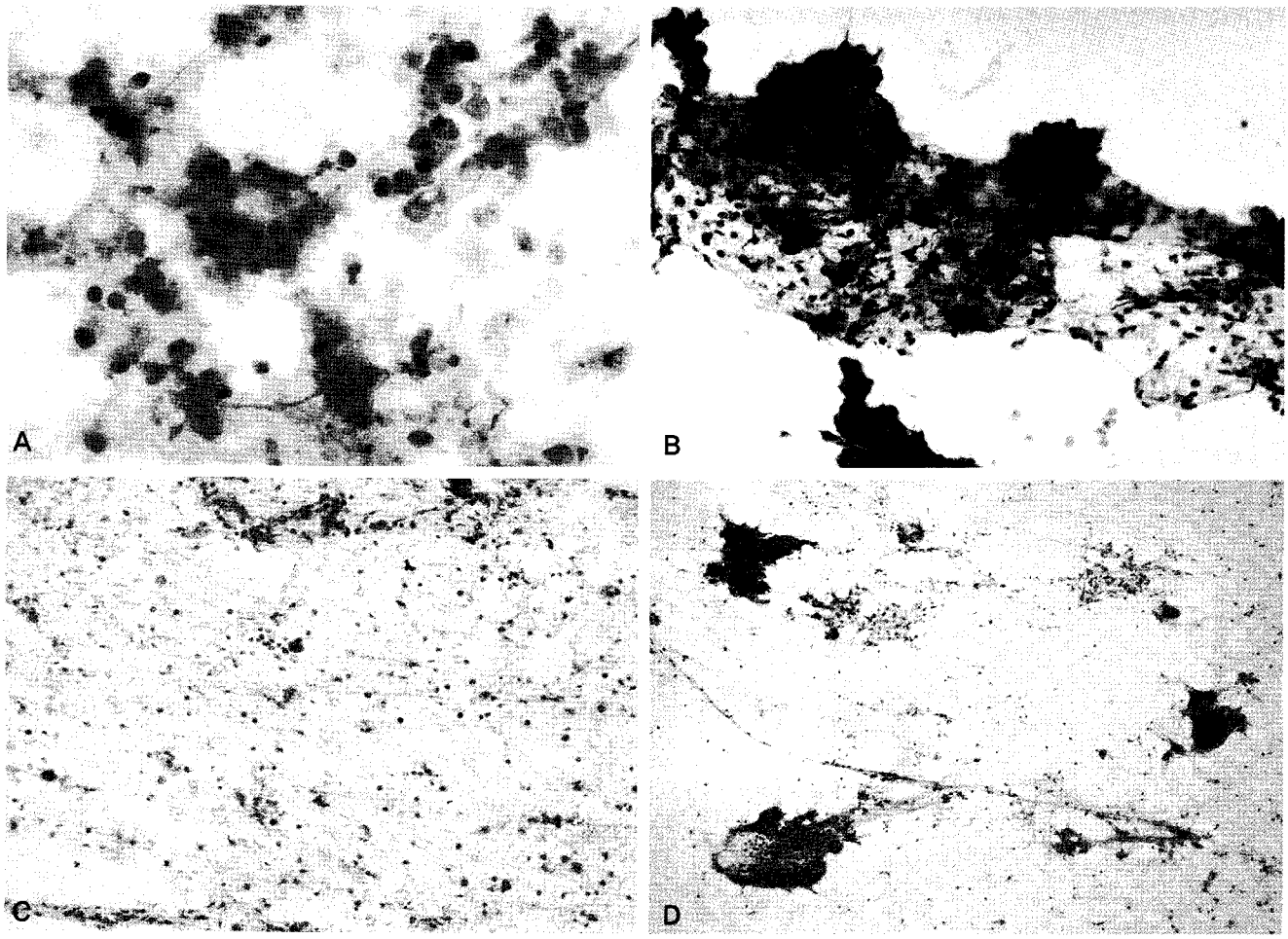

Fig. 1. Cytologic features of subacute granulomatous thyroiditis. (A) Plump transformed follicular cells, (B) epithelioid granuloma, (C) dirty inflammatory background and (D) multinucleated giant cell are seen. (Papanicolaou stain).

of 10 SGTs. Therefore, we can differentiate SGT from other thyroid diseases like lymphocytic thyroiditis or adenomatous hyperplasia, which display hyertrophic follicular cells, transformed lymphocytes, fire-flare cells and oncocytic cells.

3. Immunocytochemical stainning results (Table 3)

The CK19 and Ret immunoreactivity in the follicular cells revealed that 37.5% of the SGTs were positive for CK19 and 10% of the SGTs were positive for Ret. In contrast to the SGT results, the CK19 and Ret immunoreactivities in the follicular cells of the PTC were all positive (100%). These results were statistically significant (CK19; p=0.02, Ret; p=0.002) (Fig. 2). There was no SGT case that its follicular cells positively stained with both markers at the same time.

Table 3. Immunocytochemical Results in Subacute Granulomatous Thyroiditis and Papillary Carcinoma.

		CK19		Ret	
		SGT	PTC	SGT	PTC
Follicular cell	0	5	0	9	0
	+	2	0	0	0
	++	1	4 *	1	5 †
MGC	0	8	3	3	0
	+	0	1	5	4
	++	0	0 ‡	2	1 §

SGT; Subacute granulomatous thyroiditis
PTC; Papillary carcinoma
MGC; Multinucleated giant cells
*; p=0.02, †; p=0.002, ‡; p=0.333, §; p=0.748

The CK19 and Ret immunoreactivity in the MGCs revealed that none of the cases of SGT was positive for

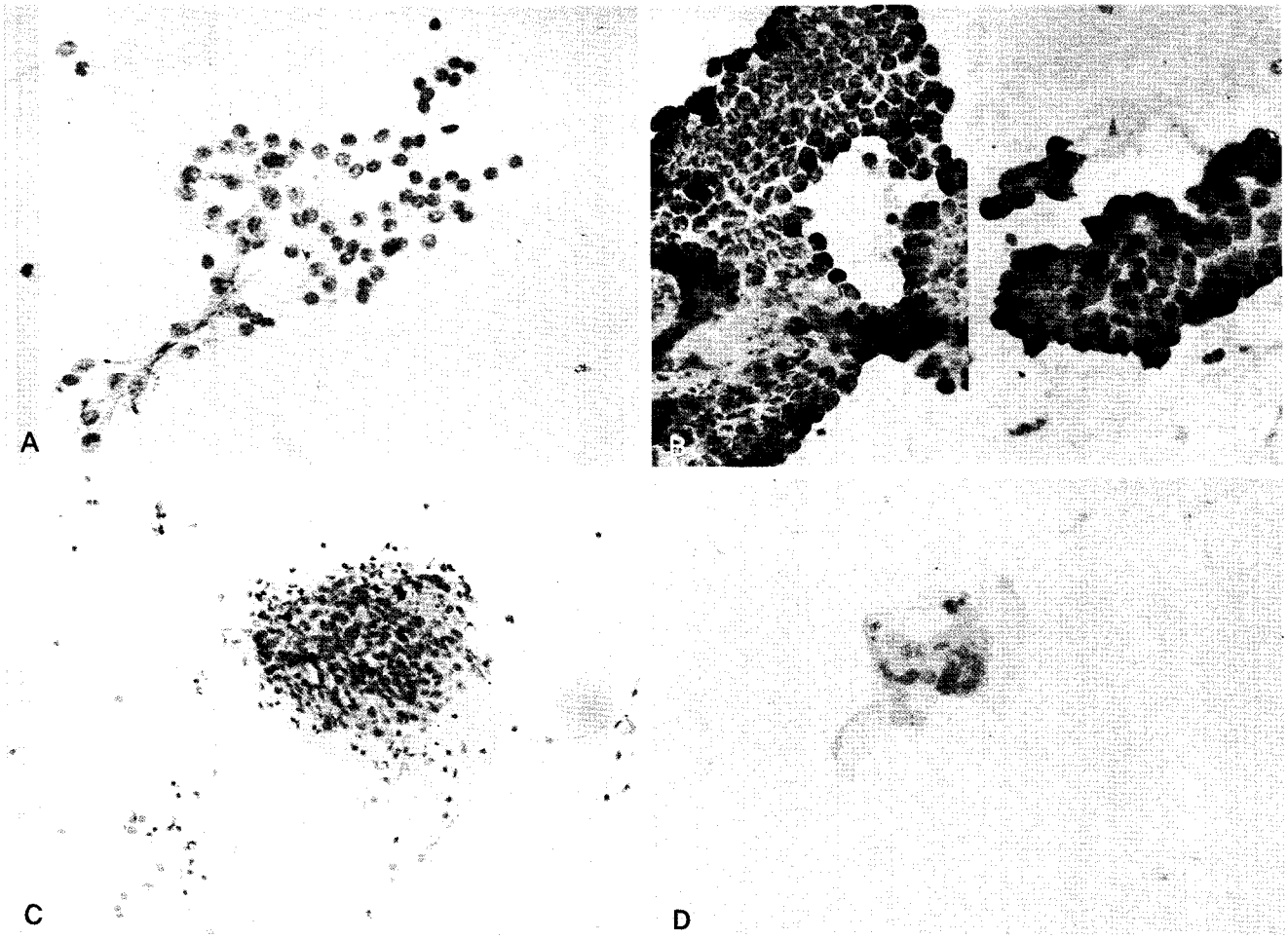

Fig. 2. Immunocytochemical stain of CK19. (A) Follicular cells of subacute granulomatous thyroiditis are negative for CK19. (B) Follicular cells of papillary carcinoma are positive for CK19. Multinucleated giant cells of subacute granulomatous thyroiditis(C) and papillary carcinoma (D) are negative for CK19.

CK19 and 70% of the cases of SGT were positive for Ret. As similar to the SGT results, only one case of the MGCs of the PTCs was positive for CK19 and 100% of the cases of the PTCs was positive for Ret(CK19; p=0.333, Ret; p=0.748) (Fig. 3).

DISCUSSION

These results suggest that immuhocytochemistry will help physicians make the differential diagnosis of SGT from thyroid malignancy. The results of CK19 and Ret in the follicular cells revealed that SGT expressed less of these proteins than did PTC. These differences were statistically significant(CK19; p=0.02, Ret; p=0.002). To diagnosis thyroid malignancy in a surgical specimen, several immunohistochemical markers such as galectin-3, HBME-1 or CK19 have been used and investigated.[8,11,12] Therefore, CK19 has also been considered by many investigators to be a useful ancillary tool for making the cytological diagnosis of papillary carcinoma, and especially in the cytologically indeterminate cases.[13-15] The sensitivity and specificity using CK19 as a single marker were as high as 92% and 97%, respectively.[15] A combination of markers, including CK19 and galectin-3, was reported to reach 100% for both the specificity and sensitivity during the management of thyroid lesions that had a cytologic diagnosis of follicular oncocytic tumor.[13] RET/PTC oncogene immunostaining has been reported to be an additional useful

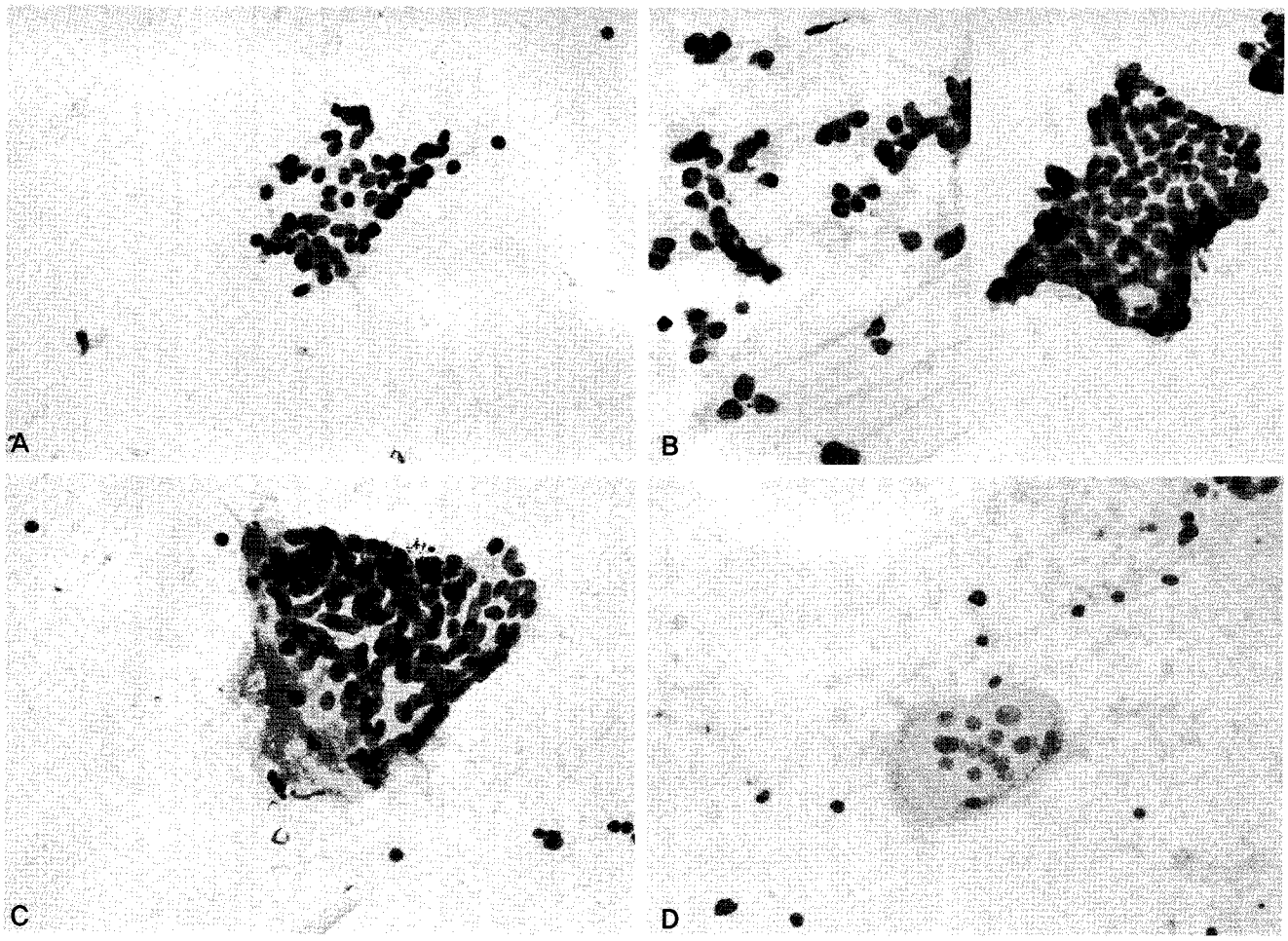

Fig. 3. Immunocytochemical stain of Ret. (A) Follicular cells of subacute granulomatous thyroiditis are weak positive for ret. (B) Follicular cells of papillary carcinoma are strong positive for ret. Multinucleated giant cells of subacute granulomatous thyroiditis (C) and papillary carcinoma (D) are weakly positive for Ret.

tool when used in combination with other antibodies, including CK19, galectin-3, and HBME-1, in the assessment of thyroid specimens and aspirates.[16,17]

However, even for the surgical specimen, there has not been any reports concerned with the ancillary tools to differentiate SGT from thyroid malignancy. Therefore, performing immunocytochemical staining for making the differential diagnosis of SGT from thyroid malignancy has never been reported.

The results of CK19 and Ret immunostaining for the MGCs revealed that only one PTC case showed positivity for CK19(p=0.333), but 100% of the PTCs and 70% of the SGTs were positive for Ret(p=0.748). The MGCs in thyroid disease has been considered as histiocyte derivatives by morphology,[18] but there has been much investigation as to whether or not the MGCs in PTC are a sort of epithelial derivative. So far, those MGC cells in PTC have been confirmed to be histiocyte derivatives.[19,20]

The cytologic literature on SGT is relatively rare, but the cytologic features of SGT has been well described.[1,4-6,10,21] One report summarized the key cytologic features of SGT with performing Giemsa staining.[10] and after that, another report has been published that used Papanicolou stain.[21] The aspirate of SGT usually displays the characteristic cytologic findings seen in their series: follicular cells with intravacuolar granules and/or plump transformed follicular cells, epithelioid granulo-

mas, multinucleated giant cells, an acute and chronic inflammatory dirty background, and the absence of the following cells: fire-flare cells, hypertrophic follicular cells, oncocytic cells, and transformed lymphocytes.[20] Our study was performed with Papanicolou stain and we were able to compare the reported cytologic features.[10,21] with those of our cases. Our cytologic results were consistent with these previous reported descriptions.[10,21]

In summary, the constant and typical cytologic features of SGT are plump transformed follicular cells, epithelioid granulomas, multinucleated giant cell and an inflammatory dirty background without fire-flare cells, oncocytic cells or transformed lymphocytes. In addition, immunocytochemical staining for CK19 and Ret is helpful to discriminate the plump transformed follicular cells and hypertrophic follicular cell from atypical malignant follicular cells. Further studies on other markers in thyroid disease are needed in the future.

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