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Cytological features of well-differentiated tumors of uncertain malignant potential: Indeterminate cytology and WDT-UMP

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Abstract. The purpose of this study was to clarify the cytopathological features of well-differentiated tumors of uncertain malignant potential (WDT-UMP), a possible borderline lesion of thyroid follicular cell tumor. We analysed the cytopathological findings of fine needle aspiration (FNA) smears from 6 cases histologically diagnosed as WDT-UMP. WDT-UMP, benign and malignant lesions were compared retrospectively and morphologically. No (0%) nuclear pseudoinclusions were found in adenomatous goiter (AG), follicular adenoma (FTA) and WDT-UMP. Nuclear pseudoinclusions were increased in number in papillary thyroid carcinoma (PTC) with indeterminate cytology (0.8%) and PTC with malignant cytology (1.2%). The incidence of nuclear grooves increased gradually from AG/FTA (0%), WDT-UMP (4.5%), PTC with indeterminate cytology (6.2%) and PTC with malignant cytology (6.5%). The nuclear area of WDT-UMP, an average of 40.0 μm^2 , was between that for benign AG/FTA and PTC with malignant cytology. The maximum/minimum axis of WDT-UMP (0.934) lied between that of AG/FTA and PTC. The degree of the nuclear circularity of WDT-UMP was less than that for PTC. WDT-UMP belong to indeterminate category between PTC and follicular adenoma morphologically, and this is one of the major reasons why some of PTC can be found in the indeterminate category. Questionable PTC-N including questionable nuclear inclusions (artifact vacuole) may be seen in WDT-UMP, but absolute or definite nuclear inclusions with sharp border are not found in our 6 cases. Therefore this group of thyroid tumors (EnFVPTC and WDT-UMP) may be found in indeterminate category more often, because of intermediate nuclear morphology and incomplete nuclear vacuoles.

Key words: Thyroid, Borderline, Well-differentiated tumor uncertain malignant potential, Cytology, Indeterminate

FINE-NEEDLE ASPIRATION (FNA) is a useful clinical procedure in decision making for thyroid nodules [1]. Recently, the term “well-differentiated tumor of uncertain malignant potential (WDT-UMP)” was proposed by Williams for non-invasive encapsulated follicular tumors with equivocal papillary thyroid carcinoma (PTC) type nuclear changes (PTC-N) [2]. PTC-N are the most reliable morphological features in the diagnosis of PTC, which include nuclear enlargement, nuclear overlapping, nuclear clearing, nuclear grooves and cytoplasmic pseudoinclusions [3]. Incidence of PTC-N

in WDT-UMP and details of the cytological features of WDT-UMP have not been reported previously because no fixed criteria for cytology of WDT-UMP exist.

Therefore, the purpose of this study was to investigate the cytopathological features of histologically proven WDT-UMP, to clarify how WDT-UMP is different from the features of adenomatous goiter (AG) and follicular adenoma (FTA) and to determine the cytological similarities to PTC. Histological criteria of WDT-UMP in our diagnosis included encapsulated follicular patterned thyroid tumor with focal unequivocal nuclear changes of PTC or diffuse equivocal PTC-N [4].

Materials and Methods

FNA smear

Cases who underwent surgical treatment for thy-

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roid lesions were reviewed in the archived files of the Department of Human Pathology in Wakayama Medical University during the period from 1997 to 2008. Cases with unsatisfactory or non-diagnostic smears were excluded from this study, therefore, three main categories were included: benign (AG/FTA, $n = 6$), indeterminate (histologically confirmed WDT-UMP ($n = 6$) or PTC ($n = 3$)) and malignant (PTC, $n = 10$) from 218 cases who underwent surgical treatment for thyroid lesions were included in this study [5, 6].

FNA cytology was performed by experienced surgeons using a 22-gauge needle attached to a 10-mL syringe, with or without ultrasound guidance. The specimen was smeared on glass slides and immediately fixed with Cytodrops (Muto Pure Ltd, Tokyo, Japan), which contains 25% methanol, 68% isopropanol and 1% polyethylene glycol and stained routinely using Papanicolaou method [7]. FNA smears were reviewed and the diagnosis was evaluated according to the Papanicolaou Society recommendation [5, 6, 8].

Computer-assisted cytometrical analysis

Two pathologists (K. K. and Z. L.) first retrospectively evaluated all histological slides of 25 cases. All 25 cases with the FNA smears and hematoxylin eosin (H&E) stained sections were reviewed (Table 1). Morphometric analysis of the smears and representative H&E sections were performed using an Olympus BX50 microscope (Olympus Optical CO., LTD, Japan) combined with a Polaroid PDMCle/OL Digital Camera (Tokyo, Japan) and WinROOF image processing software (Mitani Corp., Tokyo, Japan) for Windows [9]. Ten digitized, multispectral images for each case were obtained using the HSCOPE method and captured at 200 magnification. The nuclear features of each case were assessed on the digitized image, in accordance with the following parameters: nuclear/cytoplasmic (N/C) ratio, chromatin clearing, nuclear circularity, nuclear grooves, pseudoinclusion and nuclear areas. The nuclear circularity was evaluated by the ratio of maximum/minimum axis of the nuclei. All the parameters were counted and averaged respectively.

Statistical differences between histological subtypes were evaluated by Stat View 5.0 using the Student's *t*-test. Correlation between cytological diagnosis and histological subtypes was estimated using the Chi-square test. Probability values less than 0.05 were considered statistically significant.

Results

Patients

The clinicopathological parameters of all 25 cases with thyroid lesions are summarized in Table 1. All the patients were Japanese with a mean age of 48.4 years (range 19-74 years). Informed consent was obtained from all 25 cases. The diameter of the tumors varied from 1.0 to 1.5cm (mean = 1.2).

Cytopathological features

Among these lesions, PTCs were divided into groups according to the growth pattern histologically, encapsulated type PTC ($n = 1$), follicular variant ($n = 2$) and common type PTC ($n = 10$). The nuclear features are summarized in Table 2. No pseudoinclusions were found in AG, FTA or WDT-UMP (0%). In WDT-UMP, there were some clear vacuoles inside the nucleus (Fig. 1A), which were small, clear and different from the typical pseudoinclusion (Fig. 1B). Nuclear pseudoinclusions and nuclear grooves were more common in PTC indeterminate group (0.8%) and PTC malignant group (1.2%) (Fig. 1C and 1D) than in benign thyroid lesions, which were small and round, without pseudoinclusion and nuclear grooves (Fig. 1E). In borderline tumors, WDT-UMP (Fig. 1F), these features were less obvious than those of PTC.

Morphologically, the nuclear groove of WDT-UMP was rare, thin and faintly visible. While that of PTC was dense and running across the long axis of the nucleus. The incidence of the nuclear grooves increased gradually from AG/FTA (0%), WDT-UMP (4.5%), PTC indeterminate group (6.2%) and PTC malignant group (6.5%) (Table 1).

The nuclear area of WDT-UMP was between AG/FTA and PTC indeterminate group and PTC malignant group, with an average area of $40.0 \mu\text{m}^2$ (Table 2). In the evaluation of nucleus circularity, when the value of the maximum/minimum axis was one, the nucleus was considered as truly round (Fig. 2). When it was less than one, the nucleus was considered oval. The maximum/minimum axis of WDT-UMP (0.934) was greater than that of AG/FTA, but less than that in PTC (Table 2). The degree of the nuclear circularity of WDT-UMP was found to be statistically smaller than that of the PTC indeterminate group and PTC malignant group. These results suggest that the nucleus of WDT-UMP has more atypia than AG/FTA, but less than PTC.

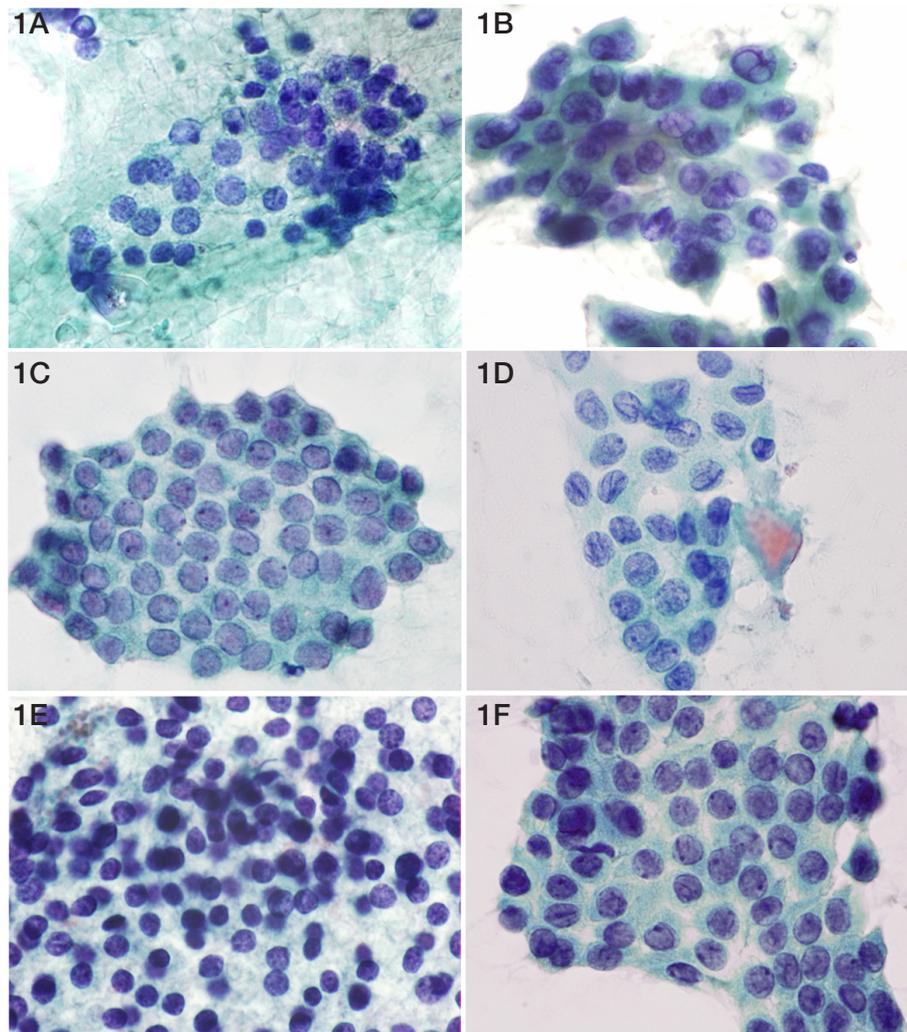


Fig. 1 (1A): Clear vacuoles in well differentiated tumor uncertain malignant potential, (1B): Pseudoinclusions in papillary thyroid carcinoma, (1C): Nuclear grooves in well differentiated tumor uncertain malignant potential, (1D): Nuclear grooves in papillary thyroid carcinoma, (1E): Nuclear features of benign thyroid lesions, (1F): Nuclear features of borderline thyroid lesions

Table 1 Clinicopathological parameters of all the thyroid lesions (n = 25)

Cytological diagnosis	Histological diagnosis	Cases	Gender (F/M)	Average age (years)
Benign	AG/FTA	6	5/1	48.5 ± 13.0
Indeterminate	WDT-UMP	6	5/1	47.2 ± 14.0
	PTC	3	3/0	49.7 ± 9.6
Malignant	PTC	10	5/5	47.8 ± 12.3

Table 2 Comparison of the nuclear features of different thyroid lesions

Cytological diagnosis	Histological diagnosis	Nuclear size*	N/C ratio	Clear vacuoles	Maximum/minimum axis	Circularity	Nuclear area (μm ²)	Nuclear groove (%)	Pseudoinclusion (%)
Benign	AG/FTA	1.811	0.342	-	1.119	0.925	36.1	0	0
Indeterminate	WDT-UMP	2.902	0.568	+	1.118	0.934	40.0	4.5	0
	PTC	3.153	0.891	+	1.203	0.919	39.6	6.2	0.8
Malignant	PTC	3.436	0.901	++	1.203	0.920	46.0	6.5	1.2

*: the average ratio of tumor cell/normal thyroid follicular cell; N/C ratio: Nuclear/cytoplasmic ratio

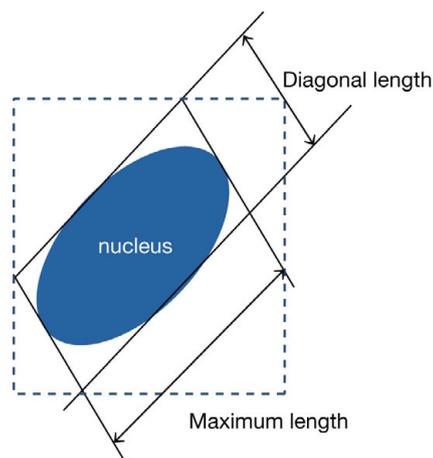


Fig. 2 When the value of the maximum/minimum axis is one, it was evaluated as round. When it is less than one, it was evaluated as circularity.

Discussion

Thyroid FNA is a reliable preoperative test that has been shown to be highly practical to detect thyroid nodules. However, the cytological diagnosis of thyroid lesions often results in overdiagnosis or underdiagnosis because of overlapping features between benign and malignant thyroid lesions; furthermore, diagnoses of indeterminate are common up to 20% of all samples [10-13]. In order to clarify the cytological aspects of WDT-UMP in our definition, we analysed FNA smears obtained from 6 patients with WDT-UMP and compared these smears with those from AG/FTA and PTC cases.

PTC is defined on the basis of its characteristic nuclear features histologically and cytologically [14]. However, there is a continuous debate about encapsulated follicular variant thyroid lesions without invasion or with equivocal capsular/vascular invasion [15-21]. This group of tumors has been suggested to have borderline morphology and does not have genetic change specific for PTC such as RET/PTC rearrangements or BRAF point mutation [17, 22, 23]. We recently demonstrated that WDT-UMP has different morphologi-

cal, immunohistochemical and molecular features with PTC [4, 24]. WDT-UMP may be one of the major reasons why PTC can be found in the indeterminate, because both WDT-UMP and PTC have overlapping morphological characteristics and PTC and follicular adenoma are unfortunately continuous lesions cytologically. This causes difficulty for cytopathologist in making an accurate diagnosis.

Questionable PTC-N including clear vacuoles (possible artifact vacuole) may be seen in WDT-UMP, but absolute or definite nuclear inclusions with sharp border are not found in our 6 cases. Therefore WDT-UMP may be found in indeterminate category more often, because of intermediate nuclear morphology and incomplete nuclear vacuoles. The present study confirmed that there are at least three special features of WDT-UMP that distinguishing WDT-UMP from both PTC and follicular adenoma morphologically. Firstly, the atypia of WDT-UMP was between AG/FTA and PTC according to the value of nuclear area and maximum/minimum axis. Secondly, the nuclear groove of WDT-UMP was thin and faintly visible. While that of PTC was dense and running across the long axis of the nucleus. Thirdly, only clear vacuole was identified in WDT-UMP, which was undoubtedly different from the pseudoinclusion present in PTC. These features are useful for accurate diagnosis of thyroid lesions, especially borderline lesions.

In conclusion, this study demonstrated the following cytological features of WDT-UMP: no pseudoinclusions and occasional clear nuclear vacuoles, 4.5% incidence of nuclear grooves, enlarged nuclear area and greater circularity than benign thyroid lesions. These results suggest that AG/FTA, WDT-UMP and PTC are continuous lesions, and WDT-UMP occupies between benign AG/FTA and malignant PTC cytologically, suggesting it is so-called borderline malignancy [24, 25].

Disclosure/Conflict of Interest

The authors declare no conflict of interest.

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