



ORIGINAL ARTICLE

Cytological features of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)

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KEYWORDS

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Introduction The noninvasive encapsulated follicular variant of papillary carcinoma (EFVPC) was recently renamed a noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) because of its unique genetic alterations and biological behavior. The objective of this report is to help cytopathologists and cytotechnologists improve diagnostic accuracy and determine the need for cytogenetic studies during adequacy evaluation of thyroid fine-needle aspirations.

Materials and methods Fifty-five cases of surgery-proven noninvasive EFVPC with corresponding cytology material were reviewed. These cases were collected over 17 years, from 1999 to 2016.

Results Thirty-four of 55 (61.8%) cases were diagnosed as follicular neoplasm or suspicious for follicular neoplasm on cytology. Eighty to ninety percent of cases showed scant colloid, cellular smears with small clusters of follicular cells with nuclear atypia including enlarged nuclei, oval-shaped nuclei, nuclear grooves, mild chromatin powdering, and rare nuclear pseudo-inclusions.

Conclusions NIFTP has unique features: cytologically similar to follicular neoplasms, and nuclear atypia falling between atypia of undetermined significance (category III) and suspicious for/and papillary thyroid carcinoma (category V/VI) (The Bethesda System for Reporting Thyroid Cytopathology).

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Introduction

In the last 20 years, the incidence of well-differentiated thyroid cancer has been increasing dramatically. Sanabria et al¹ addressed the multiple factors that contribute to this trend, such as demographic transition, disparities in health

care access, quality of the registry, mass media influence, economic and medicolegal incentives, and medical education. Nevertheless, a high proportion of the increase in the well-differentiated thyroid cancer incidence is due to exaggerated screening and a consequent overdiagnosis. As a result, overdiagnosis has become an important issue. The noninvasive encapsulated follicular variant of papillary thyroid carcinoma (EFVPTC) is an example, accounting for 10% to 20% of all thyroid cancers currently diagnosed in Europe and North America.^{2,3} It was recently renamed as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) because of its unique behavior and good prognosis.⁴ Accurate diagnosis of this entity will significantly reduce reported thyroid cancer incidence, overdiagnosis, and treatment.

EFVPTC or NIFTP behaves in an indolent manner, with a much lower frequency of extrathyroid extension, positive margins, nodal metastases, and recurrence.⁵ Castro et al reported that this tumor has PAX8-PPAR rearrangements and RAS mutations similar to the follicular adenoma/carcinoma.⁶ Rivera et al reported the K601E and G474R BRAF mutations in this tumor were different from V600E of classic papillary thyroid carcinoma.⁷ The treatment for NIFTP is different from traditional treatment for papillary thyroid carcinoma. Conservative surgery such as lobectomy rather than total thyroidectomy is recommended for this entity.⁴

Although histologic criteria have been devised and recommended by a panel of experts,⁸ diagnosing this tumor presurgically by fine-needle aspiration (FNA) is still a challenge. There are some reports describing the cytologic features of NIFTP, especially in recent years. Three cases of NIFTP were reported as benign on FNA diagnosis in Turkey.⁹ Yang et al¹⁰ compared 72 cases of NIFTP to 37 cases of EFVPTC with invasion (IEFVPTC) and reported hypercellularity in 5.6% of NIFTP cases versus 8.1% in IEFVPTC cases; diffuse papillary thyroid carcinoma (PTC) nuclear features present in 44% of NIFTP and 45.9% of IEFVPTC. Strickland et al¹¹ reported that NIFTP is distinct from benign nodules because of nuclear atypia that is more significant than atypia of undetermined significance using nuclear atypical score criteria (which include nuclear atypia using three parameters: nuclear size/shape, nuclear membrane, and chromatin). Jaconi et al reported that NIFTP was mostly diagnosed as Bethesda thyroid categories III/IV and there were overlapping features between NIFTP and FVPTC and benign nodules in 200 cases.¹² Chandler et al reported that NIFTP can be differentiated from invasive or infiltrative FVPTC by the presence of a microfollicular pattern and absence of nuclear pseudo-inclusions.¹³ Brandler et al studied 56 NIFTP, 67 classic PTC, and 30 follicular adenoma cases and concluded the cytomorphic features and surgical diagnosis of those cases may be used as clues to distinguish them on FNA aspiration.¹⁴ Bizzarro et al found the majority of NIFTPs can be discriminated from IFVPTC by cytomorphology.¹⁵ Singh et al found that

among 174 patients with surgery for PTC, only 21 patients were diagnosed with NIFTP postoperatively, and were less likely to have a preoperative malignant cytological diagnosis for NIFTP than classic PTC.¹⁶ Zhao et al's work on 50 NIFTP and 47 IFVPTC cases, however, concluded that it is unlikely to distinguish most cases using FNA biopsy.¹⁷

From the reports cited, it is clear that NIFTP diagnosis by FNA is a challenge due to its nature as a low-grade neoplasm. Nevertheless, presurgical accurate diagnosis is critical for clinician and patient to prepare surgery and treatment. Thus far, there are few reports summarizing these features in great detail. This paper attempts to define criteria to help pathologists make an accurate diagnosis. It also attempts to help pathologists or cytotechnologists with adequacy checks to determine whether they need to collect extra specimens for cytogenetic studies.

We summarize the common cytological features of this tumor in detail by reviewing 55 cases and comparing them with the 6-tiered Bethesda System for Reporting Thyroid Cytopathology (TBSRTC).

Material and methods

The cases were retrieved from the Mount Sinai Health System including Mount Sinai Hospital, Beth Israel Hospital, St Luke's Hospital, and Roosevelt Hospital in New York City between 1999 and 2016. We first identified surgical pathology cases by diagnostic criteria, then looked for cases with corresponding cytology. Only those cases with both surgical pathology and cytology were selected for our review. Surgical pathology diagnostic criteria were based on the following features: well-circumscribed/noninvasive tumors (encapsulated, partially encapsulated) composed entirely of follicles and having tumor cells with subtle degree of nuclear PTC features (ie, overlapping, hyperchromasia, peripheral micronucleoli, groove formation, irregular-prominent membrane borders), and combinations thereof without vascular/perineural invasion, lymph node metastasis, and extrathyroidal spread. All the surgical pathology cases were signed out by experienced head and neck pathologists. All the cytology cases were signed out by board certified cytopathologists and centrally reviewed by ZZ. The cytology slides for the selected cases were collected. Patient demographic information (age and sex) and nodule characteristics (location/size of the nodule) were recorded from the institutional database. This study was conducted with the approval of the institutional review boards of all institutions involved. All the cytology cases were reviewed and assigned a TBSRTC category.

Results

Forty-four (80%) of the 55 patients were female and 11 (20%) were male, with a mean age of 45.9 years (range: 19–78 years). The nodule size ranged from 0.6 cm to 4.5 cm,

with a mean size of 1.94 cm. Thirty-six (65%) of the patients had multiple nodules and 19 (35%) had solitary ones. **Table 1** shows demographics and module characteristics.

Using the TBSRTC system, no cases 0 (0%) were non-diagnostic (ND), 3 (5.5%) were benign diagnoses (BD), 5 (9.1%) demonstrated atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS), 34 (61.8%) were follicular neoplasms (FN)/suspicious follicular neoplasm (SFN), 10 (18.2%) were suspicious for malignancy (SFM), and 3 (5.5%) were malignant (M) (**Table 2**).

The nuclear features (see **Figs. 1** and **2**) of cases are summarized in **Table 3**. Nuclear enlargement with oval-shaped nuclei was seen in more than 90% of cases. The nuclear size is mostly around 1.2 to 1.5 time the size of a red blood cell. Occasional large cells 2 to 3 times the size of a red blood cell could be seen. Oval-shaped nuclei are present in 10% of the cells. Nuclear grooves are present in most of the cases. Clear chromatin is more easily seen in Papanicolaou-stained slides and lightly stained Diff-Quik (DQ) smears. Definite nuclear inclusions are uncommon but ill-defined ones can often be identified.

Cytological features (see **Fig. 3**) are summarized in **Table 4**. The majority of cases are cellular smears with many clusters of follicular cells. There are usually more than 30 groups of cell clusters present. There are both large and small clusters. Small clusters are defined as those with fewer than 50 cells in the cluster. Small clusters can be appreciated in 95% of cases and it is a significant feature of this entity. Microfollicular arrangements of the cells are often present in the small clusters. Large clusters (>200 cells) can be seen but usually is not a dominant population. Colloid is usually scant, either dense or thin. Abundant colloid or no colloid is present in a small proportion of cases.

Discussion

NIFTP represents a distinct class of thyroid tumor. It shows only some of the cytomorphologic nuclear features of PTC and its diagnosis is often controversial on FNA preparations.

Table 1 Patient characteristics.

Characteristics	NIFTP cases
Patients, n	55
Male, n (%)	11 (20)
Female, n (%)	44 (80)
Age, years	
Mean	45.9
Range	19-78
Nodule size, cm	
Mean	1.94
Range	0.6-4.5
Multiple, %	65
Solitary, %	35

Table 2 Distribution of NIFTP cases according to categories.

FNA result according to TBSRTC						
ND	BD	AUS/FLUS	FN/SFN	SFM	FM	Total
0	3	5	34	10	3	55

Abbreviations: AUS, atypia of undetermined significance; BD, benign diagnoses; FLUS, follicular lesion of undetermined significance; FN, follicular neoplasms; FNA, fine-needle aspiration; M, malignant; ND, nondiagnostic; SFM, suspicious for malignancy; SFN, suspicious follicular neoplasm; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

There is no immunohistochemical stain to distinguish the tumor from other kinds of thyroid lesions, especially follicular lesions. Therefore, the diagnosis preoperatively remains a challenge. **Table 5** summarizes the findings from the listed literature on this entity.

The size of the nodules ranged from 1.3 to 2.7 cm in the listed literature. Howitt et al also reported that the average size of nodules in their study was 2.5 cm.¹⁸ Most of the patients (65%) had multiple nodules and about one-third had a single nodule; one study, however, reported that 38 out of 51 cases were single nodules.¹³ This could be helpful in differentiating it from follicular adenoma, which is a single nodule.

Regarding cellularity, Maletta et al¹⁹ reported 54 of 96 cases were diagnosed as Bethesda category IV which is close to our results of 34 of 55 (62%) as category IV. Chandler et al¹⁴ reported that a predominantly micro-follicular pattern was associated with NIFTP. Our data showed similar findings of small clusters with fewer than 50 cells present in 52 of 55 (95%) cases. Bizzarro et al¹⁶ reported 17 of 37 (46%) cases with more than half of the clusters being small clusters containing 0 to 10 cells, and 20 of 37 (54%) cases with more than half of the clusters being large clusters containing more than 10 cells. Bizzarro et al's data may have differed from ours because they used

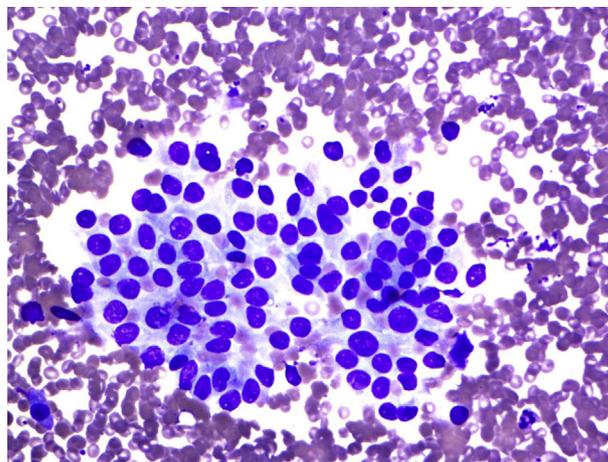


Figure 1 Nuclear features on Diff-Quik show enlarged, oval nuclei, irregular nuclear membranes, nuclear grooves, powdery chromatin, and ill-defined nuclear pseudoinclusions.

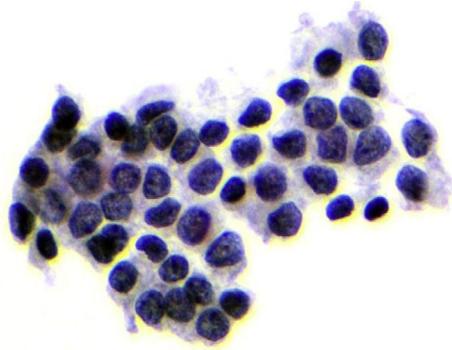


Figure 2 Nuclear features show enlarged nuclei, slightly irregular nuclear membrane, slightly chromatin clearing and nuclear grooves.

different criteria for size of cluster. Nevertheless, small clusters (0 to 10 cells) in their study could be seen in 17 of 37 cases. Easily identifiable microfollicles were seen in NIFTP as also reported by their studies.^{11,13,16,19} Colloid was seen in Maletta et al's study in 7 of 55 cases and 15 of 37 cases in Bizzarro et al's study. In our study, colloid can be seen in 48 of 55 (87%) cases, with no colloid in 7 cases (13%). The colloid in most cases is scant and dense (51%). Similar findings were reported by Brandler et al, with scant or no colloid in 53.6% of their cases, although, with abundant colloid in 28.6% of 56 cases,¹⁵ they reported slightly more abundant colloid compared with our findings.

The nuclei in 50 of 55 (91%) cases were slightly enlarged to 1.2 to 2 times the size of a red blood cell (approximately 9-15 μm). Bizzarro et al report nuclei size was less than 20 μm in 26 of 37 (70.3%) cases, and larger than 20 μm in 11 of 37 (29.7%) cases. The median size was 18.7 μm . We rarely saw cases where nuclear size reached 2 to 3 times the size of a red blood cell (15-22.5 μm). It was more common to see a few large cells 2 to 3 times the size of a red blood cell with most identified cells smaller than that. Nuclear enlargement was also seen but the exact size was not mentioned in 47 of 56 (83.9%) cases from Brandler et al,¹⁵ 19 of 48 (39.6%) cases from Chandler et al,¹³ nor in 37 of 55 (67%) from Maletta et al.¹⁹ Maletta et al used criteria for

Table 3 Nuclear features of NIFTP.

Type of nuclear atypia	Frequency
Pseudoinclusion	5/55 (9%)
Ill-defined pseudoinclusion	23/55 (41%)
Grooved nuclei	45/55 (81%)
Enlarged nuclei ^a	50/55 (91%)
Oval shape	52/55 (95%)
Crowded nuclei	51/55 (93%)
Clear chromatin	35/55 (63%)
Nuclear membrane irregularity	33/55 (60%)

^aEnlarged nuclei mostly are 1.2 to 2 times the size of a red blood cell on Diff-Quik.

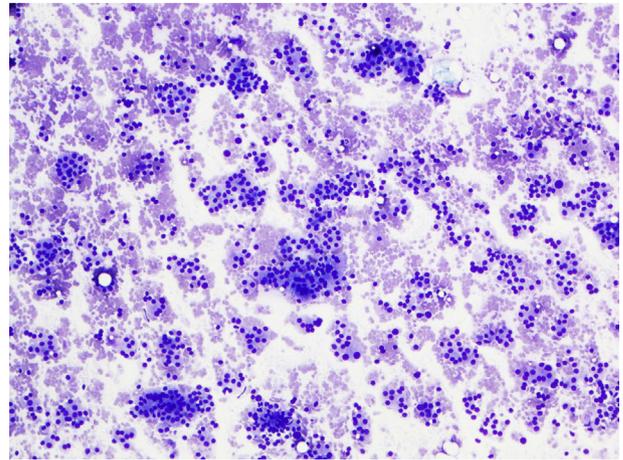


Figure 3 Cellular smear with small clusters of follicular cells.

nuclear enlargement more than twice the size of a red blood cell but did not mention whether it was in small proportion or in the majority of the population. From these reports, nuclear enlargement is one of the most common nuclear features. Bizzarro et al saw nuclei larger than 20 μm in 66.7% of invasive FVPTC compared with 29.7% of NIFTP. That nuclei size in invasive tumors is larger and more commonly present was also reported by others.^{13,14}

Oval-shaped nuclei or nuclear elongation was often mixed with round-shaped nuclei. A pure oval shape was extremely rare and we did not see one in our study. Bizzarro et al saw 19 of 37 (51.4%) cases with oval-round mixed shape and 2 of 37 (5.4%) with pure oval shape in their study. Chandler et al reported 12 of 48 cases with oval shape but did not mention mixed type or pure oval type. Nuclear grooves were seen in 45 of 55 (81%) of our cases; in 5 of 37 cases and focally in 11 of 37 cases in Bizzarro et al's report, and in 21 of 48 cases in Chandler et al's report. Nuclear grooves are very common but vary in number from case to case. When nuclear atypia is present, the nuclear grooves were usually more likely appreciated.

Table 4 Cytological features of NIFTP.

Type of feature	Frequency
Architecture	
Large clusters (>200 cells/cluster)	21/55 (38%)
Small clusters and microfollicles (<50 cells/cluster)	52/55 (95%)
Background	
No colloid	7/55 (13%)
Scant dense colloid	28/55 (51%)
Scant thin colloid	10/55 (18%)
Abundant colloid	10/55 (18%)
Cellularity	
1+ (<6 groups)	0/55 (0%)
2+ (6-12 groups)	6/55 (11%)
3+ (12-30 groups)	14/55 (25%)
4+ (>30 groups)	35/55 (64%)

Table 5 Summary of literature and comparison with our study.

Characteristic	Maletta et al	Bizzarro et al	Chandler et al	Brandler et al	This study
Mean age, years	46	35	49.6	48.1	45.9
Nodular size, cm	2.6	1.3	2.7	2.4	1.94
Nuclear, %					
Enlargement	67.3	9.7 (>20 μ m)	39.6	83.9	90.1
Oval shape	N/A	56.8	25	N/A	94.5
Membrane irregularity	85.5	75.5	12.5	10.7	60
Chromatin clearing	52.7	100	10.4	69.6	63.6
Cytology, %					
High cellularity	27.3	N/A	N/A	N/A	64
Architecture, %					
Large clusters	5.5	N/A	N/A	N/A	38
Small/microfollicles	87.3	100	37.5	73.2	94.5
Colloid, %	12.7	N/A	N/A	46.4	87

Average nodular size = 2.2 cm.

Nuclear membrane irregularity was seen in 10.7% up to 85.5% of cases in the literature. The irregularity was appreciated by most researchers but in different percentages.

Chromatin clearing is one of most important nuclear features and was seen by most authors. The percentage varies due to observers' subjectivity but can be seen in more than 50% of cases. Our finding is similar to others. In our study, however, most cases had more DQ-stained slides and fewer Papanicolaou-stained slides. It was more difficult to appreciate the chromatin pattern in DQ-stained slides than in Papanicolaou-stained slides. Because DQ stain is important in rapid adequacy evaluation, a "hypohematoxylin" staining slide is recommended. "Hypohematoxylin" means staining slides in hematoxylin for approximately 10 seconds rather than the usual 1 minute to make the chromatin appear lighter.

True nuclear inclusions were rare by most reports^{12-15,19} and generally seen in around 5% of cases. Ill-defined nuclear inclusions were seen in 23 of 55 (41%) of our cases and it was more likely seen in DQ-stained slides. Only Maletta et al reported this same phenomena of nuclear molding (in 50 of 55 of cases).

Nuclear score was used by some researchers to more accurately analyze the nuclear atypia. Nuclear score includes three parameters: nuclear size/shape, nuclear membrane, and chromatin. Score 0 was assigned if a specific nuclear feature was absent or heterogeneously present in fewer than 50% of cells. Score 1 was assigned if a given nuclear feature was clearly present in more than 50% of cells in a lesion. A total score of 0 to 3 was given. Using the nuclear score as criteria, the researchers compared NIFTP with benign and invasive FVPTC. They found that the nuclear score of NIFTP is higher than benign^{12,19,20} but lower than invasive FVPTC.^{12,19}

The current cytology of thyroid lesions diagnosis uses TBSRTC system. According to the system, cellular smears of the NIFTP cases would be categorized as IV, which was the most common diagnosis in our cases, and this diagnosis

was also reported by multiple authors.^{12,16,19} In addition, we also saw a certain degree of cytological atypia. Regarding the degree of atypia, most of our cases showed more atypia than Bethesda category III but less than category V. Those cases placed in categories V or VI, however, have relatively more nuclear atypia than the cases placed in categories III or IV in our observation. Nuclear feature was also observed by multiple researchers and often diagnosed as category III.^{13-15,18,20} Most researchers agree that the degree of atypia is more than the degree of atypia of benign or adenomatous lesions, but less than invasive PTC. Strickland et al,¹¹ Jaconi et al,¹² and Maletta et al¹⁹ used semiquantitative scores of cytologic and nuclear features to help accurately identify this entity. This could possibly be integrated into TBSRTC.

Summary

NIFTP is a low-grade thyroid neoplasm that has overlapping features with other thyroid neoplasms. The differences between it and other neoplasms are subtle and there is no single distinguishing feature. In order to diagnose it accurately, pathologists have to combine all the morphologic features and subtle unique features. The unique features were a cellular aspirate with abundant small clusters and microfollicles similar to follicular adenoma, with slightly enlarged, crowded nuclei with frequent nuclear grooves, slightly clear chromatin, and mild irregular contours similar to papillary carcinoma. Colloid was often scant and dense. Ill-defined nuclear inclusions were noted in ~40% of cases but well-defined nuclear inclusions were infrequent.

Conclusions

NIFTP has unique features that are cytologically similar to follicular neoplasm, and nuclear atypia usually fall between categories III and V/VI.

Disclosure statement

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work.

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