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Fine Needle Aspiration Cytology of Papillary Thyroid Carcinoma: Association of Cytological Features with Tumor Invasiveness in 43 Cases

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KEYWORDS

Papillary thyroid carcinoma, Fine needle aspiration, lymphovascular invasion, pseudoinelusions, Syncytial cell groups, Pleomorphic nuclei

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ABSTRACT

Background & Objective: The aim of the study was to evaluate fine needle aspiration (FNA) samples for tumor invasion in patients with papillary thyroid carcinoma (PTC) and correlate the findings with aggressive behavior in thyroid surgical specimens.

Methods: Retrospective, cross-sectional, and descriptive analysis of 43 PTC cases with available thyroid surgery and preoperative FNA samples was done. Cytological features were compared between thyroid capsule and lymphovascular invasion groups. FNA samples were examined for correlation of cytological patterns with aggressive behavior, such as thyroid capsule invasion and lymphovascular or lymph node invasion in thyroid surgery specimens.

Results: There were significant differences for the occurrence of isolated cells, pseudoinclusions, syncytial cell clusters, and large pleomorphic nuclei, which were more frequent in the invasion-positive group.

Conclusion: These cytological attributes can be indicators of aggressive tumor behavior and are important markers in assessing the invasive potential of PTC.

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Introduction

As per the Global Cancer Statistics 2022 (GLOBOCAN), thyroid cancer was identified as the seventh most common cancer overall, with three times greater incidence rates among females compared to males (1). The four main types of thyroid cancers—papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), Hürthle-cell carcinoma, medullary thyroid carcinoma (MTC), and anaplastic thyroid carcinoma (ATC)—represent more than 98% of thyroid tumors, the most prevalent being PTC (2, 3). PTC is an epithelial cancer with follicular cell differentiation and specific nuclear characteristics. Although PTC overall has an outstanding prognosis, certain clinicopathological parameters, such as older age at presentation, larger tumor size, extrathyroidal extension, male sex, greater undifferentiated or solid components, vascular invasion, and aneuploid cell populations, can lead to poorer outcomes. Some subtypes of PTC, such as the tall cell variant, diffuse sclerosing variant, solid variant, and follicular variant, are also associated with more aggressive clinical behavior (4-7).

Fine needle aspiration (FNA), usually done under the guidance of ultrasound (US), is a critical component of preoperative assessment of thyroid nodules owing to its high sensitivity and specificity for malignancy detection (8, 9). FNA is indicated in thyroid nodules that have clinical or ultrasonographic features suspicious for malignancy, such as irregular margins, microcalcifications, hypoechogenicity, or increased

vascularity, as well as nodules exceeding 1 cm in size or those with risk factors for malignancy, such as family history of thyroid cancer or history of radiation (8, 10).

This minimally invasive technique allows for the cytologic diagnosis of thyroid nodules, allowing benign and malignant lesions to be differentiated and aiding in preoperative planning (9). FNA samples of classic PTC are typically cellular and show papillary architecture, monolayered sheets, and three-dimensional groups in a background of watery or dense "ropy" colloid with associated nuclear or calcific debris, macrophages, and stromal fragments (8, 11).

The tumor cells are typically eosinophilic in cytoplasm and are large, elongated, or oval with elongated nuclei, thickened membranes, chromatin clearing, grooves, and pseudoinclusions (10). The tumors typically possess large follicular cells in monolayer sheets and in follicular clusters in thin and thick colloid backgrounds (9, 11). As FNA is a widely used diagnostic technique, the primary aim of this study was to evaluate FNA samples for signs of capsular spread of tumor (12), blood vessel and lymph node involvement (13) and correlate these with invasive behavior identified in thyroid surgery specimens. These findings are crucial for prognosis assessment and treatment planning (14).

Materials and Methods

Inclusion and exclusion criteria

This study was conducted at Imam Reza Hospital in Tabriz, Iran. The research involved individuals who had undergone FNA targeting thyroid nodules that were suspicious of malignancy on clinical examination and ultrasonographic examination. A final histopathological diagnosis of PTC was confirmed following surgical resection. Cases with indeterminate or suspicious cytological reports but without definitive histopathological proof of PTC were not included in the study.

Furthermore, those in which surgical outcomes could not be obtained or in which surgery revealed benign lesions or malignancies other than PTC were excluded. Cases with insufficient FNA samples were excluded as well.

Data collection

A total of 1,500 thyroid surgery and FNA samples collected between 2014 and 2019 were reviewed. Following the exclusion and inclusion of necessary cases, 43 cases were selected for thorough analysis.

Charts were examined in the setting of eligible patients in order to gather demographic information, such as age and sex. Information on thyroid capsule invasion, lymphovascular invasion, and lymph node involvement was gathered from surgical pathology reports.

Tumor invasiveness was defined on the basis of histopathological proof in surgical samples, namely the presence of any one or more of the following: thyroid capsule invasion, lymphovascular invasion, extrathyroidal extension, or lymph node metastasis, evaluated by experienced pathologists on routine histopathological examination.

From FNA samples, the following cytological features were evaluated by senior pathologists using standard criteria of the Bethesda System for Reporting Thyroid Cytopathology: the existence of single cells, true papillary patterns, microfollicles, nuclear grooves, pseudoinclusions, cell density, syncytial groups, large and pleomorphic nuclei, quantity of colloid, and cellularity. The 43 cases were classified into two categories depending on whether invasive characteristics were present (positive group) or absent (negative group). The variables assessed between these categories comprised demographic factors, such as age and gender, as well as the aforementioned cytological features.

Statistical analysis

Data were analyzed with SPSS version 23 (SPSS Inc., Chicago, IL, USA). The continuous data, i.e., age, were presented as mean ± standard deviation (SD) and compared among groups using the independent samples t-test. The categorical data, i.e., gender and cytological features, were presented as frequencies and percentages and compared using the chi-square test or Fisher's exact test where applicable. A p-value of < 0.05 was deemed to be statistically significant.

Results

Demographic Characteristics

This study analyzed FNA and thyroid surgical samples of 43 patients with confirmed PTC, from 2014 to 2019. The age range of the participants was 41.2 ± 13.18 years, consisting of 4 men (9.3%) and 39 women (90.7%).

Thyroid Capsule Invasion

In terms of thyroid capsule invasion, the average age was calculated as 40.0 ± 13.3 years for the positive group and 42.7 ± 13.24 years for the negative group ($p = 0.506$). The gender distribution showed 2 males (8.3%) and 22 females (91.67%) for the positive group and 2 males (10.52%) and 17 females (89.47%) for the negative group ($p = 0.607$). The frequency of single cells was significantly higher in the thyroid capsule invasion group (20/24, 83.33%) compared with the non-invasive group (7/19, 36.84%; $p = 0.004$), suggesting an association with aggressive tumor behavior. Similarly, pseudoinclusions were more common in the positive group (19/24, 79.17%) compared with the negative group (7/19, 36.84%; $p = 0.005$). Syncytial clusters were observed in 20/24 (83.33%) of the positive group versus 7/19 (36.84%) of the negative group ($p = 0.004$), and large and pleomorphic nuclei in 19/24 (79.2%) of the positive group versus 6/19 (31.6%) of the negative group ($p = 0.002$). No notable differences were observed in relation to genuine papillary patterns ($p = 0.515$),

microfollicular structures (p = 0.686), nuclear invaginations (p = 0.416), cell density (p = 0.714), colloid levels (p = 0.495), or cellular composition (p = 0.846) (Table 1).

Table 1. Comparison of variables in the study groups regarding invasion of the thyroid capsule

Variable		Thyroid capsule invasion		P-value
		+	-	
Age		40±13.3	42.74±13.24	0.506
Gender	Man	2 (8.3%)	2 (10.52%)	0.607
	Woman	22 (91.67%)	17 (89.47%)	
Presence of single cells	+	20 (83.33%)	7 (36.84%)	0.004
	-	4 (16.67%)	12 (63.16%)	
Presence of a true papillary pattern	+	15 (62.5%)	10 (52.63%)	0.515
	-	9 (37.5%)	9 (47.37%)	
Presence of microfollicles	+	15 (62.5%)	13 (68.41%)	0.686
	-	9 (37.5%)	6 (31.58%)	
Presence of nuclear grooves	+	23 (95.83%)	17 (89.47%)	0.416
	-	1 (4.17%)	2 (10.53%)	
Pseudo-inclusion	+	19 (79.17%)	7 (36.84%)	0.005
	-	5 (20.83%)	12 (63.16%)	
Cell density	+	20 (83.33%)	15 (78.95%)	0.714
	-	4 (16.67%)	4 (21.05%)	
Syncytial group	+	20 (83.33%)	7 (36.84%)	0.004
	-	4 (16.67%)	12 (63.16%)	
Large and Pleomorphic Nuclei	+	19 (79.2%)	6 (31.6%)	0.002
	-	5 (20.8%)	13 (68.4%)	
Colloid Amount	Abundant	22 (91.67%)	0 (0%)	0.495
	Low	2 (8.33%)	19 (100%)	
Cellularity	High	12 (50.0%)	9 (47.37%)	0.846
	Moderate to low	12 (50.0%)	10 (52.63%)	

Lymphovascular or Lymph Node Invasion

In case of lymphovascular or lymph node invasion, the mean age was 37.86 ± 14.77 years in the positive group and 44.71 ± 10.52 years in the negative group (p = 0.809). Gender distribution was comparable (p = 1.00). Single cells were seen far more frequently in the positive group (19/22, 86.4%) than the negative group (8/21, 38.1%; p = 0.002). Pseudoinclusions were observed in 18/22 (81.8%) of the positive group and 8/21 (38.1%) of the negative group (p = 0.005). Syncytial groups were observed in 19/22 (86.4%) of the positive group and 8/21 (38.1%) of the negative group (p = 0.002), and large and pleomorphic nuclei were observed more often in the positive group (18/22, 81.8%) compared to the negative group (7/21, 33.3%; p = 0.002). Other features, including true papillary configurations (p = 0.172), microfollicular structures (p = 0.137), nuclear indentations (p = 0.607), cell density (p = 1.00), amount of colloid (p = 1.00), and general cellular composition (p = 0.443), did not show any statistically significant difference (Table 2).

Table 2. Comparison of variables in the study groups regarding lymphovascular or lymph node invasion

Variable		lymphovascular or lymph node invasion		P-value
		+	-	
Age		37.86±14.77	44.71±10.52	0.809
Gender	Man	2 (9.1%)	2 (9.5%)	1.00
	Woman	20 (90.9%)	19 (90.5%)	
Presence of single cells	+	19 (86.4%)	8 (38.1%)	0.002
	-	3 (13.6%)	13 (61.9%)	
Presence of a true papillary pattern	+	15 (68.2%)	10 (47.6%)	0.172
	-	7 (31.8%)	11 (52.4%)	
Presence of microfollicles	+	12 (54.5%)	16 (76.2%)	0.137
	-	10 (45.5%)	5 (23.8%)	
Presence of nuclear grooves	+	21 (95.5%)	19 (90.5%)	0.607
	-	1 (4.5%)	2 (9.5%)	
Pseudo-inclusion	+	18 (81.8%)	8 (38.1%)	0.005
	-	4 (18.2%)	13 (61.9%)	
Cell density	+	18 (81.8%)	17 (81.0%)	1.00
	-	4 (18.2%)	4 (19.0%)	
Syncytial group	+	19 (86.4%)	8 (38.1%)	0.002
	-	3 (13.6%).	13 (61.9%)	
Large and Pleomorphic Nuclei	+	18 (81.8%)	7 (33.3%)	0.002
	-	4 (18.2%)	14 (66.7%)	
Colloid Amount	Abundant	1 (4.5%)	21 (95.5%)	1.00
	Low	1 (8.4%)	20 (95.2%)	
Cellularity	High	12 (54.5%)	9 (42.9%)	0.443
	Moderate to low	10 (45.5%)	12 (57.1%)	

Discussion

The current research examined the cytological characteristics found on FNA samples and invasive behavior in 43 PTC cases, paying particular attention to thyroid capsule invasion, lymphovascular invasion, and lymph node invasion. The results of significance demonstrated a greater occurrence of single cells, pseudoinclusions, syncytial groups, and large pleomorphic nuclei in cases with thyroid capsule invasion in association with lymphovascular or lymph node invasion. These features may reflect aggressive tumor behavior. Below, we discuss each significant finding individually, interpret its implications, and compare it with existing literature to contextualize our results.

Isolated cells were significantly more frequent in the thyroid capsule invasion group (83.33% vs 36.84%, p = 0.004) and in lymphovascular or lymph node invasion cases (86.4% vs 38.1%, p = 0.002). Isolated cells may be a manifestation of discohesive tumor cells with enhanced invasive potential, possibly due to reduced cell-to-cell adhesion, a feature of aggressive malignancies. The finding is in line with Lee et al., who reported single cells with eccentric nuclei in the hobnail variant of PTC, which is associated with aggressive behavior and higher recurrence (15). However, Takagi et al. noted occasional single cells in the diffuse sclerosing variant of PTC and postulated subtype-specific variability (16). The prominence of single cells in our invasive cases underscores their value as a cytologic indicator of aggressiveness.

Pseudoinclusions were much more frequent in the invasive group regarding invasion of the thyroid capsule (79.17% vs. 36.84%, p = 0.005) and lymphovascular or lymph node invasion (81.8% vs. 38.1%, p = 0.005). A feature that distinguishes PTC, pseudoinclusions refer to nuclear membrane abnormalities and are often found in conjunction with high tumor grades. Our study findings suggest that a higher frequency of pseudoinclusions in fine-needle aspiration samples could be predictive of invasive behavior. Tanaka et al. reported pseudoinclusions in the tall cell variant of PTC that were associated with poor outcomes, thus supporting our findings (17). On the other hand, Bongiovanni et al. described an absence of pseudoinclusions in the columnar cell variant of PTC, with variant-dependent patterns (18). Our high

correlation puts pseudoinclusions in the category of a helpful prognostic marker.

The presence of syncytial groups was significantly greater in the invasive group with regard to thyroid capsule invasion (83.33% versus 36.84%, p = 0.004) and lymphovascular or lymph node invasion (86.4% versus 38.1%, p = 0.002). These syncytial clusters with overlapping nuclei can be a sign of loss of normal cell architecture and possible tumor invasion. Lee et al. described syncytial clusters in hobnail variant PTC, which was correlated with aggressive behavior, as in our results (15). Similarly, Ohashi et al. described cohesive solid nests in solid component PTC, which correlated with higher recurrence (19). The prevalence of syncytial groups in our invasive cases suggests that they may serve as an indicator of aggressive behavior in PTC.

The proportion of large and pleomorphic nuclei was much higher in the invasive group in terms of thyroid capsule invasion (79.2% vs 31.6%, p = 0.002) and lymphovascular or lymph node invasion (81.8% vs 33.3%, p = 0.002). These nuclear characteristics can reflect genetic instability or aneuploidy underlying and correlating with tumor aggressiveness. Ohashi et al. described pleomorphic nuclei in PTC with solid components and correlation with recurrence like in our findings (19). Lee et al. also described pleomorphism of nuclei in hobnail variant PTC (15). Our observations are that large and pleomorphic nuclei on FNA would be valuable to identify PTC cases with high potential for invasive behavior and aid risk stratification.

No significant differences were observed with respect to genuine papillary structures, microfollicular structures, nuclear grooves, cellularity, colloid content, or overall cellularity. This lack of correlation may be due to the small sample size (n=43), which limited statistical power, or to the heterogeneity of PTC variants in our study group. For instance, Tanaka et al. described papillary structures in tall cell variant PTC and noted their absence in other more aggressive variants (17). Cameselle-Teijeiro et al. reported high cellularity and unusual colloid in the cribriform-morular variant of PTC, which contrasts with our findings (20). These differences reflect the influence of histopathological variants on cytological features.

Our findings are partly in agreement with the literature but also reveal differences, likely due to differences in PTC types, sample size, or FNA procedures. For example, Bongiovanni et al. reported columnar cell variant PTC with hypercellularity and superposition of the nuclei, not present in our findings (18). Takagi et al. referred to numerous psammoma bodies and lymphocytic infiltration of diffuse sclerosing variant PTC, not necessarily present in our series (16). These observations stress the value of application of histological subtypes for cytological result interpretation.

Study limitations include the small sample size (n=43), potentially reducing statistical power, and the lack of histopathological PTC variants analysis, potentially influencing cytological features. Tumor size and multifocality, prognostic factors, were not assessed, potentially reducing the completeness of our findings. The retrospective nature may be subject to selection bias, with exclusion of cases with unsatisfactory FNA samples. FNA technique or pathologist variation may also have affected outcomes. Larger variant-specific groups and additional prognostic variables need to be examined in the future to validate these observations.

In summary, this research identifies single cells, pseudoinclusions, syncytial aggregates, and large pleomorphic nuclei as cytologic features related to the invasiveness of PTC. These results can be used to improve preoperative risk stratification and inform clinical management. Additional research is necessary to validate these associations in other PTC subtypes and larger populations.

Conclusion

The results of this study suggest that certain cytological features seen in FNA samples, including solitary cells, pseudoinclusions, syncytial clusters, and large pleomorphic nuclei, are associated with invasive behavior in PTC, as represented by capsule invasion and lymphovascular structure or lymph node involvement. Such markers may allow the preoperative prediction of the aggressiveness of the tumor and guide clinical practice.

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None

Authors' Contributors

All authors contributed equally to the conceptualization, design, and execution of this study.

Data Availability

The datasets generated and analyzed during the current study are not publicly available; however, the data can be shared for research and authentication purposes upon reasonable request.

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Ethics Approval

Ethical Approval This descriptive, cross-sectional, and retrospective study was approved by the Tabriz University of Medical Sciences Ethics Committee in Tabriz, Iran (IR.TBZMED.REC.1398.859). Data analysis was conducted by using patient samples and records, with strict confidentiality requirements.

Conflict of Interest

The authors declared no conflict of interest.

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