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Immunohistochemical and Clinical Assessment of Low-Risk Thyroid Tumors

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ABSTRACT

Objective: Differential diagnosis and prognosis of low-risk follicular cell-derived thyroid neoplasms have been conflicting. We aimed to evaluate immunohistochemical features and prognosis of tumors in "well-differentiated tumor of uncertain malignant potential" and "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" categories.

Methods: Fifty-two low-risk thyroid tumors which were classified as well-differentiated tumor of uncertain malignant potential (n = 23) and noninvasive follicular thyroid neoplasm with papillary-like nuclear features (n = 29) with a follow-up of at least 60 months were included. Galectin-3, HBME-1, CK19, and CD56 expressions were evaluated. The control group included benign nodules (n = 53), conventional papillary thyroid carcinomas (n = 37), and encapsulated follicular variant papillary thyroid carcinomas (n = 60).

Results: During a median 84 months follow-up period, none of the patients experienced a recurrence of tumor. Expression of HBME-1 in low-risk tumors was significantly frequent than benign and infrequent than malignant tumors ($P = .001$ and $P < .001$, respectively). The frequency of galectin-3 positivity was similar between low-risk and malignant tumors ($P = .805$) and significantly higher in low-risk tumors when compared to benign nodules ($P < .001$). Expression of CK19 in low-risk tumors was significantly frequent than benign nodules and infrequent than malignant tumors ($P = .01$ and $P = .001$, respectively). The expression profile of CD56 was similar in benign nodules and low-risk tumors ($P = .361$). Total loss of CD56 in tumor was the most specific marker of malignancy (100%). Positive staining of HMBE-1 was the most sensitive marker (89.7%) for predicting malignancy.

Conclusion: Low-risk thyroid tumors had immunohistochemical features overlapping with both benign and malignant thyroid tumors and had a benign course of disease during a long follow-up period.

Keywords: Noninvasive follicular thyroid neoplasm with papillary-like nuclear features, well-differentiated neoplasm of uncertain malignant potential, HBME-1, galectin-3, CD56

Introduction

The diagnosis and classification of encapsulated thyroid tumors with follicular patterns have been controversial. The fifth edition of World Health Organization classification of thyroid neoplasms defines a group of "low-risk neoplasms" consisting of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), thyroid tumors of uncertain malignant potential, and hyalinizing trabecular tumor.[1] The term NIFTP was included in the 2017 classification for the first time and replaced the majority of the tumors previously diagnosed as "encapsulated noninvasive follicular variant papillary thyroid carcinoma" as it was previously shown that these tumors had an uneventful long-term follow-up.[2] The definition of a well-differentiated tumor of uncertain malignant potential (WDT-UMP) and follicular tumor of uncertain malignant potential (FT-UMP) remained unchanged in the new classification and they describe encapsulated follicular tumors with questionable nuclear features and/or invasion.[1]

Differential diagnosis of WDT-UMP, NIFTP, and encapsulated follicular variant papillary thyroid carcinoma (EFVPTC) depends on morphologic features and may be challenging as some histopathological findings of these tumors may overlap occasionally.[3] The diagnostic utility of immunohistochemical markers such as CK19, galectin-3, HBME-1, and CD56 has been evaluated for differential diagnosis of borderline tumors in a limited number of studies.[4-8] Results were controversial as benign, malignant, and intermediate expression profiles of immunohistochemical markers were reported in borderline tumors by different studies.[4-8]

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In the present study, we aimed to evaluate immunohistochemical features and long-term prognosis of WDT-UMP and NIFTP.

Materials and Methods

Patients

In the study, 65 patients with low-risk tumors who underwent thyroid surgery between 2006 and 2015 and had follow-up data for at least 60 months were evaluated. All specimens were categorized according to the fourth (2017) edition of World Health Organization (WHO) thyroid tumor classification at the time of diagnosis and they were reexamined for inclusion in the present study. Thirteen patients were excluded because of incomplete sampling or reclassification as malignant neoplasm. Finally, 52 submitted tumors that were classified as WDT-UMP (n = 23) or NIFTP (n = 29) were included. Demographical data, histopathological features, type of surgery, diagnostic tests performed during follow-up [serum thyroglobulin (basal/stimulated), anti-thyroglobulin antibody, neck ultrasonography, ^{131}I whole body scan, and other imaging methods if available] were assessed retrospectively.

A control group consisting of 53 benign nodules, 37 conventional papillary thyroid carcinomas (cPTCs), 35 noninvasive EFVPTCs, and 25 invasive EFVPTCs was determined retrospectively for comparison of immunohistochemical features. All tumors in the control group were diagnosed after 2017, classified according to the fourth edition of WHO classification and immunohistochemistry was performed at the time of diagnosis.[3]

The study was approved by the Ethical Committee of Ankara University Faculty of Medicine (June 2016, approval number: 11-480-16). Informed consent was obtained from all participants.

Histopathology and Immunohistochemistry

Fifty-two tumors were classified as WDT-UMP (n = 23) and NIFTP (n = 29) according to the criteria defined by WHO thyroid tumor classification (2017). All samples were evaluated by 2 histopathologists independently. Subcentimeter and oncocytic tumors were included in the NIFTP group as these tumors are included in this category in the fifth classification.[1]

The paraffin-embedded tissues for demonstration of histological characteristics of tumor were sliced into 4-µm thick sections by microtome. The standard technique with streptavidin–biotin–peroxidase method with Ventana automated immunostainer (BenchMark XT Staining Module, Ventana Medical Systems, Oro Valley, Arizona, USA) was performed. Antigen retrieval consisted of CC1 (EDTA, pH: 8) or CC2 (citrate, pH: 6) solutions (Ventana Medical Systems). Positive control tissues recommended by the suppliers of the antibodies were stained in all procedures.

MAIN POINTS

- Low-risk thyroid tumors had good prognosis with a benign course of disease during a long-time follow-up period.
- Low-risk thyroid tumors had immunohistochemical features similar to both benign and malignant tumors.
- Loss of CD56 was the most specific marker for predicting malignant thyroid tumors.
- Expression of HBME-1 was the most sensitive marker for predicting malignant thyroid tumors.

CK19 (A53-B/A2.26, NeoMarkers, Westinghouse Dr. Fremont, California, USA, 1/500 dilution), CD56 (123C3.D5, Cell Marque, RTU, Rocklin, California, USA), galectin-3 (9c4, NeoMarkers, 1/25 dilution), and HBME1 (HBME-1, Cell Marque RTU, Rocklin, California, USA) were used. Staining patterns were classified as follows: score 0: no staining, score1: focal staining, and score 2: diffuse staining. Cytoplasmic staining and nuclear staining were considered as positive for galectin-3, and cytoplasmic staining was considered as positive for CK19. Membranous staining was considered as positive for HBME-1.

Statistical Analyses

Statistical analyses were performed using Statistical Package for the Social Sciences software, version 20.0 (IBM Corp, Armonk, NY, USA). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of immunohistochemical features were calculated. Descriptive statistics are summarized as counts and percentages for categorical variables; mean and standard deviations for normally distributed continuous variables, and median (interquartile range) for ordinal or nonnormally distributed continuous variables. Categorical data were compared using the chi-square test or Fisher's exact test, where appropriate. $P < .05$ was considered statistically significant.

Results

A total of 52 patients with low-risk neoplasms (23 WDTs and 29 NIFTPs) were included. The general characteristics of patients and tumors are summarized in Table 1. None of the patients had lymph node metastasis identified at the surgery or pre/postoperative neck ultrasonography. During a median 84 months (60-144) follow-up period, none of the patients experienced a recurrence of the tumor.

The mean age at the time of diagnosis was 49.5 ± 11.9, 47.6 ± 13.5, and 48.3 ± 14.4 years in malignant, benign, and low-risk neoplasms,

Table 1. General Characteristics of Patients and Tumors in Low-Risk Neoplasm Categories

Features	
Age at diagnosis, years (mean ± SD)	48.3 ± 14.4
Gender, male/female (%)	14/38 (26.9/73.1)
Follow-up period, months [median (minimum–maximum)]	84 (60- 144)
Tumor type	
WDT– UMP, n (%)	23 (44.2)
NIFTP, n (%)	29 (55.8)
Type of surgery	
Lobectomy, n (%)	2 (3.8)
Subtotal thyroidectomy, n (%)	3 (5.8)
Total thyroidectomy, n (%)	47 (90.4)
Coexisting DTC	
mPTC, n (%)	5 (9.6)
FTC, n (%)	1 (1.9)
Radioactive iodine ablation, n (%)	18 (34.6)

Data are given as mean ± SD, median (minimum–maximum) or number (%) where appropriate.
FTC, follicular thyroid carcinoma; mPTC, papillary microcarcinoma of the thyroid; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; WDT-UMP, well-differentiated thyroid tumor of uncertain malignant potential.

respectively. The male/female ratio was 28/69, 12/41, and 14/38 in malignant, benign, and low-risk neoplasms, respectively.

Immunohistochemical features of low-risk neoplasms, cPTCs, EFVPTCs, and benign nodules are summarized in Table 2. The frequency of immunoreactivity with HBME-1and CK19 was more frequent in NIFTPs when compared to WDT-UMPs [56.0% (n = 14) vs. 18.8% (n = 3), $P = .018$; 74.1% (n = 20) vs. 41.2 (n = 7), $P = .029$, respectively]. Immunoreactivity with galectin-3 and CD56 were similar between NIFTP and WDT-UMP groups [29.6% (n = 8) vs. 47.1% (n = 8), $P = .242$; 93.8% (n = 15) vs. 100% (n = 14), $P = 1.000$, respectively].

Positive immunostaining with galectin-3, HBME-1, and CK19 were significantly frequent in malignant tumors when compared to benign nodules [38.5% (n = 37) vs. 3.8% (n = 2), $P < .001$; 89.7% (n = 87) vs. 11.3% (n = 6), $P < .001$; and 85.6% (n = 83) vs. 35.8% (n = 19), $P < .001$, respectively] (Table 3). The total loss of CD56 expression was significantly frequent in malignant tumors when compared to benign nodules [60% (n = 57) vs. 0% (n = 0), $P < .001$].

The sensitivity, specificity, PPV, and NPV of galectin-3, HBME-1, CK19, and CD56 are summarized in Table 3. Total loss of CD56 in tumor tissue was the most specific marker of malignancy followed by galectin-3 positivity. The sensitivity, specificity, PPV, and NPV of total CD56 loss for predicting malignancy were 60%, 100%, 100%, and 58.2% with 74.3% accuracy, respectively (Table 3). When focal negativity of CD56 was also considered as CD56 loss, sensitivity and PPV increased to 80% and 95%, whereas specificity and NPV decreased to 92.5%, and 72.1%, respectively. The sensitivity, specificity, PPV, and NPV of galectin-3 expression for predicting malignancy were 38.5%, 96.2%, 94.9%, and 46.4% with 59.1% accuracy, respectively.

The combination of total loss of CD56 expression with positivity of other markers did not increase the sensitivity or specificity for predicting malignancy (Table 3).

Positive expression of HMBE-1 in tumor was the most sensitive marker for predicting malignancy, followed by CK19. The sensitivity, specificity, PPV, and NPV of HBME-1 expression for malignancy were 89.7%, 88.7%, 93.6%, and 82.5% with 89.3% accuracy. The sensitivity, specificity, PPV, and NPV of CK19 expression were 85.6%, 64.2%, 81.4%, and 70.8% with 78.0% accuracy.

The frequency of HBME-1 expression was significantly higher in low-risk tumors when compared to benign nodules [41.5% (n = 17) vs. 11.3% (n = 6), $P = .001$]. The frequency of CK19 expression was significantly higher in low-risk tumors when compared to benign nodules [61.4% (n = 27) vs. 35.8% (n = 19), $P = .012$]. The frequency of galectin-3 positivity was significantly higher in low-risk tumors when compared to benign tumors [36.4% (n = 16) vs. 3.8% (n = 2), $P < .001$]. None of the benign nodules showed total loss of CD56. The expression profile of CD56 was similar between benign nodules and low-risk tumors ($P = .361$). Galectin-3, HBME-1, CK19, and CD56 expression profiles of low-risk, benign, and malignant tumors are summarized in Table 4.

The frequency of galectin-3 positivity was similar between low-risk and malignant tumors [36.4% (n = 16) vs. 38.5% (n = 37), $P = .805$]. Total loss of CD56 expression was significantly frequent in malignant tumors when compared to low-risk tumors [60% (n = 57) vs. 3.3% (n = 1), $P < .001$]. The frequency of HBME-1 expression was significantly higher in malignant tumors when compared to low-risk tumors [89.7% (n = 87) vs. 41.5% (n = 17), $P < .001$]. The frequency of CK19 expression was significantly higher in malignant tumors when compared to low-risk tumors [85.6% (n = 83) vs. 61.4% (n = 27), $P = .001$].

The frequency of galectin-3 positivity was similar between low-risk and EFVPTCs [36.4% (n = 16) vs. 23.7% (n = 14), $P = .163$]. Total loss of CD56 expression was significantly frequent in EFVPTCs when compared to low-risk tumors [53.4% (n = 31) vs. 3.3% (n = 1), $P < .001$]. The frequency of HBME-1 expression was significantly higher in EFVPTCs when compared to low-risk tumors [86.7% (n = 52) vs. 41.5% (n = 17), $P < .001$]. The frequency of CK19 expression was significantly higher in EFVPTCs when compared to low-risk tumors [88.3% (n = 53) vs. 61.4% (n = 27), $P = .001$].

Loss of CD56 was 60% (n = 57) and 1% (n = 1) in PTC and non-PTC groups, respectively ($P < .001$)

Discussion

In the present study, we observed that loss of CD56 expression was the most specific marker of PTC and EFVPTC in thyroid nodules followed by galectin-3, whereas HBME-1 expression was the most sensitive marker followed by CK19. When focal and total loss of CD56 expression in tumor tissue was considered as "CD56 loss" together, the sensitivity of CD56 loss increased, but specificity decreased slightly. Combination of CD56 loss with a positive immunohistochemical marker did not improve the sensitivity or specificity for malignancy. Benign nodules and low-risk tumors showed similar CD56 expressions, whereas total/focal loss was significantly more frequent in malignant tumors. The expressions of HBME-1 and CK19 in low-risk tumors had intermediate profiles between benign and malignant tumors. Galectin-3 expression was similar in malignant and low-risk tumors. None of the patients with low-risk tumors had lymph nodes or distant metastasis at the time of diagnosis. No recurrence was observed during the follow-up period.

Although benign courses of low-risk thyroid tumors have been emphasized, much of this information comes from small patient groups.[9-11] In the study of Liu et al[10] no recurrence was observed in 20 patients with WDT-UMP during an average 80 months follow-up. Before the establishment of the NIFTP category, a review by Chan[12] revealed that EFVTPCs had favorable prognoses and only 1 tumor-related death was reported in the literature. In the study of Piana et al,[9] among 1009 cases of thyroid carcinoma, 45 cases had noninvasive encapsulated follicular variant PTC, 11 cases had low-risk thyroid tumors, and no cancer-related mortality was reported in these patients. A study by Ganly et al[11] revealed that the biological behavior of noninvasive EFVPTC was similar to follicular adenomas (FAs), and they could be treated conservatively. An international multidisciplinary study by Nikiforov et al[2] included 109 patients with noninvasive EFVPTC and 101 with invasive EFVPTC. None of the patients with noninvasive EFVPTC experienced a recurrence of the disease during an at least 10-year follow-up period, whereas 12% of patients with invasive EFVPTC either experienced metastasis or died because of the disease. Authors proposed "NIFTP" term for noninvasive FVPTCs, because of the benign course of the disease.[2] The diagnostic criteria of NIFTP included the absence of vascular/capsular invasion, high mitotic activity (≥3/10 per high power field mitoses), necrosis, psammoma bodies, >1% true papillae, and morphologic features of other

Table 2. Expressions of CD56, CK19, HBME-1, and Galectin-3 at WDT-UMP, NIFTP, Benign, C-PTC, Invasive, and Noninvasive EFVPTC Groups

	CD56			CK19			HBME-1			Galectin-3		
	0	1	2	0	1	2	0	1	2	0	1	2
WDT-UMP	0 (0)	2 (14.3)	12 (85.7)	10 (58.8)	4 (23.6)	3 (17.6)	13 (81.2)	3 (18.8)	0 (0)	9 (52.9)	8 (47.1)	0 (0)
NIFTP	1 (6.3)	1 (6.3)	14 (87.4)	7 (26.0)	10 (37.0)	10 (37.0)	11 (44.0)	7 (28.0)	7 (28.0)	19 (70.4)	4 (14.8)	4 (14.8)
Benign	0 (0)	4 (7.5)	49 (92.5)	34 (64.2)	16 (30.2)	3 (5.6)	47 (88.7)	5 (9.4)	1 (1.9)	51 (96.2)	2 (3.8)	0 (0)
cPTC	26 (70.3)	3 (8.1)	8 (21.6)	7 (18.9)	3 (8.1)	27 (73.0)	2 (5.4)	4 (10.8)	31 (83.8)	14 (37.8)	8 (21.6)	15 (40.5)
NIEFVPTC	20 (57.1)	9 (25.8)	6 (17.1)	5 (14.3)	6 (17.1)	24 (68.6)	6 (17.1)	7 (20.0)	22 (62.9)	28 (80.0)	5 (14.3)	2 (5.7)
IEFVPTC	11 (47.8)	7 (30.5)	5 (21.7)	2 (33.5)	6 (23.2)	17 (43.3)	2 (8.0)	4 (16.0)	19 (76.0)	17 (70.8)	7 (29.2)	0 (0)

CK19, cytokeratin 19; C-PTC, classical papillary thyroid carcinoma; HBME-1, Hector Battifora mesothelial-1; IEFVPTC, invasive encapsulated follicular variant papillary thyroid carcinoma; NIEFVFTC, noninvasive encapsulated follicular variant papillary thyroid carcinoma; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; WDT-UMP, well-differentiated thyroid tumor of uncertain malignant potential.

Table 3. Diagnostic Utility of Galectin-3, HBME-1, CK19 Positivity, and CD56 Loss in Predicting PTC and EFVPTC in Thyroid Nodules

	Markers, n (%)	Benign	Malignant	*P*	Se% (95% CI)	Spe% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Acc.%
PTC vs. benign nodules	Galectin- 3 positivity	2 (3.8)	37 (38.5)	<.001	38.5 (28.8-49.0)	96.2 (87.0-99.5)	94.9 (82.7-99.4)	46.4 (36.8-56.1)	59.1
	HBME1 positivity	6 (11.3)	87 (89.7)	<.001	89.7 (81.9- 94.9)	88.7 (76.9-95.7)	93.6 (86.5-97.6)	82.5 (70.1-91.3)	89.3
	CK19 positivity	19 (35.8)	83 (85.6)	<.001	85.6 (76.9-91.9)	64.2 (49.8-76.9)	81.4 (72.5-88.4)	70.8 (55.9-83.1)	78.0
	CD56 loss*	0 (0)	57 (60.0)	<.001	60.0 (49.4-69.9)	100.0 (93.3-100.0)	100.0 (93.7-99.9)	58.2 (47.4-68.5)	74.3
	CD56 loss**	4 (7.5)	76 (80.0)	<.001	80.0 (70.5-87.5)	92.5 (81.8-97.9)	95.0 (87.7-98.6)	72.1 (59.9-82.3)	84.5
	HBME-1 (+) and CD56 (–)*	0 (0)	54 (56.8)	<.001	56.8 (46.3-66.9)	100.0 (93.3-100.0)	100.0 (93.4-100)	56.4 (45.8-66.6)	72.3
	Galectin-3 (+) and CD56 (–)*	0 (0)	27 (28.1)	<.001	28.1 (19.4-38.2)	100.0 (93.3-100.0)	100.0 (87.2-100)	43.4 (34.5-52.7)	53.7
	CK19 (+) and CD56 (–)*	0 (0)	54 (56.8)	<.001	56.8 (46.3-66.9)	100.0 (93.3-100.0)	100.0 (93.4-100)	56.4 (45.8-66.6)	72.3
EFVPTCs vs. benign nodules	Galectin–3 positivity	2 (3.8)	14 (23.7)	.003	23.7 (13.6-36.6)	96.2 (87.0-99.5)	87.5 (61.7-98.5)	53.1 (42.7-63.4)	58.0
	HBME-1 positivity	6 (11.3)	52 (86.7)	<.001	86.7 (75.4-94.1)	88.7 (76.9-95.7)	89.7 (78.8-96.1)	85.5 (73.3-93.5)	87.6
	CK19 positivity	19 (35.8)	53 (88.3)	<.001	88.3 (77.4- 95.1)	64.2 (49.8-76.9)	73.6 (61.9-83.3)	82.9 (67.9-92.9)	76.9
	CD56 loss*	0 (0)	31 (53.4)	<.001	53.5 (39.9-66.7)	100.0 (93.3-100.0)	100.0 (88.8-100)	66.3 (54.8-76.5)	75.7
	CD56 loss**	4 (7.5)	47 (81.0)	<.001	81.0 (68.6-90.1)	92.5 (81.8-97.9)	92.2 (81.1-97.8)	81.7 (69.6-90.5)	86.5
	HBME-1 (+) and CD56 (–)*	0 (0)	28 (48.3)	<.001	48.3 (34.9-61.8)	100.0 (93.3-100.0)	100.0 (87.7-99.9)	63.9 (52.6-74.1)	72.9
	Galectin-3 (+) and CD56 (–)*	0 (0)	9 (15.3)	.003	15.3 (7.2-27.0)	100.0 (93.3-100.0)	100.0 (66.4-99.9)	51.5 (41.4-61.4)	55.4
	CK19 (+) and CD56 (–)*	0 (0)	30 (51.7)	<.001	51.7 (38.2-65.1)	100.0 (93.3-100)	100.0 (88.4-100)	65.4 (54.0-75.7)	74.8
	HBME-1 (+) and CD56 (–)**	2 (3.8)	44 (75.9)	<.001	75.9 (62.8-86.1)	96.2 (87.0-99.5)	95.7 (85.2-99.5)	78.5 (66.5-87.7)	85.6
	Galectin-3 (+) and CD56 (–)**	0 (0)	12 (20.7)	<.001	20.7 (11.2-33.4)	100.0 (93.3-100.0)	100.0 (73.5-100)	53.5 (43.2-63.6)	58.6
	CK19 (+) and CD56 (–)**	2 (3.8)	45 (77.6)	<.001	77.6 (64.7-87.5)	96.2 (87.0-99.5)	95.7 (85.5-99.5)	79.7 (67.8-88.7)	86.5

*Total loss of CD56 expression.
**Total/focal loss of CD56 expression.Acc: Accuracy, NPV: Negative predictive value, PPV: Positive predictive value, Se: Sensitivity, Spe: Specificity,

Table 4. Immunohistochemical Expression Profiles of HBME-1, Galectin-3, CK19, and CD56 in Low-Risk Tumors Versus Benign Nodules, Malignant Tumors, and EFVPTCs

	Borderline, n (%)	Benign, n (%)	*P*	Malignant, n (%)	*P*	EFVPTCs, n (%)	*P*
HBME-1 positivity	17 (41.5)	6 (11.3)	.001	87 (89.7)	<.001	52 (86.7)	<.001
Galectin-3 positivity	16 (36.4)	2 (3.8)	<.001	37 (38.5)	.805	14 (23.7)	.163
CK19 positivity	27 (61.4)	19 (35.8)	.012	83 (85.6)	.001	53 (88.3)	.001
CD56 negativity	1 (3.3)	0 (0)	.361	57 (60.0)	<.001	31 (53.4)	<.001

CK19, cytokeratin 19; EFVPTC, encapsulated follicular variant papillary thyroid carcinoma; HBME-1, Hector Battifora mesothelial-1.

variants of PTC. The criteria <1% papillae were revised as the absence of well-formed papillae.[13] However, in subsequent studies using the original criteria, no adverse events were observed and the proposed 2022 classification allows less than 1% true papillae.[1] Avoidance of tumor staging, immediate completion thyroidectomy, and radioiodine ablation were advocated by the study group for NIFTP. Our results were consistent with the literature as no recurrence was observed in patients with low-risk neoplasms during a long period of follow-up.

Diagnosis of EFVPTC has been challenging due to the interobserver variability of nuclear features.[14] Immunohistochemical markers including CK19, galectin-3, HBME1, and CD56 have been studied for differential diagnosis of malignant thyroid tumors.[15-18] On the other hand, the immunohistochemical profile of low-risk neoplasms was studied in a limited number of studies and their results were conflicting.[5-7,19] The reported sensitivity and specificity of CK19 positivity in predicting thyroid malignancy was between 75.4%-96.3% and 40.4%-70.9% in previous studies.[16,18] Strong and diffuse CK19 staining pattern was significantly related to malignancy but not to tumor size.[16] In the studies of Bukhari et al[20] and Noroozinia et al,[4] CK19 positivity was associated with WDT-UMP and FVPTC diagnosis. Another study by Liu et al[10] investigated the utility of HBME-1, galectin-3, and CK19 in the differential diagnosis of follicular adenoma, follicular carcinoma, invasive EFVPTC, noninvasive EFVPTC, and WDT-UMP. Positivity of these markers was significantly more frequent in FVPTC when compared to WDT-UMP. However, no difference was observed between the expressions of these markers in WDT-UMP, follicular adenoma, and follicular carcinoma.[10] In the study of Yassin et al,[19] WDT-UMP had a moderate to strong CK19 expression, which revealed an intermediate profile between benign and malignant lesions. Although the majority of studies reported strong/diffuse immunoreactivity with CK19 in WDT-UMP, Scognamiglio et al[21] and Hofmann et al[22] reported CK19 positivity in 64%-74% of low-risk follicular lesions.[4,7] In our study, we observed that 61.4% of low-risk neoplasms had CK19 positivity and strong/diffuse staining was found in 29.5%. The frequency of immunostaining with CK19 in low-risk neoplasms was significantly higher than benign and lower than malignant tumors.

A neural cell adhesion molecule CD56 expression was found to be related to follicular tumors of the thyroid gland.[17] The data on the diagnostic utility of CD56 for thyroid tumors are limited. In the study of Mohamed and Shamlola,[6] comparison of immunohistochemical features of WDT-UMP, benign, and malignant tumors showed that WDT-UMPs were intermediate lesions that had similarities with malignant tumors. In the WDT-UMP group, CD56 was negative in 90% and CK19 was positive in 50% of tumors, whereas CD56 was positive in 96% and CK19 was negative in 84% of benign tumors. Another study compared the immunohistochemical expression profiles of CD56, HBME-1, CK19, galectin-3, and e-cadherin in PTC and WDT-UMPs. The CD56 expression was negative in 91.1%, 65%, and 8.3% of malignant, WDT-UMP, and benign tumors, respectively.[5] The most specific and sensitive marker for malignancy was CD56 and a combination of positive and negative markers such as "galectin-3+CD56" or "HBME-1+CD56" had both high specificity and sensitivity for malignancy. The authors proposed that 75% of WDT-UMPs could be classified as either benign or malignant according to CD56 negativity and HBME-1/galectin-3 positivity. On the contrary, Nechifor-Boilă et al[8] studied HBME-1, galectin-3, CD56, and CK19 in borderline thyroid tumors and observed that the immunohistochemical profile of borderline tumors was similar to the benign tumors.[19] The positivity of CD56, CK19, HBME-1, and galectin-3 was observed in 61.3%, 9.7%, 12.9%, and 16.1% of low-risk follicular derived tumors, respectively. The most sensitive marker was CD56 followed by HBME-1, whereas CK19 and galectin-3 were the most specific markers.[8] In our study, the CD56 expression profile was similar with benign nodules, and galectin-3 expression was similar with malignant tumors, whereas CK19 and HBME-1 had expression profilesbetween benign nodules and malignant tumors. The combination of CD56 loss with a positive immunohistochemical marker did not improve the diagnostic utility.

In conclusion, low-risk thyroid tumors had overlapping immunohistochemical features with both benign nodules and malignant tumors. Loss of CD56 expression was the most useful marker for thyroid malignancy. Low-risk thyroid tumors had a benign course of disease during a long follow-up period.

Data Availability Statement: Authors agree to make data and materials supporting the results or analyses presented in their paper available upon reasonable request.

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