Original paper

CD56, CD57, HBME1, CK19, Galectin-3 and p63 immunohistochemical stains in differentiating diagnosis of thyroid benign/malign lesions and NIFTP

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Detection of thyroid carcinoma has been steadily increased in the past few decades. After the recognition of NIFTP, also gain importance to differentiate benign tumors (follicular adenoma) from follicular patterned variants of papillary thyroid carcinoma (invasive and infiltrative follicular variant papillary thyroid carcinoma), and low-risk lesions of thyroid (NIFTP).

Follicular patterned proliferations of thyroid still persists as a battle for pathologists. In this study, we aimed to analyze the most commonly used immunohistochemical stains "HBME1, CK19, Galectin-3", adding the new ones "CD56, CD57, and p63".

Study groups were; nodular hyperplasia, follicular adenoma, NIFTP, infiltrative follicular variant PTC, classical variant PTC (CVPTC) and follicular carcinoma. Each group consisted of twenty cases. The sections were stained with CD56, CD57, p63, CK19, HBME1 (Mesotel cell), Galectin-3 antibody.

Although the expression of CD56 was high in benign follicular lesions, FC could not be excluded in this group. CD57 was high in malignant follicular group and NIFTP. Interestingly, p63 was found highly expressed in FVPTC, which might be promising to predict invasiveness in follicular group of lesions. CK19, Galectin-3 and HBME1 were found quietly prominent in CVPTC in concordance with the previous reports.

Key words: thyroid lesions, differential diagnosis, CK19, Galectin-3, CD56, CD57, p63, HBME1.

Introduction

Due to the sophisticated imaging modalities, detection of thyroid carcinoma has been steadily increased in the past few decades. There are some authors calling this trend "overdiagnosis hypothesis", with increases in only one histotype (papillary thyroid carcinoma – PTC) and mainly micro-PTC and NIFTP (formerly named as encapsulated follicular variant of PTC). However, increased rates of advanced stage and larger-size PTC have already contradicted this hypothesis. As a fact, PTC, the most

common endocrine cancer, is not a "pseudodisease" but a real disease in our era [1].

Thyroid surgery (total or hemithyroidectomy) is commonly performed by virtue of the hight detection rates of the thyroid nodules (up to 70%) [2, 3]. Although, the presence of capsule and/or vascular invasion were being used only in the differentiation of benign from malignant follicular tumors (Follicular adenoma (FA) vs. follicular carcinoma (FC) and Hürthle cell adenoma vs. Hürthle cell carcinoma), after the recognition of NIFTP, also gain importance to differentiate benign tumors (FA) from follicular patterned variants of PTC (invasive encapsulated follicular variant PTC), and low-risk lesions of thyroid (NIFTP) [4]. Either hemi or total thyroidectomy materials, the differentiation among these lesions is crucial to choose the optimal therapeutic method for the sake of rational patient management [5, 6].

PTC is diagnosed by the presence of nuclear characteristics including nuclear overlapping, nuclear enlargement, chromatin clearing, intranuclear pseudo-inclusions and grooves. There is no battle for the pathologists in the diagnosis of classical variant of PTC with the papillary structures and complete nuclear characteristics. On the contrary follicular patterned proliferations of thyroid is condemn to be a battle for years concerning to differentiation of benign from malignant [7]. Differential diagnosis of follicular patterned thyroid lesions represents a wide spectrum of benign and malignant entities including invasive encapsulated follicular variant papillary thyroid carcinoma (FVPTC), FA, FC, noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and nodular hyperplasia (NH). Definitive diagnosis is sometimes difficult even in histopathology specimens and requires additional studies.

CD56 is present in follicular epithelial cells of normal thyroid tissue [8, 9]. It is expressed in NK cells in activated T lymphocytes. This protein is thought to regulate cell motility and reduces tumor invasion [10, 11]. CD56 is a neural cell adhesion molecule, its expression affects the migratuar capacity of tumor cells and it is determined that its release is reduced in malignant thyroid tumors while it is released in normal thyroid epithelial cells. Loss of CD56 expression was associated with metastatic potential and poor prognostic clinical course [12].

CD57 was first identified in lymphocytes showing Natural Killer activity. There are studies showing that CD57 is expressed in thyroid tumors and other carcinomas [13]. Two studies reported that CD57 was beneficial in distinguishing thyroid follicular lesions as benign and malignant [14, 15]. These reports have also suggested that CD57 is of limited utility to evaluate follicular neoplasms of the thyroid and cannot be used as a specific marker [16, 17].

P63 is p53 homologous nuclear transcription factor. It has a negative dominant effect on the P53 gene. P63 is expressed in basal cells, squamous and myoepithelial cells, and the tumor suppressor feature is controversial [12].

In the literature, CD56, CD57, HBME-1, CK19, Galectin-3 and p63 were tested in a few of studies separately in different lesions of thyroid but not in heterogeneous diagnosis groups and not consider all of these antibodies. Several studies were planned after the recent description of NIFTP but no about all of these markers.

The most commonly used immunohistochemical markers in thyroid malignancies are Mesothelial cell antibody (HBME-1), Cytokeratin-19 (CK19) and Galectin-3. These antibodies favors papillary thyroid carcinoma. But there is no marker for differentiating thyroid diseases with high sensitivity and/or specificity [18]. Although, there are many studies in the literature have been done by molecular markers, still limited number of studies [19] were analyzed the usefulness of well-known immunohistochemical markers to differentiate these above mentioned entities. In this study, we aimed to analyze the most commonly used immunohistochemical stains "HBME1, CK19, Galectin-3", adding the new ones "CD56, CD57, and p63" in the diagnosis of nodular hyperplasia, follicular adenoma, invasive encapsulated follicular variant PTC, classical variant PTC (CVPTC), follicular carcinoma and NIFTP.

Material and methods

Patient selection

Patients diagnosed in Trakya University Department of Pathology were reviewed retrospectively. The study design was approved by the local Ethic Committee of the university hospital. Patients were selected according to who had been performed thyroidectomy. The age of the patients ranges 18 to 75. There were 120 patients that 17 male and 103 females.

Definitions of clinicopathological criteria

Histopathological reports were reviewed. Study groups were created as in the following; nodular hyperplasia, follicular adenoma, NIFTP, invasive encapsulated follicular variant PTC, classical variant PTC and follicular carcinoma. Each group consisted of twenty cases and patients were randomly selected from the thyroidectomy materials that had been diagnosed in our laboratory.

Hematoxylin-eosin stained slides were re-evaluated, and the diagnoses were confirmed. Histological subtyping in thyroid malignancy was adjusted based

on the criteria of 4th WHO classification of tumors of endocrine organs [20]. A hyperplasia of different size follicles, with no thick fibrous capsule, and with ordinary nuclear features, was diagnosed as nodular hyperplasia. Follicular adenoma was diagnosed in nodules with thick fibrous capsule, without capsule invasion and with ordinary nuclear properties in normofollicular, microfollicular or macrofollicular pattern. Follicular carcinoma was diagnosed if there was a capsule and/or vascular invasion in these nodules. For the diagnosis of classical papillary carcinoma, nuclear membrane irregularity, nuclear pseudoinclusion, nuclear groove, nuclear clarification, nuclear overlapping, papillary features with fibrovascular structures, psammoma bodies were investigated. invasive encapsulated follicular variant PTC was diagnosed when tightly packed follicular structures contained PTC nuclei with capsular invasion. NIFTP was diagnosed as follicular patterned, encapsulated or well-limited lesions with PTC-like nuclear features with the additional requierements of revised criteria [4, 19].

Immunohistochemical study

For immunohistochemical studies, 2-3-micron thick sections were taken from the paraffin blocks containing the lesion. The sections were stained with avidin-biotin complex method with CD56, CD57, p63, CK19, HBME1 (Mesotel cell), Galectin-3 antibody. Immunohistochemical stains were evaluated by two pathologists. Evaluation of immunohistochemical staining was performed with a 10× and 20× objectives in light microscope. The following staining patterns were considered positive: HBME1 and CD56 membranous staining, cytoplasmic and/or membranous staining of CD57 and CK19, nuclear and/or cytoplasmic Galectin-3 and nuclear expression of p63. Immunoreactivity was considered positive when at least 10% of the tumor cells for all markers since aberrant staining may also be present [12, 13, 18].

Statistical analyses

Data analyses were done with the STATE 13 software package program (*Version 24.0; Armonk, NY, USA: IBM Corp*.). For comparison of categorical variables, the chi-square test and Fisher's exact test were used. P values below 0.05 were considered significant. When non-parametric ROC analysis with sequential results is examined; ROC analysis for ordinal ratings (1-4) were used to calculate the area under the curve.

Results

CD56 generally was found positive in benign lesions and follicular carcinoma. CD57 was positive in malignant lesions (FVPTC, FC, CVPTC) and NIFTP and expression of P63 was prominent in FVPTC more than any other lesions in group. HBME1, Galectin-3 and CK19 were stained more commonly in CVPTC. CK19 was also highly expressed in FVPTC and NIFTP (Table I, Fig. 1).

The expression of CD56 in the lesions of current study was found as 19 (%95) in NHs, 18 (%90) in FAs, 18 (%90) in FCs, 3 (%15) in NIFTPs, 2 (%10) in FVPTCs, 1 (%5) in CVPTCs (Table I). CD56 expression was detected in 37 (92.5%) of 40 cases in the group of benign diseases consisting of nodular hyperplasia and adenoma. On the other hand, CD56 expression was seen in 21 (35%) of 60 cases with malignant tumors (FC, FVPTC and CVPTC), ($p < 0.001$) (Table II, Fig. 2). Of those 18 (85%) was FC (18/21). Follicular patterned proliferations also classified separately as NH, FA, NIFTP, FVPTC and FC. CVPTC was the only entity in non-follicular patterned proliferations. CD56 positivity was found only in 1 (5%) case of the non-follicular patterned

Table I. Positive expression of immunohistochemical antibodies in all cases

		CD56 n (%)	CD57 n (%)	P63 n (%)	HBME1 n (%)	Galectin-3 n (%)	CK19 n (%)
Benign	**n = 40**	**37 (92.5)**	**2 (5)**	**1 (2.5)**	**2 (5)**	**0 (0)**	**12 (30)**
	NH (n = 20)	19 (95)	1 (5)	0	1 (5)	0	8 (40)
	FA (n = 20)	18 (90)	1 (5)	1 (5)	1 (5)	0	4 (20)
Malignant	**n = 60**	**21 (35)**	**56 (93.3)**	**26 (43.4)**	**25 (41.7)**	**28 (46.7)**	**50 (83.3)**
	FC (n = 20)	18 (90)	19 (95)	3 (15)	0	0	11 (55)
	IFVPTC (n = 20)	2 (10)	18 (90)	17 (85)	7 (35)	11 (55)	19 (95)
	CVPTC (n = 20)	1 (5)	19 (95)	6 (30)	18 (90)	17 (85)	20 (100)
NIFTP	**n = 20**	**3 (15)**	**17 (85)**	**4 (20)**	**3 (15)**	**3 (15)**	**18 (90)**
Total	**n = 120**	**61**	**75**	**31**	**30**	**31**	**80**

NH – nodulary hyperplasia; FA – follicular adenoma; FC – follicular carcinoma; IFVPTC – infiltrative follicular variant papillary thyroid carcinoma; CVPTC – classical variant papillary thyroid carcinoma; NIFTP – noninvasive folicular thyroid neoplasm with papillary like nuclear features

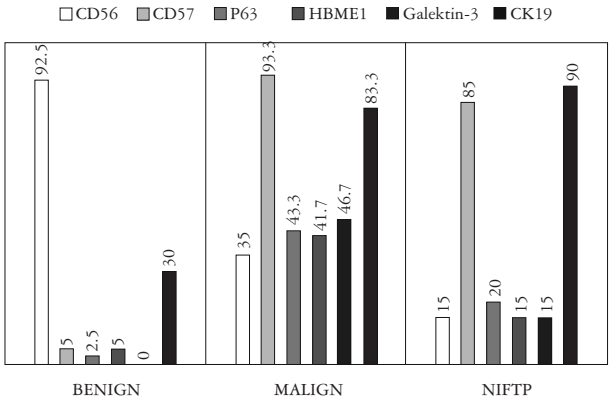

Fig. 1. Distribution of the percentages of immunohistochemical antibodies according to case groups

proliferations; whereas 60 (60%) of follicular lesions were showed statistically significance ($p < 0.001$) (Table III).

CD57 expression was common in malignant group more than in benign group. CD57 was positive in 1 (5%) of NHs, 1 (5%) of FAs, 19 (95%) of FCs, 17 (85%) of NIFTPs, 18 (90%) of FVPTCs and 19 (95%) of CVPTCs (Table I). Accordingly, CD57 positivity was found in 2 (5%) of benign group (NH and FA) and in 56 (93.3%) of malignant group ($p < 0.001$) (Table II, Fig. 2). CD57 was positive in most of NIFTPs similarly malignant group cases (Table IV). CD57, also was positive in 56 (56%) of follicular patterned proliferations and in 19 (95%) of non-follicular patterned proliferations ($p = 0.001$) (Table III).

P63 positivity was detected none of NHs, however in 1 (5%) of FAs, 3 of FCs (15%), 4 of NIFTPs (20%), 17 (85%) of FVPTCs and 6 (30%) of CVPTCs (Table I). Benign lesions (NH and FA) showed positive staining in 1 (2.5%) of them, while 26 (43.3%) of malignant group were stained ($p < 0.001$) (Table II, Fig. 2). P63 was positive small quantity of NIFTPs. According to this study, p63 was found to be positive in most malignant cases especially in FVPTC. Follicular lesions showed positive staining in 25 cases (25%), whereas non-follicular lesions showed staining in 6 cases (30%) however, this difference was not statistically significant ($p = 0.641$) (Table III).

HBME1 staining was observed in 1 (5%) of NHs, in 1 (5%) of FAs, in none of FCs, in 3 (15%) of NIFTPs, in 7 (35%) of FVPTCs and in 18 (90%) of CVPTCs (Table I). While HBME1 positivity was

Table II. P values and distribution of the percentages of immunohistochemical markers according to benign and malignant groups

EXPRESSION OF ANTIBODY	BENIGN N = 40 %	MALIGNANT N = 60 %	P VALUE*
CD56			
Negative	7.5	65	< 0.001
Positive	92.5	35	
CD57			
Negative	95	6.7	< 0.001
Positive	5	93.3	
P63			
Negative	97.5	56.7	< 0.001
Positive	2.5	43.3	
HBME1			
Negative	95	58.3	< 0.001
Positive	5	41.7	
Galectin-3			
Negative	100	53.3	< 0.001
Positive	0	46.7	
CK19			
Negative	70	16.7	< 0.001
Positive	30	83.3	

** χ^2 analysis p value ($p = 0.001$)*

Table III. Distribution of the percentages of immunohistochemical markers according to follicular and non-follicular groups

EXPRESSION OF ANTIBODY	FOLLICULAR GROUP (N = 100) %	NON-FOLLICULAR GROUP (N = 20) %	P VALUE*
CD56			
Negative	40	95	< 0.001
Positive	60	5	
CD57			
Negative	44	5	0.001
Positive	56	95	
P63			
Negative	75	70	0.641
Positive	25	30	
HBME1			
Negative	88	10	< 0.001
Positive	12	90	
Galectin-3			
Negative	86	15	< 0.001
Positive	14	85	
CK19			
Negative	40	0	0.001
Positive	60	100	

** χ^2 analysis p value ($p = 0.001$)*

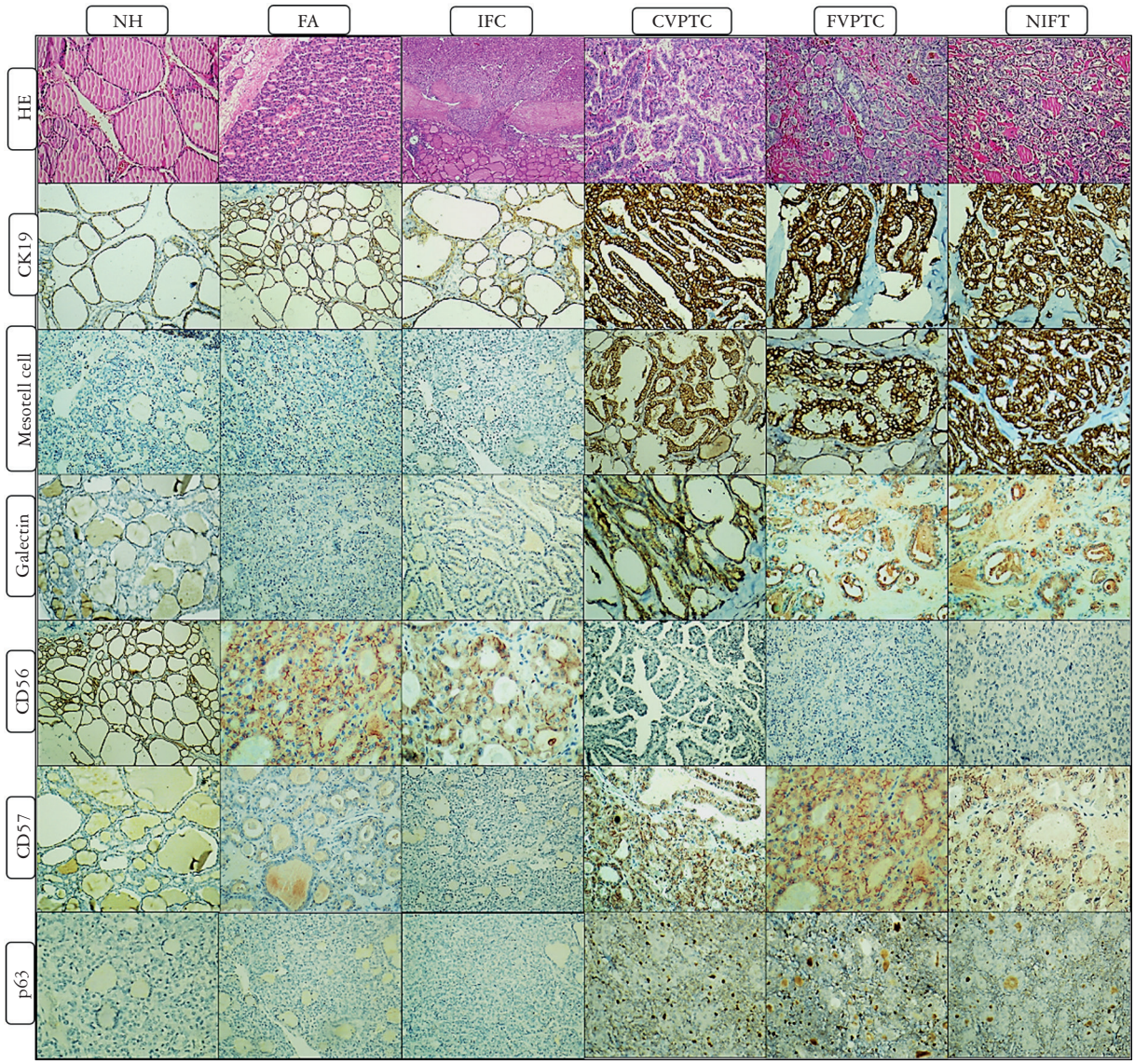

Fig. 2. Distribution of the immunohistochemical antibodies expression according to case groups

seen in 2 (5%) of benign group, 25 (41.7%) of malignant group had HBME1 positivity ($p < 0.001$) (Table II, Fig. 2). HBME1 was positive in few of NIFTPs similarly benign group cases (Table V). Twelve (12%) of follicular lesions were stained positive whereas in 18 (90%) of non-follicular lesions ($p < 0.001$) (Table III). According to these results, HBME1 was mostly positive in malignant lesions, specifically in CVPTC.

Galectin-3 represents cytoplasmic and nuclear staining. There was no expression in NHs, FAs and FCs, whereas, 3 (15%) of NIFTPs, 11 (55%) of FVPTCs and 17 (85%) of CVPTCs were positively expressed (Table I). Benign group also showed no expression, but 28 of malignant group (46.7%) were positively expressed ($p < 0.001$) (Table II, Fig. 2). Galectin-3 was positive in 3 (15%) of NIFTPs. In the group of follicular patterned vs. non-follicular patterned proliferations, the expression was 14 (14%)/ 17 (85%), respectively ($p < 0.001$) (Table III). According to this, Galectin-3 and HBME1 were more positive in malignant group, especially in CVPTC.

CK19 was positive in 8 (40%) of NHs, in 4 (20%) of FAs, in 11 (55%) of FCs, in 18 (90%) of NIFTPs, in 19 of FVPTCs (95%) and in all of CVPTCs (Table I). Twelve (30%) of benign group and 50 of malignant group (83.3%) were expressed ($p < 0.001$) (Table II, Fig. 2). CK19 was positive in most of NIFTPs similarly malignant group (Table IV). CK19 was found positive in 60 (60%) of follicular patterned proliferations, and all of non-follicular patterned proliferations ($p = 0.001$) (Table III). Accordingly, CK19 has high expression ratios in CVPTC and FVPTC and NIFTPs.

Table IV. Distribution of the percentages of immunohistochemical markers according to NIFTP and malignant groups

Expression of antibody	NIFTP (n = 20) %	Malignant group (n = 60) %	p value*
CD56			
Negative	85	65	0.091
Positive	15	35	
CD57			
Negative	15	6.7	0.253
Positive	85	93.3	
P63			
Negative	80	56.7	0.062
Positive	20	43.3	
HBME1			
Negative	85	58.3	0.030
Positive	15	41.7	
Galectin-3			
Negative	85	53.3	< 0.016
Positive	15	46.7	
CK19			
Negative	5.3	16.7	0.211
Positive	94.7	83.3	

** χ^2 analysis p value (p = 0.016)*

Table V. Distribution of the percentages of immunohistochemical markers according to NIFTP and benign groups

Expression of antibody	NIFTP (n = 20) %	Benign group (n = 40) %	p value*
CD56			
Negative	7.5	85	< 0.001
Positive	92.5	15	
CD57			
Negative	15	95	< 0.001
Positive	85	5	
P63			
Negative	80	97.5	0.021
Positive	20	2.5	
HBME1			
Negative	85	95	0.186
Positive	15	5	
Galectin-3			
Negative	85	100	0.012
Positive	15	0	
CK19			
Negative	5.3	70	< 0.001
Positive	94.7	30	

** χ^2 analysis p value (p = 0.016)*

In sum, 3 markers (CK19, CD57 and CD56) were with the highest diagnostic power in differentiating benign lesions from malignant ones (CD56: 86.98%, CD57: 98.59%, P63: 70.11%, HBME1: 71.69%, Galectin-3: 70%, CK19: 98.94%) (Fig. 3).

Discussion

Although the diagnosis of thyroid tumors is easy in most cases, it may be difficult to differentiate some malignant tumors from benign lesions. In such cases, particularly follicular lesions diagnosis can be quite difficult with histopathological examination. In this paper we investigated whether immunohistochemical markers may be used for differential diagnosis in these cases.

In this study, we investigated the staining of 6 immunohistochemical markers in 6 different diagnostic groups (nodular hyperplasia, follicular adenoma, follicular carcinoma, noninvasive follicular thyroid neoplasm with papillary-like nuclear features, invasive encapsulated follicular variant papillary thyroid carcinoma, classical variant papillary thyroid carcinoma) in the name of finding any significance from their combination in differential diagnosis of these lesions.

CD56 is used to detect neuroendocrine [21] and some hematopoietic disorders. Demellawy *et al.* [12] investigated CD56 expression in 175 benign and malignant follicular lesions and found strong membranous staining in non-PTC lesions (follicular and anaplastic carcinoma and benign lesions). Boila *et al.* [18] found CD56 absent staining 84.8% in 204 cases with PTC. Atti *et al.* [22] found 89.4% positivity in follicular pattern thyroid lesions that 32 hyperplastic nodules, 12 follicular adenomas, 3 follicular tumours of unknown malignant potential. Muthusamy *et al.* [23] found CD56 to be useful in differentiating follicular variant PTC from follicular adenoma. According to present study similar to the literature, 95% of classical variant PTCs, 90% of invasive encapsulated follicular variant PTCs, 85% of NIFTPs showed loss of staining, a high rate (91.6%) of positive staining in benign thyroid lesions and in follicular carcinoma.

Differential diagnosis of follicular pattern lesions, especially between FA and NIFTP; is based on PTC like nuclear features and is very incompatible with pathologists. In these cases CD56 can be used in the differential diagnosis.

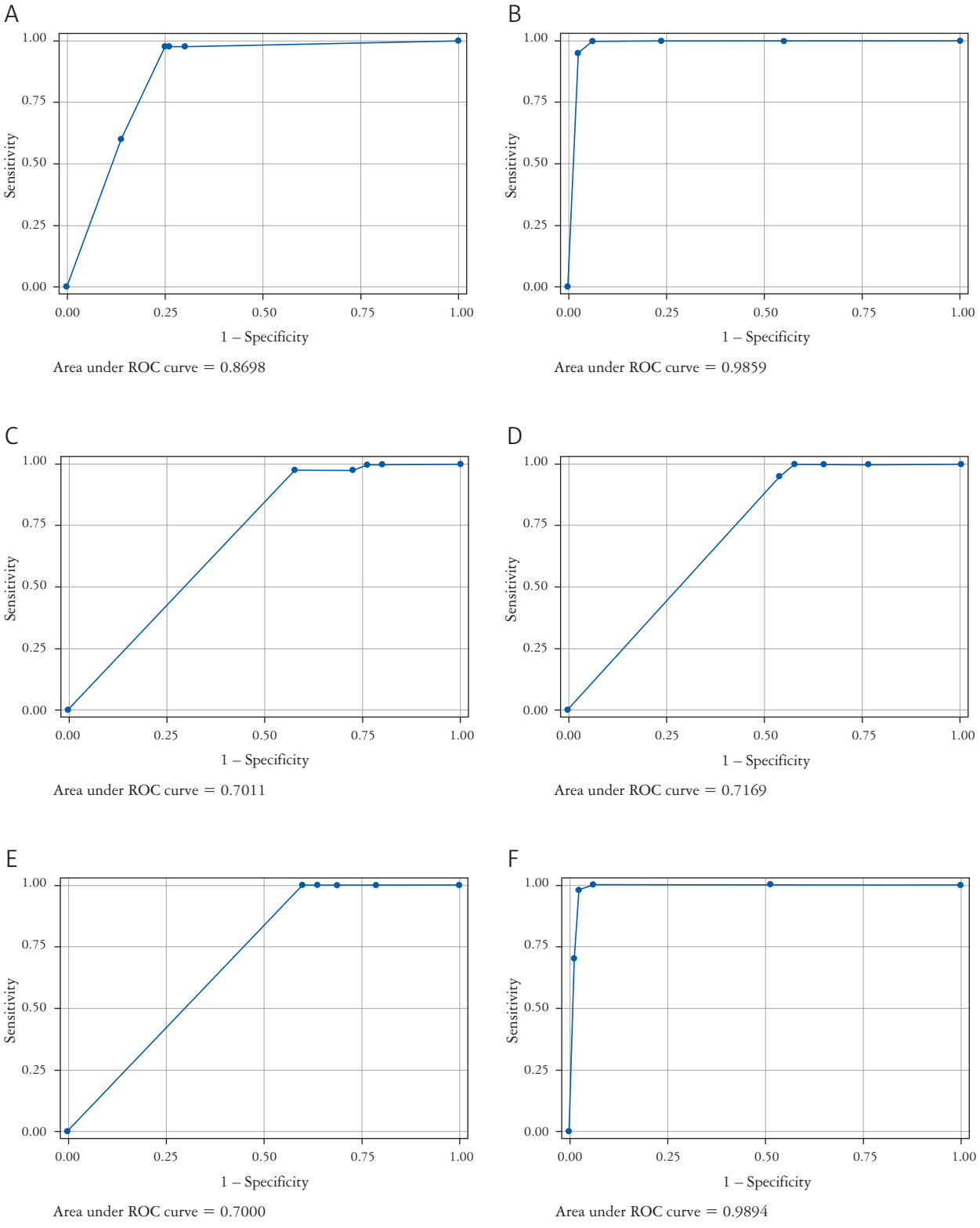

Fig. 3. ROC curves of immunohistochemical markers differentiating benign and malignant groups (CD56, CD57, P63, HBME1, Galectin-3, CK19 from left to right respectively)

In our study, CK19 was the most specific marker for PTC. It is stained most of CVPTC, FVPTC (100%, 95%) and also most of NIFTPs (94.7%). Demellawy *et al.* [12] demonstrated CK19 positivity 85% in PTCs. Boila *et al.* [3] also showed staining in 0.6% of normal thyroid tissue. In our study, CK19 positivity was detected in 40% of nodular hyperplasias. Therefore, CK19 should not be used alone in diagnosis. For FVPTC, the panel consisting of CK19 and CD57 was found to be very useful.

Khan *et al.* [13] observed CD57 stated that follicular lesions of thyroid may be used in the differential diagnosis of benign-malignant. Nasır *et al.* [24] showed CD57 was found to be 71% of follicular carcinomas and 15% of follicular adenomas. In our study, CD57 was positive in follicular lesions except NIFTP. CD57 positivity was demonstrated most of malignant group (91.3%). We found CD57 to be useful in differentiating malignant and benign lesions. Especially in follicular lesions, CD57 immunohistochemical staining can be used to with capsulary and vasculary invasion, in differential diagnosis of FA with FVPTC.

Galectin-3 is associated with malignant transformation in thyroid cells, and strong, intensive staining is observed, especially in classical variant PTC [25]. In most studies, Galectin-3 has been found to be a sensitive and reliable marker for thyroid malignancies, particularly PTC [26, 27, 28, 29]. Boila *et al.* [3] observed Galectin-3 was 94.9% in classical variant PTC and 27.8% in follicular variant PTC similarly our study. We found Galectin-3 to be useful in differentiating malignant thyroid lesions particularly nonfollicular ones from benign lesions.

In many studies in the literature; HBME-1 was observed high expression in CVPTC, whereas in FVPTC lower expression was observed [18, 30, 31, 32, 33, 36]. In our study, it was found to be similar Galectin-3, 90% positive in classical variant PTC and in 35% of infiltrative follicular variant PTC. HBME1 and Galectin-3 panel may be useful in malignant nonfollicular lesions particularly classical variant PTC.

P63 immunohistochemical staining study in thyroid tumors is present in a few cases in the literature. P63 positivity in thyroid carcinomas ranged from 6.9% to 74% [18, 34]. In one study, p63 positivity was detected 25.9% in papillary thyroid carcinomas [35]. In our study, p63 positivity was found to be 85% in FVPTC. Demellawy *et al*. [12] showed p63 and CD56 panel were very useful in distinguishing PTC from other non PTC lesions. Similarly we found p63 was usable differentiating malignant cases particularly follicular variant PTC.

As the staining level of CD56 increases, the probability of being benign increases. Other markers increase the possibility of malignancy as the level of staining increases.

Conclusions

The differential diagnosis of thyroid tumor may be occasionally difficult particularly in follicular lesions. Although morphological findings are very valuable, immunohistochemical studies may also be used in the precise diagnosis. In our comprehensive immune panel study, we found CD56 in benign follicular lesions, CD57 in follicular lesions especially in malign lesions and NIFTP. p63 in FVPTC, CK19 in FVPTC, CVPTC, and NIFTP, Galectin-3 and HBME1 in CVPTC are very useful. Especially in the differentiation of malignant and benign follicular thyroid lesions; CD56, CD57 and CK19 immunohistochemical panel may be very useful.

The authors declare no conflict of interest.

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