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CK19 IS A USEFUL MARKER IN DISTINGUISHING FOLLICULAR VARIANT OF PAPILLARY THYROID CARCINOMA FROM BENIGN THYROID LESIONS WITH FOLLICULAR GROWTH PATTERN

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Abstract

Context. Thyroid cancer is the most common endocrine malignancy. Within various subtypes of thyroid neoplasms, those with follicular growth pattern usually make diagnostic problems.

Objectives. To examine ck19 expression as a diagnostic marker in thyroid neoplasms with follicular growth pattern.

Design. In this cross sectional study, 86 patients were enrolled.

Subjects and Methods. Totally 22 follicular adenoma (FA), 18 well differentiated tumors with undetermined malignant potential (WT-UMP) and 46 follicular variants of papillary thyroid carcinoma (FV-PTC) were enrolled and examined for Ck19 expression by immunohistochemistry staining. Membranous/cytoplasmic staining patterns were considered as positive. Specimens without staining were considered as 0, < 5% positively stained cells as 1+, 5%-25% as 2+, 25%-75% as 3+ and >75% as 4+.

Result. CK19 was negative in most cases of FA while positive in most WDT-UMP and FV-PTCs, $p<0.001$. Additionally, most cases with 2+ and 3+ staining patterns were FV-PTC (75% and 81%, respectively, $p<0.001$) and none of FAs showed 3+ positivity ($p<0.001$). Additionally, most of strongly positive results in patients > 45 y/o were PTC ($p<0.001$).

Conclusion. Ck19 is a useful marker in differentiating FA from FV-PTC. We found diffuse and strong (3+) staining pattern in FV-PTC but none of FAs were so. We concluded that diffuse and strong staining for ck19 in a thyroid lesion with follicular pattern of growth, especially in a patient older than 45 y/o should raise the possibility of malignancy.

Key words: thyroid nodule, Ck19 expression, growth pattern.

INTRODUCTION

Thyroid neoplasms are one of the most common tumors in human. Follicular variants of thyroid neoplasms are the most common subtype (1). Within tumors with follicular features, follicular variant of papillary thyroid carcinoma, well differentiated tumor with uncertain malignant potential, follicular adenoma and follicular carcinoma do exist. Diagnosis of these entities is based on morphologic findings combined with architectural and nuclear features including nuclear grooves, inclusions, overlapping and other microscopic findings (2). As follicular neoplasms share these histopathologic changes and can manifest similar morphologic findings, the differential diagnosis becomes difficult in some cases (3).

Previous studies have reported significant interobserver differences in diagnosis of thyroid follicular neoplasms. These differences are because the criteria for diagnosis of each follicular neoplasm are subjective (4). Thus, it is of great importance to find some objective criteria or specific markers for definite classification of follicular neoplasms and increase interobserver consensus.

Several studies have been performed in cellular and molecular levels to find one or combination of markers specific for each terminology. Among various studied markers, some novel markers including galectin3, cyclin D1, HBME1 and cytokeratin (CK) 19 were of greater interest and revealed more promising results (5-8).

CK19 is one of the low molecular weight keratins which expressed in thyroid tissue (8, 9). But there are some controversies on its expression pattern. Some papers have shown that this marker is expressed only in malignancies but there are some studies showing its expression in benign lesions (10). Considering current controversies, in this study we tried to evaluate the expression of CK19 in thyroid neoplasms with follicular growth pattern by immunohistochemical (IHC) method.

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Table 1. Demographic data of the enrolled patients

		Total number	Gender		Size	Age	TNM stage		
			Female	Male	(cm)	(years)	T	N	M
Subtype	FA	22	17	5	1.5±3.8	41.7±12.9	-	0	0
	WT-UMP	18	12	6	2.3±2	41.4±15.9	-	0	0
	FV-PTC	46	27	19	1.6±2.8	48.1±15.4	pT1-pT3	0	0

FA: follicular adenoma, WT-UMP: well differentiated tumor with undetermined malignant potential, FV-PTC: follicular variant of papillary thyroid carcinomas.

Table 2. CK 19 staining results in each lesion

	0	1+	2+	3+	P value
FA	16 (61.5%)	3 (15.8%)	3 (15%)	0	< 0.001
WT-UMP	3 (11.5%)	9 (47.4%)	2 (10%)	4 (19%)	
FV-PTC	7 (27%)	7 (36.8%)	15 (75%)	17 (81%)	
	26	19	20	21	

FA: follicular adenoma, WT-UMP: well differentiated tumor with undetermined malignant potential, FV-PTC: follicular variant of papillary thyroid carcinomas.

MATERIAL AND METHODS

In this study, a total of 86 paraffin embedded samples of thyroid neoplasms with follicular growth pattern collected by department of pathology, Urmia University of Medical Sciences, Urmia, Iran were enrolled. The slides were re-examined by three pathologists and the samples with follicular growth pattern were selected. So, 22 follicular adenomas (FA), 18 well differentiated tumor with uncertain malignant potential (WDT-UMP) and 46 follicular variant of papillary thyroid carcinomas (FV-PTC) were enrolled. Additionally, patients' demographic data were also collected from their medical records. The pathologic diagnosis of the selected samples were confirmed and classified according to WHO classification criteria, for example, the diagnosis of papillary thyroid carcinoma was based on characteristic cytologic features including nuclear inclusions, grooves, nuclear clearing, overlapping and crowding. After selection of appropriate samples, the paraffin embedded blocks were used to obtain 4 micrometer sections and prepared for IHC staining. The IHC staining for CK19 was performed on each prepared slide according to manufacturer instructions (Dako, DAKO Corporation, Glostrup, Denmark). Briefly, after deparaffinization, the sections were rehydrated, and then the endogenous peroxidase activity was blocked using hydrogen peroxide. After antigen retrieval, the primary antibody was added, after 45 min incubation, the antibody was washed and the slides were incubated with Envision (DAKO Corporation, Glostrup, Denmark). For visualization of the stained cells, diaminobenzidine tetrahydrochloride (DAB) solution was used. The membranous/cytoplasmic staining patterns were considered as positive. Specimens without staining were considered as 0, < 5% stained cells as 1+, 5%-25% as 2+, 25%-75% as 3+ and >75% as 4+ (11).

Statistical analysis

The results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Normality of data was evaluated with the Kolmogorov-Smirnov test. The statistical differences between proportions were determined by $\chi 2$ analysis. Numerical data were evaluated using analysis of variance, followed by Tukey's post hoc test. $P < 0.05$ was considered as significant.

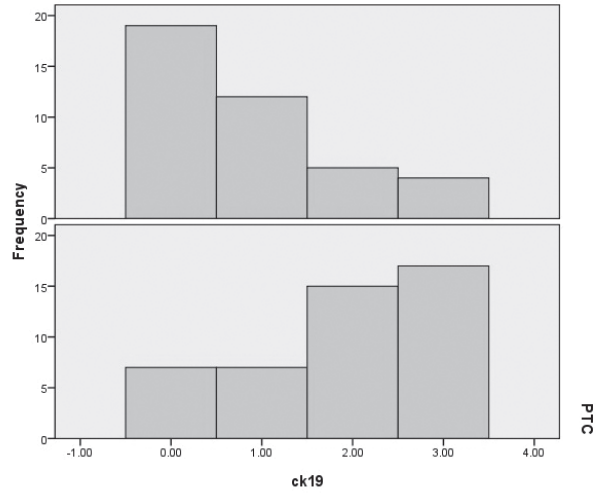

Figure 1. Ck19 intensity in benign and malignant lesion. The above diagram is for follicular adenoma (FA) showing that most of the FAs have no staining or 1+ staining intensity in immunohistochemical staining (IHC) for ck19. In contrast, follicular variant of papillary thyroid carcinoma (FV-PTC) shows mostly 3+ staining intensity for ck19 in IHC staining (the lower diagram). The y axis shows the number of lesions and the x axis shows the intensity of staining for ck19.

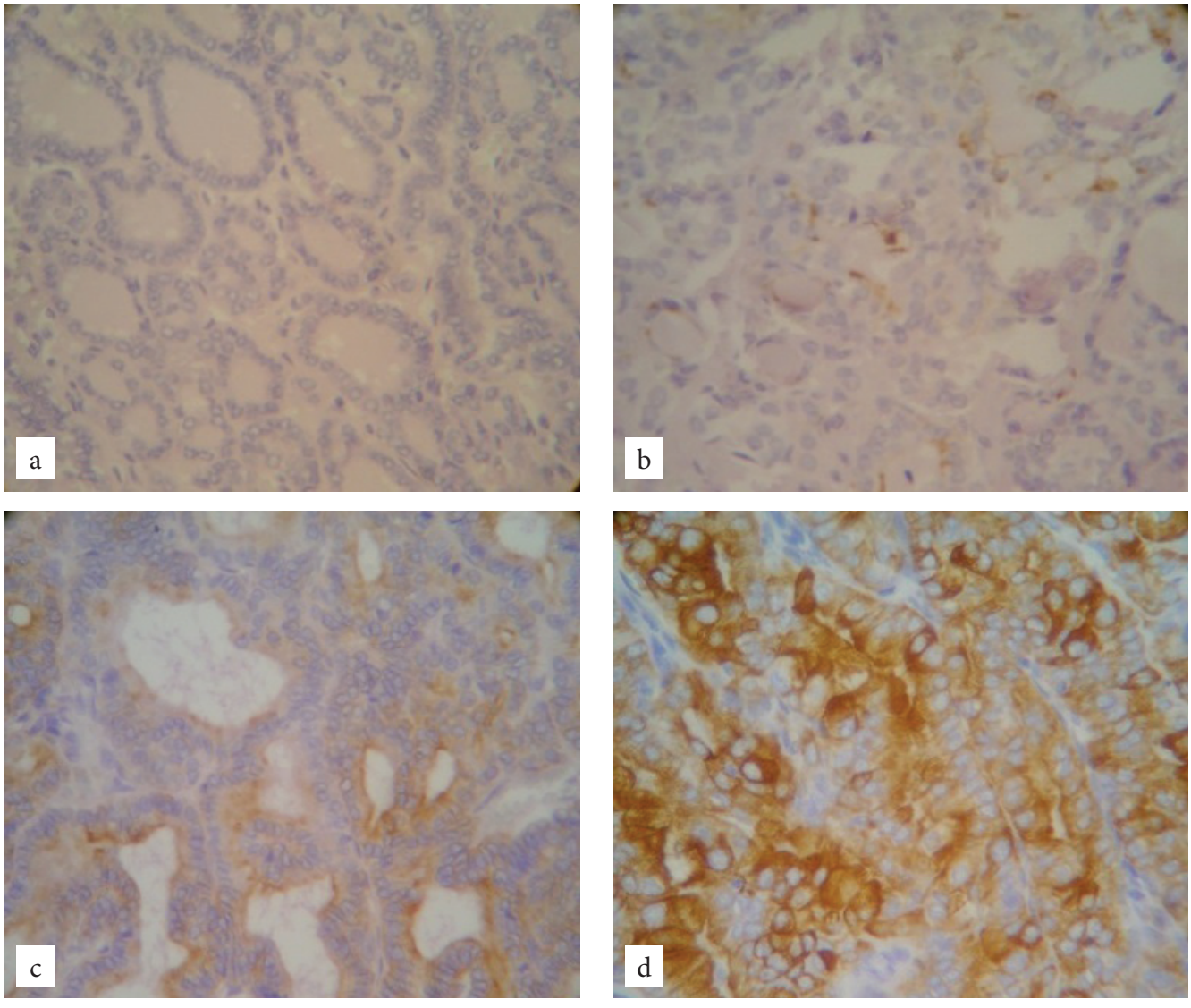

Figure 2. Immunohistochemical staining for Ck19 , HE counterstain, 40 X. a) negative staining in follicular adenoma; b) 1+ staining in follicular adenoma; c) 2+ staining in follicular variant of papillary thyroid carcinoma; d) 3+ staining in follicular variant of papillary thyroid carcinoma.

RESULTS

Among 86 cases enrolled in this study 56 were female and 30 were male (43.3 ± 15.4 vs 48.3 ± 14.3 years old, p>0.5). The average size of the lesions was 1.6±2.8 cm. The gross and microscopic findings of the neoplasms are listed in Table 1. There was no difference between patients age with different pathologic lesions. Among 86 studied cases, 26 cases were negative and 60 cases were positive for CK19 in IHC staining. The distribution of CK19 positivity and the intensity of positive results among tumor subtypes are listed in Table 2. Our data showed that the expression level of CK 19 and its intensity were increased from benign lesions to malignant FV-PTC (Fig. 1). As, about 73% of follicular adenomas compared to 15% of FV-PTC were negative for CK19 (p<0.001). Totally, positive expression of CK19 in diagnosis of FV-PTC was as

Table 3. CK19 distribution in patients older than 45 years old

	CK19		CK 19 intensity				P value
	Negative	Positive	0	1+	2+	3+	
FA	6	2	6	2	0	0	
WT-UMP	1	6	1	5	0	1	<0.001
FV-PTC	2	24	2	3	10	11	

FA: follicular adenoma, WT-UMP: well differentiated tumor with undetermined malignant potential, FV-PTC: follicular variant of papillary thyroid carcinomas.

follows: sensitivity, 84.8%; specificity, 47.5%; positive predictive value (PPV), 86.7%; negative predictive value (NPV), 69.5%. Additionally, about 81% of cases with strong positivity (3+) for CK19 were FV-PTC and 19% were WDT-UMP. None of the FA showed strong (3+) positivity for CK19 ($p<0.001$), (Figs 2-5). Among patients older than 45 years old, more than 92% of FV-PTC cases were positive for Ck19 comparing to 25% of FA ($p<0.001$). In patients older than 45 years old, more than 90% of strongly positive cases and more than 96% of cases with 2+ and 3+ positivity were FV-PTC patients ($p<0.001$)(Table 3). No association was found between tumor size or gender and CK 19 expression.

DISCUSSION

Among thyroid neoplasms those with follicular feature raise the most controversies which is due to lack of universally accepted criteria for discriminating these lesions. To overcome this defect, many studies have tried to find additional markers beside present routine histopathological findings. Cytokeratins (CK) are one of the markers widely used for this purpose. Although some studies reported encouraging results but some others were equivocal and there are still some controversies.

Primarily, high molecular weight keratins (HMWK) were studied but the results were not encouraging (11-13). Studies on low molecular weight keratin (LMWK) showed that some of them including CK7 and CK8 are strongly expressed in normal thyroid tissue (14). Some studies on CK19 showed 100% expression rate in PTC although this result was questioned by others, but suggested that high expression level of CK19 in a thyroid nodule would increase risk of malignancy (15,16). Recently, it was reported that CK19 could be found in both PTC and FA, but the prevalence and intensity of staining were significantly different (17). They have reported that CK19 was diffusely expressed in about 70% of PTCs (80% in classic type PTC and 60% in FV-PTC) but only focally in about 3% of FAs (17). Our results are in line with these findings as we found strong expression of CK19 in more than 80% of FV-PTC and just weak expression in some (15%) of FAs. If we consider only 3+ staining pattern as positive result, none of FAs in our study were positive for CK19. Additionally, we found that in patients older than 45 years, more than 90% of cases with strong positivity were FV-PTCs which indicates the importance of CK19 in older patients.

Several molecules have been studied to find the best marker for distinguishing between thyroid benign and malignant lesions. TPO, HBME-1, Gal-3 and CK19 are mentioned as best markers in several studies (2, 18, 19) and CK19 is mentioned to have high specificity for PTC. Although in some studies no utility was found for CK19 in distinguishing between follicular carcinoma and FV-PTC but there are some others showing opposite results. Additionally some authors have considered CK19 just as a sensitive but not specific marker of PTC (20, 21). There are some studies which have evaluated the specificity, sensitivity, PPV and NPV of CK19 in discriminating PTC from benign lesions. For example, Liu *et al.* (22) found sensitivity of 96.3% and specificity of 40.4%. They have also reported PPV of 72.6% and NPV of 86.8%. There is a valuable study performed by Dunderovic *et al.* (23) in which they have reviewed most of the papers on this topic. They have reviewed 24 studies containing more than four thousand patients and have calculated the overall sensitivity, specificity, PPV and NPV for CK19 in discriminating PTC from benign thyroid lesions. They have reported 75.4% sensitivity, 70.9% specificity, 80% PPV and 65% NPV for CK19 in discriminating PTC from benign lesions. Although we reported lower specificity comparing to Dunderovic *et al.* study but found very close sensitivity, PPV and NPV. It is mentioned that some of these controversies might be due to different antibodies and IHC methods used in different studies (20). As a limitation we have studied just one IHC marker and combination of CK19 with other helpful markers could improve our diagnostic accuracy. But according to literature, combination of all valuable markers including CK19, HBME-1 and TPO have just slightly improved the diagnostic accuracy (2). Additionally, combination of marker other than CK19 such as Gal3 and HBME-1 failed to improve diagnostic specificity and even decreased the specificity (2, 18, 19, 21). So, we decided to just evaluate CK19 to find out if this marker alone could distinguish between FV-PTC, FA and WDT-UMP through which we can distinguish these lesions easier with less expense than using multiple IHC markers.

In conclusion, CK19 is a useful marker in differentiating FA from FV-PTC. We found CK19 expression in FA, WDT-UMP and FV-PTC with different intensities. Diffuse and strong (3+) staining pattern was found in FV-PTC but none of FAs were so. Additionally, more than 90% of strongly positive cases in patients older than 45 year old were FV-PTC. So, we concluded that diffuse and strong staining for CK19 in a thyroid lesion with follicular pattern of growth should highly raise the possibility of malignancy and should

be examined precisely and reported with caution, especially if the lesion is in a patient older than 45 years old. As far as 15% of our FV-PTC cases showed no or weak CK19 expression, it should be considered that a negative staining result in a suspicious nodule cannot rule out FV-PTC.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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