ORIGINAL ARTICLE

Absent CD56 expression in papillary thyroid carcinoma: A finding of potential diagnostic value in problematic cases of thyroid pathology

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Background: There are inter-observer disagreements between papillary thyroid carcinoma (PTC) with other follicular lesions of thyroid in aspect of diagnosis. CD56 is present on follicular epithelial cells of the normal thyroid. We evaluated the diagnostic value of CD56 expression in PTC, follicular thyroid lesions, and follicular thyroid neoplasms. **Materials and Methods:** Seventy-three cases diagnosed as follicular lesions and 73 cases diagnosed as PTC were stained with CD56 marker. A positive membranous immunostaining in more than 10% of the neoplastic cells qualified the case as "positive (+)" for CD56. **Results:** CD56 expression was seen in 70 samples of non-papillary carcinoma lesion (95.8%) versus one case of PTC (1.3%) ($P < 0.001$, Chi-square). Therefore, CD56 was 98.6% sensitive and 95.8% specific in distinguishing PTC from other follicular thyroid lesions. **Conclusion:** CD56 is both a sensitive and specific marker for differentiating PTC from other follicular lesions of thyroid singly but it may be better to use a combination of markers for clinical evaluation of patients.

Key words: CD56, papillary thyroid carcinoma, thyroid neoplasms, thyroid nodule

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INTRODUCTION

Thyroid nodules are very common (about 5% of the general population) and are usually discovered during routine medical care. With the emergence of ultrasound, impalpable thyroid nodules can be detected in 20-67% of the general population.[1,2] Thyroid cancer represents about 5-24% of thyroid nodules and ~1-2% of all malignancies.[3] Papillary thyroid carcinoma (PTC) constitutes about 80% of all thyroid malignancies.[4]

The diagnosis of papillary carcinoma is based on nuclear morphology of a thyroid neoplasm. Chan and Saw[5] described the grooved nucleus with conventional hematoxylin and eosin (H & E) stained sections and considered it a useful diagnostic criterion for papillary carcinoma. The appreciation of the nuclear features of PTC is crucially dependent on the tissue processing (fixative, duration of fixation, and thickness of sections). Morphologic similarities between benign and malignant lesions are frequent; papillary and follicular architectures and nuclear irregularity may be seen both in benign and malignant lesions.[6-8] Moreover, severe chronic lymphocytic thyroiditis, Hashimoto's thyroiditis, and reactive atypia attributed to inflammation result in nuclear morphology similar to that of papillary carcinoma, with nuclear enlargement, chromatin clearing, and even grooves.[9,10]

In contrast, follicular variant of PTC and other variants may cause, if the nuclear features of PTC are insufficiently appreciated, severe problems in differentiation from follicular thyroid carcinoma (FTC), follicular adenoma (FA), or even multi-nodular goiter.[11] Therefore, the diagnosis of noninvasive, encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) versus follicular adenoma is prone to considerable inter-observer variability.[5,12] In over 696 cases of thyroid nodules, Nordic pathologists showed an agreement of 58%; the remaining 42% indicated a huge problem in dealing with thyroid nodules.[13]

Ancillary tests can help to reach an accurate diagnosis. Several immunohistochemical markers are of some value,[7,14,15] but there are limitations, as evidenced by the lack of specificity of several markers discussed. CD56 is present on follicular epithelial cells of the normal thyroid and it shows diffusely membranous staining.[16,17] Low or absent expression of CD56 was noted in PTC using immunohistochemistry and PCR.[18] We evaluated the diagnostic value of protein expression using antibodies against CD56 in papillary thyroid carcinoma, follicular thyroid lesions, and

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follicular thyroid neoplasms. Our aim was to study the applicability of difference in CD56 expression as a marker that distinguishes PTC, (including its variants) from other follicular thyroid lesions and neoplasms, provided that the right morphological and clinical features are fulfilled. Although, CD56 expression have been evaluated in a previous study[19] with a good sensitivity and specificity in diagnosing of PTC, but it was limited and variants of PTC were not denoted as they recommended performing a larger study to confirm this marker. Therefore, we evaluated CD56 expression in a more extensive study, with emphasis on follicular variant of PTC, recommending it for using in problematic papillary thyroid carcinoma cases.

MATERIALS AND METHODS

This study is a cross-sectional research with backward direction. Purpose of this study is to determine sensitivity and specificity of CD56 expression in papillary thyroid carcinoma.

The study is performed at Al-Zahra Hospital Pathological Lab, Isfahan, Iran. The specimen obtained from Pathology Department of Al-Zahra and Kashani hospitals. The sampling was performed by simple method and involved all cases that met the inclusion criteria, which included 73 papillary thyroid carcinoma and 73 follicular thyroid neoplasm and lesions, were surgically removed by thyroidectomy, between 2009 and 2012, fixed in formalin and embedded in paraffin (permanent specimens).

Cases were excluded if they had been previously operated upon (recurrent lesions) or were metastatic lesions to distant sites but not lymph nodes. Paraffin blocks sections (5 μm) were stained using H & E. Specimens were reviewed by two pathologists, whose diagnostic consensus was necessary for inclusion of the cases.

For the diagnosis of PTC, we followed the histological criteria proposed by Chan.[5] Follicular thyroid neoplasms include follicular and Hürthle cell adenoma and carcinoma. They are diagnosed according to the stringent criteria proposed by LiVolsi and Baloch.[20] Thyroid follicular lesions include: Autoimmune thyroid disease, chronic lymphocytic thyroiditis, and nodular goiters involving the follicular cells.

The demographic and histopathological characteristics of the specimens were retrieved from the formal pathology report and verified by two pathologists. These included age, gender, the histological classification, encapsulated or infiltrated, capsular and/or vascular invasion, and pattern of growth.

Immunohistochemical analysis

Immunohistochemistry was performed on 4-μ-thick sections that had the same diagnosis using the labeled streptavidin-biotin peroxidase complex system. Heat-induced antigen retrieval was carried out for CD56 (Dako's Target Retrieval solution, pH 6.1, steaming for 30 min at 94°C), and sections were incubated at room temperature with primary antibodies for 30-60 min. After primary antibodies, all sections were blocked for endogenous avidin and biotin by incubating with avidin solution for 20 min, followed by biotin solution for 20 min (Dako's avidin/biotin blocking system, X0590). Normal thyroid parenchyma was used as positive controls for CD56. Appropriate negative controls through substituting primary antibody with isotype-matched mouse or rabbit IgG were also included.

Assessment of immunostaining

Evaluation of the immunohistochemical staining was performed by light microscopy using a 10 × objective lens with the selective use of a 20-40 × objective lens for confirmation. A positive membranous immunostaining in 10% or more of the neoplastic cells qualified the case as "positive (+)" for CD56. The stained sections were reviewed by two independent observers and a consensus regarding controversial cases was reached with the aid of a double-headed microscope.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 16 software was used to determine any association between data with chi-square. Results were considered to be statistically significant, if the *P*-value for the null hypothesis was <0.05. Sensitivity, and specificity of CD56 were assessed by comparing CD56 results with diagnostic "Gold standard" of PTC that is H&E staining.

RESULTS

The mean age of the patients was 42.4 ± 17.0 (8-85) years, and female to male ratio of the patients was 2.9:1. There was no significant difference in age and gender between PTC and non-PTC groups ($P = 0.475$ and $P = 0.087$, respectively). Among the total of 146 patients that were included in this study, 73 cases were diagnosed as follicular lesions (38 follicular adenoma, five follicular carcinoma, 13 Hurthle cell adenoma, three Hurthle cell carcinoma, 12 multinodular goiter, and two Hashimoto's thyroiditis) and 73 cases were diagnosed as PTC. The patients' characteristics in two groups of PTC and non-PTC lesions are summarized in Table 1. In PTC cases, capsular invasion, multicentricity, and regional lymph node metastasis were seen in nine (12.3%), 30 (41.1%), and 24 (32.9%) of the cases, respectively. The average size of papillary cell carcinoma tumor was 2.9 ± 2.1 cm.

Expression of CD56 in non-papillary carcinoma lesions was membranous in 70 cases (95.8%), and cytoplasmic in three cases, consisting of two cases of follicular adenoma, and one case of Hurthle cell adenoma. In all cases, CD56 expression was found in >10% of the cells [Figure 1]. Of course, the percent of CD56 expression in cells in 5 cases of follicular carcinoma and 3 cases of hurthle cell carcinoma, was lower than other non-PTC lesion (about 10-50% vs. >50%). Evaluation of CD56 staining in the 73 PTC cases showed negative CD56 expression in 49 cases (65.7%). In 20 (27.3%) cases, CD56 expression was cytoplasmic (not membramous), and we considered them negative. In four cases (two follicular variant, one oncocytic variant, and one tall cell variant) CD56 expression was membranous. On the other hand, distribution of CD56 expression only in four cases was more than 10% of the tumoral cells, and only in one of them CD56 expression was membranous. Therefore, we considered only one case of PTC, positive (according to the inclusion criteria), which was a follicular variant of papillary carcinoma. Table 2 was summarized CD56 expression in these two groups. Biostatistic analysis on the data, showed a significant difference between two groups in CD56 expression ($P < 0.001$, Pearson Chi-Square test).

According to the distribution of CD56 expression in two groups, CD56 was 98.6% sensitive and 95.8% specific in distinguishing PTC from other follicular thyroid lesions.

DISCUSSION

CD56 or neural cell adhesion molecule (NCAM) is a homophilic membrane glycoprotein. It is an adhesion molecule from the immunoglobulin (Ig) superfamily that is expressed normally on the surface of neurons, glia, skeletal muscle cells, and natural killer cells.[21,22] CD56 expression (both protein and mRNA) is also confirmed in thyroid follicular epithelial cells and adrenal glands.[17,23] It is membranously stained in follicular epithelial cells.

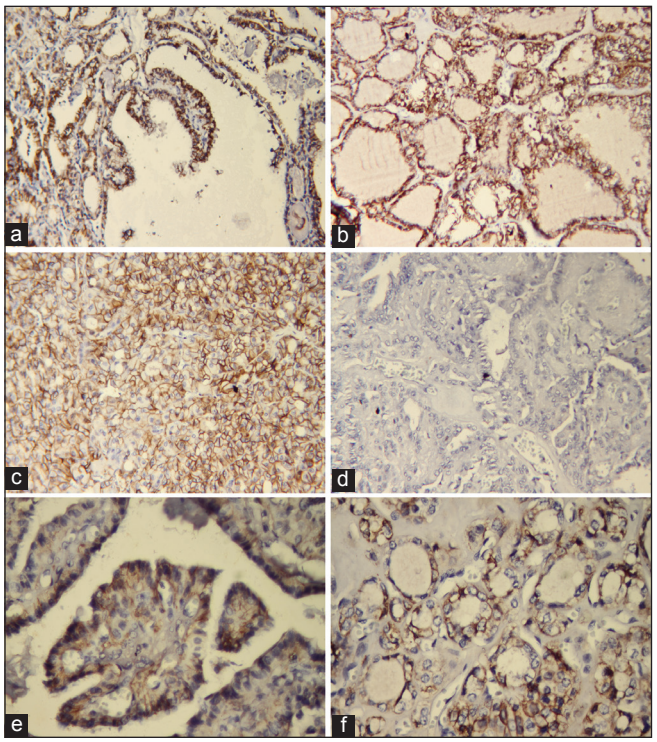

Figure 1: CD56 expression in thyroid lesions. (a) Strongly membranous expression in a nodular goiter with papillary structure (b) in a follicular adenoma, and (c) in a Hurthle cell adenoma (d) Papillary carcinoma showing negativity for CD56 (e) Focally cytoplasmic expression CD56 in papillary thyroid carcinoma (f) Focally membranous expression CD56 in a follicular variant of papillary thyroid carcinoma

Table 1: The patients' characteristics in two groups of papillary thyroid carcinoma and other follicular thyroid lesions

	Total (*n*=143)	Papillary thyroid carcinoma (*n*=73)	Non-Papillary thyroid carcinoma lesion (*n*=73)	
Age [mean±(SD)]	42.42±(17.07)	43.44±(16.93)	41.41±(17.25)	*P*=0.475
Female:male ratio	2.9:1	4.2:1	2.1:1	*P*=0.087
Subgroup diagnosis		Conventional: 49 (67.1%)	Follicular adenoma: 38 (52.0%)	
		Follicular variant: 14 (19.1%)	Follicular carcinoma: 5 (6.8%)	
		Micropapillary: 7 (9.5%)	Hurthle cell adenoma: 13 (17.8%)	
		Tall cell variant: 2 (2.7%)	Hurthle cell carcinoma: 3 (4.1%)	
		Oncocytic variant: 1 (1.3%)	Nodular goiter: 12 (16.4%)	
			Hashimoto's thyroiditis: 2 (2.7%)	

Table 2: The results of immunohistochemical staining for CD56 in various thyroid lesions

CD56	Papillary cell carcinoma (*n*=73)			Non-Papillary cell carcinoma lesions (*n*=73)		
	Distribution			Distribution		
	0%	1-10%	>10%	0%	1-10%	>10%
Intensity						
Negative	49 (67.1%)	0	0	0	0	0
Weakly cytoplasmic	0	17 (23.2%)	3 (4.1%)	0	0	3 (4.1%)
Membranous	0	3 (4.1%)	1 (1.3%)	0	0	70 (95.8%)
CD56 expression-(Final)		1 (1.3%)			70 (95.8%)	

Reduction of its expression was previously reported in papillary cell carcinoma of thyroid (PTC).[6,18,19,24,25] In this study, we showed a significant reduction of CD56 expression in PTC cases including FVPTC. It is important to remember that CD56 can be expressed in some of the tumor cells in PTC. These cells only stained weakly cytoplasmically (not membranously), and less than 10% of tumor cells were positive for this marker. However, we had cases of PTC that more than 10 percent of cells stained. Although all positive conventional PTC cells were stained weakly cytoplasmically, one case of FVPTC was stained membranously. Etem *et al*.,[26] in contrast to our results, reported that there was no significant differentiation regarding CD56 staining in follicular tumor and PTC but they accepted both cytoplasmic and membranous staining for CD56 and with any percent of distribution of tumor cells as positive.

In a study by El Demellawy *et al*.,[19] both sensitivity and specificity of 100% for CD56 expression was shown in distinguishing PTC from other thyroid follicular lesions. Ozolins *et al*.,[27] showed that CD56 expression could be negative in follicular adenoma. They showed sensitivity of 100% but specificity of 82% for CD56 in PTC diagnosis. Park *et al*.,[25] also showed sensitivity and specificity of 92.5% and 86.4%, respectively. Results of our study did not replicate sensitivity or specificity of 100%, yet it confirmed that CD56 is a single marker with high sensitivity and specificity. Abd El Atti *et al*.,[28] in their study used combined CD56 and claudin-1 expression in the differentiation between the FVPCs and other follicular nodules. The combined use of CD56 and claudin-1 (claudin-1+/CD56-) showed specificity (100%), positive predictive value (100%) and sensitivity (81.3%) in this differentiation. But, in single use of CD56, sensitivity and specificity were 81.3% and 89.4%, respectively. Lee *et al*.,[29] in a recent study on thyroid tumors with a prominent hyalinizing trabecular pattern showed, CD56 expression was negative in six out of 16 cases. They showed expression of CK19 or Galectin-3 (two markers for distinguishing of PTC from other thyroid lesions) in four out of six cases with negative CD56 expression and decided to categorize them as PTC. Whereas, in several studies including ours, CD56 sensitivity and specificity were not 100%, it may be better to use a combination of markers for clinical evaluation of patients.

The previous studies showed that changes in CD56 expression may affect the migratory potential of tumor cells.[30] A poor prognosis in colonic and pancreatic carcinomas and astrocytoma was associated with loss of CD56 expression.[31,32] Cavallaro *et al*.,[33] showed that CD56 stimulates β1-integrin-mediated cell-matrix adhesion by activating fibroblast growth factor receptor (FGFR) signaling; therefore, its low expression can increase the probability of metastasis through stimulation of lymphangiogenesis. Moreover, Scarpino *et al*.,[18] reported that modifications of CD56 expression in PTC can cause down-regulation of vascular endothelial growth factor D (VEGF-D) and VEGF-C, factors that stimulate lymphangiogenesis. Kim *et al*.,[34] in another study, showed homeobox B9 (a transcription factor) regulates transcription of CD56 in PTC. Furthermore, the silencing of HoxB9 nuclear expression was correlated with tumor stage and extrathyroidal extension, whereas the loss of CD56 expression was not correlated with clinicopathologic features. All these findings are evidences that explain why papillary thyroid carcinoma characteristically metastasizes to regional lymph nodes, whereas follicular thyroid carcinoma commonly spreads hematogenously.[35] In this study, because only one case of PTC showed CD56 expression (according to inclusion criteria) comparison of this effect on tumor extension was impossible.

In conclusion, CD56 is both a sensitive and a specific marker for differentiating PTC from all other follicular lesions of the thyroid, individually but it may be better to use a combination of markers for clinical evaluation of patients. We recommend further studies to explore the potential role of CD56 in tumor progression in PTC.

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