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Diagnostic Values of CD56 Marker in Differentiating Papillary Thyroid Carcinoma from Benign Follicular Lesions

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Keywords:

All procedures have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was signed prior to participation in the study.

Introduction:

Thyroid cancer is the most common endocrine malignancy that is increasing thorough the last years (Jemal *et al.*, 2011; Sipos & Mazzaferri, 2010). It has been shown that the annual incidence increment of thyroid cancer between the years 2000 to 2009 is 6%, which is the highest among all types of cancers (Sipos & Mazzaferri, 2010). Although the mortality rate of this cancer is low, the rate of relapse is high, which increases the treatment failure rate and mortality (Tuttle *et al.*, 2010; DeLellis, 2010). According to the Iranian Cancer Institute, the prevalence of thyroid cancer is 1.8%. The mean age of patients is 43 years and the ratio of women to men is 1.8. Thyroid cancer in Iran is the seventh most common cancer in women and 14th in men and the eleventh most common cancer in both genders (Ghamari, 2019). Various types and subtypes of thyroid cancers vary in histological characteristics, cellular origin, and prognosis. There are two types of thyroid cells (follicular thyroid cells and Para follicular cells), thyroid cancers derived from them. Most thyroid cancers are derived from follicular thyroid cells, which include papillary thyroid cancer (PTC), follicular thyroid cancer, low differentiation thyroid cancer,

Abstract

Thyroid cancer is the most common malignancy in the endocrine system. Occasionally, the distinguishing of malignant tumors from benign lesions may be difficult through histological evaluation. Nowadays, Ancillary assays such as immunohistochemistry are used for this purpose. The present study was conducted to evaluate the CD56 diagnostic value in distinguishing benign cases from malignant thyroid tumors. The paraffin blocks of patients with a pathologic diagnosis of benign lesions and papillary thyroid carcinoma were included. The findings from the histological evaluation were compared to the CD56 immunohistochemistry results. Anti-CD56 monoclonal antibodies were used for immunohisto-chemically staining. Less than 10% staining was considered negative. CD56 marker was positive in 20% of patients with thyroid malignancy. The marker positivity in the benign lesions was significantly higher than those with thyroid malignancy i.e., the 100% of patients with benign thyroid lesions showed a high expression of CD56. The Sensitivity and specificity of CD56 marker in distinguishing non-malignant from malignant lesions were 100% and 80%, respectively.

and anaplastic thyroid cancer. Follicular thyroid cancer and papillary thyroid cancer are classified as well-differentiated thyroid cancers. Medullary thyroid carcinoma derived from Para follicular cells accounts for a small percentage of thyroid cancers (Sipos & Mazzaferri, 2010). The prognosis of thyroid malignancies is related to histology, gender, age, degree of involvement and race (Lukas *et al.*, 2012; Xing *et al.*, 2005) The differentiation of benign follicular lesions from malignant tumors is very important in choosing an appropriate therapeutic approach to disease (Mazzaferri & Kloos, 2012). Diagnosis of follicular cells derived thyroid tumors and differentiating them from other lesions, especially follicular variants of papillary thyroid carcinoma is a challenging topic in the pathology field. Even though PTC diagnostic criteria have been established more than 50 years ago, there is still a controversy among skilled pathologists (Rosai, 1971). Although the golden standard method to diagnosis all types of thyroid nodules is Hematoxylin and eosin (H&E), due to the morphological overlap between follicular and neoplastic lesions, accurately diagnosis is difficult in some cases (Lloyd, 2004). Hence, differentiation of the benign thyroid lesions Malignant tumors may be difficult even for skilled pathologists, and

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repeatability of diagnosis is not acceptable between pathologists. Therefore, finding objective markers for the diagnosis of malignant thyroid tumors, especially the differentiation of benign lesions from malignant tumors, is very important, since inaccurate diagnosis of thyroid carcinoma can lead to complete thyroidectomy and lifelong needing to treatment (Gaffney, 2003). Ancillary studies, including Immunohisto-chemistry, may be helpful in these cases. Markers that have been proposed to facilitate the diagnosis of thyroid cancers include Cytokeratin 19, Galectin-3, CD56, CD44, and nm23 (El Demellawy, 2009).

Cd56 is a neural cell adhesion molecule, and therefore the expression of this molecule affects the tumor cells' ability to migrate. Studies have shown that loss of CD56 markers associated with metastases and poor prognosis in some cancers. The CD56 is already expressed as normal follicular epithelial thyroid cells (Zeromski, 1998). The absence or reduction of CD56 expression has been observed in PTC immunohistochemistry (Scarpino, 2007). In the current study, we have aimed to evaluate the diagnostic value of CD56 expression in the differentiation of PTC from benign follicular thyroid lesions.

Methodology:

Study design: this case-control study was carried out on patients suspicious to thyroid malignancy referred to Imam Khomeini Hospital. The case group included malignant thyroid tumors and the control group consisted of patients with benign thyroid lesions. Demographic data included age and sex were extracted from the patient files.

Histological evaluation: samples from thyroidectomy and neck lymph nodes were cut and macroscopic characteristics, especially tumor size, were determined. Two millimeters slices were prepared from tumor and neck lymph nodes. The tissue processing was done automatically by a tissue processing machine. three-micron sections were prepared from paraffin blocks. The tumor type and metastasis were diagnosed by H&E staining.

Immunohistochemically assessment: 3-micron slide was prepared for immunohistochemically staining. First, the slide was placed at 60°C for one hour. After paraffin removal, the slides were placed in three Xylenol dishes for 10 minutes and then in three alcohol containers at concentrations of 100, 96 and 70% (each container for five minutes). Then, H₂O₂ 3% was applied on slides for 10 minutes and rinsed with water. In the next step, the citrate buffer (pH:6) applied on the slides and autoclaved in 126°C and 15 atmospheres for one minute. After washing the slides with a TBS buffer, the peroxidase block solution was applied for 5 min. In the next step, the slides were covered with an anti-CD56 antibody for one hour. Then, the slides were stained with hematoxylin for 1 min.

Statistical analysis: analyses were performed by IBM® SPSS® Statistics 19. For all data, descriptive statistics

including mean, standard deviation and frequency were calculated. Independent t-test or Mann-Whitney was used to compare the means. Comparison of the proportions was done by chi-square test. The p-value less than 0.05 was considered significant.

Results:

In this study, 30 patients with thyroid lesions were evaluated. The mean age of patients was 38.2 in the range of 21 to 62 years, where 80% of the patients were women. The most commonly diagnosed lesion was classical PTC with a frequency of 26.7% followed by nodular goiter (16.7%) and follicular PTC (table-1). The mean age of patients in different types of thyroid abnormalities did not show any significant difference ($p > 0.88$). CD56 marker was positive in 20% of patients with thyroid malignancy. However, the CD56 positivity had no significant correlation with the type of malignancy ($p > 0.7$) (Fig.-1 & 2). Also, the tumor stage did not correlate with the CD56 marker positivity ($p > 0.3$). However, the CD56 positive rate was significantly higher in patients with benign thyroid malignancies than those with thyroid malignancy, so that 100% of patients with benign thyroid lesions express CD56 broadly with High intensity ($p < 0.0001$) (table-2) (Fig.-2). The sensitivity and specificity of the CD56 marker in differentiating benign lesions from malignant tumors were 100% and 80%, respectively (Table-3). The multivariate regression analysis has shown that just the type of thyroid lesion is associated with the expression of the CD56 marker (table-4).

Discussion:

The neural cell adhesion molecule (NCAM), also known as CD56 is a hemophilic binding glycoprotein. CD56 is a member of the immunoglobulin superfamily expressed on the neuron, glial cell, skeletal muscle, and natural killer cells (Hoos *et al.*, 2002; Lanier *et al.*, 1989). Additionally, CD56 may be expressed on the thyroid follicular epithelial cells and adrenal glands (Zeromski *et al.*, 1998, 1999). On the other hand, a lack of CD56 expression on the thyroid papillary cells has also been reported by Zeromski *et al.* (1998; 1999); El Demellawy *et al.* (2009) and Scarpino *et al.* (2007). In the present study, we have compared the expression of CD56 in various types of thyroid lesions, including benign and malignant tumors.

Our results indicated that although in 100% of benign lesions CD56 brightly expressed, only 3 patients with malignant thyroid tumors expressed the CD56 marker. Our findings also showed that the sensitivity and specificity of the CD56 marker in distinguishing benign thyroid lesions from malignant tumors were 100% and 80%, respectively. These findings are in line with other previous studies. In a study by El Demellawy *et al.* (2009), it was shown that the sensitivity and specificity of CD56 in differentiating PTC from other follicular thyroid lesions is 100%. Shah Ebrahimi *et al.* (2013) also showed the sensitivity and specificity of

Table-1: Patients' primary medical record

Variables	Frequency	Percent
Age (Mean±S.D, Range)	38.2±12.7, 21-62	
Gender		
Male	6	20.0
Female	24	80.0
Diagnosis		
PTC Classic	8	26.7
PTC Follicular	4	13.3
PTC micropapillary carcinoma	3	10.0
Hashimoto's thyroiditis	3	10.0
Follicular adenoma	2	6.7
Graves disease	2	6.7
Nodular goiter	5	16.7
lymphocytic thyroiditis	3	10.0

Table-2: The association of CD56 marker positivity with thyroid lesion properties

Variables	(p-values)			
A	B	Pos	Neg	Total
Malignant lesions (X²,df=2, p>0.713)				
PTC Classic	Count	1	7	8
	Percent	12.5%	87.5%	100.0%
PTC Follicular	Count	1	3	4
	Percent	25.0%	75.0%	100.0%
PTC micropapillary carcinoma	Count	1	2	3
	Percent	33.3%	66.7%	100.0%
Malignancy (X²,df=2, p<0.0001)				
Malignant	Count	3	12	15
	Percent	20.0%	80.0%	100.0%
Non-malignant	Count	15	0	15
	Percent	100.0%	0.0%	100.0%
Tumor stage (x²=2.14, df=2, p>0.343)				
1a	Count	1	6	7
	Percent	14.3%	85.7%	100.0%
1b	Count	0	3	3
	Percent	0.0%	100.0%	100.0%
3	Count	2	3	5
	Percent	40.0%	60.0%	100.0%

Table-3: Diagnostic accuracy of CD56 marker in distinguishing non-malignant thyroid lesions.

● Sensitivity	1.000
● 95% confidence interval	0.7820 to 1.000
● Specificity	0.8000
● 95% confidence interval	0.5191 to 0.9567
● Positive Predictive Value	0.8333
● 95% confidence interval	0.5858 to 0.9642
● Negative Predictive Value	1.000
● 95% confidence interval	0.7354 to 1.000

Table-4: Multivariate regression analysis

Ind. var.	Coefficient	S.E.	t	P	rp	rsp
(Constant)	2/2642					
Age	-0/007540	0/006732	-1/120	0/2729	-0/2146	0/1793
Diagnosis	-0/1058	0/03193	-3/314	0/0027	-0/5449	0/5305
Gender	-0/08857	0/2148	-0/412	0/6835	-	
0/08060	0/06600					
Ind. var.- Independent variable, rp-rpartial, rsp-rsemipartial

Figure-1: Lack of CD56 expression in classic Ptc

Figure-2: CD56 marker expressed on follicular adenoma on 100% of cells with 4+ intensity

CD56 marker for diagnosis non-PTC lesions are 94.8% and 92.3%, respectively. In another study, Migita *et al.* (1991) compared the expression of CD56 at the molecular level in Graves and PTC lesions and showed that none of the PTC cases express CD56 markers. In a study conducted by Scarpino *et al.* (2007) the lack of CD56 expression on the PTC cells suggests its diagnostic value in distinguishing PTC from other thyroid lesions including follicular variants.

In the present study, 1 of the classic PTC patients, one follicular PTC samples and one micro papillary PTC were positive against anti-CD56. Similarly, Mokhtari *et al.* (2013) in a similar study have shown that the CD56 could be expressed in 1.3% of PTC cases. While in contrary to our findings, 4.2% of non-PTC cases were negative for CD56. Moreover, Erdogan-Durmus *et al.* (2016) showed that 95.7% (not 100%) of PTC cases exhibited no CD56 expression.

Conclusion:

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The findings of this study in the complement of previous studies suggested the CD56 marker as a good diagnostic marker in distinguishing benign cases from malignant thyroid cancers. Our findings also indicate that CD56 expression is not affected by the disease stage, age, and gender. However, our findings could not estimate the prognostic value of CD 56 marker, suggested that future studies evaluate the effect of CD56 on the disease outcome.

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