

Original article

The Role of CD56 in distinction of PTC from other thyroid neoplasms

Omid Emadian Saravi¹, Zhila Torabizadeh², Saeed Amirkhani³

1- Associate Professor of Pathology, Pathology Department of Imam Khomeini hospital, Sari, Iran.

2- Orthopedic Research center, Mazandaran University of Medical Sciences, Sari, Iran.

2- Associate Professor of pathology, Gut and liver Research Center, Pathology Department of Imam Khomeini hospital, Sari Faculty of Medicine, Mazandaran University of medical Sciences.

3- Resident of Pathology, Pathology Department of Imam Khomeini hospital, Sari Faculty of Medicine, Mazandaran University of medical Science.

Corresponding author: Dr Zhila Torabizadeh

Email: zhtorabi@yahoo.com

Abstract

Objective: Thyroid nodules which are common in population consist of mostly benign nodules and less malignant neoplasms. Differentiating malignant nodules from each other arises a drastic problem in making a correct diagnosis. Thus, this study was done to investigate the role of CD56 and its specificity and sensitivity in different types of thyroid neoplasms especially papillary carcinoma.

Methods: 73 paraffin embedded-blocks of thyroid masses (nodules) were studied for CD56 immunohistologically from Imam Khomeini hospital, Sari, Mazandaran. The 4-scaled semiquantitative method (0-3) was applied to estimate CD56 expression in tumor cells; less than 10% (0) or negative staining, 10-33% (1), 33-66% (2), more than 66% (3).

Results: In this study 24.7% were male and 75.3% were female. 13.7%, 68.5% and 17.8% of patients were less than 20, between 20- 50, and over 50 years old respectively. Simultaneously 50 (68.5%) patients had both right and left lobes nodules. 12 (16.4%), 6 (8.2%) and 1 (1.4%) patients had nodules in right and left lobes and isthmus respectively. Consequently, 4 (5.5%) patients showed left and right lobes, and isthmus nodules simultaneously. There was no statistically significant difference between the sexes and ages and also anatomical regions in patients with and without PTC. 30 out of the 73 patients had papillary carcinoma (PTC) which 4 out of them expressed CD56 while 37 out of 43 (86%) patients who had non-PTC showed positive results for CD56. Sensitivity and specificity of negative result of CD56, positive predictive value (PPV) and negative predictive value (NPV) in the diagnosis of papillary carcinoma were 86.6%, 86%, 81.2% and 86.3% respectively.

Conclusion: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy (OA) of negative result of CD56 in the diagnosis of PTC were 86.6%, 86.04%, 81.25%, 90.24% and 86.3% respectively. This study showed that CD56 was a valuable sensitive and specific marker in differentiating PTC from other thyroid tumors.

Keywords: Papillary thyroid carcinoma, Other thyroid masses, CD56

Introduction

Palpable nodules in the thyroid are found in approximately 5% of the general population. By using ultrasound, these nodules are detected in 20-67% of the general population (1, 2) such nodules are mostly benign and 5-24% of thyroid nodules are malignant which totally account for 1-2% of the malignancies. (3) Malignancy originated from thyroid follicular epithelium is the most common malignancy of the endocrine system in which hormonal and environmental factors play a role (4) Papillary thyroid cancer (PTC) include above 80% of all thyroid cancers, which based on the

histopathological view including nuclear clearing, overlapping, grooves and pseudo-inclusion (5) 13 microscopic subtypes of papillary carcinoma has been detected so far and the most common subtype is the follicular variant with distant metastases, such as to the lungs and bones with multicentricity, vascular invasion and spread of nodular pattern of spreading. (4, 6)

The differential diagnosis of thyroid nodules can be difficult because of morphologic overlappings, So that in the absence of follicular structures, accurate distinction of adenomatous nodules with follicular

variants can be challenging (5) in addition , because of histologic differentiations of neoplastic tissue, using imaging techniques such as ultrasound cannot be helpful . As a result, it has been reported that up to 85% of thyroid nodules with suspicious cytology which were under surgery are benign lesions (7, 8) That is why in recent years several studies have been conducted with special immunohistochemical methods and molecular techniques .among these molecules we can mention CD56 . CD56 is expressed as a neural cell adhesion molecule (NCAM) CD56 is normally expressed in the thyroid gland and it has been found that it can affect the invasive features of the tumor (9), the absence of this molecule in papillary carcinoma is reported in various studies (10-15) while it is reported as d positive in most cases of papillary carcinoma (13, 16, 17) therefore probably it can be used as a sensitive and specific marker for differentiation of PTC from other neoplasms. In the study of Mikyung et al in South Korea, CD56 had sensitivity and specificity 95% and 72/7% respectively for detection of PTC (18) also in the study of Dina et al CD56 is reported as a sensitive marker for the diagnosis of PTC. (13) Although in a study in Cyprus conducted by Hylya there was no difference in the expression of this molecules in papillary and follicular neoplasms. (19) since the importance of distinction between thyroid neoplasms, and due to conflicting reports about molecular markers, this study was applied on paraffin blocks of thyroid tumors in histology achieve of pathology department of Hospital Khomeini in order to investigate the role of CD56 and as and its specificity and sensitivity differentiating between thyroid tumors .

Method

This was an analytic study which was designed to determine the role of CD56 in differentiation of thyroid neoplasms and was applied on the thyroid tissue samples archived in the pathology department of Imam Khomeini hospital from 2001 to 2012. Our estimation of sample size was 73 due to previous studies (13, 15, 17, 18) The paraffin blocks stained with hematoxylin and eosin (H & E) relating to thyroid neoplasms were derived from the archive. samples were reviewed verified by two pathologists independently. Diagnosis, type and grade of thyroid pathological samples was performed by an expert and experienced pathologist, according to the World Health Organization WHO classification of tumors. (20) The paraffin blocks were evaluated by Immunohistochemical methods to detect CD56 . Immunohistochemistry was performed on samples with diameter of 4µm using by standard techniques (streptavidin-biotin-peroxidase technique) with

regard to the appropriate positive and negative controls using primary CD56 antibodies. Multiple microscopic fields of the were analyzed in terms of staining and the results were reported as semi-quantitative estimation of the percentage of tumor cells stain absorption. Internal positive control presence of NK cell was considered as internal positive control for the CD56 and presence of endothelial cells in tissue were considered as negative control, respectively. Staining less than 10% was considered as zero (0) . 10-33% 1 , 33-66% 2 and more than 66% of) was considered 3. Zero was considered as negative staining and 1 to 3 were considered positive results. The data was recorded in the statistical software SPSS 18.0 for Windows. parametric data was reported as mean \pm SD and non-parametric data was described descriptively . t student test (for quantitative data) and Chi square and fisher exact test (for qualitative data) were used to compare the obtained data in each group. The sensitivity and specificity and positive and negative predictive value of CD56 expression was also reported using the ROC curve. P value less than 0.05 was considered statistically significant.

Results

The number of participants in our study was 73 patients, including 18 male (% 24/7) and 55 female (% 75/3). 10 patients (13/7%) were under 20 years, 50 patients (% 68/5) and between 20 to year and 50 13 patients (17/8%) were above 50 year (Figure 1).

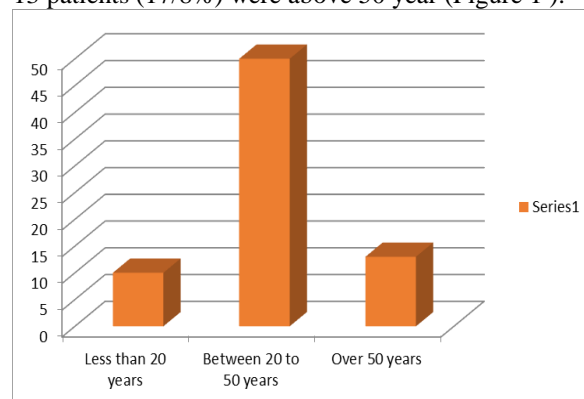


Figure 1: The frequency of patients according to age

anatomic locations of thyroid nodules , in order of frequency, were right and left lobe simultaneously in 50 patients (%68/5), right lobe in 12 (%16/4), left lobe in 6 patients (8/2%), simultaneous right lobe, left lobe and isthmus in 4 patients (5% / 5) and involvement of isthmus in one patient (1/4) (Figure 2).

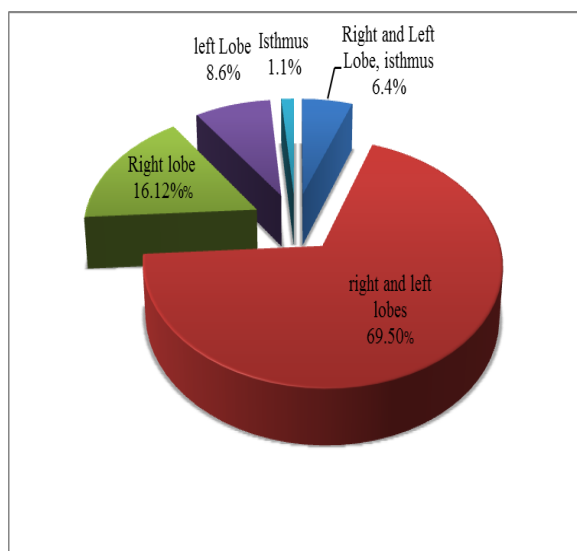


Figure 2 : The frequency of thyroid nodules

The pathological examination of the nodules showed that 30 patients (41/1%) had PTC. Of these 30 patients with PTC, 4 patients were male (% 13/3) and 26 were female (% 86/7). There was no statistically significant difference between genders in terms of PTC development ($P = 0.061$). Of these 30 patients, 7 patients (23/3%) were under 20 years, 19 patients (% 63/3%) between 20 to 50 years and 4 patients (% 13/3) were above 50 year. There was no statistically significant difference between the age of affected patients ($P = 0.12$). Other than PTC, 8 cases (19/04%) were Hashimoto, 17 follicular adenomas (40/47%), 11 cases nodular goiter (% 26/19%) 1 colloid goiters (2/38%), 2 medullary carcinoma (% 4/76), 2 follicular carcinoma (4/76%) and 1 Hertell cell (2/38%)

PTC nodules in patients who were diagnosed in 20 patients (% 7/66) simultaneously on both the right and left lobes, in 4 patients (% 3/13) in the right lobe, in 3 patients (10%) in the left lobe in 2 patients (% 7/6) at the same time in the right lobe, left isthmus and in one patient (% 3/3) at the isthmus there. Significant differences between patients with and without a diagnosis of PTC anatomical site, there was no mass ($P = 0.72$).

Nodules in 20 patients of PTC (% 66/7) were located simultaneously in both the right and left lobes, 4 patients (% 13/3) in the right lobe, 3 patients (10%) in the left lobe in 2 patients (% 6/7) simultaneously in the right lobe, left isthmus and in one patient (% 3/3) at the isthmus. No significant differences between patients with and without a diagnosis of PTC in terms of anatomical site was seen ($P = 0.72$).

The CD-56 study in the patients, 41 patients (% 56/2) were CD-56 positive and in 32 patients (% 43/8) CD-56 was negative. Of the 41 patients who were positive for CD-56, CD-56-positive level in 18 patients (% 43/9) was 2+ , in 14 patients (34/14%) +1, in 7 patients (17/07%) weakly positive and in 2 patients (4/87%) was 3+ (Figure 3).

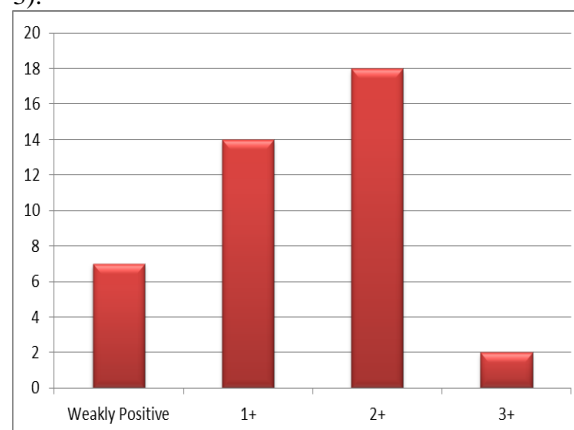


Figure 3: The frequency of CD56 positive

in the pathological evaluation of the patients in the study, CD-56 was positive in 4 patients of PTC (% 13/3), while it was negative in other 26 patients with PTC (% 86/7) (image 1 and image 2).

Furthermore , of 43 patients who were not diagnosed as PTC, CD-56 was positive in 37 patients (86%) and negative in 6 patients (14%). CD-56 positive rate was significantly lower in patients with PTC than other patients. Possibility of negative CD-56 with PTC was 40/8 folds higher than other patients (OR: 40.08, 95% CI: 10.27-156.32, $P < 0.0001$).

Due to the fact that among the 30 patients with PTC 26 patients and among 43 patients with other diagnoses 6 patients were negative for CD-56, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy (OA) of CD-56 negative markers in the diagnosis of PTC, is 86/6%, 86/04% , 81/25% , 90/24% and 86/3%, respectively.

Discussion

Thyroid cancer is the most common cancer of endocrine system and the seventh most common malignancy, and thyroid papillary carcinoma is the most common cancer of thyroid. In recent decades, a significant increase has occurred in papillary thyroid cancer incidence because of the improvement of medical care system. the pathological diagnosis of these cancers is easy in most cases . However, follicular variant of papillary thyroid carcinoma may be wrongly mistaken with follicular adenoma.

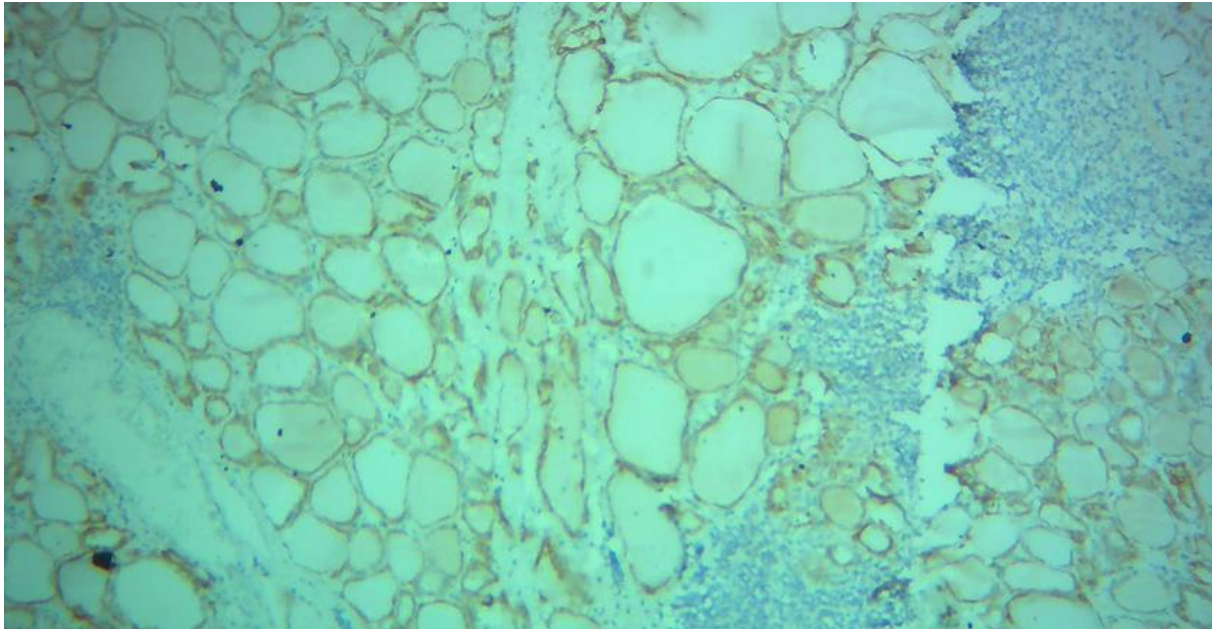


image 1: thyroid pathology specimens CD56- positive

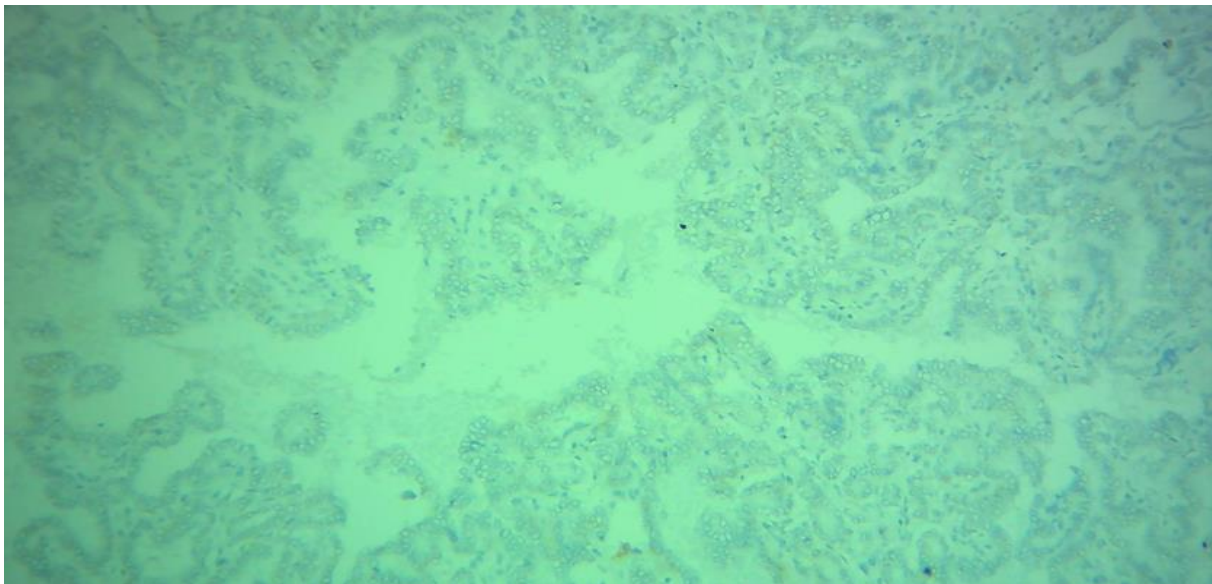


image2 : Thyroid pathology specimens CD56 negative

It may also be difficult to distinguish minimally invasive follicular adenomas and carcinomas. Differentiation of pseudo-papillary hyperplastic epithelial from true papillary cancer can be an important diagnostic problem. Immunohistochemistry recently is of high consideration due to its ability to differentiate benign and malignant thyroid lesions (21)

CD56 or neural cell adhesion molecule (NCAM) is a homogeneous glycoprotein. It is one member of a large family of adhesion molecules of immunoglobulins (Ig), which is normally found on the surface of cells, neurons, glia, skeletal muscle cells and natural killer cells (NK cell). (22) The

expression of CD56 in thyroid follicular epithelial cells and adrenal gland has been proved (9, 23, 24) In follicular epithelial cell it is stained in the membrane. reduction of its expression in papillary thyroid carcinoma (PTC) has been reported previously (10, 11, 13, 15) This study showed a decreased expression of CD56 in PTC. It should be kept in mind that CD56 is expressed in some neoplastic cells of PTC. The staining of such cells is cytoplasmic and not of membrane. Usually less than 10% of tumor cells are positive for this marker (25) in the study of Etem et al there was no significant difference in the expression of CD56 between PTC and follicular tumors. [19] However, in the study of El-DEMELLAWY et al, in

differentiating PTC from other follicular lesions was 100% (11) also in the study of Mokhtari et al this sensitivity and specificity was and 98/6%, 95/8%, respectively. (25)

Our study, as well as the other two recent studies, demonstrates the efficacy of CD56 in differentiating of PTC from other tumors (sensitivity and specificity, respectively, % 86/6 and 86/04%). This study showed that CD56 is a sensitive and specific marker for differentiation of PTC from other lesions.

Suggestions

It is suggested that CD56 as well as other markers be used in order to differentiation of the lesions for their higher levels of sensitivity and specificity and thus facilitating the diagnosis making. It is also recommended that in future studies be designed to evaluate the prognostic role of CD56 in PTC.

References

- Gharib H, Papini E. Thyroid nodules: clinical importance, assessment, and treatment. *Endocrinol Metab Clin North Am.* 2007;36 (3):707-35.
- Topliss D. Thyroid incidentaloma : the ignorant in pursuit of the impalpable. *Clin Endocrinol (Oxf).* 2004;60 (1):18-20.
- Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the US, 1985-1995. *J Cancer.* 1998;83 (12):2638-48.
- DeLellis R, Williams E. Thyroid and parathyroid tumors. *Pathology and Genetics of Tumours of Endocrine Organs IARC WHO Classification of Tumours* DeLellis RA, Lloyd RV, Heitz PU, and Eng C (eds) Lyons, France: IARC Press. 2004;12(2):51-6.
- Nasr MR, Mukhopadhyay S, Zhang S, Katzenstein A-LA. Immunohistochemical markers in diagnosis of papillary thyroid carcinoma: utility of HBME1 combined with CK19 immunostaining. *Mod Pathol.* 2006;19 (12):1631-7.
- Lloyd RV, Erickson LA, Casey MB, Lam KY, Lohse CM, Asa SL, et al. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *The American journal of surgical pathology.* 2004;28 (10):1336-40.
- Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED-1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor- γ , and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. *European Journal of Endocrinology.* 2008;158(3):375-84.
- Haugen BR, Woodmansee WW, McDermott MT. Towards improving the utility of fine-needle aspiration biopsy for the diagnosis of thyroid tumours. *Clin Endocrinol (Oxf).* 2002;56 (3):281-90.
- Zeromski J, Lawniczak M, Galbas K, Jenek R, Golusiński P. Expression of CD56/N-CAM antigen and some other adhesion molecules in various human endocrine glands. *Folia histochemica et cytobiologica/Polish Academy of Sciences, Polish Histochemical and Cytochemical Society.* 1997; 36 (3):119-25.
- Park WY, Jeong SM, Lee JH, Kang HJ, Sin DH, Choi KU, et al. Diagnostic value of decreased expression of CD56 protein in papillary carcinoma of the thyroid gland. *J Basic and Applied Pathology.* 2009;2 (2):63-8.
- El Demellawy D, Nasr AL, Babay S, Alowami S. Diagnostic utility of CD56 immunohistochemistry in papillary carcinoma of the thyroid. *J Pathology-Research and Practice.* 2009; 205(5):303-9.
- Migita K, Eguchi K, Kawakami A, Ida H, Fukuda T, Kurata A, et al. Detection of Leu-19 (CD56) antigen on human thyroid epithelial cells by an immunohistochemical method. *J Immunology.* 1991;72(2):246.
- El Demellawy D, Nasr A, Alowami S. Application of CD56, P63 and CK19 immunohistochemistry in the diagnosis of papillary carcinoma of the thyroid. *J Diagn Pathol.* 2008; 3(5):213-18.
- Scarpino S, Di Napoli A, Melotti F, Talerico C, Cancrini A, Ruco L. Papillary carcinoma of the thyroid: low expression of NCAM (CD56) is associated with downregulation of VEGF-D production by tumour cells. *The Journal of pathology.* 2007; 212(4):411-9.
- Zeromski J, Biczysko M, Stajgis P, Lawniczak M, Biczysko W. CD56 (NCAM) antigen in glandular epithelium of human thyroid: light microscopic and ultrastructural study. *Folia histochemica et cytobiologica/Polish Academy of Sciences, Polish Histochemical and Cytochemical Society.* 1998;37 (1):11-7.
- Casey MB, Lohse CM, Lloyd RV. Distinction between papillary thyroid hyperplasia and papillary thyroid carcinoma by immunohistochemical

staining for cytokeratin 19, galectin-3, and HBME-1. *J Endocr Pathol.* 2003; 14 (1):55-60.

17. Cavallaro U, Niedermeyer J, Fuxa M, Christofori G. N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. *J Nat Cell Biol.* 2001;3 (7):650-7.

18. Shin MK, Kim JW, Ju Y-S. CD56 and high molecular weight cytokeratin as diagnostic markers of papillary thyroid carcinoma. *Korean Journal of Pathology.* 2011;45 (5):477-84.

19. Etem H, ÖZEKİNCİ S, Mizrak B, ŞEN TÜRK S. The role of CD56, HBME-1, and p63 in follicular neoplasms of the thyroid. *J Turk Patoloji Derg.* 2010;26(3):238-42.

20. DeLellis RA. Pathology and genetics of thyroid carcinoma. *J Surg Oncol.* 2006;94 (8):662-9.

21. Abdelzaher E, Rizk AM, Allam M. A Logistic Regression Model Predicting Malignancy in Follicular Thyroid Lesions Based on CD56 Expression and Patient's Age. *Journal of Interdisciplinary Histopathology.* 2014; 2 (4):205-12.

22. Hoos A, Stojadinovic A, Singh B, Dudas ME, Leung DH, Shaha AR, et al. Clinical significance of molecular expression profiles of Hürthle cell tumors of the thyroid gland analyzed via tissue microarrays. *The American journal of pathology.* 2002;160 (1):175-83.

23. Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. *The Journal of experimental medicine.* 1989;169 (6):2233-8.

24. Zeromski J, Dworacki G, Jenek J, Niemir Z, Jezewska E, Jenek R, et al. Protein and mRNA expression of CD56/N-CAM on follicular epithelial cells of the human thyroid. *Int J Immunopathol Pharmacol.* 1998;12 (1):23-30.

25. Mokhtari M, Eftekhari M, Tahririan R. Absent CD56 expression in papillary thyroid carcinoma: A finding of potential diagnostic value in problematic cases of thyroid pathology. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences.* 2013;18(12):1046-9.