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Research Paper

Immunohistochemical Expression of Galectin-3 in Thyroid Neoplasms and Its Correlation with CD56

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ABSTRACT:

BACKGROUND:

The incidence of thyroid cancer has been increasing significantly since the mid-1990s.Some studies have revealed that in thyroid gland, the galectin-3 plays a role in the pathogenesis of well-differentiated carcinoma. For that, it is may be one of the markers that may help to assist in distinguishing thyroid neoplasms.

OBJECTIVE:

To detect the frequency of immunohistochemical expression and diagnostic value of Galectin-3 in different types of thyroid neoplasms. To correlate the expression of galectin-3 findings with immunoprofile data of CD56. To correlate the result of immunohistochemical expression of Galectin-3 with the age and gender of the sampled cases.

PATIENTS AND METHODS:

A retrospective case series study was intended in fifty surgically removed cases of primary thyroid neoplasm whose CD56 status had been tested by immunohistochemical technique. The galectin-3 protein was tested on formalin fixed paraffin embedded section by immunohistochemical method using Ab-1, isotype IgG1, clone 9C4, Mouse Monoclonal Antibody.

RESULTS:

Galectin-3 was positive in 52 % of the cases. Relation of galectin-3 with the histological type of neoplasm and malignant tumors versus benign was significant (P= 0.000152 , P=0.000712 respectively). The galectin-3 was expressed in most of non-follicular pattern (p =0.00001). In this study, 42% of the cases were galectin-3 positive/ CD56 negative and 38% of the cases were galectin-3 negative/CD56 positive, the association of galectin-3 with CD56, was significant (P=0.000023).

CONCLUSION:

Galectin-3 immune stain marker considered as a useful marker in differentiating malignant tumors with non-follicular pattern, especially papillary thyroid carcinoma, from a benign tumors. Significant association was found between Galectin-3 and CD56. Galectin-3 can be used as a positive marker while the CD65 is a negative marker for diagnosis of thyroid carcinoma.

KEYWORDS: thyroid cancer, galectin-3, papillary carcinoma, CD56.

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INTRODUCTION:

The most common malignancy of the endocrine system is the thyroid cancer (TC)[(1,2)], which forms approximately 3.4% of all cancers diagnosed annually[(1)]. The incidence of TC has been increased in significant number since the mid-1990s [(3)]. The diagnosis of thyroid tumors is usually based on hematoxylin and eosin (H&E) stained slides, however the diagnosis of thyroid lesions, is difficult in some cases because of the overlapping of histomorphological features [(4)].

Previous studies assess the diagnostic role of several markers in thyroid tumors, such as Galectin-3(Gal-3), (HBME-1), CK19, CD44, CD57, Cyclin D1 and P27 [(5)], but still the definitive markers or panels for thyroid lesions are not yet determined [(6)]. The Gal-3 is a 31 kDa protein, it is a member of Gal-3 family that shows a strong affinity for β-galactoside[(7)]. It has been proposed to modulate

cell adhesion and cell growth through its influence on the cell cycle[7]. Some studies in thyroid gland have revealed that, Gal-3 has a role in the pathogenesis of TC, especially in papillary TC, therefore, it may be one of the markers that may play important role in distinguishing thyroid neoplasms[8]. The CD56, is a neural cell adhesion molecule, that helps in cell-to-cell adhesion[9, 10]. In the thyroid tissue, CD56 is released in normal thyroid epithelial cells and it is highly expressed in the membrane of the thyroid follicular cells[6], while in malignant cells, the release of CD56 is reduced and loss of its expression was associated with poor prognostic clinical course [11].

PATIENTS AND METHODS:

In this retrospective case series study, fifty cases of primary thyroid neoplasm whose CD56 status had been tested by immune-histochemical technique were included. The blocks of the cases from the 1st of October 2018 till the 1st of November 2019 were collected from Al-Jumhori teaching hospital, Nineveh private hospital, Al-Rabee private hospital and other private laboratories in Mosul city, and the period of study was conducted from 1st of January 2021 till the 1st of November 2021. Sections from paraffin embedded tissue were taken on clean slides and stained with H&E, then examined under the light microscope. Histological typing was determined according to the WHO classification. The most appropriate block of each case was chosen for immune-histochemical staining with Gal-3. Other information regarding the age of the patient, gender and CD56 status were obtained from the medical record. This study was performed by using the primary antibody which is Gal-3 Ab-1, isotype IgG1, clone 9C4, Mouse Monoclonal Antibody, supplied by Thermo scientific company/UK. The tissue processed according to the method prescribed by the manufacture company and treated using automated procedure.

Interpretation of immunohistochemistry staining: Immunoreactivity for Gal-3 was evaluated and scored manually. Slide from small intestine was used as positive control. Both the quantity and quality of the staining of the tumor cells in an average of 10 microscopic fields (magnification x400) were demonstrated. The diffuse brown pigmentation of cytoplasm with or without the nuclear staining was detected in the tumor cells. The propotional score was: Negative (no staining or staining in < 10 % of the neoplastic cells), focal staining (staining in 10% - 50% of the neoplastic cells and diffuse staining (staining >50% of the neoplastic cells)). Intensity score was graded as: 0 (no staining), 1+ (slight staining), 2+ (moderate staining) and 3+ (intense staining)). The cases were regarded as positive, when the neoplastic cells showed a specific staining (cytoplasmic staining with or without nuclear stain) of more than 10% of the neoplastic cells with slight, moderate or intense staining [12].

Statistical analysis:

The data of this study was analyzed by the using of Chi-square and Fisher exact test when indicated. The association was considered statistically significant when "P value was less than 0.05" with confidence interval of 95%.

RESULTS:

In this study, the age of patients were ranged from 15 to 73 years (mean±SD =39.56± 6.25), 46 cases were females and only 4 cases were males with female to male ratio was about 11.5:1. Immunohistochemical study of these cases for Gal-3 revealed that 26 cases (52 %) were positive. Other patients' characteristics are summarized in table (1). The variable percentage of neoplastic cells expressed the Gal-3 staining and intensity of this stain is shown in photo (1). In this study, no significant relation was found between the Gal-3 and the age of the patients (P value=0.1168), also no significant association (P=0.6105) was detected between the Gal-3 and patient′s gender. Regarding the relation of Gal-3 expression with the histological types of thyroid neoplasms, as shown in photos (2,3&4), it was significant with P=0.000152, in the follicular adenoma (FA) 17 cases out of 21 were the Gal-3 negative, while the majority of PTC (19 out of 24 cases) were positive for Gal-3, as shown in table (2). In the current study, 27 cases were malignant tumors, the Gal-3 was positive in 20 cases of them, while most of benign tumors (17 out of 23 cases) were negative, this association was also significant (P=0.000712). In comparing the pattern of tumors, the follicular versus those with non-follicular, the Gal-3 immune marker was positive in 5 out of 25 cases of tumors with follicular pattern, while 21 out of 24 cases of non-follicular pattern were positive, that the Gal-3

was negative in most of follicular patterned tumors (P=0.00001). According to this, Gal-3 was more positive in malignant group, especially of non-follicular pattern. The CD56 was negative in 21 cases of the malignant tumor, while the positivity of this marker was found more in the benign cases, this association was statistically significant (P=0.000077).

In this study, 21(42%) cases were Gal-3 positive/CD56 negative and 19 (38%) cases were Gal-3 negative/CD56 positive, this association was significant (P=0.000023), as shown in table (3).

Table 1: The descriptive analysis of clinicopathological results of the sampled cases.

		Data		No.	Percentage
Age in years		10-19		1	2%
		20-29		10	20%
		30-39		13	26%
		40-49		14	28%
		50-59		10	20%
		60-69		1	2%
		70-79		1	2%
Gender		Female		46	92%
		Male		4	8%
Histological Types	**Benign tumors**	FA		21	42%
		Oncocytic cell adenoma		2	4%
	Malignant tumors	PTC	Conventional PTC	20	40%
			Follicular variant PTC	4	8%
		Anaplastic TC		2	4%
		Medullary TC		1	2%
CD56		Positive		24	48%
		Negative		26	52%
Gal-3		Positive		26	52%
		Negative		24	48%

Table 2: Relation of Galectin-3 expression with the variable clinico-pathological data of the sampled cases.

Data		Galectin-3 Positive No.(%)	Galectin-3 Negative No. (%)	Total No.(%)	P- value
Age in years	10-19	0(0%)	1(2%)	1(2%)	0.1168
	20-29	7(14%)	3(6%)	10(20%)	
	30-39	3(6%/)	10(20%)	13(26%)	
	40-49	8(16%)	6(12%)	14(28%)	
	50-59	7(14%)	3(6%)	10(20%)	
	60-69	1(2%)	0(0%)	1(2%)	
	70-79	0(0%)	1(2%)	1(2%)	
Gender	Female	23(46%)	23(46%)	46(92%)	0.6105
	Male	3(6%)	1(2%)	4(8%)	
Histopathological types	Follicular adenoma (FA)	4(8%)	17(34%)	21(42%)	0.000152
	Oncocytic cell adenoma	2(4%)	0(0%)	2(4%)	
	Conventional PTC	18(36%)	2(4%)	20(40%)	
	Follicular variant PTC (FVPTC)	1(2%)	3(6%)	4(8%)	
	Anaplastic TC (APTC)	1(2%)	1(2%)	2(4%)	
	Medullary TC (MTC)	0(0%)	1(2%)	1(2%)	
Thyroid tumors	Benign tumors	6(12%)	17(34%)	23(46%)	0.000712
	Malignant tumors	20(40%)	7(14%)	27(54%)	
Pattern	Follicular (Follicular adenoma, and follicular variant PTC)	5(10%)	20(40%)	25(50%)	0.00001
	Non-follicular (Oncocytic cell adenoma, conventional PTC and anaplastic TC)	21(42%)	3(6%)	24(48%)	

Table 3: Relation of CD56 expression with the thyroid tumors and Galectin-3.

Data		CD56 Positive No. (%)	CD56 Negative No. (%)	Total NO.(%)	P- value
Thyroid tumors	Benign tumors	18(36%)	5(10%)	23(46%)	0.000077
	Malignant tumors	6(12%)	21(42%)	27(54%)	
Galectin-3	Positive	5(10%)	21(42%)	26(52%)	0.000023
	Negative	19(38%)	5(10%)	24(48%)	

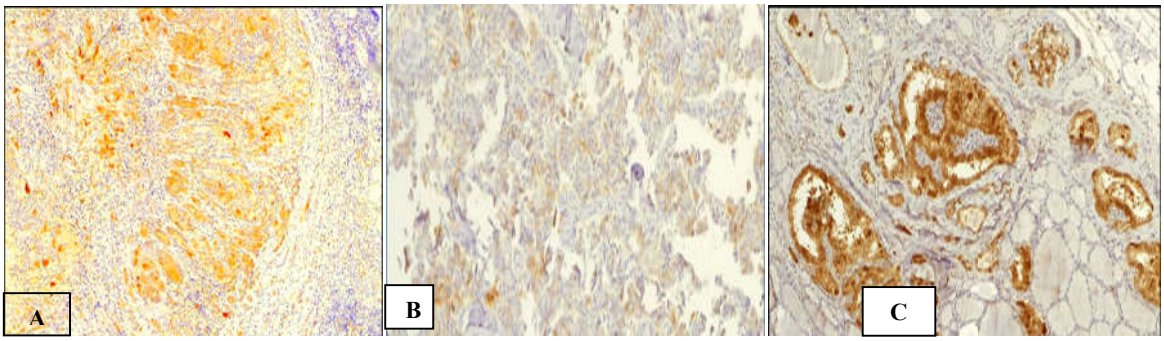

Photo 1: Variable intensity of immune staining for galectin-3: A (+) intensity ,B and C are (++) and (+++) intensity respectively. (A&B X40 and C X100 magnification).

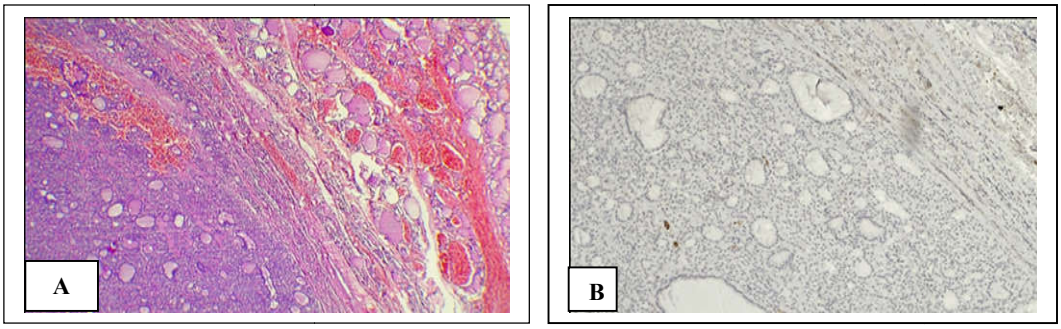

Photo (2): Follicular adenoma: A, H&E B, negative immune staining for Gal-3 (A and B X 40 magnification).

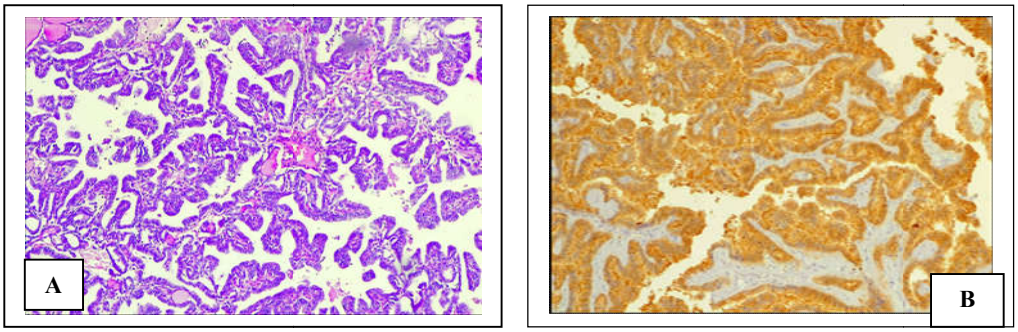

Photo (3): Papillary thyroid carcinoma A: H & E staining, B: positive immune-histochemical staining for Gal-3 (A and B X100 magnification).

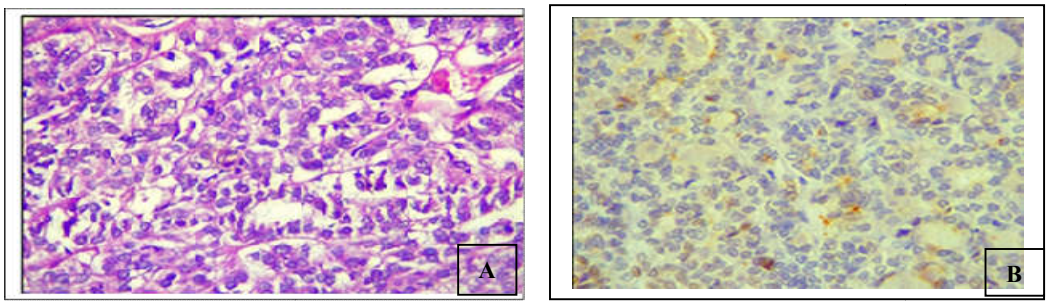

Photo (4): Follicular variant PTC: A: H & E staining, B: negative immune staining for Gal-3 (A and B X400 magnification).

DISCUSSION:

A lot of efforts have been directed to detect the new diagnostic markers for improving the diagnosis of TC, and Gal-3 was one of the most extensively-studied marker so far[12,13,14,15]. Large number of biological studies have detect that the cytoplasmic localization of Gal-3 in tumor cell play critical role in tumor genesis and metastasis, compared with nuclear localized Gal-3[16]. So in the current study, with or without nuclear stain, the presence of cytoplasmic staining was depended to determine the positivity of Gal-3. In this study no statistical significant was found between the age and gender of the patients with Gal-3 immune marker, these results were similar to that found by Weinberger PM et al[14] and Dunđerović D et al[17]. In general, the Gal-3 expression had been reported in 58% to 100% of TC [16]. In studies were done on PTC by El-kateb M. I. et al [18] and by Sumana B S [19], the Gal-3 was positive in 93.3% and in 91.3% respectively and in the conventional variant of PTC the Gal-3 positivity was identified in 82% to 100%[16], these results are near to the result of current study that was 79% of all cases of PTC and 90% of PTC conventional variant were positive. Few studies have reported Gal-3 expression in PTC by histological subtype. In follicular variant PTC, the positivity of Gal-3 was widely varied, ranged 33%-100% [16], this is disagree with the result in this study which showed Gal-3 positivity in 25% of the cases, this may be due to small number of follicular variant PTC subtype were included in this study, however, studies found that the expression of Gal-3 in follicular variant PTC tended to be lower than that in the conventional variant PTC[16] and this similar to what was found in the current study.

Regarding the follicular adenomas, the Gal-3 was negative in most of cases, this result near to that of Borkar PV[12]. Only 2 cases of oncocytic cell adenoma were included in this study and both of them were positive for Gal-3 and this is agreement with that of Borker PV[12]. This may be due to that, in response to stimuli, the Gal-3 will be translocate either from the nucleus or cytosol to the mitochondria [20], so accumulation of the Gal-3 in the mitochondria which is increased in oncocytic cell tumors is responsible for this tumor type to be immunohistochemically positive for Gal-3. The role of Gal-3 in the diagnosis of medullary TC (only 1 case) and anaplastic carcinomas 2 cases (1 case was positive and other was negative) can't be assessed due to small number of these cases. So other studies with more cases of these rare subtypes are needed to evaluate the role of Gal-3 in their diagnosis. Although no cases of follicular TC were included in this study, but studies were done by Dunderović D et al[17] and Sumana BS[19] found that the expression of Gal-3 in follicular adenoma was not statistically different from that its expression in follicular TC. In this study the Gal-3 expression was more expressed in non-follicular pattern thyroid neoplasm than follicular pattern this is similar to the result of Tastekin E et al[11]. Regarding the Gal-3 expression in relation to the benign and malignant tumors, Gal-3 was expressed more in malignant than in benign tumors, this association was statistically significant, this is similar to that of a study was done in Baghdad/Iraq by Al-Jubouri RSM[21], same result was detected by Tastekin E et al[11] , Borkar PV[12], Weinberger PM et al[14], Dunđerović D et al [17], Mandal et al[22] and Dhal A[24].

Regarding the CD56 expression in thyroid neoplasm, it decreases or totally losts in cases of TC especially PTC[17]. This is similar to the result of current study and other studies[11,23]. In this study highly significant association was found between the Gal-3 and CD56 that most of cases with Gal-3 positive cases, were negative for CD56, while most of cases were negative to Gal -3, were positive to CD56. On the basis of previous studies, they found that the uses of more than one marker would increase diagnostic value of thyroid neoplasms[25]. As CD56 expression is normally present in follicular cells of the thyroid tissue, while malignant cells, revealed a higher rate of loss of CD56 expression, so unlike other IHC markers, CD56 is can be considered as a negative marker[6]. So Gal-3 can be used as a positive marker while the CD65 is a negative marker for diagnosis of TC.

CONCLUSION:

Galectin-3 could be utilized as a marker for the diagnosis of thyroid tumors, and its combination with CD56 may enhance its value with the highest performance for the diagnosis TC, especially PTC. However the importance of such combination of markers should be confirmed in a study including a larger series of cases including different thyroid lesions and tumor subtypes especially the border line neoplastic lesions and follicular TC.

REFERENCES:

1. Prete A, Borges de Souza P, Thyroid Censi S, Muzza M, Nucci N, Sponziello M. Update on fundamental mechanisms of Cancer. *Front Endocrinol (Lausanne)*. 2020;11:1-10. doi:10.3389/fendo.2020.00102.
2. Aliyev E, Ladra-González MJ, Sánchez-Ares M, Nallib IA , Piso-Neira M, Rodríguez-Carnero G, et al. Thyroid papillary microtumor : Validation of the (Updated) porto proposal assessing sex hormone receptor expression and mutational BRAF gene Status. *Am J Surg Pathol*. 2020; 44:1161-72.
3. Arrangoiz R, Cordera F, Caba D, Moreno E, Luque-De-Leon E, Muñoz M. Thyroid cancer keywords. Int J Otolaryngol Head Neck Surg. 2019;8:217-70.
4. Zargari N, Mokhtari M. Evaluation of Diagnostic Utility of immunohistochemistry markers of TROP-2 and HBME-1 in the diagnosis of thyroid carcinoma. Eur Thyroid J. 2019;8:1-6. doi:10.1159/000494430
5. De Rose F, Braeuer M, Braesch-Andersen S, Otto AM, Steiger K, Reder S, et al. Galectin-3 targeting in thyroid orthotopic tumors opens new ways to characterize thyroid cancer. J Nucl Med. 2019;60:770-76. doi:10.2967/jnumed.118.219105
6. Pyo JS, Kim DH, Yang J. Diagnostic value of CD56 immunohistochemistry in thyroid lesions. Int J Biol Markers. 2018;33:161-67.
7. Tang W, Huang C, Tang C, Xu J, Wang H. Galectin-3 may serve as a potential marker for diagnosis and prognosis in papillary thyroid carcinoma: A meta-analysis. Onco Targets Ther. 2016;9:455- 60. doi:10.2147/OTT.S94514
8. Albi E, Curcio F, Lazzarini A, Floridi A, Cataldi S, Lazzarini R , et al. How Micro gravity changes galectin-3 in thyroid follicles. Biomed Res Int. 2014;2014:1-5.
9. Abdullah MI, Junit SM, Ng KL, Jayapalan JJ, Karikalan B, Hashim OH. Papillary thyroid cancer: Genetic alterations and molecular biomarker investigations. Int J Med Sci. 2019;16:450- 60. doi:10.7150/ijms.29935
10. Parsa AA, Gharib H. Update on thyroid nodule management. US Endocrinol. 2019;15:32- 8. doi:10.17925/USE.2019.15.1.32.
11. Tastekin E, Keskin E , Can N, Canberk S, Mut A, Erdogan E , et al. CD56, CD57, HBME1, CK19, Galectin-3 and p63 immunohistochemical stains in differentiating diagnosis of thyroid benign/malign lesions and NIFTP . Pol J Pathol . 2019;70:286-94. doi: 10.5114/pjp.
12. Borkar PV, Gowrinath K, Sandeep K. Intensity and grading of galectin-3 expression as a sole marker to differentiate benign and malignant thyroid neoplasms. Nat. J of Lab Med. 2019;8:1-4. doi:10.7860/ NJLM/ 2019/ 40370.
13. Ozcinar B, Es B, Ertugrul B, Ha E. Galectin-3 expression in thyroid papillary cancer. Austin J Surg . 2019; 6:1-5.
14. Weinberger PM, Adam BL, Gourin CG, Moretz WH, Bollag RJ, Wag BY, et al. Association of nuclear, cytoplasmic expression of galectin-3 with β-catenin/Wnt-pathway activation in thyroid carcinoma. Arch Otolaryngol - Head Neck Surg. 2007;133:503-10.
15. Bartolazzi A, Sciacchitano S, D'Alessandria C. Galectin-3: The impact on the clinical management of patients with thyroid nodules and future perspectives. Int J Mol Sci. 2018;19. doi:10.3390/ijms19020445
16. Chiu CG, Strugnell SS, Griffith OL,Jones SJM ,Gown AM, Walker B, et al. Diagnostic utility of galectin-3 in thyroid cancer. Am J Pathol. 2010;176:2067-81.
17. Dunderović D, Lipkovski JM, Boričic I, Soldatović I, Božic V, Dubravka Cvejić D, et al . Defining the value of CD56, CK19, galectin 3 and HBME-1 in diagnosis of follicular cell derived lesions of thyroid with systematic review of literature. Diagn Pathol. 2015;10. doi:10.1186/s13000-015-0428-4
18. Elkateb MI, Ahmed M, Mohammed S, Mostafa M. Evaluation of thyroid nodules using thyroid peroxidase and galectin-3:an immunohistochemical study. Zagazig Univ Med J. 2013;19:1-8. doi:10.21608/zumj.2013.4263
19. Sumana BS, Shashidhar S, Shivarudrappa AS. Galectin-3 immunohistochemical expression in thyroid neoplasms. J Clin Diagnostic Res. 2015;9:EC07-EC11.
20. Harazono Y, Kho DH, Balan V, et al. Galectin-3 leads to attenuation of apoptosis through Bax heterodimerization in human thyroid carcinoma cells. Oncotarget.2014;5:9992-10001. doi:10.18632/oncotarget.2486

21. Al-Jubouri RSM ,Mohamed BJ ,Naji RZ . Detection of some biomarkers in Iraqi patients with thyroid carcinoma. Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 2017;12:34-9. e-ISSN:2278-3008, p-ISSN:2319-7676.

22. Mandal AP, Saha R, Mitra S. Immunohistochemical expression of galectin-3 and cytokeratin 19 in the Spectrum of Thyroid Neoplasms J Clin Diagnostic Res. 2021;15:19-23.

23. Ahmed AJ. Immunohistochemical expression of CD56 in thyroid neoplasms in Nineveh province .A thesis submitted to the Scientific Council of Pathology in partial fulfillment of the requirement for the degree of fellowship of the Iraqi Board for Medical Specialization in Pathology/Histopathology,2020

24. Dhal A, Agrawal S, Mishra DP.Evaluation of galectin-3 expression in non-neoplastic and neoplastic lesions of thyroid.JMSCR 2021;9: 61-67.

25. Bozdogan Arpaci R. Thyroid tumors with encapsulated follicular pattern : A diagnostıc dilemma. Adv Cytol Pathol. 2017;2:90-7.