IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. IV (Jul – Aug 2017), PP 34-39
www.iosrjournals.org

Detection of Some Biomarkers in Iraqi Patients with Thyroid Carcinoma

ReyadhSalim Mohammed[1],Ban JasimMohamad[2],RanaZuhair Naji[3]
[1]*College of Dentistry/IbnSina University of Medical and Pharmaceutical Sciences,Baghdad, Iraq.*
2Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[3]*Center Public health laboratory, Histopathology Department, Baghdad, Iraq*
Corresponding Author: ReyadhSalim Mohammed

Abstract:
Background:*Thyroid cancer, the most common endocrine malignancy, has increased dramatically in the lastyears.Cathepsin D and Galectin-3 have been implicated in the regulation of cellular growth, differentiation and malignant transformation in various organs including thyroid gland. It has been extensively studied as an immunohistochemical (IHC) of these markers in thyroid malignancy.*
Aim: *To assess the diagnostic role of galectin-3 (Gal-3) and cathepsin D (CD) in thyroid neoplasm.*
Materials and Methods: *In this study we evaluated Cathepsin D and Galectin-3expression in 43 of thyroid tissue cases, (23) thyroid malignant,(12) benign and (8) control cases. The final common diagnosis arrived at after histopathological evaluation of H&E stained sections by pathologist was considered the final H and E standard diagnosis. The malignant neoplasms included Papillary Thyroid Carcinoma (PTC), Follicular Thyroid Carcinoma (FTC), Medullary Thyroid Carcinoma (MTC), Anaplastic Thyroid Carcinoma (ATC) andHürthle cell cancer (HCC). The benign neoplasms included Follicular Adenoma (FA). IHC staining for CD and Gal-3 was performed for all cohort.*
Statistical Analysis: *Statistical analysis was done by using Pearson and Spearman correlations to calculate p-value to statistical significance.*
Results: *CD and Gal-3 expression were significantly higher in malignant thyroid neoplasms as compared to benign neoplasms ($p<0.01$). As well highly significant correlation of expression between galectin-3 and cathepsin D ($p<0.01$).*
Conclusion: *Diffuse and strong IHC staining for galectin-3 and cathepsin D in malignant, very weak stain score 1 inbenign tumour and no stain in control. These markers can be used in the diagnosis of thyroid neoplasms with equivocal morphologic features.*
Keywords: *Thyroid cancer, IHC, cathepsin D, Galectin -3.*

Date of Submission: xx-xx-xxxx Date of acceptance: xx-xx-xxxx

---

I. Introduction

Thyroid carcinoma is the most common endocrine malignancy and it comprises 3% of all newly diagnosed malignancies(Jemal*et al*.,2010), follicular carcinoma represents more than 90% of thyroid carcinoma, the papillary thyroid carcinoma (PTC) accounts for a majority of these new diagnoses, other types includes follicular thyroid cancer (FTC, up to 11%), Hürthle cell cancer (HCC, 3%) and anaplastic thyroid cancer (ATC, 2%), PTC and FTC are commonly referred to together as differentiated thyroid cancer (DTC), while ATC represents un-differentiated thyroid cancer. The other type of thyroid carcinoma that originated form non-follicular thyroid cell (calcitonin producing cells) called medullary thyroid carcinoma which accounts 4% of thyroid cancers (Brown *et al*.,2011**)**.In addition to morphology, much attention has been paid to the prognostic, predictive, and therapeutic potential. In this regard, the diagnostic approach to these tumorsshould include IHC markers that can aid in better assessment of morphologic details (Fisher and Asa, 2008).

Galectinsare a family of lectins that are predominantly localized in the cytoplasm, translocate to the perinuclear membrane, nucleus and/or get secreted from the cytoplasm (Aiad*et al*., 2008; Cvejic*etal*., 2005; Nangia-Makker,2007).Galectin-3 are participates in cellular functions such as cell proliferation, apoptosis, cellular transformation, tumour progression and metastasis of cancer cells. In the recent times Galectin-3 has received notable recognition for its usefulness as a diagnostic marker for thyroid cancer (Chiu *et al*., 2010), the utility assessed by studies have reported encouraging results(Weber *et al*., 2004; Hermann *et al*., 2002).

Cathepsin D (CD) is an aspartic lysosomalendopeptidase, it is present in almost cells and tissues of mammals. Overexpression of this protease has been related with the progression of several human cancers such as thyroid carcinomas, squamous cell carcinoma, renal cell, carcinoma, glioma brain tumors, laryngeal

carcinoma, breast cancer, lung cancer, ovarian carcinoma (Jacobson-Rabe*et al*., 2011, Tan *etal*., 2013).CathepsinD play an important role in cancer, it is promote tumor growth directly by acting to degrade and remodel the basement membrane and interstitial stroma surrounding the primary tumor and indirectly by activation of other enzymes or in collaboration with other cathepsins in the proteolysis process (Derocq*et al*.,2012). The levels of cathepsin D in tumors were documented to be higher than in adjacent normal tissue (Szajda*et al*., 2008).In this study we aim to evaluate the expression of cathepsinD and galectin-3 in thyroid carcinoma.

II. Materials And Methods

A retrospective study of 43 paraffin blocks of thyroid tissue cases were selected from archive files of Histopathology department in the Central Public Health Laboratory, Baghdad, Iraq. H and E stained sections were re-examined by consultant pathologist and the final diagnosis of the tumors types in accordance with World Health Organization classification. These cases included 23 cases were malignant, 12 benign and 8 cases were control.

Immunohistochemistry

Immunohistochemical evaluation of galectin-3and cathepsin D were performed on representative histologic sections of the thyroid neoplasms.Sections were de-waxing in xylene and rehydrated through ethanol. Antigen retrieval was done with Tri-sodium citrate buffer (pH 6.0 to 6.2) using microwave oven. Slides were brought to room temperature. Slides were incubated in peroxidase – blocking solution (Dako, ready- to- use) for 20 min. Non-specific binding of antibodies was blocked by the addition of 2.5% normal horse serum, from (ImmPRESS™, Vector, USA).Primary antibody for cathepsin D was diluted (1:1000) and for Gal-3 was diluted (1:200)using antibody diluant (ready-to-use, Code No.ab64211Abcam, Cambridge, UK), and incubated for 1hour at room temperature.After that secondary antibodies (Anti Rabbit. peroxidase, Cat. No. MP-7401, ImmPRESS™ Vector, USA) for cathepsin D and (anti mouse peroxidase , Cat. No. MP-7402,ImmPRESS™ Vector, USA) for galectin- 3were applied to the slides and incubated for 30 minutes at room temperature in a humidified chamber. The colorimetric detection of reaction was achieved by the diaminobenzidine (DAB) Peroxidase Substrate method. Then, sections were counterstained with haematoxylin, dehydrated, and mounted.

ImmunohistochemicalScoring System

Galectin-3

Scoring for all the immune expression results were assessed with the aid of a specialist pathologist**.** Positive galectin-3 staining was considered when any cytoplasmic brown staining was seen according to scoring system submitted by Weber *et al.* (2004) and Hermann *et al*.(2002). This system was evaluated semiquantitatively by using two parameters evaluated; percentage of tumor cell stained and the intensity of stain. The staining intensity score (IS) was graded on a scale of 0 to 3 where 0, 1+, 2+, and 3+ denote no staining, weak/slight staining, moderate staining and intense staining respectively, and the proportion score (PS) of stained cells were interpreted as 1+ (< 5% of cells), 2+ (5% to 50% of cells) and 3+ (>50% of cells).Positive result denotes the specific staining of more than 5% of the tumour cells with slight, moderate or intense staining.

Cathepsin D

The positive cells was scored semiquantitatively according to Allgayer*et al*.(1997), this system depend on the number of positive stain tumor cells; Score 0: negative; Score 1: 30% positive tumor cells; Score 2: 30–70% positive tumor cells; Score 3: 70% positive tumor cells.

Statistical Analysis

Statistical analysis was performed using SPSS 20.Descriptive statistic for age and sex, Spearman and Pearson correlationsfor ordinal variables to determine correlations of biomarkers with histological type as well between expressions of two markers. A *P* value of <0.01 was considered as significant.

III. Results

Thirty five of Iraqi patients with thyroid tumors were studied. Patient's age ranged from (20-75) years with a mean of 36.91±11.17 years. Females constituted 26 (61%) while male 17(39%) of the cases.The malignant neoplasms were more common were23 (64%), 12,5,4,1 and1 were PTC, MTC, FTC, ATC and HCC respectively,while benign were 12 (28%). Eight samples were used ascontrol cases 8 (19%) (figures1 and 2).This study demonstrated positive immunostaining of cathepsin D and galectin-3 in cytoplasm of cancer cells as a brown diffuse pigmentation (Figure-3).Results of IHC expression of cathepsin D andgalectin-3in thyroid tissue specimens are summarized in table 1.

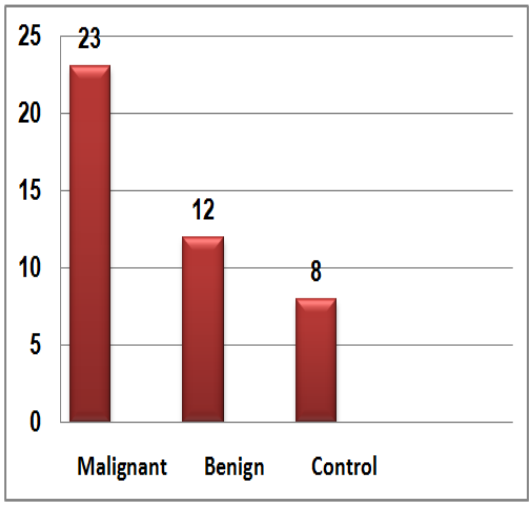


Fig.1. Distribution of histopathological diagnoses of cases

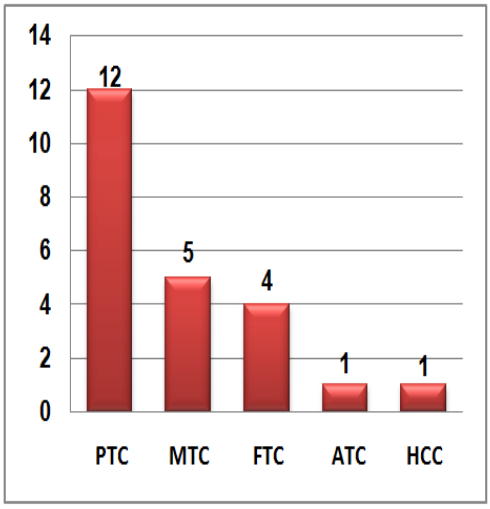
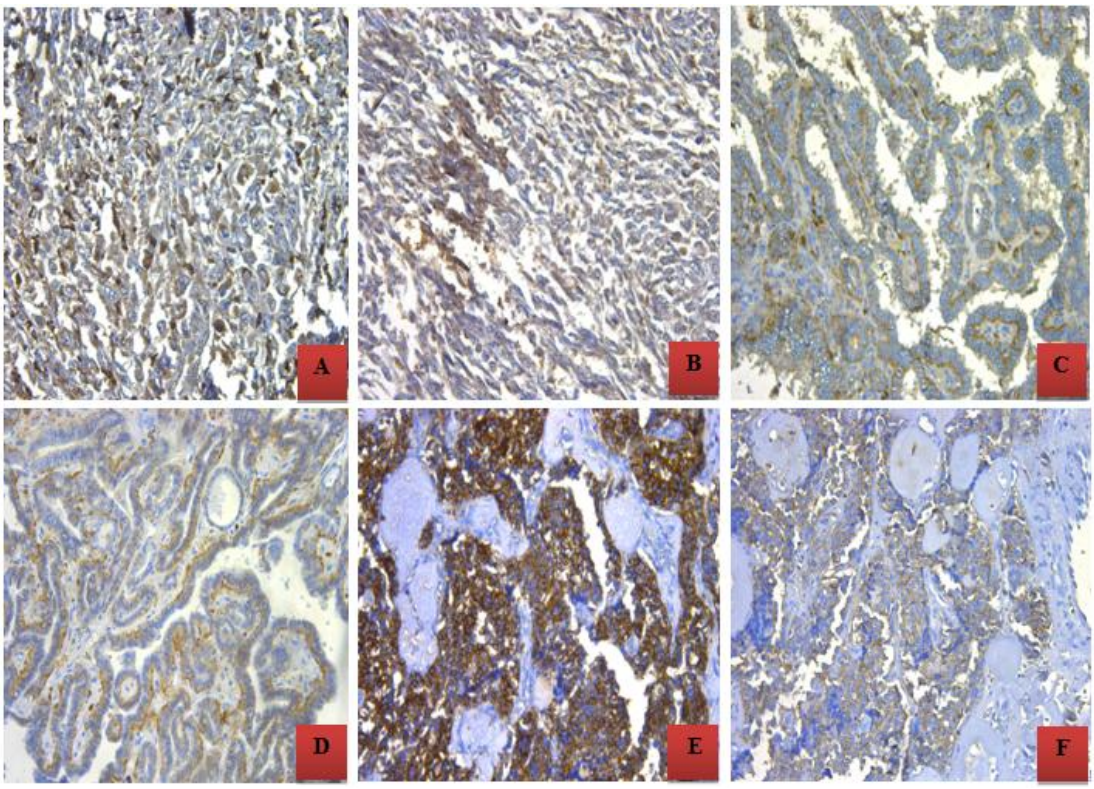


Fig.2. Distribution of histopathologicaldiagnoses of malignant

Table1. Immunohistochemical expression of galectin-3 and cathepsin D according to histological classification.

Histological Type	Total	Galectin-3				Cathepsin D			
		0	1	2	3	0	1	2	3
PTC	12	7	1	4	0	5	3	2	2
FTC	4	2	0	1	1	0	0	3	1
MTC	5	1	0	3	1	1	0	2	2
ATC	1	0	0	0	1	0	0	0	1
HCC	1	1	0	0	0	0	0	1	0
Benign	12	4	8	0	0	5	7	0	0
Control	8	8	0	0	0	8	0	0	0



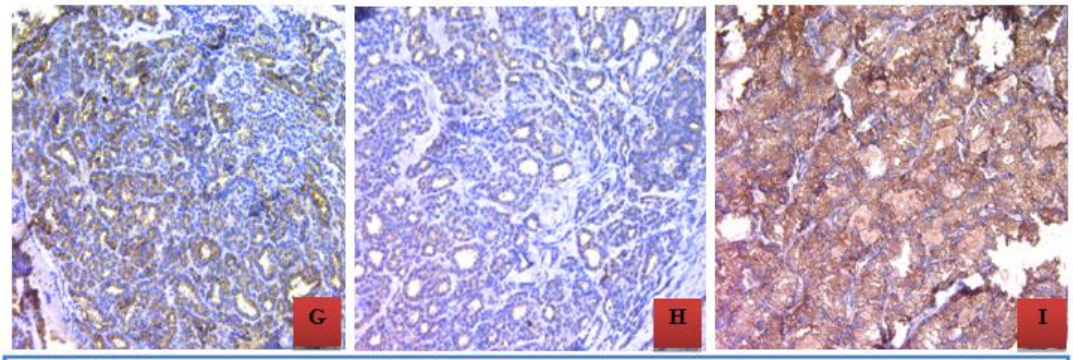


Figure3.

(A) ATC, IHC of CD, score 3 200x, (B) ATC, IHC of Gal3, score 3 200x, (c) PTC, IHC of CD, score 2 200x, (d) PTC, IHC of Gal3, score 2 200x, (e) MTC, IHC of CD, score 3, 200x, (f) MTC, IHC of Gal3, score 3, 200x, (g) FTC of CD, score 3, 200x, (h) FTC of Gal3, score 2 200x (i). PTC, IHC of Gal3 score 3, 200x.

Abbreviation: **(ATC)** Anaplastic thyroid carcinoma, **(PTC)** Papillary thyroid carcinoma, **(MTC)**Medullary thyroid carcinoma, **(FTC)** Follicular thyroid carcinoma. **(CD)**, cathepsin D, **(Gal-3)**galectin-3, **(IHC)** Immunohistochemistry

Score for Galectin-3: I Score: Intensity score graded as 0 (no staining),1+ (slight staining), 2+ (moderate staining), or 3+ (intense staining) P Score: Proportion of stained cells scored as 1+ (<5%of cells), 2+ (5%-50% of cells), or 3+ (>50% of cells),Positive result= specific staining of more than 5% of the tumour cells with slight, moderate or intense staining.

Score for Cathepsin D: Score 0: negative; Score 1: 30% positive tumor cells; Score 2: 30–70% positive tumor cells; Score 3: 70% positive tumor cells.

Cathepsin D (CD) was the most sensitive marker in this study, more half of malignant cases were positive for this marker followed by galectin-3.This study found that the majority of the follicular thyroid carcinoma and medullary thyroid carcinoma were positive to these markers. The benign cases showed from no stain for cathepsin D and galectin-3 (negative expression) to weak positive stain, while all normal tissues were negative expression for these two markers, (table 1). Statistical analyses showed highly significant correlation between cathepsin D and galectin-3, *p* value (0.001).Present results revealed that a highly significant correlation between immunohistochemical expression of cathepsin D with histological type *p* value (0.001). Table 2.

Table 2. Association of histological type with expression of cathepsin D

Histological Type	No. of Cases	IHC Expression of Cathepsin D		*P* Value
		Positive	Negative	
Malignant	23 (53%)	17	6	0.0001*
Benign	12 (28%)	7	5	
Control	8 (19%)	0	8	
Total	43 (100%)	24	19	

(*p< 0.01)

As well as the current study showed that a highly significant relationship between immunohistochemicalexpression of galectin-3 with histological type *p*value(0.005). Table.3

Table 3. Association of histological type with expression of galectin-3

Histological Type	No. of Cases	IHC Expression of Galectin 3		*P* Value
		Positive	Negative	
Malignant	23 (53%)	12	11	0.005*
Benign	12 (28%)	8	4	
Control	8 (19%)	0	8	
Total	43 (100%)	20	23	

(*p< 0.01)

IV. Discussion

Follicular epithelial cell-derived thyroid cancers are the most common and increasingly incident endocrine malignances, in this study the follicular carcinoma constitutes the vast majority than non-folllicular carcinoma, these results were agree with previous study by Brown *et al*.,(2011). PTC wereconstitutedthe majority of the malignant cases this results agreed with (Chen *et al*.,2009), they reported that the PTC increasing in new diagnoses of thyroid carcinoma. In this study,cathepsin Dand galectin-3 were specifically expressed by malignant neoplasm, showing that these markers could be used as a support biomarker for thyroid cancer.Galectin-3 has important roles in variety biological events, such as differentiation, proliferation, cell adhesion, growth, apoptosis and regulation of immune system. Also galectin-3 is expressed in various cells and plays an important role in tumorgenesis, angiogenesis, tumor metastasis and malignant transformation (Lee *et*

al., 2009; Sakaki*et al*., 2010; Zhang *et al*.,2009).Galectin-3, has received significant recent attentionfor its utility as a diagnostic marker for thyroid cancerrepresents the well-studied molecular candidate for thyroid cancer diagnosis. The current result showed that the cytoplasmic expression of galectin-3 was found in carcinomas and not found in normal thyroid tissue while a very weak expression in adenoma cases,this study documented a significant correlation between malignant cases with galectin-3 expression, most of authors reported very low galectin-3postivity in adenomas making this molecule a useful marker in distinguishing thyroid carcinoma and adenomas (Barroeta*et al*., 2006), similar observation was noted by Mehrotra*et al*. (2004).Study by Chui *et al*. (2010),they were found thathighly expressed of galectin-3in thyroid cancinoma, but not expressed in normal thyroid tissue, and very low frequently in benign thyroid cases, so they found thatgalectin-3 might also represent an attractive target for therapy of thyroid cancer. As well,Baru*etal*.(2010) were studied the immunohistochemical expression of galectin-3in the diagnosis and differential diagnosis of malignant and normal thyroid lesions. They suggested that, the use of galectin-3might provide significant contributions in the differential diagnosis of malignant thyroid tumors. According to current results and previous studies we can conclude that theimmunohistochemical expression of galectin-3 may be highly related with malignancies and could be used to differentiated malignancies from normal tissues but not differentiated between malignant and benign.

Cathepsin D (CD) has different biological functions, its playing an important in tumor progression such as, proliferation, angiogenesis, invasion and metastasis (Tan *et al*., 2013; Leto*et al*.,2004).The current study revealed that the cathepsin D was positive expression in majority of malignant cases confirming its sensitivity as amarker for malignancies, 7 out of 12 (58%) of PTC were positive, all four cases of FTC were positive, 4 out of 5 (80%) of MTC were sensitive for cathepsin D, Hürthle cell cancer was positive for cathepsin D expression, the benign cases were low weak and negative expression, while negative expression in control tissues,this results agreed with (Tan *et al*., 2013), they were found elevated of cathepsin D in thyroid carcinoma and other types of solid tumors. The current results found highly significant correlation between cathepsin D expression with malignant tumors. These results closed with study of (Szajda*etal*., 2008), they reported that the levels of cathepsin D in tumors were higher than in adjacent normal tissue. From these findings we can conclude the role of cathepsin D in the progression of this tumors comparison with normal tissue and adenoma cases. It can be suggested that the cathepsin D considered a diagnostic marker for thyroid lesions because it specific for malignancies. As well, the current result found high significant association between immunohistochemical expression of cathepsin D and galectin-3, no previous study was assessed this association but we can be concluded form these results that the cathepsinD and galectin3 play an important role in tumor progression.Experimental and clinical data showed a correlation between galectin-3 expression and tumor progression and metastasis (Balan*et al*,. 2010),as well cathepsin D is submit to assist early stages of tumorgenesis such as cell proliferation and local invasion and metastasis (Tan *et al*., 2013). From above we can explain this highly association because these two makers were expressed in proliferation, invasion and distal metastasis of thyroid tumor cells.

V. Conclusion

In conclusion, cathepsin D and galectin-3 are good biomarkers of malignancies especially in supporting the diagnosis of thyroid carcinoma. If thyroid carcinomas can give negative expression for both (cathepsin D and galectin-3) or for one of them (cathepsin D and galectin-3), hence we cannot depend on this negative biomarkerexpression as a diagnostic method to detect thyroid malignancy.Finally, this current result can be recommended to add cathepsin D and galectin-3 to routinely diagnosis markers in our land for detecting of thyroid malignancies cases.

Acknowledgments

The authors are grateful to CPHL(Central Public Health Laboratory, Baghdad-Iraq) for assistance and helpful this study. The authors also wish to thank the technicians of the Histopathology unit in(CPHL) for their very precious technical help.

References

[1] Fischer S, Asa SL. Application of immunohistochemistry to thyroid neoplasms.Arch Pathol Lab Med. 2008;132:359-72.
[2] Aiad, H.A., Kandil, M.A., Assad, N.Y., El-Kased, A.M. and El-Goday, S.F. Galectin-3 immunostaining in cytological and histopathological diagnosis of thyroid lesions. Journal of the egyptian nat. Cancer Inst. 2008;20:36-46.
[3] Cvejic, D., Savin, S., Petrovic, I., Selemetjev, S., Paunovic,I.andTatic, S., et al. Galectin-3 and proliferating cell nuclear antigen (pcna) expression in papillary thyroid carcinoma. ExpOncol. 2005;27(3):210-14.
[4] Nangia-Makker, P., Nakahara, S., Hogan, V. andRaz, A. Galectin-3 in apoptosis, a novel therapeutic target. J BioenergBiomembr.2007;39(1):79-84.
[5] Chiu, C.G., Strugnell, S.S., Griffith, O.L., Jones, S.J.M., Gown, A.M. and Walker, B., et al. Diagnostic Utility of Galectin -3 in Thyroid Cancer.The American Journal of Pathology. 2010;176 (5):2067-81.

[6] Weber, K.B., Shroyer, K.R., Heinz, D.E., Nawaz, S., Said, M.S. and Haugen, B.R. The use of a combination of galectin-3 and thyroid peroxidase for the diagnosis and prognosis of thyroid cancer.Am J ClinPathol. 2004;122:524-31.

[7] Herrmann, M.E., LiVolsi, V.A., Pasha, T.L., Roberts, S.A., Wojcik, E.M. andBaloch, Z.W. Immunohistochemical expression of galectin-3 in benign and malignant thyroid lesions.ArchPathol Lab Med. 2002;126:710–13.

[8] Chen, A.Y., Jemal, A. and Ward, E.M. Increasing incidence of differen-tiated thyroid cancer in the United States, 1988-2005. Cancer 2009;115(16):3801-7.

[9] Jemal, A., Siegel, R., Xu, J., et al. Cancer statistics. CA Cancer J Clin. 2010;60:277–300.

[10] Jacobson-Raber, G., Lazarev, I., Novack, V., Mermershitein, W., Yael, B., Geffen, D., Vardy, N. and Ariad, S. 2011. The prognostic importance of cathepsin D and E-cadherin in early breast cancer: a single-institution experience, Onco. Lett., 2(6):1183–1190.

[11] Derocq, D., Prébois, C., Beaujouin, M., Laurent-Matha, V., Pattingre, S., Smith, G. and Liaudet-Coopman, E.Cathepsin D is partly endocytosed by the LRP1 receptor and inhibits LRP1-regulated intramembrane proteolysis. Oncogene. 2012;31(26):3202-3212.

[12] Szajda,S., Snarska, J., Jankowska, A., Roszkowska-Jakimiec,W., Puchalski, Z. and Zwierz, K. Cathepsin D and carcino-embryonic antigen in serum, urine and tissues of colon adenocarcinoma patients, Hepatogastroenterology, 2008; 55(82-83):388–393.

[13] Tan,GJ., Peng,ZK., Lu,JP., Tang,FQ. Cathepsins mediate tumor metastasis. World J BiolChem2013; 26; 4(4): 91-101.

[14] Fisher, K.E., Neill,S.G., LalehEhsani,L., Caltharp,S.A., Momin, T. Siddiqui, M.T.andCohen,C. Immunohistochemical Investigation of BRAF p.V600E Mutations in Thyroid Carcinoma Using 2 Separate BRAF Antibodies. ApplImmunohistochemMolMorphol 2014;22:562–567.

[15] Allgayer,H., Babic,R., Grutzner,K.U., Beyer, B., Tarabichi, A., Schildberg, F.W. andHeiss,M.M. AnImmunohistochemical Assessment of Cathepsin Din Gastric Carcinoma. Cancer 1997;80:179–87.

[16] Chiu, C.G., Strugnell, S.S., Griffith, O.L., Jones, S.J., Gown, A.M., Walker, B., Nabi, I.R. and Wiseman, S.M. Diagnostic utility of galectin-3 inthyroid cancer. Am J Pathol 2010, 176:2067-2081.

[17] Barut, F., OnakKandemir, N., Bektas, S., Bahadir, B., Keser, S. and Ozdamar, S.O.Universal markers of thyroid malignancies:galectin-3, HBME-1, and cytokeratin-19. EndocrPathol 2010,21:80-89.

[18] Rebecca, L., Brown, R.L., Jonas A. de Souza,J.A. and Cohen, E.E.W. Thyroid Cancer: Burden of Illness and Management of Disease.
Journal of Cancer 2011; 2:193-199

[19] Lee, J., Moon, C., Kim, J., Jung, C., Lee, K.H., Joo, H.G., Ahn, M. and Shin T: Immunohistochemical localization of galectin-3 in thegranulomatous lesions of paratuberculosis-infected bovine intestine. J Vet Sci 2009, 10:177-180.

[20] Sakaki M, Fukumori T, Fukawa T, Elsamman E, Shiirevnyamba A, Nakatsuji H, Kanayama HO: Clinical significance ofGalectin-3 in clear cell renal cell carcinoma. J Med Invest 2010,57:152-157

[21] Zhang, H.Y., Jin, L., Stilling, G.A., Ruebel, K.H., Coonse, K., Tanizaki, Y., Raz, A. and Lloyd, R.V.RUNX1 and RUNX2 upregulate Galectin-3expression in human pituitary tumors. Endocrine 2009, 35:101-111

[22] Mehrotra, P.,Okpokam, A.,Bouhaidar, R., Johnson, S.J., Wilson, J.A., Davies, B.R.andLennard,T.W.J. Galectin-3 does not reliably distinguish benign from malignant thyroid neoplasms. Histopathology 2004, 45, 493–500.

[23] Barroeta J.E., Baloch, Z.W., Lal, P., Pasha, T.L., Zhang, P.J.and LiVolsi, V.A. Diagnostic value of differential expression of CK19, galectin-3, HBME-1, ERK, RET, and p16 in benign and malignant follicular-derived lesions of the thyroid: an immunohistochemical tissue microarray analysis. Endocrine Pathology,2006; 17 225–234.

[24] LetoG,Tumminello, F.M., Crescimanno, M., Flandina,C.andGebbia, N. Cathepsin D expression levels in nongynecological solid tumors: clinical and therapeutic implications. ClinExp Metastasis 2004; **21**: 91-106.

[25] Balan,V., Nangia-Makker,P. andRaz, A.Galectins as Cancer Biomarkers. Cancers 2010, 2, 592-610.

Reyadh Salim Mohammed. "Detection of Some Biomarkers in Iraqi Patients with Thyroid Carcinoma." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 12.4 (2017): 34-39.