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Benign ve malign folliküler patternli tiroid lezyonlarının ayırıcı tanısında galektin 3, PTEN ve tiroid peroksidaz ekspresyonu
Galectin 3, PTEN and thyroid peroxidase expression in the differential diagnosis of benign and malignant follicular patterned thyroid lesions

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Öz

Giriş: Folliküler paternli tiroid lezyonlarının oranı son yıllarda belirgin bir şekilde artmıştır. Folliküler paternli tiroid lezyonları olan papiller tiroid karsinomu folliküler varyant, folliküler karsinom, folliküler adenom ve adenomatöz nodüllerin ayrımında sıklıkla yorum farklılıkları ile karşılaşırız. Amacım bu folliküler paternli lezyonları birbirinden ayırabilmektir.

Yöntem ve Gereçler: Çalışmamda hastanenin patoloji arşivlerindeki 119 olgu incelendi. Bu olgularda galektin 3, PTEN ve tiroid peroksidazın ekspresyonu değerlendirildi. Bu çalışmaya 27 papiller tiroid karsonomu folliküler varyant, 23 folliküler karsinom, 34 folliküler adenom ve 35 adenomatöz nodül olgusu alındı. İmmunohistokimyasal olarak galektin ve tiroid peroksidaz ile sitoplazmik boyanma, PTEN ile nükleer ve sitoplazmik boyanma değerlendirildi.

Bulgular: Malign lezyonlar ile kıyaslanan galektin 3'ün boyanma yüzdesi ve yoğunluğu, benign lezyonlarda belirgin olarak daha düşüktü. PTEN ile boyanmanın yoğunluğu ve yüzdesi, malign lezyonlar ile kıyaslandığında benign lezyonlarda daha yüksek idi. Malign lezyonlar ile kıyaslandığında tiroid peroksidazın boyanma yüzdesi ve yoğunluğu benign lezyonlarda belirgin olarak daha yüksek idi.

Tartışma ve Sonuç: Bu immunohistokimyasal boyalar birbiri ile karışan, özellikle papiller tiroid karsonomu folliküler varyant, folliküler karsinom, folliküler adenom ve adenomatöz nodülün ayırıcı tanısında beraberce kullanılabilir.

Anahtar Kelimeler: Galektin 3, PTEN, tiroid peroksidaz.

Abstract

Introduction: The rate of follicular patterned thyroid lesions has significantly increased in the recent years. We frequently encounter differences of interpretation in the differentiation of papillary thyroid carcinoma follicular variant, follicular carcinoma, follicular adenoma and adenomatous nodule which are follicular patterned thyroid lesions. My aim is to distinguish these follicular pattered lesions from each other.

Material and Methods: In my study, 119 cases were viewed from the pathology archives deposited of hospital. In these cases, it was evaluated expressions of galectin 3, PTEN and thyroid peroxidase immunohistochemical markers. Immunohistochemical evaluation revealed cytoplasmic staining with galectin 3 and thyroid peroxidase, nuclear and cytoplasmic staining with PTEN.

Results: The intensity and percentage of staining with galectin 3 compared with the malignant lesions were lower markedly in benign lesions. The intensity and percentage of staining with PTEN were higher in benign lesions compared with the malignant lesions. The intensity and percentage of staining with thyroid peroxidase compared with the malignant lesions were higher markedly in benign lesions.

Discussion and Conclusion: These immunohistochemical stains may be used together especially in the differential diagnosis of the papillary thyroid carcinoma follicular variant, follicular carcinoma, follicular adenoma and adenomatous nodule confused with each other.

Keywords: Galectin 3, PTEN, thyroid peroxidase.

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Introduction

Papillary thyroid carcinoma follicular variant (PCFV) is a papillary carcinoma consisting nearly entirely of follicles. The diagnosis was largely made considering nuclear properties seen in classically papillary carcinomas. Follicular carcinoma (FC) could be defined in a generic sense as any malignant thyroid tumor exhibiting evidence of follicular cell differentiation. This tumor is diagnosed considering capsular penetration or vascular invasion, or both. Follicular adenoma (FA) was described as encapsulated benign tumor showing the differentiation of follicular cells. This tumor show not capsular penetration or vascular invasion, and adenomatous nodule (AN) is diffuse enlargement of the thyroid with varying degrees of nodularity consisting of follicles.

In this study, follicular patterned thyroid lesions were compared in terms of intensity, percentage and type of staining with galectin 3 and PTEN, percentage and type of staining with thyroid peroxidase (TPO) (1-3).

Material and Methods:

Pathological reports and slides were obtained from the total or subtotal thyroidectomy materials stained with hematoxylin-eosin of 119 materials. This materials reported from hospital records revaluated. There was a single thyroid nodule in all of patients. Histologically, PCFVs were detected on the basis of nuclear clearing, nuclear groves, nuclear pseudoinclusions, multilayered polygonal cells and follicular architecture stained with cytokeratin 19. FCs were identified when clearly shown capsular penetration or vascular invasion, or both. Thick of capsules by slides of tissue sampling were shown. FAs were identified when not clearly shown capsular penetration or vascular invasion, or both. Fibrous capsule of varying thickness by slides of tissue sampling were shown. ANs were identified on the basis of round, uniform and normochromatic nucleus features. Despite the fact that there was sometimes a thin capsule there was not usually capsule. These materials were diagnosed as 27 PCFV, 23 FC, 34 FA, 35 AN. Slides that represent the lesions were selected for immunohistochemical study. It was checked whether cases were stained with antibodies before not stained cases prepared for immunohistochemical evaluation. It was used classical variant of papillary thyroid carcinoma in stain with Galectin 3, endothelial cells in stain with PTEN and thyroid tissue in the tumor neighborhood in stain with TPO. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded 5 µm-thick tissue sections. Galectin-3 (Neomarkers, monoclonal, 1 : 30 diluted), PTEN (Mob 369, klon 28H6 isotype Ig G1, Kappa, monoclonal, 1 : 45 diluted), TPO (DAKO, Klon MoAb47, M 7257, monoclonal, 1 : 30 diluted) antibodies were applied. The sections were deparaffinized, rehydrated in graded alcohols. Antigen retrieval was performed in a microwave oven for 15 minutes in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with a

3% H2O2-methanol solution, and the slides were incubated in 10% normal goat serum for 30 minutes to prevent non-specific staining. They were then incubated for 2 hours at room temperature with an appropriately diluted primary antibody.

The following mouse monoclonal antibodies were used: galectin-3, PTEN, TPO. Thereafter, the sections were incubated with biotinylated goat anti-polyvalent (Labvision) for 15 minutes and streptavidin peroxidase, (Labvision) for 15 minutes. 3-amino-9-ethyl carbazole-AEC (Labvision) was used as a chromogen, and the sections were counterstained with Mayer's hematoxylin. The preparations were evaluated under light microscope by a semiquantitative method. Although galectin 3 and TPO show cytoplasmic staining, PTEN shows cytoplasmic and nuclear staining. It was divided two parts into collected scores of the intensity and percentage of cytoplasmic and nuclear staining with PTEN and obtained outcome score. Immunohistochemical staining results of each a tumor group were evaluated with scoring systems that they may be meaningful. The intensity and percentage of staining with galectin 3 and PTEN, the percentage of staining with TPO were scored as follows.

The percentage of cytoplasmic staining with galectin 3;

Score 0 (0); 0-10% staining in the area of the lesion,

Score 1 (1+); 11-50% staining in the area of the lesion,

Score 2 (2+); 51-90% staining in the area of the lesion,

Score 3 (3+); 91-100% staining in the area of the lesion.

The intensity of cytoplasmic staining with galectin 3, cytoplasmic and nuclear staining with PTEN;

Score 0 (0); no staining in the area of the lesion,

Score 1 (1+); poor staining in the area of the lesion,

Score 2 (2+); moderate staining in the area of the lesion,

Score 3 (3+); strong staining in the area of the lesion.

The percentage of cytoplasmic staining with galectin 3, cytoplasmic and nuclear staining with PTEN;

Score 1 (1+); 0-25% staining in the area of the lesion,

Score 2 (2+); 26-50% staining in the area of the lesion,

Score 3 (3+); 51-75% staining in the area of the lesion,

Score 4 (4+); 76-100% staining in the area of the lesion.

The percentage of cytoplasmic staining with TPO;

Score 1 (1+); 0-80% staining in the area of the lesion,

Score 2 (2+); 81-100% staining in the area of the lesion.

SPSS 15.0 was used for statistical analysis. Spearman's rho test was used for paired group comparisons. Value of $p < 0.01$ was considered to be significant. Descriptive statistics were used for all group comparisons.

Table 1: Intensity of cytoplasmic staining in PCFV, FC, FA and AN with galectin-3

		INTENSITY OF STAINING WITH GALECTIN-3				**TOTAL**
		0	1+	2+	3+	
PCFV	n	1	3	10	13	27
	%	3.70	11.11	37.03	48.14	100
FC	n	10	6	5	2	23
	%	43.47	26.08	21.73	8.69	100
FA	n	30	4	0	0	34
	%	88.23	11.76	0	0	100
AN	n	35	0	0	0	35
	%	100	0	0	0	100
Total	n	76	13	15	15	119

Table 2: Intensity of cytoplasmic staining in malignant and benign lesions with galectin-3

		Intensity of staining with galectin-3				**Total**
		0	1+	2+	3+	
Malignant lesions (PCFV+FC)	n	11	9	15	15	50
	%	22	18	30	30	100
Benign lesions (FA+AN)	n	65	4	0	0	69
	%	94.20	5.79	0	0	100
Total	n	76	13	15	15	119

Table 3: Percentage of cytoplasmic staining in PCFV, FC, FA and AN with galectin-3

		PERCENTAGE OF STAINING WITH GALECTIN-3				TOTAL
		0	1+	2+	3+	
PCFV	n	1	2	11	13	27
	%	3.70	7.40	40.74	48.14	100
FC	n	10	5	5	3	23
	%	43.47	21.73	21.73	13.04	100
FA	n	30	4	0	0	34
	%	88.23	11.76	0	0	100
AN	n	35	0	0	0	35
	%	100	0	0	0	100
Total	n	76	11	16	16	119

Table 4: Percentage of cytoplasmic staining in malignant and benign lesions with galectin-3

		Percentage of staining with galectin-3				Total
		0	1+	2+	3+	
Malignant lesions (PCFV+FC)	n	11	7	16	16	50
	%	22	14	32	32	100
Benign lesions (FA+AN)	n	65	4	0	0	69
	%	94.20	5.79	0	0	100
Total	n	76	11	16	16	119

Table 5: Intensity of nuclear and cytoplasmic staining in PCFV, FC, FA and AN with PTEN

		INTENSITY OF STAINING WITH PTEN				TOTAL
		0	1+	2+	3+	
PCFV	n	4	13	7	3	27
	%	14.81	48.14	25.92	11.11	100
FC	n	2	9	8	4	23
	%	8.69	39.13	34.78	17.39	100
FA	n	2	6	15	11	34
	%	5.88	17.64	44.11	32.35	100
AN	n	1	3	12	19	35
	%	2.85	8.57	34.28	54.28	100
Total	n	9	31	42	37	119

Table 6: Intensity of nuclear and cytoplasmic staining in malignant and benign lesions with PTEN

		Intensity of staining with PTEN				Total
		0	1+	2+	3+	
Malignant lesions (PCFV+FC)	n	6	22	15	7	50
	%	12	44	30	14	100
Benign lesions (FA+AN)	n	3	9	27	30	69
	%	4.34	13.04	39.13	43.47	100
Total	n	9	31	42	37	119

Table 7: Percentage of nuclear and cytoplasmic staining in PCFV, FC, FA and AN with PTEN

		PERCENTAGE OF STAINING WITH PTEN				TOTAL
		1+	2+	3+	4+	
PCFV	n	5	12	7	3	27
	%	18.51	44.44	25.92	11.11	100
FC	n	3	8	8	4	23
	%	13.04	34.78	34.78	17.39	100
FA	n	1	6	16	11	34
	%	2.94	17.64	47.05	32.35	100
AN	n	1	2	13	19	35
	%	2.85	5.71	37.14	54.28	100
Total	n	10	28	44	37	119

Table 8: Percentage of nuclear and cytoplasmic staining in malignant and benign lesions with PTEN

		Percentage of staining with PTEN				Total
		1+	2+	3+	4+	
Malignant lesions (PCFV+FC)	n	8	20	15	7	50
	%	16	40	30	14	100
Benign lesions (FA+AN)	n	2	8	29	30	69
	%	2.89	11.59	42.02	43.47	100
Total	n	10	28	44	37	119

Table 9: Percentage of cytoplasmic staining in PCFV, FC, FA and AN with TPO

		PERCENTAGE OF STAINING WITH TPO		TOTAL
		1+	2+	
PCFV	n	19	8	27
	%	70.37	29.62	100
FC	n	16	7	23
	%	69.56	30.43	100
FA	n	11	23	34
	%	32.35	67.64	100
AN	n	0	35	35
	%	0	100	100
Total	n	46	73	119

Table 10: Percentage of cytoplasmic staining in malignant and benign lesions with TPO

		Percentage of staining with TPO		Total
		1+	2+	
Malignant lesions (PCFV+FC)	n	35	15	50
	%	70	30	100
Benign lesions (FA+AN)	n	11	58	69
	%	15.94	84.05	100
Total	n	46	73	119

Results

In benign lesions were seen markedly of galectin-3 expression loss in terms of the intensity and percentage of cytoplasmic staining compared with the malignant lesions. It was seen be expressed a more proportion in PCFV than FC in terms of the intensity and percentage of cytoplasmic staining of galectin-3 expressed highly in both PCFV and FC. AN being in benign lesions showed no staining with galectin-3 in terms of the intensity and percentage of cytoplasmic staining. Spearman's rho test revealed a statistically significant difference in benign and malignant lesions in terms of staining with galectin-3 ($p < 0.01$) (Table 1, Table 2, Table 3, Table 4, Figure 1).

It was seen 2+ and 3+ staining in benign lesions, 1+ staining in malignant lesions in terms of the intensity and percentage of cytoplasmic and nuclear staining with PTEN, markedly. Spearman's rho test revealed a statistically significant difference between malignant lesions and benign lesions ($p < 0.01$) (Table 5, Table 6, Table 7, Table 8, Figure 2, Figure 3).

It was seen 2+ staining in benign lesions, 1+ staining in malignant lesions in terms of the percentage of cytoplasmic staining with TPO, markedly. In other words, although TPO showed significantly expression in benign lesions, ıt showed markedly expression loss in malignant lesions. Spearman's rho test was revealed a statistically significant difference between malignant lesions and benign lesions ($p < 0.01$) (Table 9, Table 10, Figure 4).

Figure 1: Cytoplasmic galectin-3 expression in papillary carcinoma follicular variant (IHC; x400).

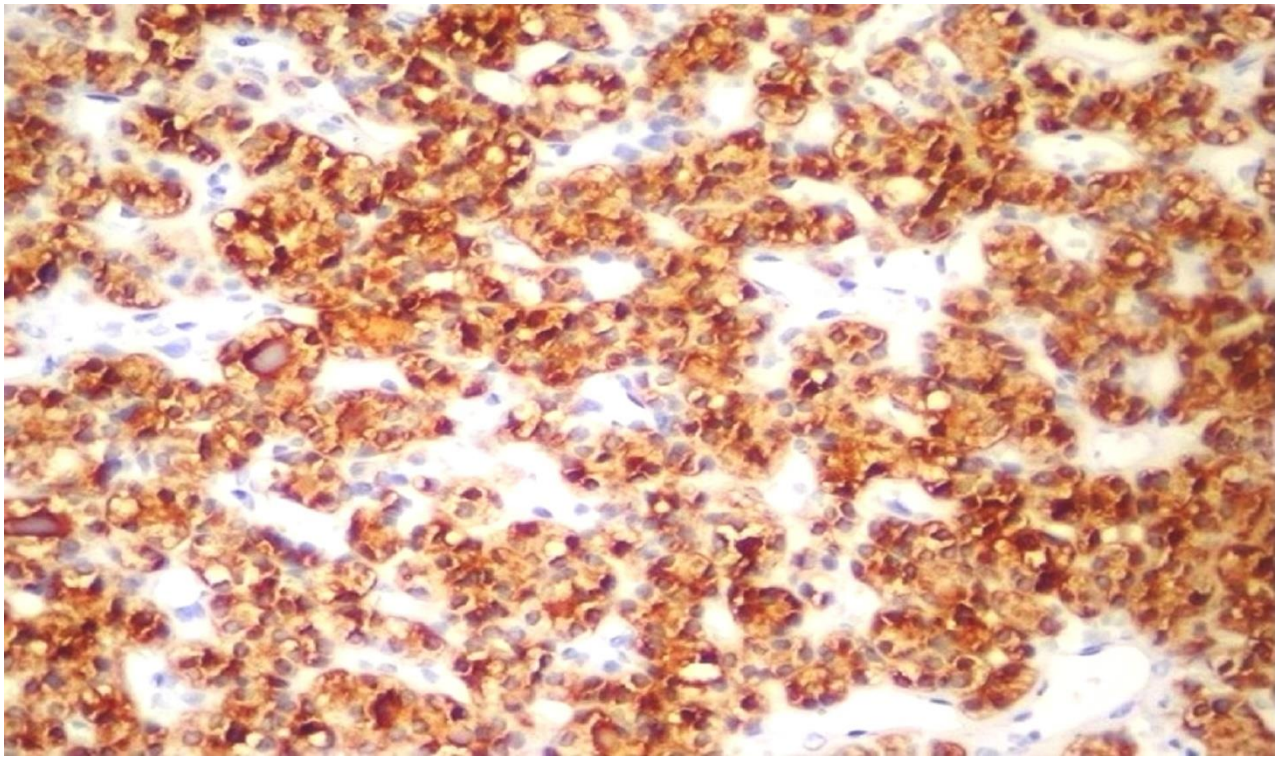


Figure 2: Nuclear PTEN expression in papillary carcinoma follicular variant (IHC; x400).

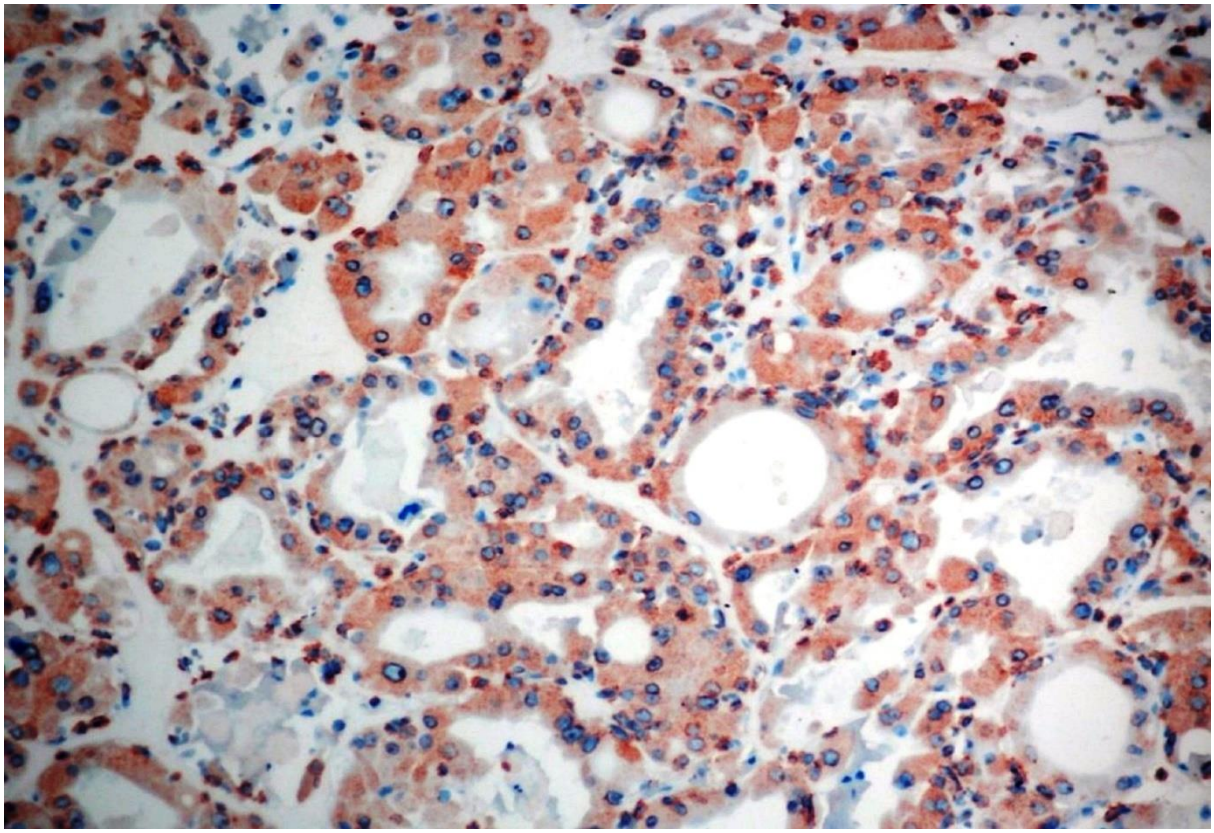


Figure 3: Cytoplasmic PTEN expression in papillary carcinoma follicular variant (IHC; x400).

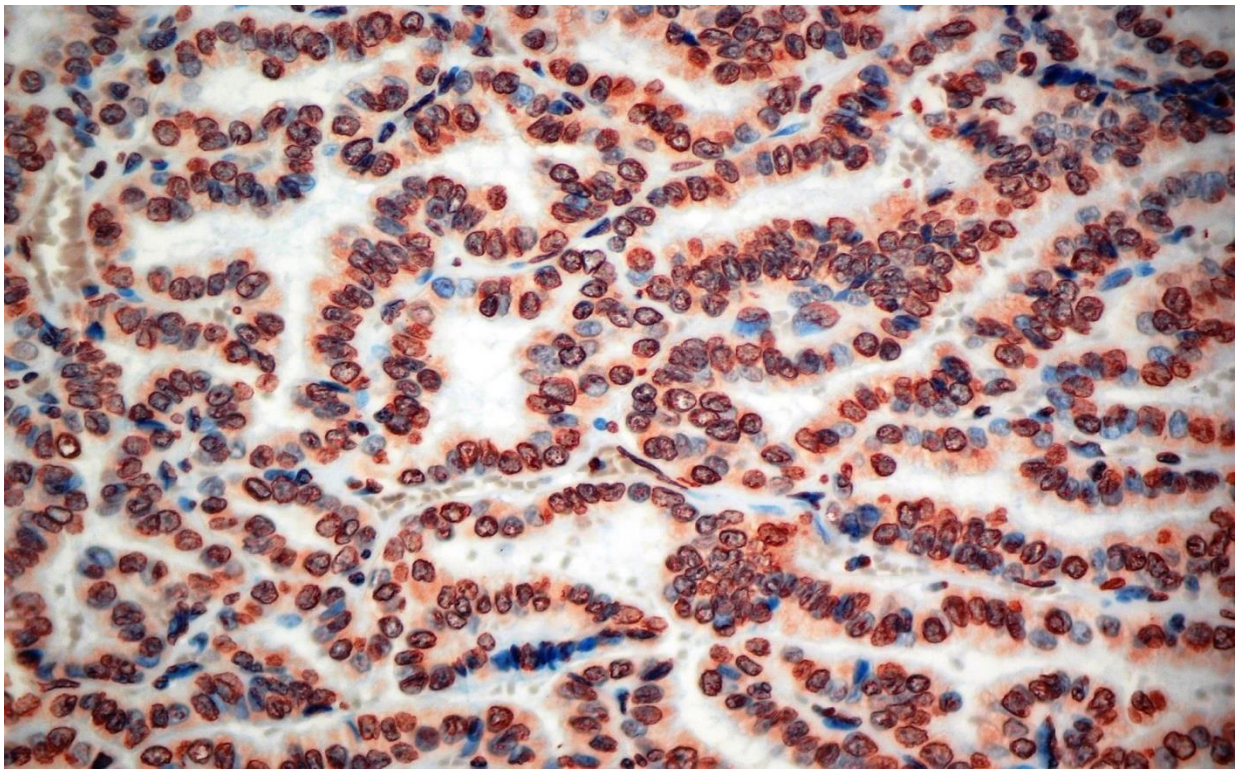
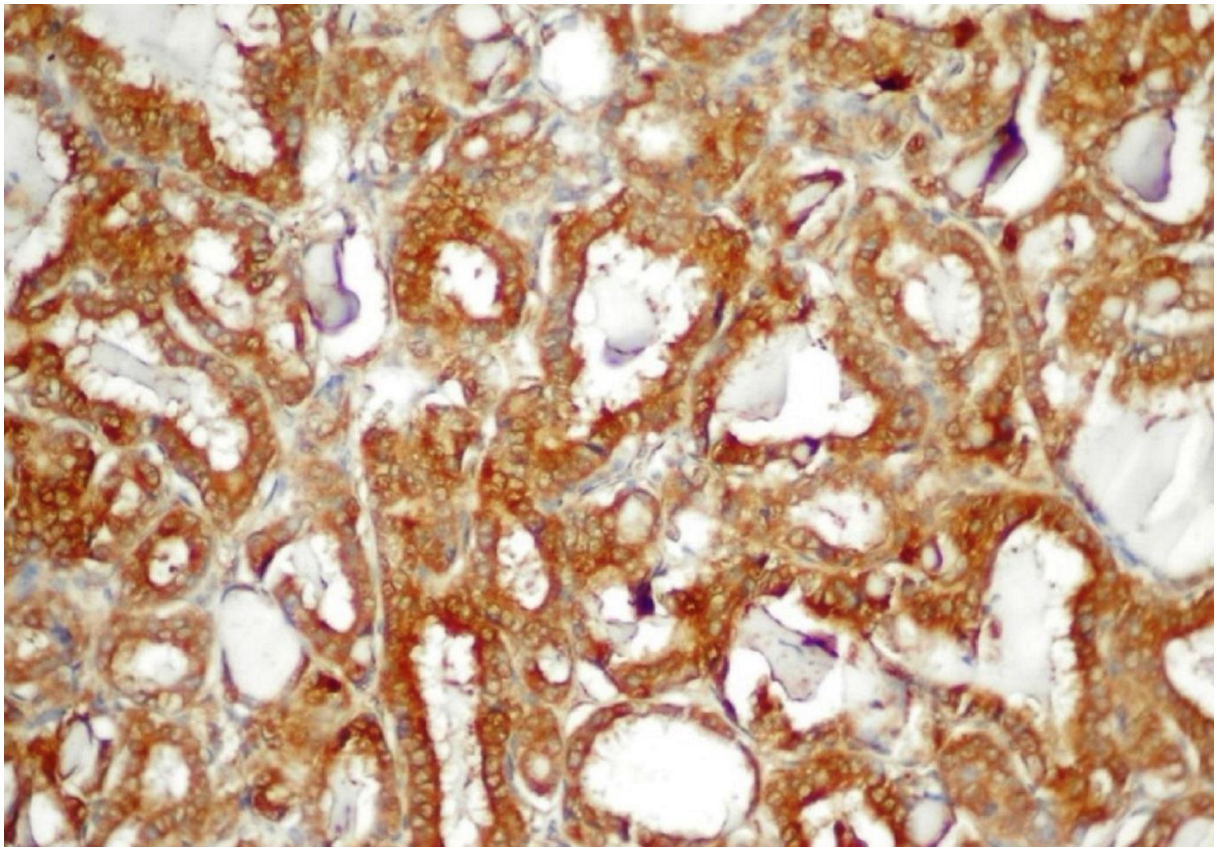


Figure 4: Cytoplasmic TPO expression in papillary carcinoma follicular variant (IHC; x200).



Discussion and Conclusion

Primary benign thyroid tumors or adenomatous nodules which resemble tumors are encountered generally. Multinodular goitre or thyroiditis have more than 50% of clinically evident single nodules. Well-differentiated encapsulated tumors of the thyroid gland with a follicular architecture may cause diagnostic difficulties. Because the presence of incomplete or equivocal capsular penetration and or focal nuclear changes such as clearing, grooves ,overlapping and pseudoinclusions which reminiscent the possibility of the follicular variant of papillary carcinoma may suggest the suspicion of follicular carcinoma. Depending on the severity of these changes and the bias of the observer, terms such as atypical adenoma, 'hybrid' carcinoma, and more recently well-differentiated carcinoma not otherwise specified were proposed (4-6).

The immunohistochemical method in evaluating thyroid tumors is used for the diagnosis and differential diagnosis. So far, it is show by studies which used of immunohistochemical marker in order to diagnosis for thyroidal lesions (7). Galectin-3 consisting of amino acids is β-galactosidase binding lectin. Galectin 3 which is more prominent in the cytoplasm is also found in the epithelium, in the nucleus and on immunized cell surfaces. Despite the fact that it is related to biological and

pathological states including apoptosis, inflammation, cell development and cell adhesion, is not known exactly role (8,9). Value of galectin-3 expression in discriminating benign and malignant thyroid nodules have shown in many studies. These studies with the aim of studying its reliability as a diagnostic indicator, particularly in differentiating problematic cases have been done on the IHC expression of galectin-3 on thyroid lesions which are inconclusive with routine H&E staining technique. PCFV and minimally-invasive FC include these studies. By doing so, it is supposed to facilitate surgical management and treatment. In a series of recent studies, galectin-3 in most malignant thyroid neoplasm has been found overexpressed. But, galectin-3 in normal and non-malignant tissue was not detectable (10). In my study, it is agreed as a useful marker to differentiate malignant thyroid lesions from benign thyroid lesions. In a study performed by Saggiorato et al. (8), FA and minimal invasive FC were evaluated histopathologically and strong galectin-3 reactivity in the case previously diagnosed as FA was found. Thereupon, serial cross-sections were performed and vascular invasion was seen. Conclusively, cytoplasmic (+) staining of galectin-3 should always be a warning and a great number of sampling should be made for malignancy criteria. In another study, cytoplasmic and nuclear staining with galectin-3 were seen in malignant neoplasms and no staining with galectin-3 was observed in benign thyroid lesions (11). In a study performed by Pisani et al. (12), specific cytoplasmic staining with galectin-3 was observed in a suspicious cell population in fine needle aspiration biopsy of a thyroid nodule. Occult papillary carcinoma was found in the operation material of this case and galectin-3 was concluded as a marker of malignancy. In another study, cytoplasmic and nuclear staining with galectin-3 were seen in malignant neoplasms and no staining with galectin-3 was observed in benign thyroid lesions (11). In a study performed by Mataraci et al. (13), when they compared malignant and benign lesions in terms of intensity of staining with galectin-3, they found +3 staining in 61.4% of malignant lesions and no staining was seen in 41.7% of benign lesions. In my case as compatible with the above studies, malignant and benign lesions were compared with percentage and intensity of staining with galectin-3, and a higher rate of staining was found in malignant lesions and a lower rate of staining was found in benign lesions, markedly.

PTEN deleted on chromosome 10 is the phosphatase and tensin homologue and is a tumor suppressor gene involved in the genesis of 40% to 75% of endometrioid adenocarcinomas. In contrast to p53, in which expression is up-regulated in most cases of p53 mutation, PTEN mutation results in immunohistochemical loss compared to normal tissues. PTEN loss has only rarely been documented in serous carcinomas (14,15).

Germline mutations of the PTEN tumor-suppressor gene located in chromosomal region 10q23.3 cause autosomal dominant multiple hamartoma and tumor syndrome. The tumor-suppressor gene PTEN encodes a 403-amino acid protein which belongs to the family of protein

tyrosine phosphatases and is emerging as the most frequently altered tumor suppressor gene other than p53 (16). A loss/reduction of PTEN expression which is a tumor-suppressor gene has been observed in thyroid neoplasms, with an inverse relationship with aggressiveness (17). It was reported that decreased of PTEN expression in approximately 40% of thyroid tumors (18,19). When PCFV, FC, FA and AN were compared with the percentage and intensity of staining with PTEN, in my study as compatible with earlier mentioned, decreased of PTEN expression in malignant lesions were shown.

TPO expressed mainly in the thyroid is an enzyme in the thyroid liberating iodine for addition onto tyrosine residues on thyroglobulin for the production of both triiodothyronine (T_3) and thyroxine (T_4) (20). It is TPO that is encoded by the TPO gene in humans. Expression of TPO which is associated with morphological differentiation and functional state of follicular cells is under the control of thyroid-stimulating hormone (3). TPO is not expressed in malignant thyroid lesions. Even if it is expressed, it has been reported that occur focal or lower levels of expression (21-23). But, it has been reported also it's be expressed at normal levels (24).

In my study as compatible with earlier mentioned, malignant and benign lesions were compared with percentage of staining with TPO, and a higher rate of staining was found in benign lesions and a lower rate of staining was found in malignant lesions, markedly.

Galectin-3, PTEN and TPO may be used together especially in cases where malignant and benign follicular patterned thyroid lesions are confused with each other.

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