The Role of Immunohistochemistry in Differential Diagnosis of Follicular Patterned Lesions of Thyroid

Tiroidin Folliküler Paternli Lezyonlarının Ayırıcı Tanısında İmmünohistokimyanın Rolü

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ABSTRACT

Objective: In the present study we aimed to assess the role of galectin-3, cytokeratin 19, thyroid peroxidase and CD44v6 in distinguishing benign from malignant follicular lesions.

Material and Method: Fifty-four malignant and 50 benign lesions were evaluated and classified according to World Health Organization 2004 histological classification. Galectin-3, cytokeratin 19, thyroid peroxidase and CD44v6 were performed immunohistochemically and the slides were evaluated by two independent investigators. Sensitivity, specificity and diagnostic accuracy were assessed for each antibody tested.

Results: Sensitivity, specificity and diagnostic accuracy were as follows respectively: Galectin-3: 59,25%, 84% and 71,15%; Cytokeratin 19: 70%, 82% and 75,4%; Thyroid peroxidase: 61%, 70% and 65,4%; CD44v6: 20,4%, 88% and 52,9%.

Conclusion: The negativity for Galectin-3 and Cytokeratin 19 can not exclude malignancy but positivity can be thought as a sign of malignant feature or potential for lesions in which there is strong suspect of malignancy. Thyroid peroxidase immunostaining failed to differantiate benign from malignant oxyphilic tumors but decreased expression can be used as a malignancy marker together with Galectin-3 and/or Cytokeratin19 positivity in suspicious cases. CD44v6 does not seem to be reliable in distinguishing benign from malignant follicular patterned thyroid lesions.

In conclusion, our approach is to take as much new samples or serial sections as possible in cases without clear-cut evidence of malignancy but with histological and immunohistochemical suspicion. Follicular variant papillary carcinoma has different criteria for malignancy and it should be always kept in mind while evaluating a benign-looking lesion with immunohistochemical signs that favor malignancy.

Key Words: Thyroid follicular neoplasia, Galectin-3, CD44v6, Thyroid peroxidase, Cytokeratin 19

ÖZ

Amaç: Bu çalışmada, Galektin-3, Sitokeratin 19, CD44v6 ve tiroid peroksidazın, tiroidin benign ve malign folliküler lezyonlarının ayrımındaki yerini ortaya koymak amaçlanmıştır.

Gereç ve Yöntem: 54 malign ve 50 benign lezyon Dünya Sağlık Örgütü 2004 sınıflaması ölçütlerine göre değerlendirilerek yeniden gruplandırıldı. İmmünohistokimyasal olarak Galektin-3, sitokeratin 19, tiroid peroksidaz ve CD44v6 uygulanan olgularda boyanma dağılımı iki bağımsız araştırmacı tarafından değerlendirildi. Sensitivite, spesifite ve tanısal doğruluk değerleri belirlendi.

Bulgular: Sensitivite, spesifite ve teşhiste değerlilik değerleri sırasıyla Galektin-3 için %59,25, %84 ve %71,15; sitokeratin 19 için %70, %82 ve %75,4; tiroid peroksidaz için %61, %70 ve %65,4 ve CD44v6 için %20,4, %88 ve %52,9 olarak saptandı.

Sonuç: Galektin-3 ve sitokeratin 19'un negatifliği maligniteyi ekarte ettirmemekle birlikte, özellikle histolojik açıdan maligniteden kuşkulanılan ancak tüm ölçütlerin karşılanmadığı olgularda pozitif boyanma malign özellik ya da potansiyel lehine değerlendirilebilir. Tiroid peroksidaz oksifilik tümörlerde benign/malign ayrımında başarısız olmakla birlikte, klinik ve histolojik açıdan malignite kuşkusu varlığında, azalmış ekspresyonu, Galektin-3 ve Sitokeratin19 pozitifliği ile birlikte malignite lehine yorumlanabilir. CD44v6 benign-malign ayrımında kullanılabilecek bir belirleyici olarak görünmemektedir.

Bizim yaklaşımımız histolojik olarak malignite şüphesi olan ancak kesin malignite bulgusu saptanamayan olgularda, immünohistokimyasal olarak malignite lehine sonuca varıldığında mümkün olduğunca çok parça ve kesitle olgunun detaylı incelenmesidir. Folliküler varyant papiller karsinom farklı malignite kriterleri taşımakta olup malignite lehine immünohistokimyasal sonuçlar elde edilen benign görünümlü olgularda akla gelmelidir.

Anahtar Sözcükler: Tiroid folliküler neoplazileri, Galektin-3, CD44v6, Tiroid peroksidaz, Sitokeratin 19

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INTRODUCTION

Solitary thyroid nodules are a frequent finding with a prevalence of 4-7% in the general population. Only the minority of these nodules (5-10%) turn out to be malignant. Identification of these malignancies preoperatively is a clinical challenge (1). Fine needle aspiration biopsies (FNAB) have been well-established to be highly accurate to discriminate malignant from benign lesions (2). However, this diagnostic procedure has limitation in differentiating benign follicular lesions from malignant ones (1-4). "Follicular patterned" thyroid lesions, a group that includes follicular adenomas (FA), follicular carcinomas (FC), the follicular variant of papillary carcinomas (FVPC) and even non-neoplastic, nodules of goiters (adenomatous nodules-AN), represent a major diagnostic dilemma in thyroid pathology also in surgical materials (5-9). Within this group FVPC represents a special group with its specific histological appearance- such as nuclear overlapping, intranuclear pseudoinclusions, optically clear nuclei and grooves. Diagnostic problems can arise where these features are present focally or multifocally rather than being diffusely distributed throughout the lesion (10).

In order to overcome the diagnostic limitations in follicular patterned lesions, several markers -such as galectin-3, HMBE-1, cytokeratin 19, CD44v6, thyroid peroxidase, S100, CD57 and CD10- have been proposed, in both surgical and FNA cytology specimens, (10-14). Among these, we aimed to assess the role of galectin 3 (Gal-3), cytokeratin 19 (CK19), CD44v6 and thyroid peroxidase (TPO) in distinguishing benign from malignant follicular lesions.

Galectin-3 is a member of the beta galactosidase binding lectin family which has been implicated in numerous biological and pathological processes including cell growth, adhesion, inflammation and apoptosis (10,14-16). Most previous studies in thyroid tissue have found Gal-3 expression to be feature of malignant but not benign or normal tissue (10,17-18).

Cytokeratin 19 is the lowest molecular weight cytokeratin and is found on a diverse range of normal epithelia and tumors. Strong and uniform expression of CK19 has been reported in all types of thyroid papillary carcinoma (10). The rate of immunoreactivity of CK19 in follicular carcinoma varies between 0%-100% in previous reports (10).

Thyroid peroxidase is involved in two different reactions in the biosynthesis of thyroid hormone: the iodination of tyrosine residues and the oxidative coupling of two iodothyrosine residues on thyroglobulin (18, 19). Its expression is associated with morphological differentiation and functional status of follicular cells (19-21). It has been reported that TPO is not expressed or expressed only focally in thyroid carcinomas (21-26).

CD44 is a polymorphic family of immunologically related cell-surface glycoproteins, which have a functional role in regulating several physiological and pathophysiological processes, including cell-cell, cell-matrix interactions, cell migration, and tumor growth and progression (8,13,27,28). CD44 can be expressed on the cell surface as a standard receptor (CD44s), as well as multiple isoforms (CD44v), the expression of which is qualitatively and quantitatively altered during tumor growth and progression (8,27,29). CD44v6 is not expressed on non-neoplastic thyroid tissue, and it can be used in the differential diagnosis of FA and FC (28,30).

MATERIALS AND METHODS

Tissue Specimens: H&E sections of 104 cases were evaluated from 1996 to 2005, according to WHO 2004 histological classification criteria (30). The cases consisted of 36 FA (15 were oncocytic variant), 17 minimally invasive follicular carcinoma (MIFC) (8 were oncocytic variant), 12 widely invasive follicular carcinoma (WIFC) (4 were oncocytic variant), 24 FVPC (1 was oncocytic variant), 1 well-differentiated carcinoma (WDC), and 14 AN (5 were oncocytic). One paraffin block having the most representative tumor area and including adjacent thyroid tissue wherever possible was selected for the immunohistochemical studies of each tumor.

Immunohistochemistry: Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections of 5 μm thick by using a manual biotin-free immunoperoxidase procedure with monoclonal mouse antibodies against human Galectin-3 (Neomarkers, MS-1756-R7/30 min incubation), cytokeratin 19 (Neomarkers, MS-198-R7/30 min incubation), CD44v6 (Neomarkers, MS-1093-R7/60 min incubation) and thyroid peroxidase (DAKO, clone MoAb47, M 7257; diluted 1:25/30 min incubation). For all antibodies tested, antigen retrieval treatment (3 min, in 10 Mmol/l citrate buffer solution (pH:6.0), using a domestic pressure cooker) was performed and immune complexes were then detected with the EnVision+ system (DAKO, K4001/30 min) to prevent endogenous biotin activity and visualized by diaminobenzidine precipitation. Slides were counterstained with Mayer's hematoxyline and mounted.

For Gal-3 renal cell carcinoma, vascular endothelium and histiocytes, for CK19 esophagus, for CD44v6 skin, and for TPO adjacent thyroid tissue was used as positive control. Negative controls were obtained by omitting the primary antibody.

Immunohistochemical Evaluation: All slides were evaluated by two independent investigators, blinded with respect to the histological diagnosis. The cells were regarded as positive for Gal-3, CK19 and TPO when immunoreactivity was clearly observed in their cytoplasm and for CD44v6 when immunoreactivity was observed in their cell membrane and cytoplasm. Immunoreactivity was graded as 0 (no staining), 1 (less than 10% of lesion positive), 2 (11-49% of lesion positive), 3 (more than 50% of lesion positive) for Gal-3, CK19 and CD44v6. For TPO, percentage of positive stained cells were assigned and positivity in less than 80% of cells was accepted as a sign of malignancy as previously reported (23).

Statistical Analyses: Sensitivity, specificity and diagnostic accuracy were assessed for each antibody tested. Sensitivity was defined as true positive/(true positive + false negative) and specificity as true negative/ (true negative + false positive). Diagnostic accuracy was defined as (frequency x sensitivity) + (1- frequency x specificity). The frequency was determined by calculating the ratio of malignant cases to all cases.

Chi-square and Fisher's exact tests were used for categorical data comparison (SPSS 13.00).

RESULTS

The results of immunohistochemical examination are summarized in Tables I, II. Sensitivity, specificity, diagnostic accuracy values are shown in Table III.

Galectin-3

With Gal-3 cytoplasmic staining was accepted as positive. Vascular endothelium and macrophages were also positive for Gal-3 as well as thyrocytes with oncocytic cytoplasm in lymphocytic thyroiditis areas and germinal centers of lymphoid follicles.

Among 50 benign cases, 8 cases (16%) were positive and among 54 malignant cases, 32 cases (59,25%) were positive

Table I: The intensity of the immunoreactivity in each diagnostic category

	AN	FA	MIFC	WIFC	FVPC	WDC
Total	14	36	17	12	24	1
Gal3*	0	8	6	9	16	1
No staining	14	28	11	3	8	0
<10% (1)	0	5	3	2	2	0
11-49% (2)	0	3	1	6	6	1
>50% (3)	0	0	2	1	8	0
CK19*	4	5	6	8	23	1
No staining	10	31	11	4	1	0
<10% (1)	4	4	1	4	6	0
11-49% (2)	0	1	4	1	11	1
>50% (3)	0	0	1	3	6	0
TPO*[a]	0	15	10	7	15	1
<=80%	0	15	10	7	15	1
>80%	14	21	7	5	9	0
CD44v6*	4	2	3	1	7	0
No staining	10	34	14	11	17	1
<10% (1)	1	1	0	0	2	0
11-49% (2)	1	1	2	1	2	0
>50% (3)	2	0	1	0	3	0

***AN:** adenomatous nodule, **FA:** follicular adenoma, **MIFC**: minimal invasive follicular carcinoma, **WIFC:** widely invasive follicular carcinoma, **FVPC:** follicular variant papillary carcinoma, **WDC:** well-differentiated carcinoma.*
** Number of cases which were positive out of total cases; [a] Immunoreactivity less than 80% of tumor cells was accepted as malignant feature.*

with Gal-3 (p<0.001). Positive stained benign cases were all FA. None of the ANs were positive with Gal-3.

Positivity of Gal-3 in each diagnostic category was compared with each other and the results were as follows; FCs expressed Gal-3 more than FAs and the difference was statisticaly significant (p=0.0013) (34.8% of positive cases were FA, 65.7 % were FC). Similarly FVPCs expressed Gal-3 more than FAs and the difference was again significant (p=0.001) (33.3% of positive cases were FA, 66.7% were FVPC) (Figures 1A-D; 2A-D).

Oncocytic and non-oncocytic subgroups of each lesion did not differ in positive staining with Gal-3 (p<1) (Tables I, III).

Cytokeratin 19

With CK19, cytoplasmic staining was accepted as positive. Nine out of 50 benign cases (18%) and 38 out of 54 malignant cases (70.3%) were positive with CK19. Benign follicular lesions and FAs alone showed significant statistical difference from malignant follicular patterned lesions in positive staining (p<0.0001 for both). The expression of CK19 in FCs and FVPCs was at least three-fold more than FAs (p=0.001 and p<0.001 respectively) (Figure 1,2). Among malignant cases, percentages of the CK19 expression in FVPC, WIFC and MIFC were 96%, 66% and 35% respectively (Table II).

Positivity of CK19 in oncocytic and non-oncocytic variants of each lesion was not different (p>0.05) (Table II).

Thyroid Peroxidase

We observed cytoplasmic staining with TPO. Immunoreactivity in less than 80% of tumor cells was observed in 15 of (30%) 50 benign cases, and 33 of (61.1%) 54 malignant cases (Figures 1A-D; 2A-D). All adenomatous

Table II: Immunoreactivity of Gal-3, CK19, TPO and CD44v6 in oncocytic and non-oncocytic subgroups of each diagnostic category

Lesion	Sub-group	Cases and %	Gal-3 n (%)	CK19 n (%)	TPO* <80% n (%)	CD44v6 n (%)
AN	AN	9 (100)	0	2 (22.2)	0	2 (22.2)
	AN-onc	5 (100)	0	2 (40)	0	2 (40)
	AN total**	**14 (100)**	**0**	**4 (28.6)**	**0**	**4 (28.6)**
FA	FA	21 (100)	5 (24)	2 (9.5)	4 (19)	2 (9.5)
	FA-onc	15 (100)	3 (20)	3 (20)	11 (73.3)	0
	FA total**	**36 (100)**	**8 (22,2)**	**5 (13.9)**	**15 (41.6)**	**2 (5.5)**
FC	MIFC non-onc	9 (100)	5 (55.5)	6 (66.6)	4 (44.4)	3 (33.3)
	WIFC non-onc	8 (100)	6 (75)	5 (62.5)	4 (50)	1 (12.5)
	MIFC-onc	8 (100)	1 (12.5)	0	6 (75)	0
	WIFC-onc	4 (100)	3 (75)	3 (75)	3 (75)	0
	MIFC total**	**17 (100)**	**6 (35.3)**	**6 (35.3)**	**10 (58.8)**	**3 (17.6)**
	WIFC total**	**12 (100)**	**9 (75)**	**8 (66.6)**	**7 (58.3)**	**1 (8.3)**
	FC total** (MIFK+WIFC)	**29 (100)**	**15 (52)**	**14 (48)**	**17 (58)**	**4 (14)**
FVPC	FVPC	23 (100)	15 (65.2)	22 (91.6)	15 (65.2)	6 (26.1)
	FVPC-onc	1 (100)	1 (100)	1 (100)	0	1 (100)
	FVPC total**	**24 (100)**	**16 (66.6)**	**23 (95.8)**	**15 (62.5)**	**7 (29.1)**
WDC**		**1 (100)**	**1 (100)**	**1 (100)**	**1 (100)**	**0**

***AN:** adenomatous nodule, **FA:** follicular adenoma, **FC:** follicular carcinoma, **MIFC:** minimal invasive follicular carcinoma, **WIFC:** widely invasive follicular carcinoma, **FVPC:** follicular variant papillary carcinoma, **WDC:** well-differentiated carcinoma, onc: oncocytic, non-onc: non-onkocytic, **Gal-3:** galectin-3, **CK19:** cytokeratin 19, **TPO:** thyroid peroxidase, **CD44v6:** CD44 variant 6*

**Immunoreactivity less than 80% of tumor cells was accepted as malignant feature; **Number of cases which were positive out of total cases.*

Table III: Sensitivity, specificity and diagnostic accuracy values of Gal-3, CK19, TPO and CD44v6 in differentiating benign (AN + FA) from malignant (FC+ FVPC + WDC) follicular patterned lesions

Marker	Sensitivity	Specificity	Diagnostic accuracy
Gal-3	59.25%	84%	71.15%
CK19	70%	82%	75.4%
TPO	61%	70%	65.4%
CD44v6	20.4%	88%	52.9%

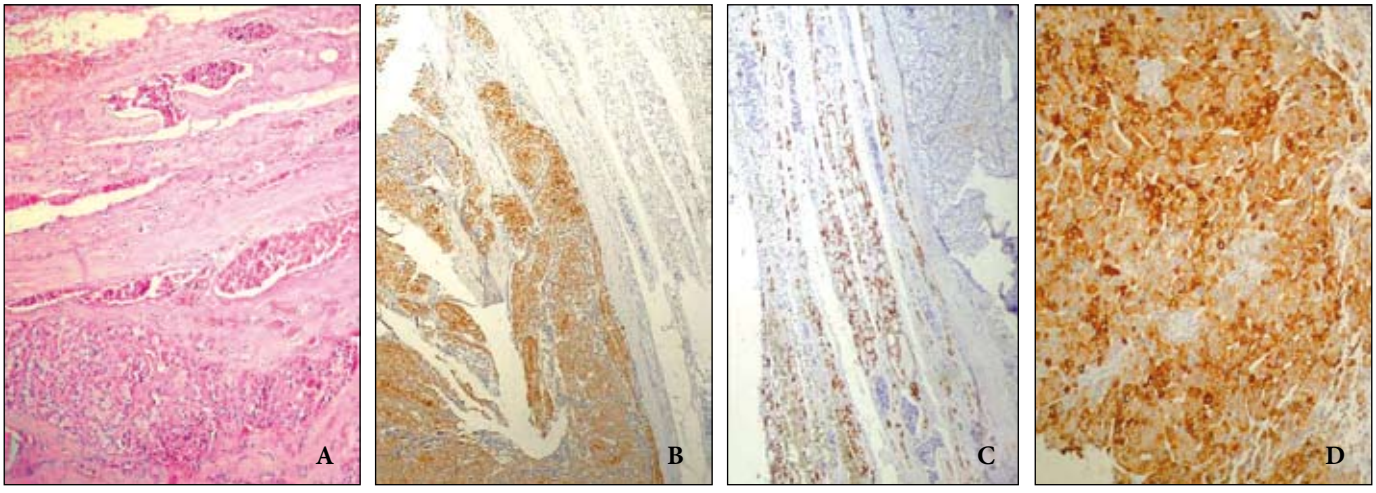

Figure 1: Follicular carcinoma **(A-D)**. Multipl foci of vascular invasion were present **(A)**. Gal-3 was positive in the neoplasm but negative in adjacent normal thyroid tissue **(B)**. With TPO, an opposite staining pattern was seen **(C)**. Strong and diffuse positivity for CK19 was found **(D)**.

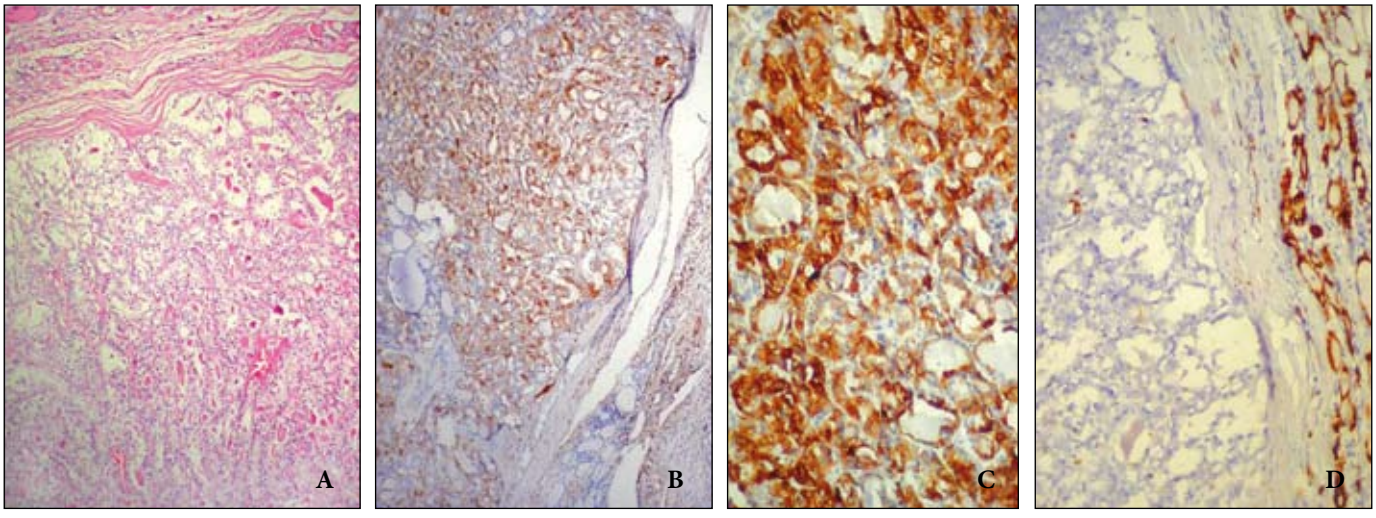

Figure 2: Follicular variant of papillary carcinoma **(A-D)**. Tumor cells were immunoreactive for Gal-3 **(B)** and CK19 **(C)**, but negative for TPO **(D)**. Positivity for TPO was found in the adjacent normal thyroid tissue (**D**).

nodules expressed TPO in more than 80% of tumor cells. When 80% was used as the cut-off point, there was a statistically significant difference in positive staining between benign and malignant cases (p=0.001).

Decreased expression was observed in oncocytic lesions. 26.7% of oncocytic variant of FAs and 81% of non-oncocytic FAs showed immunoreactivity in more than 80% of tumor cells (Figure 3). Thus the specificity decreased to 30.55% for distinguishing follicular adenoma from malignant follicular lesions (Table III).

When 80% was used as cut-off point, there was no statistiacally significant difference in staining intensity of

TPO with FA and FC, and with FA and FVPC (p>0.05) (Tables I, II).

CD44 variant-6

CD44 variant-6 (CD44v6) which showed membranous staining in epidermis and skin appandages of control block, was overexpressed in 17 of 104 cases. It showed membranous and in some cases membranous+cytoplasmic staining. Among the cases which showed positive reaction, 6 were in the benign group (12% of benign cases) and 11 were in malignant group (20.3% of malignant cases) (Figure 4A,B). It was positive in 4 of 14 adenomatous nodules. In one of the positive stained cases there were inflammatory cells within and around the nodule. In other cases there were hyalinization and myxoid changes. Among 29 FCs 4 were positive with CD44v6 (3 MIFC, 1 WIFC). A WIFC with bone metastases was negative. No relation was found between positive staining and tumor type, invasion or metastases (p=0.552) (Table I,III)

DISCUSSION

Thyroid nodules are common clinical problem in general population. Most of these lesions are benign (1, 3). FNAB helps to differentiate benign from malignant nodules and to design treatment plans (32). However, based on current criteria, 10-25% of these FNAB specimens reported as follicular pattern and patients underwent surgery for definite diagnosis. Among there, 75-80% are classified as benign lesions after surgery (2-4,32,33). However follicular patterned lesions of thyroid may pose a diagnostic challenge even to the most experienced pathologists, in histological sections as in cytology (5-10). There are two different problems in the diagnosis of follicular patterned lesions: the distinction of MIFC from FA or AN, and the confident identification of FVPCs (10,34). Nuclear features encountered in follicular nodules often show some, but not all, of the nuclear features of PC, making a clear cut diagnosis difficult. Making a decision on vascular or capsular invasion is also often difficult even in serial sections (34-35).

In order to overcome the problem several markers of malignancy have been investigated in both surgical and FNA cytology specimens, but they all present some advantages and some limitations (10,11-14).

Galectin-3 is one of the most promising markers in thyroid pathology. There are divergent results about usefulness of Gal-3 in differantial diagnosis of follicular patterned lesions in the literature. Most previous studies in thyroid tissue have found Gal-3 expression to be feature of malignant but not benign or normal tissue (2,10,17,36). In contrast some

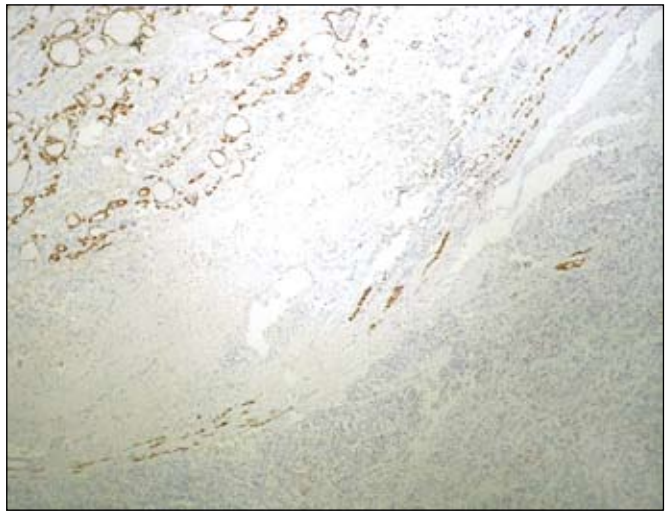

Figure 3: Follicular adenoma oncocytic variant was negative with TPO(*), while adjacent thyroid tissue was positive

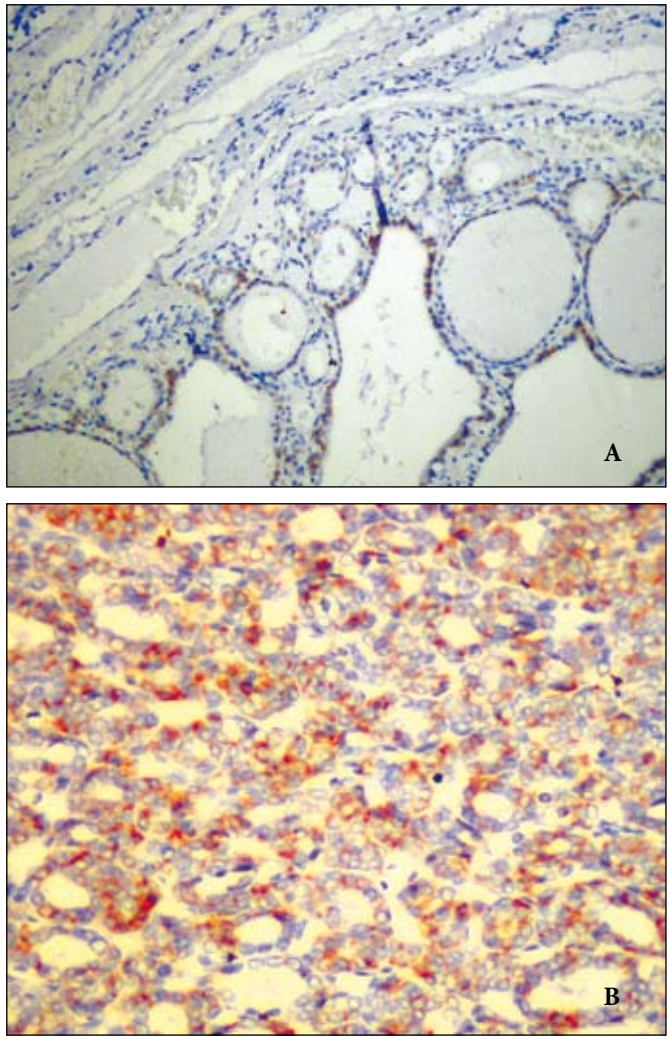

Figure 4: Positivity with CD44v6 in adenomatous nodule **(A)** and follicular variant of papillary carcinoma **(B)**.

have reported that it was not useful in differential diagnosis (37,38).

Sensitivity, specificity and diagnostic accuracy values are reported as 79-99%, 36-98% and 59-99% respectively in the literature (3,9,12,17,27,38-40). In the present study sensitivity was 59,25%, spesificity was 84% and diagnostic accuracy was 71,15%. Although sensitivity was not so high there were statistically significant difference in positive staining of benign and malignant follicular lesions.

Neither adenomatoid nodule was reactive with Gal-3 while eight (22,2%) of 36 FAs showed positivity with Gal-3. Previous studies report positivity in 0-33% of FA cases (2,10). Although no invasion of the capsule or blood vesels was detected in these positive cases, we cannot be sure whether they are true positivities in benign disease or a true reflection of invasive potential not demostrated histologically in sampled blocks. In one of our positive stained cases, positivity was observed in large, atypical cells that were comprising a distinct area in a nodule showing features of a typical FA. This area was positive with Gal-3 and CK19, and negative with TPO while the other parts showing opposite staining pattern (Figure 5A-D). It has been suggested that some of these positive cases, like in our case, might constitude cases undergoing malignant transformation (2,41). This needs to be studied further by using molecular and genetical techniques.

Staining intensity and percentage of positive cases in FVPC and WIFC were higher than in MIFC, as previously reported by Kedem et al (9). Recently Ito et al (41) demonstrated that Gal-3 expression level significantly increased with increasing degrees of vascular or capsular invasion by

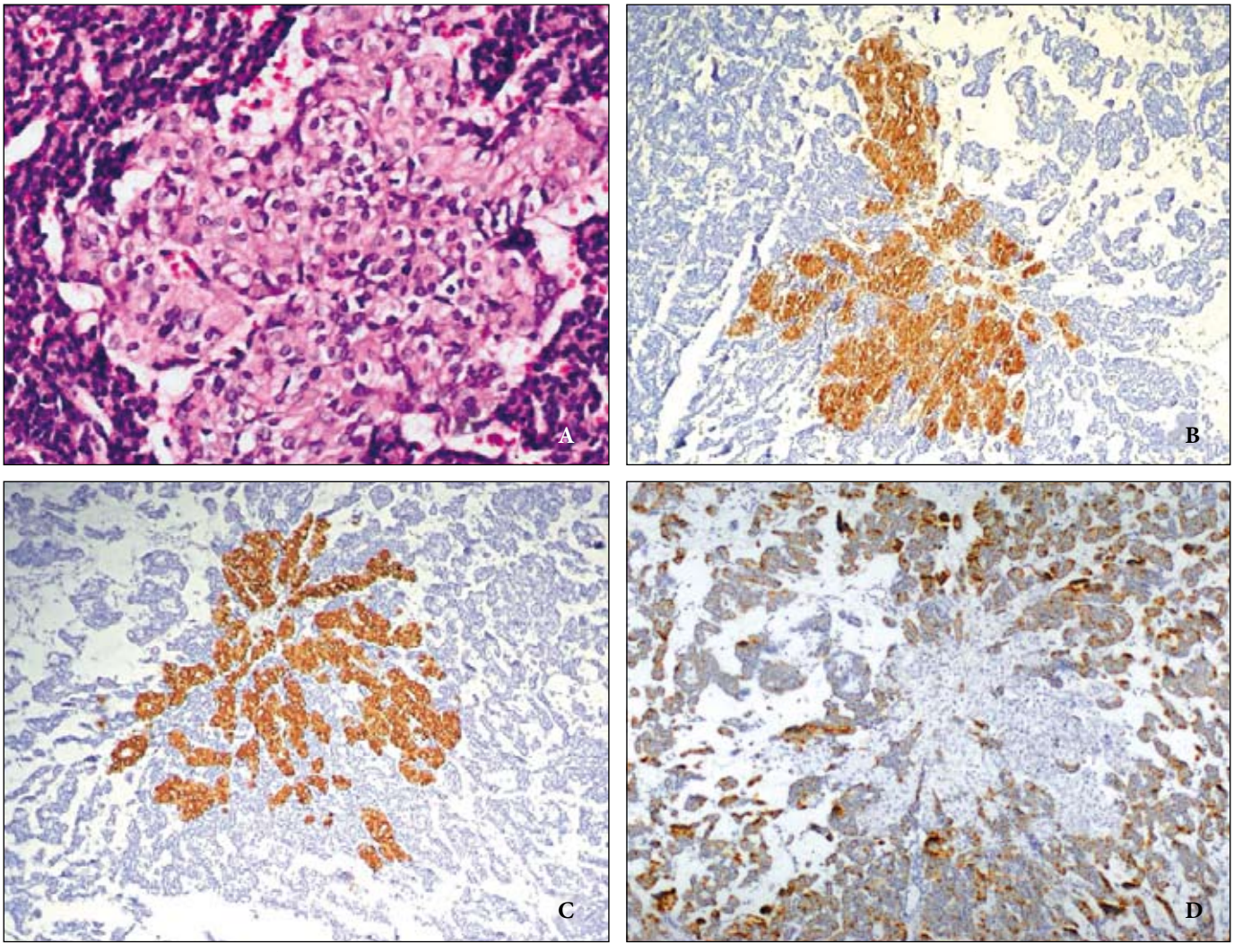

Figure 5: Large, atypical cells forming a distinct area in a nodule which was showing features of a typical FA**(A)**, were positive with Gal-3 **(B),** and CK-19 **(C),** and negative with TPO **(D)**.

follicular tumors. These findings support the idea that Gal-3 has a role in development of malignancy (9).

Oncocytic and non-oncocytic subgroups of each lesion did not differ in positive staining with Gal-3.

Cytokeratin 19 is one of the most frequently used markers in thyroid pathology. Strong and uniform expression of CK19 has been reported in all types of thyroid papillary carcinoma (10). Beesley et al (10) and Rorive et al (42) reported diffuse and strong expressivity of CK19 in a follicular patterned lesion with focal papillary carcinoma-like nuclear features, supported the diagnosis of FVPC. Immunoreactivity rates of CK19 in follicular carcinoma change between 0 % - 100% in the literature (10). In the present study, FVPCs expressed CK19 more than FAs and FCs, as reported in previous studies (10,35,43-46). It was positive in 23 of 24 FVPCs while 14 of 29 FCs and 4 of 36 FAs were positive with CK19. FC cases also expressed CK19 more than FAs and the difference was statistically significant. Oncocytic and non-oncocytic subgroups of each lesion were not differing in positive staining with CK19.

Four of 14 ANs, and 5 of 36 FAs were positive with CK19. All these positive cases were showing focal papillary carcinoma like nuclear features which were not enough for the diagnosis of FVPC andtheir staining intensity was weak except one case. Making a decision of malignancy therefore requires considering not only the positivity but also staining intensity.

Thyroid peroxidase has been reported to be not expressed or expressed only focally in thyroid carcinomas (21-26).

As previously reported, positivity in less than 80% of tumor cells was accepted as a sign of malignancy (23, 26). Sensitivity, specificity and diagnostic accuracy of TPO were 61%, 70%, 65.4% respectively. All ANs expressed TPO more than 80% of cells, but in FAs among 36 case 15 showed decreased expression with TPO. 11 of these 15 cases were oncocytic variant. As previously reported, TPO immunostaining failed to differantiate benign from malignant oxyphilic tumors (23). Qualitative changes might have occured in TPO while oncocytic properties were gained. We therefore could not visualize the expression by the antibody used.

CD44 variants, especially CD44v6, have been reported to be overexpressed during tumor growth and progression (8, 27, 29). It has been reported that this can be used in differential diagnosis of FA and FC (13,28,30). There were several studies reporting the use of CD44v6 with a second marker, i.e. Gal-3, in the differential diagnosis of follicular patterned lesions (8,27,30).

In the present study, CD44v6 was overexpressed in 17 of 104 cases. Among the cases which showed positive reaction, 6 were in the benign group and 11 were in the malignant group. Among 29 FCs, 3 MIFC and 1 WIFC were positive with CD44v6. A case which has bone metastases was negative with CD44v6. Oncocytic and non-oncocytic subgroups of each lesion did not differ in positive staining with CD44v6. No relation was found between immunoreactivity of CD44v6 and tumor type, invasion or metastases. It therefore does not seem to be reliable in distinguishing benign from malignant follicular patterned thyroid lesions even with a second marker.

To summarize, positivity of Gal-3 and CK19 can be thought as a sign of malignant feature or potential for the lesions of which there is strong suspect of malignancy in H&E stained sections. Integration of Gal-3 and CK19 immunoreactivity with both clinical and histological findings represents a reliable approach to the thyroid neoplasm. TPO is not a reliable marker in differential diagnosis of benign and malignant follicular lesions when it is used alone. In case of a clinical or histological suspicion, its decreased expression can be used as a malignancy marker together with Gal-3 and/or CK19 positivity. It should however be noted that decreased expression is also seen in benign oncocytic lesions. CD44v6 does not seem to be reliable in distinguishing benign from malignant follicular patterned thyroid lesions.

In conclusion, although the routine sections are the gold standard for determining malignancy in follicular lesions, our approach is to take as much new samples or serial sections as possible in cases without clear-cut evidence of malignancy but with immunohistochemical and histochemical suspicion. FVPC should always be kept in mind in a benign looking lesion with immunohistochemical signs that favors malignancy.

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