The Value of Cytokeratin-19 Immunohistochemistry in the Differential Diagnosis of Papillary Thyroid Carcinomas

Tiroid Papiller Karsinomlarının Ayırıcı Tanısında İmmünohistokimyasal Sitokeratin-19 Yararlılığı

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ABSTRACT Objective: The gold standard for diagnosis of papillary thyroid carcinoma is conventional histology, which depends on the characteristic nuclear features, regardless of whether papillary architecture is present or not. This study was carried on to evaluate the utility of cytokeratin-19 in the diagnosis and differential diagnosis of papillary thyroid carcinoma. **Material and Methods:** Expression of cytokeratin-19 was tested on formalin-fixed, paraffin-embedded tissues from 380 surgically resected thyroid lesions including hyperplasic nodules (n= 243), granulomatous thyroiditis (n= 3), lymphocytic (n= 53) and Hashimoto's thyroiditis (n= 11), follicular adenomas (n= 17), Hurthle cell adenomas (n= 4), well-differentiated thyroid tumor with follicular architecture of uncertain malignant potential (n= 1), papillary carcinomas (n= 45), follicular carcinoma (n= 1), insular carcinoma (n= 1), and medullary carcinoma (n= 1). The immunoreactivity was scored as negative, 1+, 2+, 3+, and 4+, based on the extent of the reaction regardless of previous diagnosis. **Results:** Positive reaction with cytokeratin-19 was denoted in all of the 45 cases of papillary carcinomas with scores of 4+, 3+ and 2+ and the ratios were 57.8% (26/45), 33.3% (15/45) and 8.9% (4/45), respectively. There seemed to be a strong diffuse cytoplasmic reactivity with cytokeratin-19 in papillary thyroid carcinomas. The sensitivity and specificity for cytokeratin-19 in papillary carcinomas among neoplastic thyroid lesions were 91.8% and 86.2%, respectively. **Conclusion:** In addition to careful histological evaluation, cytokeratin-19 seems useful for the diagnosis of papillary thyroid carcinomas.

Key Words: Carcinoma, papillary; cytokeratin-19; thyroid nodule

ÖZET Amaç: Tiroid papiller karsinomun tanısı için altın standart, papiller yapıların varlığı ya da yokluğunu dikkate almaksızın, karakteristik nükleer özellikleri temel alan geleneksel histolojidir. Çalışmamız, tiroid papiller karsinomun ayırıcı tanısında ve tanıda sitokeratin-19 yararını değerlendirmek için yapılmıştır. **Gereç ve Yöntemler:** Sitokeratin-19 ekspresyonu, hiperplasik nodül (n= 243), granülomatöz tiroidit (n= 3), lenfositik (n= 53) ve Hashimoto's tiroiditi (n= 11), foliküler adenoma (n= 17), Hurthle hücreli adenoma (n= 4), malignite potansiyeli belli olmayan foliküler iyi-diferansiye tiroid tümörü (n= 1), papiller karsinom (n= 45), foliküler karsinom (n= 1), insuler karsinom (n= 1) ve medullar karsinomu (n= 1) içeren cerrahi olarak çıkartılmış 380 tiroid lezyonundan hazırlanmış formalinle tespit edilmiş parafine gömülü dokularda test edilmiştir. İmmünreaktivitesi, önceki tanılarına bakılmaksızın reaksiyon yoğunlukları temel alınarak negatif, 1+, 2+, 3+, 4+ olarak skorlanmıştır. **Bulgular:** 45 papiller karsinom olgusunun tamamında, sitokeratin-19 ile 4+, 3+ ve 2+ skorlarında pozitif reaksiyon tespit edilmiştir ve oranları da sırasıyla %57.8 (26/45), %33.3 (15/45) ve %8.9 (4/45)'dur. Tiroid papiller karsinomlarında, sitokeratin-19 ile difüz, kuvvetli sito-plazmik reaksiyon olduğu gösterilmiştir. Neoplastik tiroid lezyonları arasında papiller karsinom için sitokeratin-19 duyarlılığı ve seçiciliği sırasıyla %91.8 ve %86.2 olarak bulunmuştur. **Sonuç:** Dikkatli histolojik değerlendirmeyle beraber, sitokeratin-19'un, tiroid papiller karsinomların tanısında yararlı olduğu görülmektedir.

Anahtar Kelimeler: Papiller karsinom; sitokeratin-19; tiroid nodülü

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Diagnosis and pathologic classification of thyroid lesions are based on the microscopic appearance of the specimens. Despite general acceptance of the World Health Organization (WHO) classification of thyroid tumors, the histological diagnosis of thyroid cancer remains one of the most conflicting areas in surgical pathology. The differential diagnosis between benign and malignant follicular tumors and differential diagnosis between papillary and follicular carcinomas may especially raise difficulties.[1]

Papillary thyroid carcinoma is the most common malignancy of thyroid follicular epithelium.[1-8] The gold standard for diagnosis of papillary thyroid carcinoma is conventional histology, which depends on the characteristic nuclear features, regardless of whether papillary architecture is present or not.[2,5,9-12] However, the interpretation of nuclear features may be quite subjective and interobserver disagreements among pathologists are well documented.[11,12] Additionally, morphologic similarities between benign and malignant lesions are frequent; the papillary architectures may be present in both benign and malignant lesions.[2,9]

The prognosis and management of thyroid carcinomas depend on their diagnoses.[2,13] Several studies showed that the use of monoclonal anti-keratin antibodies in tumor pathology may be helpful in differential diagnosis or estimating prognosis of thyroid tumors.[1,3,11,13-15] Cytokeratin-19 (CK-19) is a low molecular weight keratin and is found in a diverse range of normal epithelium and tumors. CK-19 was reported to be strongly and diffusely expressed in papillary carcinoma, whereas it is usually absent or focally expressed in benign follicular nodules.[3,11,12,14,15] This study was carried out to evaluate the contribution of CK-19 in the diagnosis and differential diagnosis of papillary thyroid carcinoma.

MATERIAL AND METHODS

Tissue Specimens

A total of 380 thyroidectomy specimens were included in this study. Of these, 360 were diagnosed as benign and malign thyroid nodules in the Department of Pathology, Zonguldak Karaelmas University Faculty of Medicine between 2001 and 2006 and 20 were diagnosed as papillary thyroid carcinomas in the Department of Pathology in İstanbul Lütfi Kırdar Kartal Training and Research Hospital. All histological sections were fixed in formalin, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin (HE). Lesions that consisted of follicles displaying significant variety in size and lined by flattened epithelium were described as hyperplasic nodules. Thyroid tumors were classified according to the WHO criteria as Hurthle cell adenoma, follicular adenoma, and well-differentiated thyroid tumor with follicular architecture of uncertain malignant potential, papillary carcinoma, follicular carcinoma, insular carcinoma, and medullary carcinoma.[16]

Immunohistochemistry

Expression of CK-19 was tested on formalin-fixed, paraffin-embedded tissues from 380 surgically resected thyroid lesions, including hyperplasic nodules (n= 243), granulomatous thyroiditis (n= 3), lymphocytic (n= 53) and Hashimoto's thyroiditis (n= 11), follicular adenomas (n= 17), Hurthle cell adenomas (n= 4), well-differentiated thyroid tumor with follicular architecture of uncertain malignant potential (n= 1), papillary carcinomas (n= 45), follicular carcinoma (n= 1), insular carcinoma (n= 1) and medullary carcinoma (n= 1).

For immunohistochemical studies, immunostaining was performed by the streptavidin-biotin-peroxidase complex technique. The sections in paraffin were collected on slides; the paraffin was removed and the sections were rehydrated. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. The sections were incubated with primary antiserum, including MS-1671-P1, CK-19 Ab-4 mouse monoclonal antibody (Lab Vision Corporation, clone BA17, Fremont, CA). After washing in phosphate-buffered saline, the tissues were first incubated with a biotin-conjugated secondary antibody and then with the streptavidin-biotin system for 30 min at room temperature. The reactions became visible after immersion of the specimens in diaminobenzidine tetrahydrochloride. The sections were counterstained with HE stain, then rinsed and mounted.

Immunohistochemical Evaluation

CK-19 expression was only cytoplasmic. The immunoreactivity was scored as negative (less than 10% cells positive); 1+ (poor-focal cells positivity); 2+ (strong-focal cells positivity); 3+ (poor-diffuse cells positivity); and 4+ (strong-diffuse cells positivity) based on the extent of the reaction regardless of previous diagnosis.

Statistical Analysis

Statistical analyses were carried out by SPSS for Windows 11.0 (SPSS Inc; Chicago, III). Diffuse staining patterns with 3+ and 4+ scores were accepted as significantly positive, and sensitivity (true positive/true positive + false negative) and specificity (true negative/true negative + false positive) of CK-19 were assessed in both malignant lesions as a whole and in papillary thyroid carcinomas in particular.

RESULTS

The expression of CK-19 in various thyroid lesions was documented in Table 1. All of the 45 cases of papillary carcinomas were positively reacted with CK-19; the immunoreactivity scores were 4+, 3+ and 2+ and the ratios were 57.8% (26/45), 33.3% (15/45) and 8.9% (4/45), respectively (Figures 1 and 2). Here, to prevent confusion about 2+ scored group, it was to be said that this group was the micropapillary variant of papillary carcinomas. 100% (1/1) of well-differentiated thyroid tumor with follicular architecture of uncertain malignant potential, 5.9% (1/17) of follicular adenoma including areas of degeneration and 3.8% (2/53) of lymphocytic thyroiditis were reacted 4+ (Figures 3 and 4). 11.8% (2/17) of follicular adenoma, 18.2% (2/11) of Hashimoto's thyroiditis and 1.9% (1/53) of lymphocytic thyroiditis displayed reaction 3+. No CK-19 expression were found in 100% (243/243) of hyperplasic nodules, 1.9% (1/53) of lymphocytic thyroiditis, 33.3% (1/3) of granulomatous thyroiditis, 52.9% (9/17) of follicular adenomas, 75.0% (3/4) of Hurthle cell adenomas, and 100% (1/1) of follicular carcinoma. Other cases were showed focal reactions.

The sensitivity and specificity for CK-19 expression were 87.5% and 97.5%, respectively, in malign thyroid lesions. In papillary carcinomas among neoplastic thyroid lesions, the sensitivity

TABLE 1: Expression of CK-19 in various thyroid lesions.

Diagnosis	CK-19 Score 0 % (n)	1 % (n)	2 % (n)	3 % (n)	4 % (n)
Carcinoma					
Papillary (n= 45)	-	-	8.9 (4)	33.3 (15)	57.8 (26)
Follicular (n= 1)	100 (1)	-	-	-	-
Insular (n= 1)	-	-	100 (1)	-	-
Medullary (n= 1)	-	-	100 (1)	-	-
Neoplasms of undetermined Malignant potential (n= 1)	-	-	-	-	100 (1)
Adenoma					
Hurthle cell adenoma (n= 4)	75.0 (3)	-	33.3 (1)	-	-
Follicular adenoma (n= 17)	52.9 (9)	11.8 (2)	17.6 (3)	11.8 (2)	5.9 (1)
Non-neoplastic Thyroid					
Granulomatous thyroiditis (n= 3)	33.3 (1)	-	66.7 (2)	-	-
Hashimoto's thyroiditis (n= 11)	-	36.4 (4)	45.5 (5)	18.2 (2)	-
Lymphocytic thyroiditis (n= 53)	1.9 (1)	13.2 (7)	79.2 (42)	1.9 (1)	3.8 (2)
Hyperplasic nodules (n= 243)	100 (243)	-	-	-	-
Total (n= 380)					

CK-19: Cytokeratin-19,

0 (less than 10% cells positive), 1+ (poor-focal cells positivity), 2+ (strong-focal cells positivity), 3+ (poor-diffuse cells positivity), 4+ (strong-diffuse cells positivity).

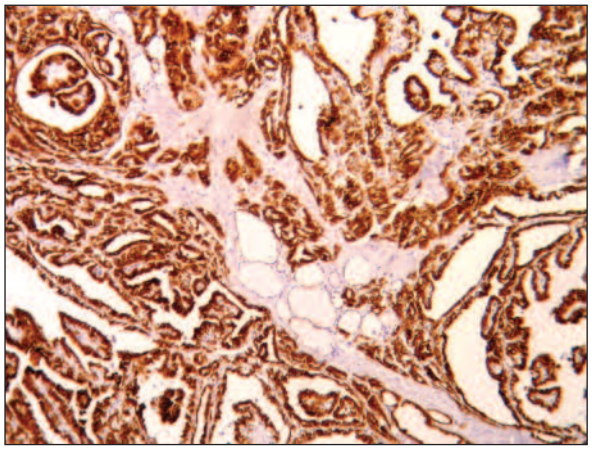

FIGURE 1: Diffuse-strong CK-19 expression in classic variant of papillary thyroid carcinoma (B-SA, DAB, x200).

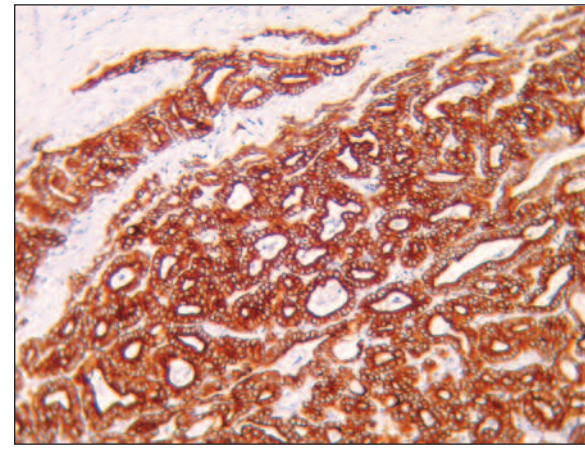

FIGURE 2: Follicular variant of papillary thyroid carcinoma showing diffuse-strong CK-19 reaction (B-SA, DAB, x200).

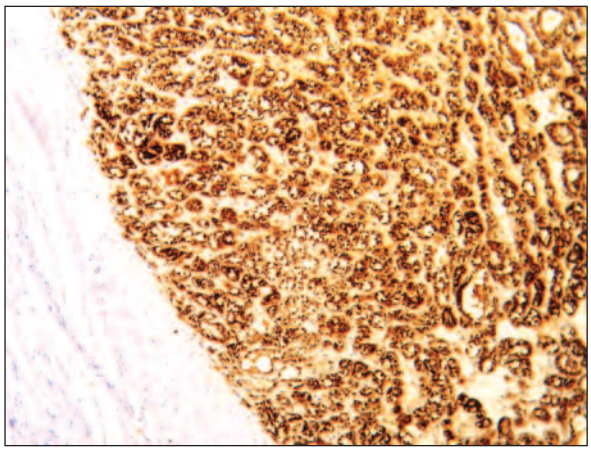

FIGURE 3: CK-19 expression in follicular adenoma (B-SA, DAB, x100).

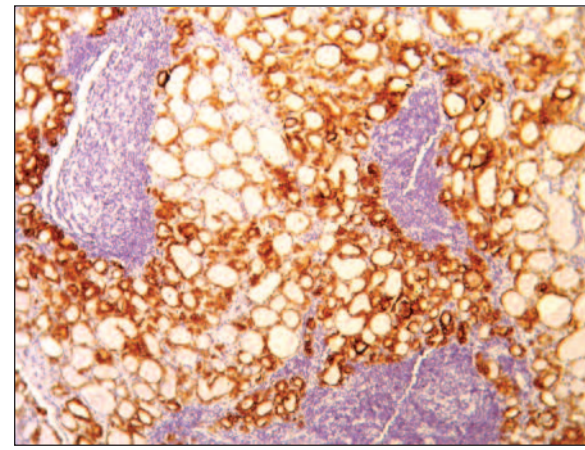

FIGURE 4: CK-19 expression in lymphocytic thyroiditis (B-SA, DAB, x100).

and specificity for CK-19 expression were 91.8% and 86.2%, respectively.

DISCUSSION

It is estimated that, 4% of the adult population is affected by one or more palpable thyroid nodules. Most of these lesions are benign, so the indication for their surgical removal should be as limited as possible.[17-19] Thyroid cancers are among frequently observed tumors.[20] Although the differential diagnosis between papillary carcinoma and benign lesions is easily made by histological examination, some lesions exhibiting hyperplasic features represent a diagnostic problem. In fact, overlapping morphological features between benign and malignant lesions are frequent, and follicular and papillary patterns are seen in both. Several critical features of malignancy, for example the optically clear nuclei of papillary carcinoma are open to subjective interpretation and nuclear clearing has also been reported in hyperfunctioning lesions.[11,13,17]

Immunohistochemistry was introduced to the practice of pathology in the early 1970s, but in thyroid pathology, its use has been restricted to the differential diagnosis between follicular and C-cell derived neoplasms. The detection of new markers of malignancy, which may distinguish malignant from benign lesions regardless of the presence of

capsular or vascular invasion, has provided interesting insights into the role of immunohistochemistry in thyroid neoplasms. Therefore, these antibodies can play an important role in the malignant transformation of thyroid cells and they are highly expressed in thyroid carcinomas. CK-19, which is one of these markers is expressed in all follicular cell-derived carcinomas and is denoted with higher expression in papillary thyroid carcinomas as in our study.[11,13,18]

The role of CK-19 in the diagnosis of papillary thyroid carcinoma is still controversial.[2,4,17] This may be partially due to the subjectivity involved in assessing positive expression.[2] CK-19 was shown to have higher expression in papillary thyroid carcinomas than in benign follicular lesions of the thyroid like in this study; the results vary among studies.[2,4,6,11,15] Sahoo et al found CK-19 expression in all benign tumors although in the majority, CK-19 is expressed in <5% of tumor cells.[21] Other investigators reported diffuse expression in papillary thyroid carcinoma when compared with the focal expression in other tumors and nodular goiters.[2,11,21]

Cheung et al demonstrated diffuse CK-19 immunoreactivity in 66% (91/138) of papillary carcinomas, 50% (3/6) of insular carcinomas and 29% (2/7) of Hurthle cell carcinomas. In the same study, focal strong reaction was present in 14 out of 75 benign lesions; the majority of the immunoreactivity occurred in areas of degeneration, indicating the reactive nature of CK-19 positivity.[9,11,13] Park et al reported that almost all papillary carcinomas including follicular variants of papillary carcinomas showed strong and diffuse CK-19 immunoreactivity.[11] CK-19 expression accounted for 85% of papillary thyroid carcinomas and 26% of non-papillary thyroid carcinomas and non-neoplastic thyroid lesions in the study by Demellawy et al[12] In the present study, diffuse CK-19 expression was demonstrated in 100% of papillary carcinomas including the micropapillary variant, 100% of neoplasms of undetermined malignant potential, 17.7% of follicular adenoma, 18.2% of Hashimoto's thyroiditis and 5.2% of lymphocytic thyroiditis. Follicular adenoma exhibited diffuse immunoreactivity for CK-19 in our study. One follicular adenoma (1/35) exhibited diffuse positivity for CK-19 in the study by Cheung et al Some benign follicular lesions expressed CK-19; the significance of this remains unclear. Cheung et al suggested that this reaction pattern was consistent with focal CK-19 reactivity in areas of degeneration, usually at the site of previous fine-needle aspiration biopsy. No CK-19 expression was determined in 4 follicular carcinomas by Cheung et al[9] The present study is concordant with these findings.

In the present study, CK-19 was also detected in lymphocytic thyroiditis supporting previous studies.[7,15] Lymphocytic infiltration or classic Hashimoto's thyroiditis is reported in association with 30-58% of papillary thyroid carcinomas, 20% of follicular carcinomas and 14% of follicular adenomas. Focal expression of CK-19 was noted in 17-65% of Hashimoto's thyroiditis cases with papillary thyroid carcinoma-like nuclear alterations by Prasad et al[22] Unfortunately, in our study, CK-19 was also focally expressed in Hashimoto's thyroiditis, lymphocytic thyroiditis and some benign tumors as confirmed in previous studies.[2-4,6,7,14,15] In addition, CK-19 was also expressed in squamous metaplasia.[4,14,15,22]

Similar to the results in our study, CK-19, which appears as diffuse cytoplasmic reactivity was shown to be a sensitive marker for papillary carcinomas by other authors also.[2-4,6,7,14,15,23,24] Besides careful histological evaluation, CK-19 seems useful for the diagnosis of papillary thyroid carcinomas. Although focal CK-19 reaction may be found in benign lesions like in this study, diffuse positivity is characteristic of malignancy and papillary thyroid carcinomas.[4,24] CK-19 expression may be helpful in differential diagnosis.[24]

In conclusion, we suggest that the analysis of thyroid lesions by the immunoperoxidase method using monoclonal antibody against CK-19 provides a valuable additional tool in routine surgical pathology in differential diagnosis of papillary thyroid carcinomas.

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