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Diagnostic utility of cytokeratins 7, 10 and 20 in renal cell carcinoma and oncocytoma

Sitokeratin 7, 10 ve 20'nin böbrek hücreli karsinom ve onkositom tanısındaki yeri

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ABSTRACT

Renal cell carcinoma is the most frequent renal epithelial tumor having various subtypes differing in their prognosis and therapeutic response. In most cases it is possible to distinguish subtypes on the basis of histology alone, however, there are diagnostic difficulties for the tumors having granular/eosinophilic cells which create morphologic similarities not only between the subtypes of renal cell carcinoma, but also, between a benign tumor and renal oncocytoma. To achieve correct diagnosis, immunohistochemical analysis focused on cytokeratin (CK) proteins has been used increasingly. We examined the diagnostic utility of CK7, CK10, and CK20 in the classification of renal epithelial tumors based upon an immunohistochemical analysis. The study included tissue macroarray (4 mm) blocks of 83 renal cell carcinomas (62 clear cell, 6 chromophobe, 13 papillary, 2 unclassified subtype), and 6 renal oncocytomas. Fuhrman nuclear grade of the tumors, divided into low (grades 1, 2) and high nuclear grade (grades 3, 4) was negatively correlated with CK7 expression (p=0.001). Diffuse and significantly higher CK7 expression was found in "non-clear cell" (chromophobe and papillary) subtypes than in clear cell renal cell carcinomas (p=0.001). Of 6 renal oncocytomas, 4 was focally positive for CK7. The results demonstrate that, diffuse and strong CK7 immunoreactivity supports the diagnosis of "non-clear cell" subtype versus clear cell renal cell carcinoma and renal oncocytoma. Seldom CK20 reactivity of the tumors did not show any significance, and the tumors were totally unreactive to CK10 which eliminates diagnostic utility of CK20 and CK10 in the classification of renal epithelial tumors.

Key words: Cytokeratin 7, cytokeratin 20, cytokeratin 10 renal cell carcinoma, renal oncocytoma

ÖZET

Böbrek hücreli karsinom erişkin böbrek epitelyal tümörlerinin büyük kısmını oluşturmakta, prognoz ve tedaviye cevap farklılığı gösteren değişik histopatolojik subtipler içermektedir. Böbrek hücreli karsinom subtiplerinin birbirinden ve onkositomdan ayırımı önemlidir. Böbrek hücreli karsinom subtipleri genellikle rutin histopatolojik incelemeyle ayırt edilebilirken, başlıca granüler/eozinofilik hücre içeren örnekler olmak üzere tanı güçlüğü yaratan durumlar yaşanabilmektedir. Böbrek hücreli karsinom subtipleri ile malign ve benign böbrek tümörlerinin ayırımında yer alabilecek sitokeratin proteinleri bulmak üzere giderek artan immünhistokimyasal çalışma yapılmaktadır. Bu çalışmada böbrek hücreli karsinom subtiplerinin kendi aralarında ve böbrek onkositomu ile ayırt edilmelerinde CK7, CK10 ve CK20'nin yeri araştırılmıştır. Çalışmada 62 "şeffaf" hücreli, 6 kromofob, 13 papiller ve 2 sınıflandırılamayan böbrek hücreli karsinom subtipleri ile 6 böbrek onkositomu yer almıştır. Tümörlerde Fuhrman nükleer grade tayin edilmiş ve tümörler düşük ve yüksek nükleer grade gösterenler olmak üzere iki grupta değerlendirilmiştir. Makroarray bloklara uygulanan immünhistokimyasal analiz CK7 ekspresyonu ile nükleer grade arasında zıt ilişki göstermiş (p=0.001), "non-şeffaf" hücreli subtiplerin (kromofob ve papiller) "şeffaf" hücreli subtipe göre belirgin artmış CK7 ekspresse ettiği gösterilmiştir (p=0.001). Onkositom 4 tümörde fokal dağınık CK7 pozitifliği geliştirmiştir. Tümörler CK10 ile tümüyle nonreaktif izlenirken, CK20 ile çok sınırlı ekspresyon göstermiştir. Bu çalışma, yaygın ve kuvvetli CK7 ekspresyonunun "non-şeffaf" hücreli böbrek hücreli karsinom subtiplerini "şeffaf" hücreli böbrek hücreli karsinom ve böbrek onkositomundan ayırmada önemli yeri olduğunu, CK7 ekspresyonu ile nükleer grade arasında zıt ilişki bulunduğunu göstermiş, CK10 ve CK20'nin ayırımda yeri olmadığı saptanmıştır.

Anahtar sözcükler: Sitokeratin 7, sitokeratin 20, sitokeratin 10 renal hücreli karsinom, renal onkositom

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INTRODUCTION

Renal epithelial neoplasms include malig-

nant renal cell carcinoma (RCC), the most common malignancy of adult kidney (1), and benign renal oncocytoma (RO). RCC comprises phenotypically and genetically heterogeneous tumor subtypes. International aggreement is achieved on the histological classification of RCC which is based on the light microscopic appearance and also consistent with prevailing genetic alterations (2). Tumor stage at presentation and histologic nuclear grade (NG) have been widely accepted as principal prognostic indicators of RCC. Studies have shown that these tumors have dictinctive microscopic, molecular features and clinical presentations relevant with metastatic tendency and potential response to therapy. The results indicated a poorer survival rate for patients with clear cell RCC (CRCC) compared with patients with "non-clear cell" RCC subtypes (3-6). The need for the application of the appropriate therapies and the development of targeted therapies for specific tumor subtypes makes the accurate classification of renal epithelial tumors more critical. Differential diagnosis is generally easy for cases with characteristic morphological features, but sometimes differentiating RCC subtypes from each other remains problematic on morphologic grounds. On the other hand, a benign renal cortical neoplasm, i.e. RO, which accounts for 3% to 7% of renal cortical neoplasms, may closely mimic a renal carcinoma in terms of histologic features and clinical presentation. A careful microscopic examination of a well sampled tumor will allow correct diagnosis in majority of the cases, but ancillary methods are necessary in certain situations. A discriminatory immunoreactivity that would confidently distinguish RCC subtypes from each other or from RO has not been identified yet. With this regard, we used various CKs; CK7, CK10, and CK20, in the differentiation of RCCs and RO which have led to various conflicting results in the literature.

MATERIALS and METHODS

Renal epithelial tumors operated between 1995 and 2002 were retrieved from the archives of Pathology Department. Hematoxylin and eosin (HE) stained slides were reevaluated and the tumors were reclassified according to the 2004 WHO classification (2). The clinical information were obtained from the patients' medical records and the macroscopic features of the tumors were recorded from pathology reports. The tumors were graded according to the Fuhrman's grading system, and were grouped as low NG (LNG; grades 1 and 2) or high NG (HNG; grades 3 and 4). Immunohistochemical (IHC) evaluation by a tissue macroarray technique (TMA) was performed on 83 cases of RCC, and 6 cases of RO. TMAs were prepared using a manual tissue-arraying instrument with a diameter of 4 mm. For the "recipient" paraffin blocks the representative areas of each tumor with characteristic histomorphology and highest NG were selected. Two to 5 tissue cylinders were punched out from each "donor" paraffin blocks. A total of 324 tumor tissue cylinders were mounted into 27 'recipient' TMA blocks with a capacity of 12 tissue cylinders of each. Antibodies against CK7, CK10, and CK20 were reacted on 4 µm thick sections of TMA blocks, using standard streptavidin-biotin peroxidase technique as shown in Table 1. Counterstain was performed with Mayer's hematoxylin, simultaneous positive and negative controls were processed. For each antibody presence of cytoplasmic and/or membranous staining was considered positive. The degree of intensity (I) of the staining was

Table 1. Details of the immunohistochemical analysis.

Antibody	Clone	Dilution	Pretreatment	Incubation time	Source
CK 7	OV-TL 12/30	1:50	Protease	90 min	Novocastra
CK 10	LHP1	1:50	Protease	90 min	Novocastra
CK 20	Ks20.8	1:50	Trypsin	90 min	Novocastra

CK7: Cytokeratin 7, CK10: Cytokeratin 10, CK20: Cytokeratin 20, min: minute.

semiquantitatively graded on a scale of 0 to 3; none (0), mild (1), moderate (2), and strong (3), and for the distribution (D) of the staining; none (0), focal (<10%) (1), patchy (10-50%) (2), and diffuse (>50%) (3). A staining score (IxD; 0-9) was calculated. The result of staining was evaluated as "positive expression" (stained) when the staining rate of the marker was >1 or "negative expression" (not stained) when it was ≤1. Statistical analysis was performed using SPSS 9.0 for Windows program. The difference in numerical data between groups was analysed using Mann-Whitney U Test or T Test. The relationship between IHC markers and histological variables were evaluated using chi-square test. P value of <0.05 was considered statistically significant throughout the analysis.

RESULTS

Among the RCC patients, there were 26 women (31.3%) with median age of 53.8 years (26-75 years) and 57 men (68.7%) with median age of 57.3 years (30-77 years) who were all treated by radical nephrectomy. The median age of all patients with RO treated by partial nephrectomy, was 60.6 years (41-74 years). The re-evaluation of the HE stained slides of the tumors revealed 62 CRCC (74.7%), 6 chromophobe RCC (ChRCC) (7.2%), 5 type 1 papillary RCC (PRCCT1) (6%), 8 type 2 papillary RCC (PRCCT2) (9.7%), and 2 (2.4%) "unclassified" RCC.

The expression of CK7 was different between CRCC and "non-clear cell" RCC subtypes; 84.2% of "non-clear cell" RCCs (ChRCC, PRCC) were found CK7 positive, while expression rate was 27.4% (17 of 62 tumors) for CRCCs (p=0.001). Of 13 PRCCs, 10 were found to be CK7 positive (%76.9) while, all ChRCCs were immunoreactive for CK7 (100%). The expression of CK7 was 100% and 62.5% (5 of 8 tumors) in PRCCT1 and PRCCT2, respectively (Table 2). In ChRCC, and PRCC the CK7 immunoreactivity was diffuse and strong throughout the cytoplasm and in the cell membranes of the tumor cells (Figure 1), whereas the distribution was patchy with strong reactivity in CRCC (Figure 2a, b). The immunoreactivity of CK7 in RO was noted in 4 of 6 tumors that was found only in scattered cells (Figure 2c, d). RO was entirely CK20 negative. CK20 was widely negative in RCC subtypes. Out of 62 CRCCs, 3 tumors were CK20 immunoreactive (4.8%) while only 1 PRCCT2 was positive (5.2%) in the group of "non-clear cell" RCCs (1

Table 2. Relation of CK7 and CK20 expression with RCC subtypes.

	CRCC	ChRCC	PRCCT1	PRCCT2	"*p*" value
CK7					
Negative	45 (72.6%)	.	.	3 (37.5%)	0.03
Positive	17 (27.4%)	6 (100%)	5 (100%)	5 (62.5%)	
CK20					
Negative	59 (95.1%)	6 (100%)	5 (100%)	7 (87.5%)	>0.05
Positive	3 (4.8%)	.	.	1 (12.5%)	

RCC: Renal cell carcinoma, CRCC: Clear cell RCC, ChRCC: Chromophobe RCC, PRCCT1: Papillary RCC type 1, PRCCT2: Papillary RCC type 2

Table 3. CK7 expression rates in RCC subtypes in relation with nuclear grades.

	CK7 expression		
	Negative	**Positive**	**Total**
Clear Cell RCC			
LNG (n)	18	10	28
% within NG	64.3%	35.7%	100.0%
% within CK7	40.0%	58.8%	45.2%
HNG (n)	27	7	34
% within NG	79.4%	20.6%	100.0%
% within CK7	60.0%	41.2%	54.8%
Total (n)	45	17	62
% within NG	72.6%	27.4%	100.0%
% within CK7	100.0%	100.0%	100.0%
Non-clear Cell RCC			
LNG (n)	1	10	11
% within NG	9.1%	90.0%	100.0%
% within CK7	33.3%	62.5%	57.9%
HNG (n)	2	6	8
% within NG	25.0%	75.0%	100.0%
% within CK7	66.7%	37.5%	42.1%
Total (n)	3	16	19
% within NG	15.8%	84.2%	100.0%
% within CK7	100.0%	100.0%	100.0%

Chi-square test crosstabs, RCC: Renal cell carcinoma, NG: Nuclear grade, LNG: Low nuclear grade, HNG: High nuclear grade

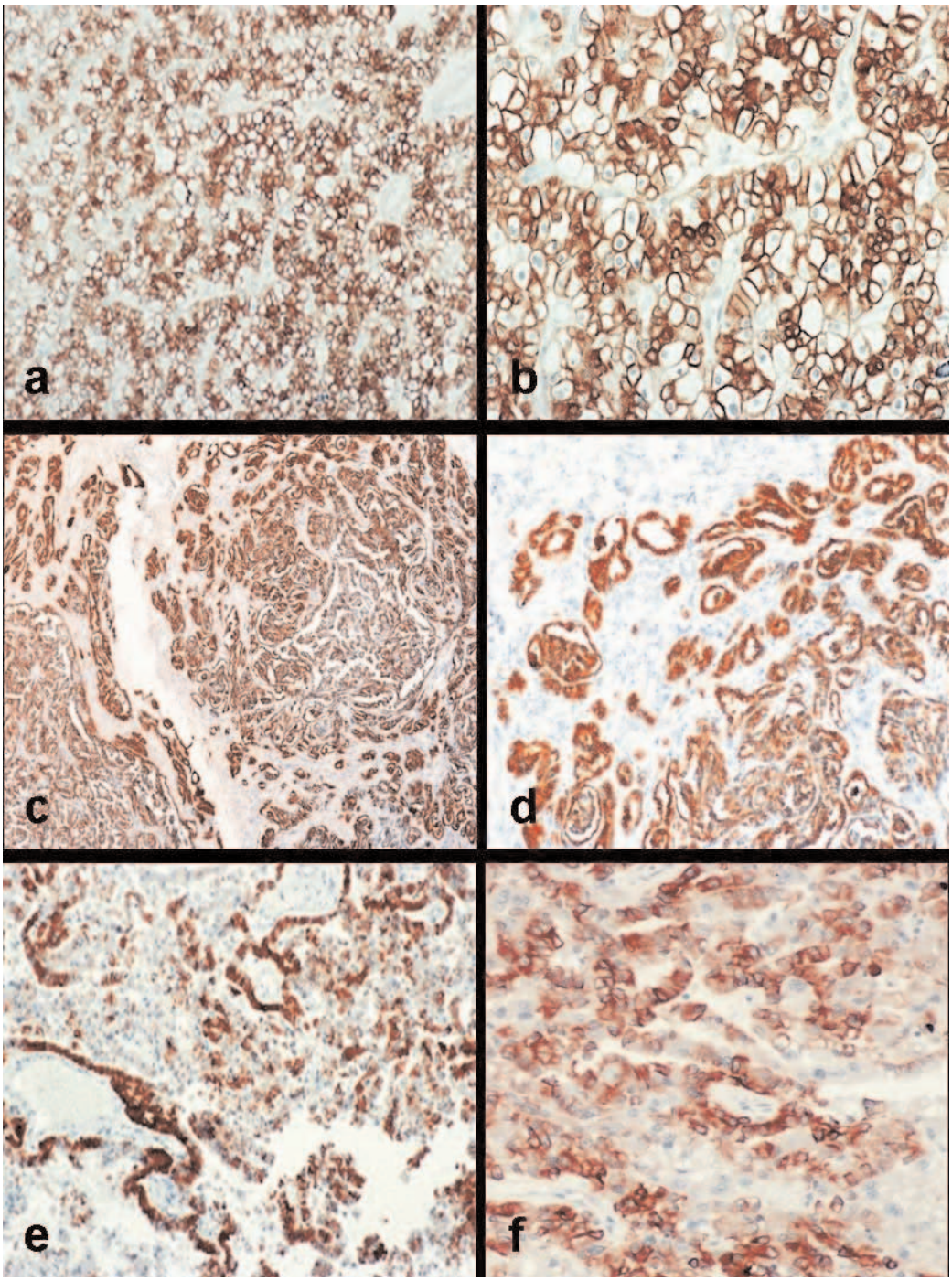

Figure 1. CK7 expression pattern in "non-clear cell" RCC subtypes. Diffuse and strong CK7 expression in chromophobe RCC with intense membranous staining (a, b. peroxidase, x200, x400), in papillary type 1 (c, d. peroxidase, x100, x400), and in papillary type 2 RCC (e, f. peroxidase, x200, x400).

of 19 tumors). In unclassified RCC group, one of the two tumors was positive for CK7 and all were negative for CK20. CK10 was entirely negative in all tumor subtypes.

As shown in Table 3, CK7 expression was related with nuclear differentiation in RCCs (p=0.039). CK7 was positive in 52.5% of the LNG and 30.2% of HNG tumors. Higher per-

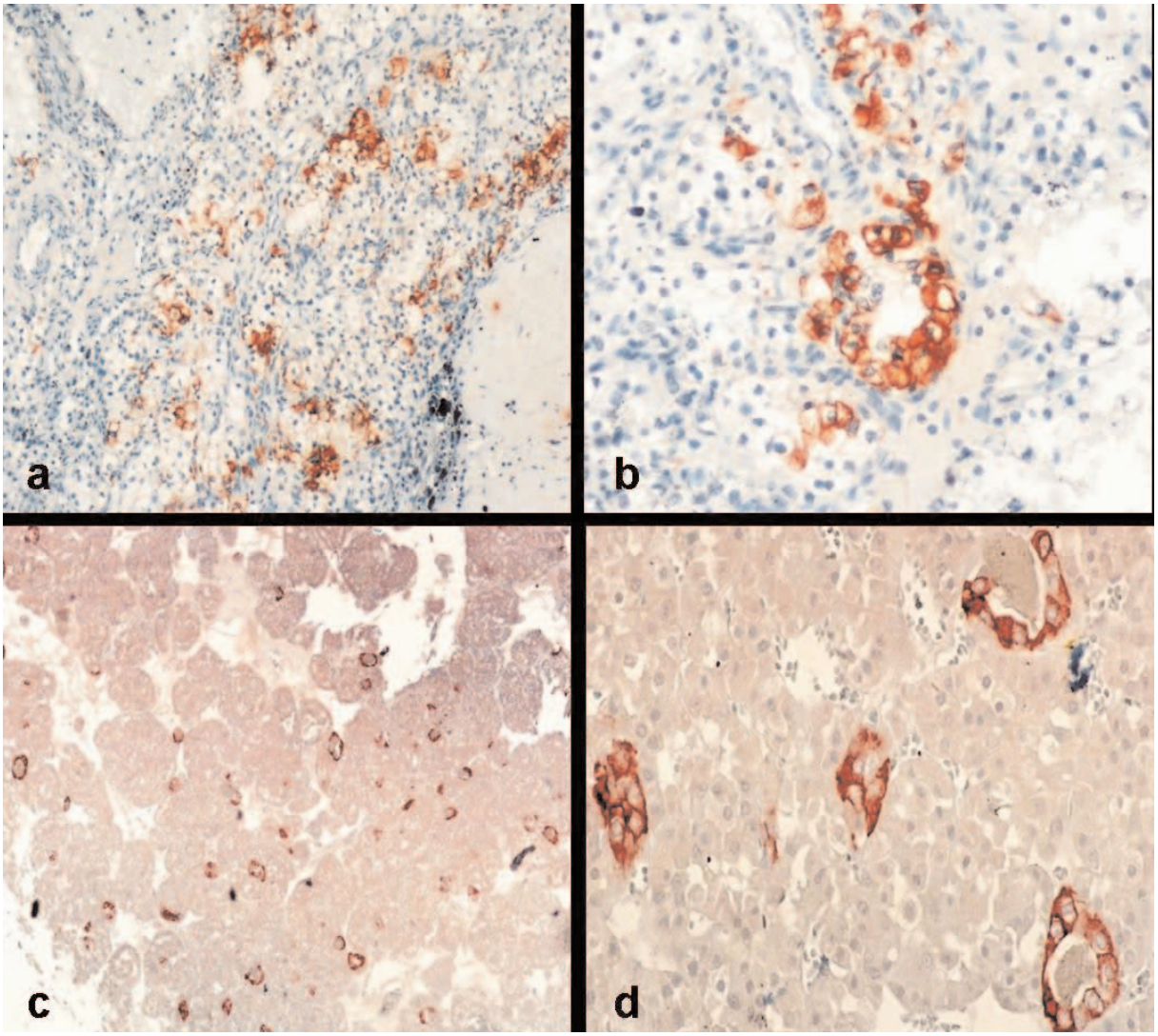

Figure 2. Patchy and strong (a, b. peroxidase, x200, x400), and scattered (c, d. peroxidase, x100, x400) CK7 expression in clear cell RCC and in renal oncocytoma, respectively.

centage (61.8%) of CK7 positive tumors showed LNG differentiation.

DISCUSSION

Cytokeratins are a family of intermediate filaments that are characterize of epithelial differentiation, and up to date at least 20 distinct CK subsets have been identified expressed by different epithelia and their neoplastic transformations (7). Majority of the studies on CK expression of renal tissues and renal tumors have examined a limited CK panel such as CK7 and CK20. There have been conflicting results on the expression of CK7 in renal epithelial tumors in the literature while some authors have recommended CK7 as a differential marker in distinguishing ChRCC from RO (8-10), the others (11-13) may insist on its role in the differentiation of "non-clear cell" RCC and RO from CRCC. Reports on the CK7 expression of CRCC have suggested a consensus of general negativity with the positivity in a range of 4.8-10.5% (12,14). PRCC and ChRCC were reported to have extensive CK7 expression with a range of 43-100% for ChRCCs (10,15,16), and a superiority

of expression for PRCCT2 over PRCCT1 (13,14). Reports on the expression of CK7 in RO also had conflicting results with focal, diffuse and negative staining patterns (8,10,14,16,17). In the present study differential diagnostic value of CK7 was exhibited between CRCC and "non-clear cell" RCC subtypes. "Non-clear cell" RCCs had significantly higher, more diffuse and intense CK7 expression rates (100% for ChRCCs and 76.9% for PRCCs), while the positivity rate was 27.4% for CRCCs demonstrating patchy and intense expression. Not only the percentage of the immunoreactive cells, but also the staining pattern with patchy distribution differed between these subtypes. The positivity rate of CK7 (27.4%) in the CRCC group is slightly higher according to the literature (8,12), but the relation with the other subtypes retains its significance. Skinnider et al (8), and Mazal et al (12) reported CK7 positivity rates for CRCC, ChRCC, PRCC as 20%, 73%, 86%, and 8%, 88%, and 77%, respectively. We conclude that; because both CRCC and non-clear RCCs may reveal the absence of CK7, strong and diffuse CK7 expression pattern becomes meaningful to support "non-clear cell" RCC diagnosis versus CRCC. This staining pattern may also indicate ChRCC diagnosis resembling RO, which has been widely reported to show focal scattered staining or absence of expression. The study revealed a scattered focal immunoreactivity of CK7 in 4 of 6 ROs, while the remaining two was diffusely negative. Skinner et al (8) reported a negative CK7 staining in 10 ROs whereas a low rate of immunoreactivity in 66 ROs has been shown by Langner et al (14). Focal scattered staining pattern, they had found, was consistent with our observation in ROs (Figure 2).

CK7 immunoreactivity in PRCC has commonly been reported with differing staining rate and intensity in type 1 and 2 tumors (13,14). Both studies have revealed a strong expression of the marker in the relatively benign PRCCT1, compared with the weak expression in the more agressive type 2 tumors. In the present study, CK7 was immunoreactive in total of 5 PRCCT1 tumors and 5 of 8 PRCCT2 tumors with a diffuse pattern and strong intensity. Although this diffuse and strong expression of CK7 in PRCCT2 is not consistent with the previous reports, the present study revealed a significant correlation between CK7 expression and nuclear differentiation ($p=0.039$), which supports the relation of CK7 expression with tumor aggressiveness. CK7 was expressed in 52.5% (20 of 39 tumors) of the tumors with LNG, and 30.2% (13 of 42 tumors) of the tumors with HNG. This strong association may be the reason for the conflicting results in the literature for the differentiation of RCC subtypes. We may refer that if CK7 expression is compared between the subtypes with the same NG, more significant and persuading results would be achieved, and it would be more meaningful in diagnosing tumor subtypes, regardless of negative impact of cellular differentiation.

CK20 has been shown to have a limited expression in normal tissues and neoplasms. CK20 expression in renal tubular epithelial tumors has been seldomly reported, and identified in 0-7.7% of RCCs (9,14,15,19). Langner et al (14) have reported a general lack of CK20 expression in a series of 233 renal tumors, with a positivity in only 2 of 8 PRCCT2s. Kim et al (9) have observed CK20 immunoreactivity in 4 of 20 PRCCs, without indicating their subtypes. Both studies have revealed the absence of CK20 in RO (9,14). Our results are similar to those found in these reports in that we detected only one PRCCT2 showing CK20 expression, and negativity for all cases with RO. But in our series in a total of 62 CRCCs, more than 50% of cells of 3 tumors were moderately stained with CK20. This confusing result shows the possibility of positive CK20 expression in CRCC. Literature findings have also shown confusing results for RO, and paucity of studies have reported CK20 expression in Ross. Stopyra et al have found the coordinate staining of CK7 and CK20 as a useful diagnostic tool in distinguishing RO from

RCC (20). In our opinion, CK20 does not appear to show a consistent immunoreactivity neither in RCC nor in RO, and does not seem to be a reliable differentiating marker for renal tubular neoplasms.

Literature contains scarce number of reports for CK10 expression in renal epithelial tumors which absolutely have shown the absence of the marker (14). None of the tumors in our study had immunoreactivity with CK10, as well. Thus CK10 seems to have no role in renal epithelial tumor differentiation.

We conclude that, CK7 immunohistochemistry is a useful diagnostic tool in distinguishing "non-clear" RCC from CRCC with diffuse and strong expression of CK7 supporting "non-clear" RCC diagnosis. The extent of CK7 expression may indicate the aggressiveness of the tumor, and with this regard, better organized studies consisting of larger series of RCCs with the same NG are needed to be evaluated. CK20 and CK10 immunohistochemistry seem to be an unreliable analytical method in differentiating renal tubular neoplasms.

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