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Original Research

The significance of BerEp4 and cytokeratin 19 expressions in epithelial tumors of kidney and renal pelvis

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ABSTRACT

Objective: The differential diagnosis in the epithelial tumors of kidney and renal pelvis can be problematic due to their overlapping morphologic features. This is much more complicated in some conditions such as renal oncocytoma (RO) versus chromophobe renal cell carcinoma (ChRCC) and RCC versus urothelial carcinoma of the renal pelvis (UC-RP). The purpose of this study was to assess the potential contributions of BerEP4 and cytokeratin 19 (CK19) expressions in the differential diagnosis of these challenging cases. **Materials and Methods:** A total of 57 cases consisted of 11 chromophobe (ChRCC), 18 clear cells RCC (CCRCC), 12 papillary RCC (PRCC), and 2 unclassified RCCs, 1 multilocular cystic renal cell neoplasm (MCRCN) with low malignant potential, 7 UC-RP, and 6 ROs were stained against BerEp4 and CK19 antibodies using automated immunostainer. **Results:** All ROs demonstrated membranous BerEP4 expression, but no CK19 expression. Unlike ROs, most ChRCCs exhibited diffuse and strong CK19 expression, but no or focal and weak BerEP4 expression. This distinctive opposite expression pattern was highlighted in hybrid oncocytic chromophobe tumor (HOCT). CCRCCs showed highly variable expression patterns for both markers. PRCC type 1 tumors demonstrated diffuse and strong BerEp4 and CK19 expressions. PRCC Type 2 exhibited BerEP4 and CK19 expressions, but their expressions were focal and weaker than for PRCC Type 1. MCRCN demonstrated diffuse and strong BerEP4 expression, but no expression for CK19 unlike cystic CCRCC, which is strongly positive for both markers. Epithelioid cells in unclassified RCC showed strong CK19 and weak BerEP4 expression, whereas spindle cells in the tumor did not express CK19 and BerEp4 or exhibited scattered and weak expressions. UC-RP showed diffuse and strong CK19 expression, but no or scattered BerEP4expression was seen in the tumor. We also evaluated CK19 and BerEP4 expression in non-neoplastic adjacent kidney and renal pelvis. **Conclusion:** This study revealed that (1) BerEP4 and CK19 exhibit variable and distinctive immunoprofiles in epithelial tumors of kidney and renal pelvis, (2) an immunoprofile of BerEP4 (+)/CK19 (−) favors RO in contrast to an opposite profile for ChRCC, (3) The heterogeneous expressions of BerEP4 and CK19 in low grade RCC with eosinophilic cytoplasm favors HOCT, (4) PRCC type 1 strongly express both biomarkers, and (5) although RCC subtypes express CK19 in a variable proportion and intensity, diffuse and strong CK19 expression favors UC-RP.

KEY WORDS: BerEP4, cancer, differential diagnosis, immunohistochemistry, keratin 19, kidney, renal pelvis, tumor

INTRODUCTION

Kidney and renal pelvis tumors account for approximately 3.7% of all cancers and represent the third most common malignant tumor of the genitourinary tract followed by bladder and prostate cancers [1]. They account for approximately 3% of all cancer deaths in males [1]. They may remain asymptomatic until they are advanced or metastatic disease. Most of them are incidentally detected on imaging studies performed for due to unrelated reasons [1].

Different histologic subtypes and new distinct entities of renal epithelial tumors have been described and categorized in the International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia in 2013 [2]. Diagnosing of new entities and subtyping of renal cell carcinoma (RCC) are clinically important because of different biological behaviors reflecting their distinct molecular and genetic features. Newer drugs targeting signal transduction pathways have been developed for the treatment of advanced, metastatic, and recurrent RCC and urothelial carcinoma (UC) discovered during their follow-up [3-7]. These targeted therapies are available thanks to knowledge on the molecular and genetic changes in hereditary RCC syndromes [3]. Therefore, the differential diagnosis of the epithelial tumors of kidney and renal pelvis becomes crucial for these targeted therapies. However, their differential diagnosis can be problematic due to their overlapping morphologic features. This problem

is especially obvious between some tumors such as renal oncocytoma (RO) versus chromophobe RCC (ChRCC) and renal cell RCC versus UC of the renal pelvis (UC-RP). Furthermore, this dilemma is magnified in the diagnosis of small biopsy specimens and metastatic advanced disease. As a result, immunohistochemistry becomes crucial for the diagnosis of epithelial tumors of kidney and renal pelvis in several problematic conditions such as differentiation of renal epithelial from non-renal epithelial tumors, histologic subtyping of renal tumors, accurate diagnosis of renal epithelial tumor in needle/small biopsy samples, and diagnosis of metastatic advanced RCC and UC-RP.

In diagnostic pathology, several biomarkers, most of which are recommended by the ISUP [8,9], are available for these diagnostic goals:

For RCC, RCC marker (RCCMa), vimentin, kidney-specific cadherin (Ksp-Cadherin), cytokeratins (especially pan CK, CK7, CK20, and high molecular weight cytokeratin [HMWCK]), anti-mitochondrial antibody (AMA), TFE3, TFEB, CD10, alpha-methylacyl coenzyme A racemase, parvalbumin, carbonic anhydrase IX, PAX2, PAX8, CD138, and survivin are most helpful [8-28].

For UC, GATA3, CK20, p63, HMWCK, and CK5/6 are most useful [9].

These biomarkers may not be always helpful for accurate diagnosis of these tumors. They may be limited in some conditions because (1) they are variably expressed not only by epithelial tumors of kidney and renal pelvis but also by tumors of other organs. (2) They have been seen in variable percentages of only some specific subtypes of renal epithelial tumors. (3) They may have some restrictions in small biopsies due to their heterogenic expressions of biomarkers in the tumor. (4) They may not support preliminary histological diagnosis due to technical reasons [29]. Thus, additional diagnostic biomarkers may be needed.

BerEP4 is a cell membrane glycoproteins expressed on the basolateral surface of normal epithelia and in various carcinomas including kidney. It plays a role as an epithelial cell adhesion molecule, in conjunction with other cell adhesion molecules such as tumor-associated calcium signal transducer 1, cluster of differentiation 326 (CD326), (epithelial specific antigen [clone VU-1D9]), and MOC 31 [30-36]. Several studies suggest that these cell adhesion molecules, including BerEP4, may be a potential target in the treatment of advanced RCC in [30-33]. In diagnostic pathology, BerEP4 has been widely used for the differentiation of malignant mesothelioma from adenocarcinoma [34].

Cytokeratins are members of a large family of molecules serving as biomarkers for epithelial differentiation, which are retained during neoplastic transformation [27,28]. Cytokeratins are largely expressed in normal renal tubules and urothelium, and tumors of kidney and renal pelvis but with well-defined site-specific restrictions in term of keratin subtypes. Therefore, a wide-spectrum cytokeratin antibody specific for both low and HMWCKs may be required in RCC and UC [27,37,38]. Cytokeratin 7 (CK7) and 19 (CK19) are simple epithelial cytokeratins and widely used as diagnostic biomarkers for diagnosis of many tumors including kidney tumors. However, most studies in this area have been focused on CK7 [27,28,37,38]. Cytoplasmic positivity with membrane accentuation for CK7 is largely seen in some RCC subtypes including chromophobe, papillary and collecting duct RCCs, and UC [27,28,37,38].

Immunohistochemical studies regarding BerEP4 and CK19 expression in epithelial tumors of kidney and renal pelvis are limited [30-33,37-39].

The purpose of this study was to assess the potential contribution of CK19 and BerEP4 expressions in the differential diagnosis and the determining new therapeutic strategies of this group of tumors.

MATERIALS AND METHODS

A total 57 of cases consisted of 6 ROs, 11 ChRCC, 18 clear cell RCC (CCRCC), 12 papillary RCC (PRCC), 2 unclassified RCCs, 1 multilocular cystic renal cell neoplasm (MCRCN) with low malignant potential, and 7 UC-RP were included to the study [Table 1]. Two of 11 ChRCC had a distinct oncocytoma component and were further classified as hybrid oncocytic chromophobe tumor (HOCT). Two of 18 CCRCC had cystic changes, but otherwise displayed typical features of RCC another four CCRCC had sarcomatoid component that was consisted of pure spindle (2 cases) or pure rhabdoid (1 case) or both (1 case) components in a variable proportion.

Consecutive sections obtained from selected paraffin blocks for each case were stained for BerEP4 (CD326; 1/50 dilution; ScyTek, Utah, USA) and CK19 (A53-B/A2.26; ready-to-use; Cell Marque, California, USA) antibodies using the Benchmark XT automated stainer (Ventana Medical Systems, Tucson, AZ, USA). The detection of the staining reactions for BerEP4 and CK19 was obtained with an ultraview Universal DAB detection system (Ventana Medical Systems, Tucson, AZ, USA) which is a multimer-technology based detection system.

For each case, the staining intensity was semi-quantitatively scored as follows: 0 = no stain, 1 = weak, 2 = moderate and 3 = strong. The staining extent was recorded as an estimated percentage of positive tumor cells and defined as the presences of specific staining in more than 5% of the tumor cells [32]. In addition, the renal tubules in the non-neoplastic adjacent kidney and renal pelvic urothelium distant from the tumor were used as an internal positive control, and their staining features compared with that in the tumor.

Statistically, Pearson's Chi-square test and Fisher's exact test were used for all comparisons [Table 1]. A $P < 0.05$ was considered statistically significant for all comparisons.

Table 1: The staining features and comparative statistical results of BerEP4 and CK19 in epithelial tumors of kidney and renal pelvis

Tumor histologic type	BerEP4					CK19					Combined BerEP4 and CK19
	Number of positive cases/ total cases (%)	*P**	Percent of mean stained cells±SD (min-max)	*P**	Mean staining intensity±SD	Number of positive cases/ total cases (%)	*P**	Percent of mean stained cells±SD (min-max)	*P**	Mean staining intensity±SD	*P***
RO	6/6 (100)	0.017	95.83±4.91 (90-100)	0.001	3±0	0/6 (0)	0.017	0	0.001	0	0.001
ChRCC***	4/11 (36)		19.09±26.72 (0-85)		0.7±1.0	7/11 (63)		56.81±44.84 (5-100)		2.2±1.4	
CCRCC	15/18 (83)		16.11±19.36 (0-80)		1.1±0.7	15/18 (83)		23.88±29.18 (0-95)		1.9±1.3	
PRCC											
Type 1	5/5 (100)	1.0	74.00±31.10 (20-95)	0.001	3±0	5/5 (100)	0.454	94.00±8.21 (85-100)	0.001	3±0	0.001
Type 2	6/6 (100)		36.66±29.77 (15-75)		2.8±0.4	4/6 (66)		59.16±46.52 (0-100)		2±1.5	
Solid variant	1/1 (100)		45±0		2±0	1/1 (100)		90±0		3±0	
MCRHN	1/1 (100)		100±0		3±0	0/1 (0)		0		0	
RCC unclassified	2/2 (100)		92.50±3.53 (90-95)		1.5±0.7	2/2 (100)		85.00±7.07 (80-90)		2.5±0.7	
All RCC subtypes	34/44 (75)	0.017	54.76±34.23 (0-100)	0.001	2.01±0.9	34/44 (75)	0.320	58,40±35.54 (0-100)	0.001	2.08±1.02	0.001
UC-RP	2/7 (28)		10.00±7.07 (5-15)		1.5±0.7	7/7 (100)		87.14±17.28 (60-100)		2.9±0.4	

CCRCC: Clear cell renal cell carcinoma, ChRCC: Chromophobe renal cell carcinoma, MCRHN: Multilocular cystic renal cell neoplasia with low malignant potential, PRCC: Papillary renal cell carcinoma, RO: Renal oncocytoma, UC-RP: Urothelial carcinoma of renal pelvis. *Fisher's exact test value. The result is significant at<0.05. **Pearson's chi-square test values. The result is significant at<0.05. This group was composed of comparative statistical results of both BerEP4 and CK19 according to percentage of mean stained cells. ***Two of them were consisted of hybrid oncocytic chromophobe tumor

RESULTS

BerEP4 and CK19 were successfully detected in routinely processed selected tissue sections with appropriate positive and negative controls. Immunostaining results were summarized in Table 1.

RO

All ROs displayed diffuse and strong membranous BerEP4 expression, whereas no expression for CK19 was noted in all cases [Table 1 and Figure 1a-d]. However, scattered membranous staining for CK19 was demonstrated in two of all RO cases, which are most probably non-neoplastic tubules trapped in the tumor [Figure 1d].

ChRCC

Among 11 cases of ChRCC, seven are typical, BerEP4 was negative in each of them, whereas CK19 was positive in all of them [Table 1 and Figure 2a-d].

Two of these 11 cases were HOCT: The RO in them demonstrated an immunoprofile of BerEP4 (+, diffuse and strong membranous)/CK19 (−), in contrast to the ChRCC component (BerEP4−/CK19+, diffuse and strong membranous) of the tumor [Figure 3a-d]. Opposite staining pattern of BerEP4 and CK19 highlighted RO and ChRCC components of HOCT, respectively. Another case showed diffuse and strong

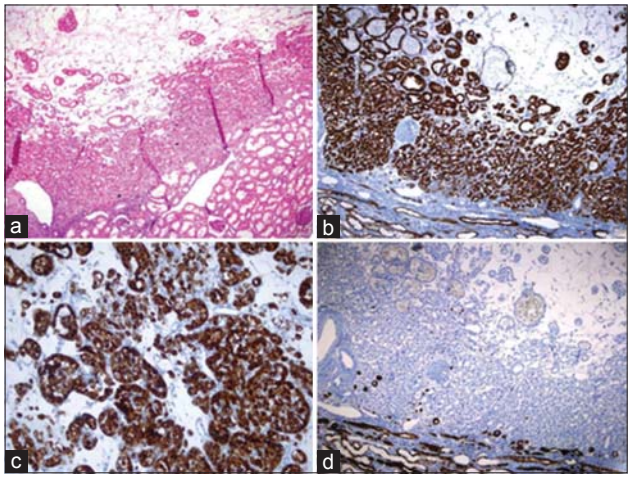

Figure 1: Renal oncocytoma (RO): (a) Tumor is composed of eosinophilic cells forming nests and tubule-like structures. There is loose connective tissue in the center of the tumor. Non-neoplastic adjacent kidney is seen in the right bottom corner of the tumor separated with well-defi ned border (H&E, ×100). (B) Strong and diffuse BerEP4 expression observed in RO at low magnifi cation. In addition, all renal tubules in the adjacent non-neoplastic kidney express BerEP4 in a variable proportion (IHC, ×100). (c) Membranous BerEP4 expression in RO is highlighted at high magnifi cation (IHC, ×200). (d) No CK19 expression is detected in the tumor and proximal tubules in the adjacent kidney, whereas it is expressed in distal tubules and collecting ducts. CK19 is noted in rare tubular structures within the tumor, which may represent entrapped non-neoplastic renal tubules (IHC, ×100)

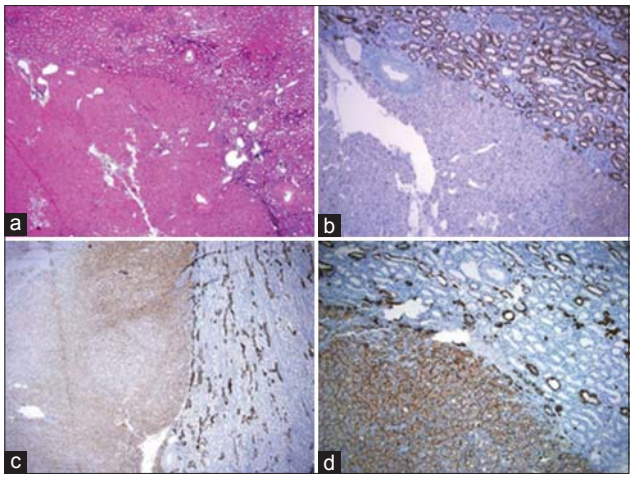

Figure 2: Chromophobe renal cell carcinoma (ChRCC): (a) The tumor separated from adjacent non-neoplastic kidney (in the upper right corner) with lobulated pushing border is composed of eosinophilic cells forming solid nests (H&E, ×40). (b) No BerEP4 expression observed in the tumor, whereas all renal tubules express BerEP4 in a variable proportion (increasing from proximal tubules to collecting ducts). Glomerular capillary and epithelial cells are not express BerEP4, too (IHC, ×100). (c) The tumor, and distal tubules and collecting ducts in the non-neoplastic adjacent kidney strongly and widely express CK19 (IHC, ×40). (d) Cytoplasmic positivity with membrane accentuation for CK19 is highlighted in neoplastic and non-neoplastic adjacent kidney at high magnification (IHC, ×100)

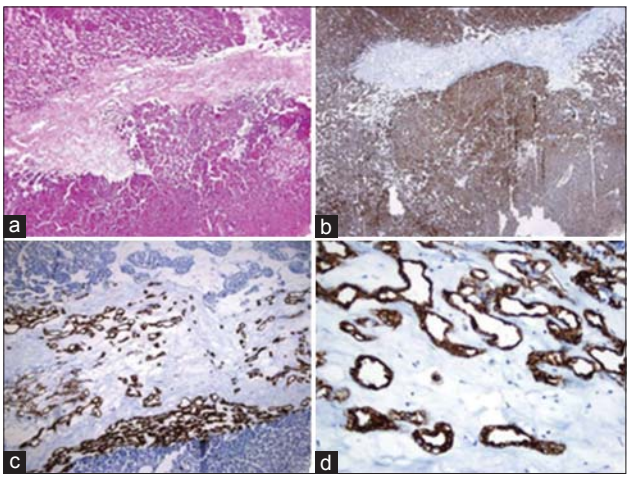

Figure 3: Hybrid oncocytic chromophobe tumor (HOCT): (a) The tumor is consisted of two components; one is composed of tubular structures embedded in the loose fibrous tissue in the center of the tumor (ChRCC morphology), the other one is composed of eosinophilic cells forming solid nests in the periphery of the tumor (RO morphology) surrounding the tumor in the center (H&E, ×20). (b) BerEP4 exclusively is expressed in the peripheral zone of the tumor (in RO component), but no BerEP4 expression is detected in the center of the tumor (IHC, ×20). (c) CK19 expression is exclusively seen in the center of the tumor (in ChRCC component), whereas no CK19 expression is observed in the periphery of the tumor (IHC, ×100). (d) Cytoplasmic positivity with membrane accentuation for is highlighted CK19 in the center of the tumor (ChRCC morphology) at high magnifi cation (IHC, ×400)

membranous BerEP4 expression just like RO, whereas focal and moderate to strong CK19 expression was observed in different areas of the tumor just like ChRCC. Again, this case might have been diagnosed as HOCT instead ChRCC.

Three of these 11 cases may represent the eosinophilic variant of ChRCC. They displayed a heterogeneous staining pattern with focal and weak to moderate membranous BerEP4 expression without CK19 expression, which was typical for RO.

CCRCC

CCRCC demonstrated highly variable expression patterns for both BerEP4 and CK19:

1. BerEP4 and CK19 were both expressed in solid areas of CCRCC: Focal and weak to moderate membranous BerEP4 and CK19 expression in a highly variable proportion were observed in 15 cases, whereas they were completely negative in 3 cases which were CCRCC with sarcomatoid component that composed of pure *spindle* or *spindle* and focal *rhabdoid* cells [Table 1 and Figure 4a-d].
2. Two cases had marked cystic changes: The cystic areas demonstrated strong and diffuse cytoplasmic positivity with membrane accentuation for both BerEP4 and CK19. The cystic and tubular areas were more differentiated with low nuclear features than another part of the tumor. The solid areas accounting for a small portion of the tumor showed highly variable focal and weak BerEP4 and CK19 expression.
3. Four cases had predominantly sarcomatoid component that was consisted of pure spindle (2 cases) or pure rhabdoid (1 case) cells, or spindle and focal rhabdoid (1 case) cells. BerEP4 and CK19 were both negative in spindle cells of the tumor, whereas they showed variable expression in rhabdoid cells of the tumor: BerEP4 was negative in both cases consisted of pure rhabdoid cells, and mixed rhabdoid and spindle cells, but CK19 was positive in one case that consisted of pure rhabdoid cells, and negative in another case that composed of a mixture of rhabdoid and spindle cells.
4. Diffuse and strong membranous CK19 expression was expressed in one case, which had low-grade morphology.

PRCC

All PRCCs Type 1 exclusively exhibited diffuse and strong BerEp4 and CK19 expressions, but a number of CK19 positive cells were more than BerEP4 [Table 1 and Figure 5a-e]. All PRCCs Type 2 demonstrated BerEP4 expressions like PRCC Type 1, but the amount and intensity of positive cells were lower than PRCC Type 1. Nevertheless, most PRCC Type 2 exhibited diffuse CK19, but the number of positive cases and the staining intensity of positive cells were lower than PRCC Type 1. Solid variant of PRCC showed BerEP4 and CK19 expressions, but the number of CK19 positive cells was more than BerEP4. This finding was similar to PRCC Type 1.

MCRCN

MCRCN exhibited diffuse and strong membranous BerEP4 expression [Table 1 and Figure 6a-c], but no expression for CK19 in the tumor [Figure 6d and e] unlike the two cystic CCRCC.

Unclassified RCC

One case of unclassified RCCs was consisted of a mixture of epithelioid cells mimicking collecting duct and/or renal medullary carcinomas, and spindle cells. The part of the tumor that was composed of epithelioid cells demonstrated strong CK19 and weak BerEP4 [Table 1 and Figure 7a-e], whereas scattered low CK19 and BerEp4 expressions were

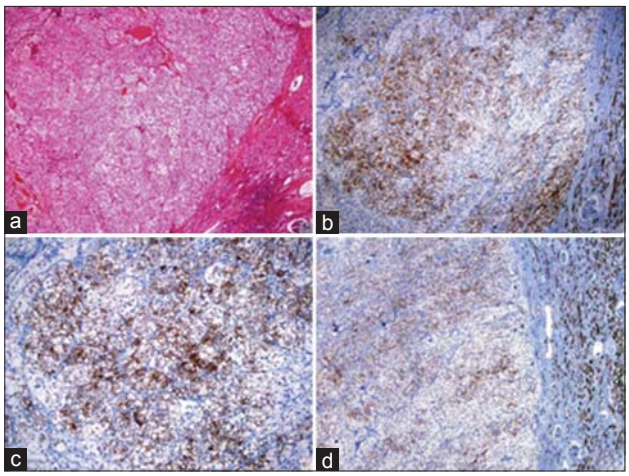

Figure 4: Clear cell renal cell carcinoma (CCRCC): (a) The tumor separated from non-neoplastic adjacent kidney (in the bottom right corner) with lobulated pushing border is composed of clear cells forming solid nests surrounded by delicate fi brovascular stroma (H&E, ×100). (b) BerEP4 expression in a highly variable proportion and intensity is seen in CCRCC. In addition, all renal tubules in non-neoplastic adjacent kidney exhibited BerEP4 expression in a variable proportion (increasing from proximal tubules to collecting ducts), but it is not detected in glomerular capillary and epithelial cells (IHC, 100). (c) Variable membranous BerEP4 expression in the tumor is highlighted at high magnifi cation (IHC, ×400). (d) Focal and weak membranous CK19 expression like BerEP4 was observed in CCRCC. In non-neoplastic adjacent kidney, CK19 is also expressed in distal tubules and collecting ducts, whereas it is not detected in proximal tubules, glomerular capillary and epithelial cells (IHC, 100)

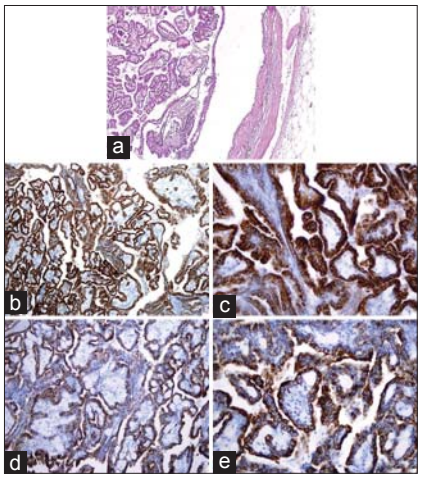

Figure 5: Papillary renal cell carcinoma (PRCC), Type 1: (a) The tumor is composed of papillary structures which have fibrovascular cores infiltrated by foamy cells (H&E, 100). (b) Strong and diffuse BerEP4 expression is observed in the tumor at low magnification (IHC, ×40). (c) Strong membranous BerEP4 expression in the tumor is highlighted at high magnifi cation (IHC, ×400). (d) Strong and diffuse CK19 expression is seen in the tumor at low magnification (IHC, ×100). (e) Cytoplasmic positivity with membrane accentuation for CK19 is highlighted in the tumor at high magnification (IHC, ×400)

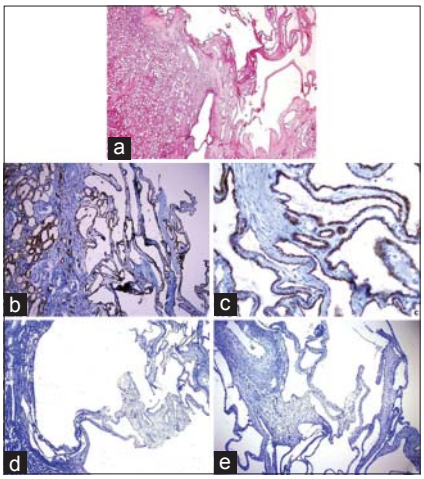

Figure 6: (a-e) Multilocular cystic renal cell neoplasm (MCRCN)*:* (A) The tumor is composed of multilocular spaces lined with monolayer attenuated fl at or plumped clear cells (H&E, ×40). (B) Diffuse and strong BerEP4 expression is detected in the tumor. In addition, all renal tubules in non-neoplastic adjacent kidney exhibited BerEP4 expression in a variable proportion (increasing from proximal tubules to collecting ducts) but it is not detected in glomerular capillary and epithelial cells (IHC, ×100). (C) Membranous BerEP4 expression in the tumor is highlighted at high magnifi cation (IHC, ×400). (D) No CK19 expression is observed in MCRCN at low magnifi cation (IHC, ×40). (E) No CK19 expression is seen in the tumor at high magnifi cation (IHC, ×100)

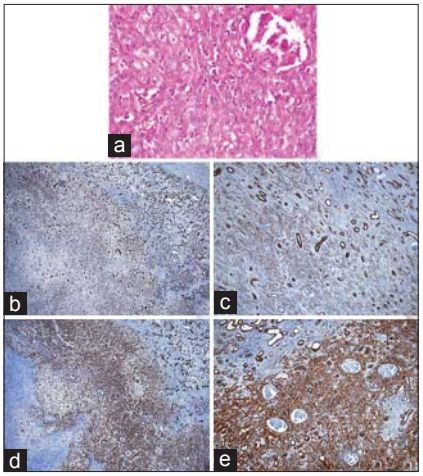

Figure 7: Unclassified renal cell carcinoma (RCC): (a) Infiltrative tumor which is composed of epithelioid and spindle cell areas is seen in the kidney (H&E, 400). (b) The tumor infi ltrating between normal renal tubules express BerEP4 (IHC, ×40). (c) The tumor demonstrates weaker BerEP4 expression than renal tubules in non-neoplastic adjacent kidney at high magnifi cation. No BerEP4 expression is detected in glomerular capillary and epithelial cells (IHC, 100). (d) Similarly, CK19 expression is seen in the tumor and renal tubules at low magnifi cation (IHC, ×40). (e) CK19 expression is equally observed in the tumor and renal tubules at high magnifi cation unlike BerEP4. No CK19 expression is detected in glomerular capillary and epithelial cells (IHC, ×100)

observed in the part of the tumor that was composed of spindle cells.

Another unclassified case was consisted of two separate components, which were UC-like areas and RCC-like areas that were composed of a mixture of epithelioid and spindle cells. The tumor demonstrated variable expression patterns: BerEP4 was totally negative in UC-like areas of the tumor, whereas BerEP4 expression was weak in spindle cells and moderate in epithelioid cells of RCC-like areas of the tumor. Diffuse and strong CK19 expression was seen in both RCC and UC-like areas. CK19 exhibited stronger expression in epithelioid cells than spindle cells of RCC-like areas of the tumor, whereas biomarkers (P63, thrombomodulin, and uroplakin III) using for diagnosis of UC were negative in RCC-like areas contrary to UC-like areas of the tumor.

UC-RP

UC-RP cases included five high and two low-grade UCs [Table 1 and Figure 8a-c]. Although BerEP4 was negative in five cases, other two cases, both high-grade UC, demonstrated scattered weak BerEP4 expression [Figure 8b]. CK19 expression was diffuse and strong in all UCs, regardless of grade [Figure 8d].

Non-neoplastic Kidney and Renal Pelvis

The immunohistochemical features of BerEP4 and CK19 in non-neoplastic adjacent kidney and renal pelvis were summarized in Table 2.

Strong and diffuse basolateral membranous BerEP4 expression was seen in all renal tubules in a variable proportion (increasing from proximal tubules to collecting ducts) at adjacent kidney, whereas no expression in glomerular capillary and epithelial cells [Figure 1b, 2b, 4b, 6b, and 7b]. Similarly, renal pelvic umbrella cells showed strong and diffuse membranous BerEP4 expression, but no expression in the rest of the renal pelvic urothelium [Figure 8c].

Strong and diffuse membranous CK19 expression was observed in distal tubules and collecting duct in the kidney, whereas no expression in proximal tubules, glomerular capillary, and epithelial cells [Figure 1d, 2c, 4d, and 7d]. All cell layers of the renal pelvic urothelium demonstrated strong and diffuse membranous CK19 expression [Figure 8e].

Comparative Statistical Results

For comparing, Pearson's Chi-square and Fisher's exact tests applied to most challenging tumor groups such as RO versus ChRCC, PRCC Type 1 versus PRCC Type 2, and all RCC subtypes versus UC-RP [Table 1].

Comparing the BerEP4 and CK19 expressions between RO (BerEP4+/CK19−) and ChRCC (BerEP4−/CK19+) demonstrated a statistically significant difference in term of both frequency of positive cases ($P = 0.017$, $P = 0.017$, respectively) and percentage of stained tumor cells ($P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively) [Table 1].

Between PRCC Type 1 (moderate to high BerEP4+/high CK19+) and PRCC Type 2 (low BerEP4+/moderate CK19+), there was no difference in staining frequency ($P = 1.0$, $P = 0.454$). However, the percentage of positive tumor cells evaluated for individual markers or in combination showed statistically significant difference between them ($P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively) [Table 1].

Comparing the BerEP4 and CK 19 expressions in between all RCC subtypes (high BerEP4+/moderate CK19+) and UC-RP (low BerEP4+/high CK19+) in term of staining frequency showed a statistically difference for BerEP4 ($P = 0.017$), but not CK19 ($P = 0.320$). In addition, the percentage of positive tumor cells evaluated for individual markers or in combination showed statistically significant difference

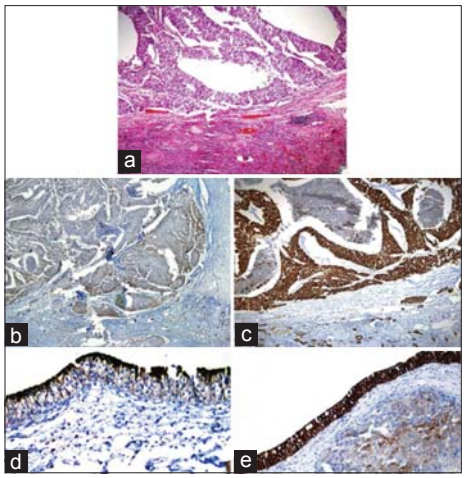

Figure 8: Urothelial carcinoma of the renal pelvis (UC-RP): (a) High-grade UC filling the cavity of the renal pelvis and infiltrating adjacent atrophic kidney consists of solid islands with central tumor necrosis (H&E, 100). (b) BerEP4 expression is weak and scattered in the periphery of the neoplastic solid islands. In addition, BerEP4 is detected in all renal tubules at adjacent kidney but it is not observed in glomerular capillary and epithelial cells (IHC, ×40). (c) Strong and diffuse CK19 expression is observed in the tumor and in renal tubules at adjacent kidney, whereas there is no expression for CK19 in glomerular capillary and epithelial cells (IHC, ×100). (d) BerEp4 is only expressed in umbrella cells of urothelium (IHC, ×200). (e) Strong and diffuse CK19 expression is exclusively seen in all cell layers of urothelium (IHC, ×200)

Table 2: BerEP4 and CK19 expression in normal kidney and renal pelvis

Organs	Sites	Staining intensity	
		BerEP4	CK19
Kidney	Proximal tubules	Weak to moderate	No stain
	Distal tubules	Strong	Strong
	Collecting ducts	Strong	Strong
Renal pelvis	Umbrella cells only	Strong	Strong (all)
	Rest of the urothelial cells	No stain	

CK19: Cytokeratin 19

between them ($P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively) [Table 1].

DISCUSSION

Most epithelial tumors of the kidney and renal pelvis can be diagnosed by routine light microscopy. However, overlapping morphology among them, problematic benign mimickers, or rare tumor subtypes is not infrequent, requiring ancillary studies for recognition [1,2,8-28]. Overlapping morphologic features in the epithelial tumors of kidney and renal pelvis are especially problematic in needle/small biopsies and in the metastatic disease. Despite improvements in molecular pathology, immunohistochemistry is still useful as an ancillary method in diagnosing kidney and renal pelvis tumors [27,28]. A large number of diagnostic immunohistochemial biomarkers are now available and has help elevated the level of diagnostic accuracy, critical for appropriate therapy. However, diagnostic conundrum remains for at least a few tumor types and additional markers would be desirable

Studies on BerEP4 and CK19 expression in renal and urologic tumors are limited, and most of them consisted of tissue microarray (TMA) [30-39]. This study utilized consecutive whole tissue sections, and included many RCC subtypes, previously underestimated, and also UC of renal pelvis, a significant differential diagnostic problem, thus significant expanding from previously studies.

Pan *et al.*, in a study of renal tumor expression of BerEP4, CD10, RCCMa and MOC 31 antibodies, observed BerEp4 expression in 28.8% of CCRCCs ($n = 236$), 92.8% of ChRCCs ($n = 28$), 66.6% of PRCCs ($n = 27$), 20% of CDCs ($n = 5$), and 28.5% of ROs ($n = 7$) [31] [Table 3]. Went *et al.* using a different clone of antibody against BerEP4 (clone VU-1D9, Novocastra) demonstrated BerEP4 in 18% of CCRCCs ($n = 147$), 75% of ChRCCs ($n = 12$), and 55% of PRCCs ($n = 20$) but not in ROs ($n = 3$) in their study [32]. Zimpfer *et al.* using the same clone like in our study (DAKO) revealed BerEP4 in 36.3% of CCRCCs ($n = 642$), 78.3% of ChRCCs ($n = 68$), 81.3% of PRCCs ($n = 20$), 43.3% of unclassified RCCs ($n = 30$), and 37.8% of ROs ($n = 45$) [33].

In the presented study, BerEP4 expression was detected in 83% of CCRCCs ($n = 18$), 36% of ChRCCs ($n = 11$), 93.3% of PRCCs ($n = 12$), 100% of unclassified RCCs ($n = 2$), 100% of MCRCN ($n = 1$), 100% of ROs ($n = 6$), and 28% of UC-RPs ($n = 7$). Our results are similar to those of Zimpfer *et al.* [33]; but differ, albeit in a minor way, from other previous studies. These differences may be resulted from several reasons as described below [Table 3].

Langer *et al.* studied various cytokeratins (K1-K20, HMW, KL1, MNF116, AE1/AE3, and Lu5) in TMA sections of RCC subtypes and RO [Table 4] and observed that CK19 expressed in 11.6% of CCRCCs ($n = 125$), 22.7% of ChRCCs ($n = 22$), 65% of PRCCs ($n = 20$), and 40.9% of ROs ($n = 66$) [37]. Skinnider *et al.* studied various keratins (CK5/6, CK7, CK8, CK13, CK14, CK17, CK18, CK19, CK20, and HMWCK) and vimentin in whole tissue sections in renal epithelial neoplasms and reported that CK19 expressed in 13% of CCRCCs ($n = 15$), 7% of ChRCCs ($n = 15$), 67% of PRCCs ($n = 15$), 67% of collecting duct carcinomas ($n = 5$), 100% of RMCs ($n = 3$), 100% of tubulocystic carcinomas ($n = 3$), 67% of mucinous tubular and spindle cell carcinomas ($n = 3$), 10% of ROs ($n = 10$), and 92% of UC-RPs ($n = 12$) [38]. Mertz *et al.* studied various cytokeratins (CK7, CK8, CK18, and CK19) in TMA sections of 126 CCRCCs and stated that CCRCCs expressing of CK7 and CK19 were associated with better outcome [39]. This study was planned to investigate the prognostic value and possible contribution of CK7 and CK19 on targeted therapy in RCC. However, this study was composed of only CCRCC, no with other RCC subtypes and UC-RP.

This study revealed that CK19 expressed in 83% of CCRCCs ($n = 18$), 63% of ChRCCs ($n = 11$), 88.6% of PRCCs ($n = 12$), 100% of unclassified RCCs ($n = 2$), 0% of MCRCN ($n = 1$), 0% of ROs ($n = 6$), and 100% UC-RPs ($n = 7$). Our results regarding CK19 expression in epithelial tumors of the kidney and renal pelvis revealed that there were partly some differences compared to previous studies. Again, these differences may be resulted from several reasons as described below in detail. However, our CK19 results regarding to UC-RP were similar to results of Skinnider *et al.* study [38] [Table 4].

Our results regarding BerEP4 and CK19 in ROs and ChRCCs were partially different from the previous studies. This difference may be multifactorial. It may be due to technical reasons including the utilization of different antibody clones

Table 3: The Comparison of the results of EpCAM expressions in epithelial tumors of kidney and renal pelvis

Studies	Tag of EpCAM	Tissue sections	% of EpCAM expression (total cases)							
			RO	CCRCC	ChRCC	PRCC	RCC unclassified	MCRHN	CDC	UC-RP
Pan *et al.* (2004)	BerEP4 clone, DAKO	TMA, 2 mm	28.5 (7)	28.8 (236)	92.8 (28)	66.6 (27)	ND	ND	20 (5)	ND
Went *et al.* (2005)	ESA, clone VU-1D9, Novocastra	TMA, 0.6 mm	0 (3)	18 (147)	75 (12)	55 (20)	ND	ND	ND	ND
Zimpfer *et al.* (2014)	BerEP4 clone, DAKO	TMA, 0.6-1 mm	37.8 (45)	36.3 (642)	78.3 (68)	81.3 (20)	43.3 (30)	ND	ND	ND
Present study (2016)	BerEP4 clone [CD326], ScyTek	Consecutive whole	100 (6)	83 (18)	36 (11)	93,3 (12)	100 (2)	100 (1)	ND	28 (7)

CCRCC: Clear cell renal cell carcinoma, CDC: Collecting duct carcinoma, ChRCC: Chromophobe renal cell carcinoma, MCRHN: Multilocular cystic renal cell neoplasia with low malignant potential, ND: Not done, PRCC: Papillary renal cell carcinoma, RO: Renal oncocytoma, TMA: Tissue microarray, UC-RP: Urothelial carcinoma of renal pelvis

from different commercial sources. It may be due to tissue sampling, highlighting a relative strength of the current study. TMA was used almost exclusively in the previous studies, in contrast to the whole tissue sections in the current study [Tables 3 and 4]. The expressions of biomarkers in RCC are often heterogeneous. Therefore, whole tissue sections rather than TMA should provide more realistic information on biomarker expressions [27,28]. It may reflect different threshold for positivity. Finally, it may reflect inherent diagnostic difficulty. It is well recognized that ChRCC, HOCT, and RO morphologically overlap, leading to different tumor classification among studies.

Although HOCT occurs in patients with Birt-Hogg-Dubé syndrome (BHD) and in association with renal oncocytomatosis without BHD, it arises sporadically in patients without evidence of BHD and renal oncocytomatosis, which is exceedingly rare. The patients with BHD usually have characteristic clinical features such as skin tumors (fibrofolliculoma or trichodiscoma) and pulmonary lesions (bullae and spontaneous pneumothorax), whereas the patients with sporadic or with renal oncocytomatosis have no specific clinical symptoms. There tumors have mixed morphology with dual population of eosinophilic cells in RO and CHRCC cytomorphology. In this study, all three HOCT cases with addendum diagnosis in one case of CHRCC had no specific clinical history about BHD or renal oncocytomatosis. HOCT show slightly distinct heterogenic immunoprofiles and significant molecular genetic heterogeneity in all three clinicopathologic groups [40-42]. Poté *et al.* reported that CK7 were positive in scattered cells or small clusters in sporadic HOCT but it was expressed by cells in CHRCC-like areas in BHD patients [40]. Similarly, we detected that CK19 and BerEP4 expression were inversely in scattered cells or small clusters in two HOCT, whereas clear-cut CK19 (in CHRC-like areas) and BerEP4 (in RO-like areas) staining was seen in only one HOCT. Several studies revealed that multiple numerical aberrations of chromosomes 1, 2, 6, 9, 10, 13, 17, 21, and 22 are higher in sporadic HOCT rather than that in the setting of oncocytomatosis. FLCN gene mutation is detected in HOCT in patient with BHD, but absent in the other two clinical settings [40-42]. We did not perform molecular genetic study on our cases.

Using immunohistochemistry, accurate histopathological diagnosis is crucial in urologic tumors to determine the appropriate molecular targeted therapy. Over the past decade, investigators have been focused on various targeted therapies such as mammalian target of rapamycin (mTOR) inhibitors (everolimus and temsirolimus) and tyrosine kinase inhibitors (sunitinib, sorafenib, pazopanib, and bevacizumab) for inhibition of vascular endothelial growth factor (VEGF) pathway for the treatment of metastatic CCRCCs [43]. Recently, clinical trials on the treatment of metastatic CCRCCs have suggested that an inhibitor of VEGF receptor, MET and AXL (cabozantinib), and a programmed cell death-1 checkpoint inhibitor (nivolumab) [43]. Although most investigations on molecular targeted therapies are related to CCRCC, all these targeted drugs have been tested in non-CCRCC, especially in PRCC with ongoing clinical trials, which are mostly phase II step [43]. Similarly, for molecular targeted therapies of muscle invasive UC, there are several ongoing clinical trials (preclinical, phase I and phase II studies) such as pan-fibroblastic growth factor receptor inhibitors, mTOR inhibitors-for patients with tuberous sclerosis complex mutations-targeting to block PI3K/AKT/mTOR pathway, ado-trastuzumab emtansine for HER-2 positive patients [44]. As a result, differential diagnosis and accurate diagnosis in urologic tumors, especially advanced disease, are crucial to determine the appropriate targeted therapies. Immunohistochemistry is still best diagnostic tool in the diagnosis of urologic tumor in this context.

We also evaluated normal kidney and renal pelvis structures for BerEP4 and CK19 [Tables 5 and 6]. Our results regarding BerEP4 expression revealed that there was difference in proximal tubules compared to the results of the previous studies [Table 5]. However, Göttlinger *et al.* and Balzar *et al.* [35,36] proposed that BerEP4 expression increases from proximal to distal tubular epithelium and collecting duct, just like our findings contrary to the results of Pan *et al.* and Zimpfer *et al.* [31,33]. This study revealed that CK 19 expression was different in urothelial epithelium of renal pelvis, which all layers of urothelial epithelial cell were strongly positive for CK19 contrary to the results of Skinnider *et al.* study [38], which it expressed in only umbrella cells, but no expressed in the rest of the urothelium [Table 6]. However, our CK19 results regarding adjacent kidney structures were similar to the results of Skinnider *et al.* study [38]. Moreover, there was no any difference in the both biomarker expressions of the normal tubules with that in different types of renal cell neoplasm.

Table 4: The Comparison of the results of CK19 expressions in epithelial tumors of kidney and renal pelvis

Studies	Tag of CK19	Tissue sections	% of CK19 expression (total cases)										
			RO	CCRCC	ChRCC	PRCC	RCC unclassified	MCRHN	CDC	RMC	TC	MTSCC	UC-RP
Langer *et al.* (2004)	Clone RCK 108, DAKO	TMA, 0.6 mm	40.9 (66)	11.6 (125)	22.7 (22)	65 (20)	ND	ND	ND	ND	ND	ND	ND
Skinnider *et al.* (2005)	Clone BA17, DAKO	Consecutive whole	10 (10)	13 (15)	7 (15)	67 (15)	ND	ND	67 (4)	100 (3)	100 (3)	67 (3)	92 (12)
Present study (2016)	A53-B/A2.26, Cell Marque	Consecutive whole	0 (6)	83 (18)	63 (11)	88,6 (12)	100 (2)	0 (1)	ND	ND	ND	ND	100 (7)

CCRCC: Clear cell renal cell carcinoma, CDC: Collecting duct carcinoma, ChRCC: Chromophobe renal cell carcinoma, MCRHN: Multilocular cystic renal cell neoplasia with low malignant potential, MTSCC: Mucinous tubular and spindle cell carcinoma, ND: Not done, PRCC: Papillary renal cell carcinoma, RMC: Renal medullary carcinoma, RO: Renal oncocytoma, TC: Tubulocystic carcinoma, TMA: Tissue microarray, UC-RP: Urothelial carcinoma of renal pelvis

Table 5: The comparison of the results of BerEP4 expressions in normal kidney and renal pelvis

Studies	Kidney				Renal pelvis	
	Proximal tubules	Distal tubules	Collecting ducts	Glomerular epithelial cells	Umbrella cells only	Rest of the urothelial cells
Pan *et al.* (2004)	-	+	+	-	ND	ND
Zimpfer *et al.* (2014)	-	+	+	-	ND	ND
Present study (2016)	+, weak to moderate	+	+	-	+	-

ND: Not done

Table 6: The Comparison of the results of CK19 expressions in normal kidney and renal pelvis

Studies	Kidney				Renal pelvis	
	Proximal tubules	Distal tubules	Collecting ducts	Glomerular epithelial cells	Umbrella cells only	Rest of the urothelial cells
Skinnider *et al.* (2005)	+	+	+	-	+	-
Mertz *et al.* (2008)	-	+	+	ND	ND	ND
Present study (2016)	+	+	+	-	+	+

ND: Not done, CK19: Cytokeratin 19

Although a large number of biomarkers for diagnosis and classification of renal tumors and tumors of urothelial origin have been currently used in diagnostic pathology, a number of diagnostic problems remain, and therefore, additional biomarkers are still needed. In that aspect, this study revealed that renal epithelial tumors as well as UC displayed distinct immunohistochemical pattern of expression for these two markers. Despite some discordance with the previous studies, which have some limitations as mentioned above, our findings expand current knowledge in this context and the most important thing is that these two markers may help to solve a number of thorny diagnostic problems, especially the differentiation of CHRCC and RO that current markers may not help, compared and contrasted our findings to previous studies.

CONCLUSION

This study revealed point of interest as follows (1) BerEP4 and CK19 exhibit variable and distinctive immunoprofiles in epithelial tumors of kidney and renal pelvis, (2) an immunoprofile of BerEP4 (+)/CK19 (−) is favor of RO unlike ChRCC, (3) the heterogeneous expressions of BerEP4 and CK19 in different areas of low-grade RCC with eosinophilic cytoplasm favors HOCT; however, if the tumor is high grade (ISUP Grade 3 or 4), the diagnosis is favor of CCRCC, (4) PRCC Type 1 is strongly and widely expressed by BerEP4 and CK19, and (5) although RCC subtypes express CK19 in a variable proportion and intensity, diffuse and strong CK19 expression is favor of UC-RP. However, these results need to be proven in more wide series composed of whole tissue sections rather than TMA sections.

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