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Immunohistochemical Expression of Napsin A, CD82 and Cyclin D1 in Some Renal Tumors

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ABSTRACT

Background: most of renal neoplasms are of epithelial origin and they are malignant. Renal cell carcinoma (RCC) accounts for approximately 2% of all cancers. The disease resulted in more than 100,000 yearly deaths worldwide. Histologic diagnosis of renal neoplasms is usually straight forward by routine light microscopy. However, immunohistochemistry may be essential in several contexts, including differentiating renal from non-renal neoplasms, differentiating subtypes of primary renal epithelial neoplasms and diagnosing rare types of renal neoplasms or metastatic RCC in biopsy specimens. **Aim of the work**: multiple therapeutic options tailored to an individual patient are now being offered. In view of these developments, availability of a robust and dependable panel of immunohistochemical stains becomes even more important because pathologists are frequently asked to render diagnosis on limited material. **Material and methods**: in this study a total number of 50 cases of some types of renal cell tumors were immunohistochemically stained for Napsin A, CD 82 and Cyclin D1. Results: these cases included 18 cases of ccRCC (36%), 16 cases of PRCC (32%), 8 cases of ChRCC (16%) and 8 cases of oncocytoma (16%).**Conclusion:** we concluded that napsin A may be useful in differentiating between ccRCC and PRCC (particularly type 1 which showed more vacuolated or clear cytoplasm). CD82 may be useful in differentiating between ChRCC, which was CD82 positive and oncocytoma, which was CD82 negative. Cyclin D1 had no significant value in the differentiation of different types of renal epithelial tumors.

Recommendation: we recommended the usage of Napsin A in differentiating between ccRCC and PRCC and CD82 in differentiation between ChRCC and oncocytoma. More studies are needed to evaluate napsin A in differentiating between ChRCC and oncocytoma.

Keywords: RCC, napsin A, CD82, cyclin D1.

INTRODUCTION

Most of renal neoplasms are of epithelial origin and they are malignant. Renal cell carcinoma (RCC) accounts for approximately 2% of all cancers. The disease resulted in more than 100,000 yearly deaths worldwide. More than 90% of the renal cell neoplasms arise from the renal tubules [1].

RCC is divided into 5 main histologic subtypes: clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (PRCC), chromophobe renal cell carcinoma (ChRCC), collecting duct and unclassified RCC. ccRCC, PRCC and ChRCC are the 3 most common types, comprising 70% to 80%, 14% to 17%, and 4% to 8% of all RCCs, respectively. Benign renal tumors comprise 10% and include several benign entities, most of which are renal oncocytoma, angiomyolipoma and papillary adenoma [2] .

Histologic diagnosis of renal neoplasms is usually straight forward by routine light microscopy. However, immunohistochemistry may be essential in several contexts, including differentiating renal from non-renal neoplasms, differentiating subtypes of primary renal epithelial neoplasms and diagnosing rare types of renal neoplasms or metastatic RCC in biopsy specimens [2] . This is because renal cell carcinoma is the most common 'recipient' of the curious phenomenon of metastasis of a cancer into another cancer. Lung carcinoma is the most common 'donor', the resulting microscopic appearance leading to interesting problems of interpretation. The opposite phenomenon also occurs, the most spectacular example being renal cell carcinoma metastasizing into a CNS hemangioblastoma in patients with von Hippel–Lindau (VHL) disease [3] .

Napsin-A expression was demonstrated in 86 of 222 (39%) ccRCCs and 16 of 21 (76%) PRCCs with a strong and diffuse staining pattern observed in PRCCs and a relatively weak and focal positivity in ccRCCs. It was positive in 3% of ChRCC. The expression of napsin-A was also found to be inversely correlated to aggressive local tumor characteristics [4].One study revealed immunopositivity for CD82 in 78% (69/88) of ChRCCs, but in only 3% (6/220) of ccRCCs. All 90 oncocytomas and 171 PRCCs were immunonegative

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for CD82. So, CD82 may be an excellent marker for distinguishing ChRCCs from other types of renal cell tumours, especially from oncocytoma [5].In one study, cyclin D1 was positive in oncocytoma (17 of 21, 81%), but negative in the ChRCC (0/23) and ccRCC (0 of 30) [6].

AIM OF THE WORK

This study aimed to assess the use of napsin A, CD82 and cyclin D1 immunohistochemocal staining as differentiating markers in some types of renal tumors and their relation to nuclear grade which may predict the prognosis.

MATERIALS AND METHODS

The material of this work included 50 paraffin blocks of selected primary renal tumor cases that had been retrieved retrospectively from the Pathology Department of Al -Azhar University Hospitals during the period from August 2105 to May 2017. **The study was approved by the Ethics Board of Al-Azhar University.**

Renal tumor blocks included 18 cases of ccRCC (36%), 16 cases of PRCC (32%), 8 cases of ChRCC (16%) and 8 cases of oncocytoma (16%). The clinical information's of the patients was obtained from their pathology reports and patient charts. The study protocol was approved by the Ethical Committee of Faculty of Medicine, Al-Azhar University. Four micron thick serial sections were cut from paraffin blocks of all cases and stained with hematoxylin and eosin (H&E) and all cases were re-evaluated and tumors were graded according to WHO classification of tumors of the kidney**.** Other sections were cut and placed on charged poly-L-lysine pretreated glass slides then taken for ready to use immunohistochemical staining as follows: for **Napsin A** (Rabbit polyclonal antibody, clone 1404205-A,ready to use, Cell Marque, California) lung adenocarcinoma was used as a positive control, lymphoid tissue was used as negative control), for **CD82** (Mouse monoclonal antibody, clone 5B5, dilution 1/80, Leica Biosystems, Newcastle) tonsillar tissue was used as a positive control, fat was used as negative control) and for **Cyclin D1** (Rabbit polyclonal antibody, clone SP4, ready to use, Cell Marque, California) lymph node was used as a positive control, fat was used as negative control). Avidin-Biotin immunoperoxidase Complex technique (ABC)was used.

The whole sections were scanned and evaluated at 10x magnification of the light microscope. Cytoplasmic staining was considered for Napsin A, membranous staining was considered for CD82 and nuclear staining was considered for Cyclin D1.Napsin A staining was scored as weak (1+) or intense (2+), A tumor was considered negative if less than 10 % of the tumor cells were stained. The case was interpreted as positive when cytoplasmic staining of more than 10% of the tumor cells was observed [7].To assess CD82 expression, both the extent and intensity of immunostaining were thought. The staining intensity score was graded as follows: none, 0; weak, 1; moderate, 2; and strong, 3. The extent of positive staining was graded as follows: <25 %, 1; 25–50 %, 2; and >50 %, 3. Then the score was determined by multiplying the extent and intensity of immunostaining to reach a range of scores from 0 to 9. Immunostaining was thought positive when the score was >3 [8].To assess cyclin D1 expression, both the extent and intensity of immunostaining were taken in consideration. The extent of immunoreactivity was graded according to the percentage of positive tumour cells as diffuse (>70%), focal (>5% to 70%), or negative (<5). The intensity of staining was graded as strong (3+), moderate (2+), and weak (1+) [9,10].

Data management and analysis were performed by using statistical analysis Systems. Data were summarized by using means and standard deviations. Categorical data were summarized as percentages. Comparisons between groups with respect to numeric variables were done using the Mann-Witney nonparametric test. Comparisons between categorical variables were done by the chisquare test or Fisher's exact test for small sample size. All p-values are two-sided. P-values < 0.05 were considered significant.

RESULTS

The benign renal epithelial tumors included 8 (16 %) out of 50 cases, all of them were oncocytoma. The malignant renal epithelial tumors included 42 (84 %) out of 50 cases. 18 (36%) were ccRCC, 16 (32 %) were PRCC including 8 (16%) cases of type 1 and 8 (16%) cases of type 2 tumors and 8 (16 %) were ChRCC. The age of benign renal epithelial tumors ranged from 46 to 59 years (mean age 51.5) while, the age of malignant tumors ranged from 19 to 85 years (mean age 53.5). The difference between the mean age of benign and malignant renal epithelial tumors was statistically insignificant (P

value = 0.647).Six out of 8 cases (75%) of benign epithelial tumors (oncocytoma) were females. 26 out of 42 cases (61.9%) of malignant epithelial tumors were males. The correlation between the sex of the benign and malignant renal epithelial tumors was statistically insignificant (P value = 0.185).According to WHO/ISUP grading system for ccRCC and PRCC, 13 out of the 34 (26.2%) cases of ccRCC and PRCC were of nuclear grade I, 17 cases (50%) were of nuclear grade II, 3 cases (8.8%) were of nuclear grade III and 1 (2.9%) were of nuclear grade IV. This study showed negative napsin A expression in all (8 out of 8) (100%) oncocytoma cases. As regard malignant tumours, 7 out of 18 cases of ccRCC were positive (Figure 1), 8 out of 8 cases of PRCC type 1 were positive, 6 out of 8 cases of PRCC type 2 were positive (Figure 1) and 8 out of 8 cases of ChRCC were positive (Table 1). The difference between napsin A expression in studied benign and malignant and between types of malignant epithelial tumors was statistically significant (P value = 0.001 and 0.005 respectively). The relation between napsin A expression and the nuclear grading of ccRCC and PRCC showed that 57.1% of napsin A positive tumors were of grade I, while 42.9% were of grade II. Both grade III and IV tumors were napsin A negative. This correlation between napsin A expression and nuclear grading was not statistically significant (P value = 0.059).

Table 1: napsin A expression in studied malignant renal tumors

Histological type	Napsin A			*Total*
	Negative	Weakly positive	intensely positive	
Clear cell RCC	11	3	4	**18**
Papillary RCC type 1	0	2	6	**8**
Papillary RCC type 2	2	2	4	**8**
Chromophobe RCC	0	5	3	**8**
Total	**13 (31%)**	**12 (28.6%)**	**17 (40.5%)**	**42 (100%)**

This study showed negative CD82 expression in all (8 out of 8) (100%) oncocytoma cases (figure 2). Positivity in malignat tumors was found in 12 out of 18 cases of ccRCC, 0 out of 8 cases of PRCC type 1, 2 out of 8 cases of PRCC type 2 and 6 out of 8 cases of ChRCC (figure 2) (table 2). The difference between the CD82 expression in studied benign and malignant epithelial tumors was statistically insignificant (P value = 0.096) while the difference between CD82 expression in studied types of malignant epithelial tumors was statistically significant (P value = 0.006). The relation between CD82 expression and the nuclear grading of ccRCC and PRCC showed that 28.6% of CD82 positive tumors were of grade I, 50% were of grade II, 21.4% of CD82 were of grade III and none of grade IV tumors were CD82 positive. This correlation between CD82 expression and nuclear grading was statistically significant (P value = 0.007).

Table 2: CD82 expression in studied malignant epithelial renal tumors

Histological type	CD82 expression				
	Negative	Weakly positive	Moderately positive	Strongly positive	*Total*
Clear cell RCC	6	2	10	0	**8(100%)**
Papillary RCC	8	0	0	0	**8(100%)**
Papillary RCC	6	0	2	0	**8(100%)**
Chromophobe	2	0	4	2	**8(100%)**
Total	**22(52.4)**	**2(4.8%)**	**16 (38.1)**	**2(4.8%)**	**42(100%)**

This study showed positive cyclin D1 expression in 4 out of 8 (50%) oncocytoma. 14 out of 18 cases of ccRCC, 4 out of 8 cases of PRCC type 1, 2 out of 8 cases of PRCC type 2 and 4 out of 8 cases of ChRCC were positive (table 3). The difference between cyclin D1 expression in studied benign and malignant and between types of malignant epithelial tumors was statistically insignificant (P value = 0.409, 0.220 respectively).

Table 3: cyclin D1 expression in studied malignant epithelial renal tumors

Histological type	Cyclin D1 expression				Total
	Negative	Weakly positive	Moderately positive	strongly positive	
Clear cell RCC	4	3	7	4	8(100%)
Papillary RCC type 1	4	2	2	0	8(100%)
Papillary RCC type 2	6	0	2	0	8(100%)
Chromophobe RCC	4	2	2	0	8(100%)
Total	8(42.9%)	7 (16.7%)	13 (31%)	4(9.5%)	42(100%)

The relation between cyclin D1 expression and the nuclear grading of ccRCC and PRCC showed that 35% of cyclin D1 positive tumors were of grade I, 45% were of grade II, 15% were of grade III and 5% were of grade IV tumors. This correlation between cyclin D1 expression and nuclear grading was statistically insignificant (P value = 0.425).

Figure 1: napsin A expression in ccRCC (left) showing weak granular cytoplasmic staining and intense staining in PRCC (right)

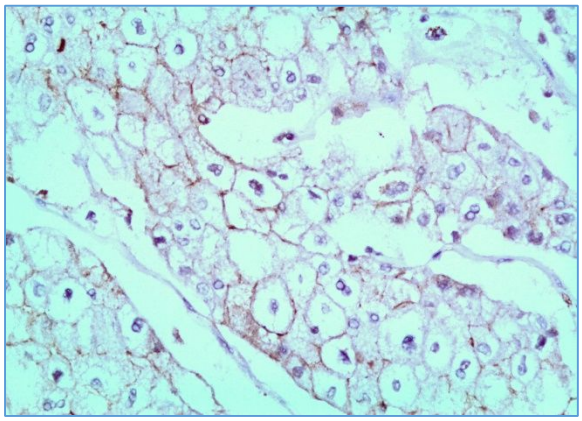

Figure 2: CD82 expression in ChRCC (left) showing moderate membranous staining and negative staining in oncocytoma (right)

DISCUSSION

Our study is consistent with results of **Latic *et al.*** [11] as regard age distribution of relevant renal tumor types' who stated that age of 74 malignant renal tumours ranged between 33 and 85 years (mean 59.29). Also, **Wu *et al.*** [12] found that the mean age of 2941 malignant cases was 56 years. Results of this study agree with the study held by **lima *et al*.**[13] who found that 75 out of 109 (68.8 %) malignant renal tumors were males and **Chevarie-Davis *et al*.** [14] who found that 99 out of 132 (75%) PRCC cases were males. Results of **Latic *et al.*** [11] showed that 48 out of 74 (64.86%) ccRCC, PRCC, ChRCC and collecting duct carcinoma were males.Results of our research are in agreement with those reported by **Zhu *et al*.** [15] as regard ccRCC and PRCC who found that 10 out of 23 (43.5%) of ccRCC and 31 out of 37 (83%) of PRCC were napsin A positive. However, our results are contradictory to their study as regard ChRCC and oncocytoma. They found that 5 out of 45 (11.1%) of ChRCC and 13 out of 23 (56.5%) of oncocytoma were immunoreactive for napsin A. The difference may be due to different number of cases studied. Our results are supported by **Xu *et al*.** [4] who found that 41 out of 90 (45.6%) cases of ccRCC and 16 out of 21 (76%) of PRCC were napsin A positive, in addition to the more intense staining in PRCC than ccRCC. Their results also in agrrement with our study in that the expression of napsin A was also found to be inversely correlated to high nuclear grade.

Bishop *et al*. [16] held a study on 118 cases of ccRCC, PRCC and ChRCC and stated that napsin A was positive in most of PRCC (79%), about one third (34%) of ccRCC, and in a single case of ChRCC (3%).

This was consistent with our study as regard ccRCC and PRCC but was different as regard ChRCC. Our results are also consistent with results of **Kadivar and Boozari** [17] who found that napsin A was positive in 7 out of 8 (87.5%) PRCC cases, 5 out of 15 (33.3) ccRCC cases and negative for all 3 oncocytomas. In contrast to our study, they found that 1 out of 7 (14.3%) ChRCC was napsin A positive.Our results are consistent with those of **Ohe *et al*.**[8] who found that 18 out of 20 (90%) ChRCC cases were CD82 positive and 1 out of 9 (10%) oncocytoma cases was positive.

Our results are also consistent with those of **Yusenko and Kovacs** [5] who found that positive CD82 immunoreactivity in 69 out of 88 (78%) of ChRCC and negative in all oncocytomas (90 cases). Their results are nearly similar to our results as regard PRCC, as they stated that all PRCCs (171 cases) were negative. In contrast to our study, they found that 6 out of 220 (3%) ccRCC was CD82 positive. This variation may be due to the difference in number of cases.

Also, **Kauffman *et al*.** [18] found that positive CD82 expression in 27 out of 31 (87%) ChRCC which is in agreement with our study. Their results are nearly like our results, as regard PRCC and oncocytoma as they stated that all PRCCs (35 cases) were negative and only 2 out of 28 (7%) oncocytomas were positive. In contrast to our study, they found that 1 out of 48 (2%) ccRCC was CD82 positive. This variation may be due to the difference in number of cases. **Kwon *et al*.** [19] showed that 98 out of 644 (15.2%) ccRCC were positive for CD82 immunostain, in disagreement with our study. This may be due to wide difference in sample size.

Iribe *et al*. [20] found that all cases (11) of ChRCC and only 2 out of 11 (18.2%) oncocytomas were positive for CD82 immunostain. This augment our findings. They disagree with our study as regard ccRCC as they found no positive cases among studied 12 ccRCC. There were no available previous studies that reported the relation between CD82 positivity and tumor grade in ccRCC and PRCC to compare our findings with them. Results of the current study are nearly similar to those of **Zhao *et al*.** [6] who found that 17 out of 21 cases of oncocytoma (81%) was positive for cyclin D1, but our study was inconsistent with them regarding ccRCC and ChRCC as they showed that all ccRCC and ChRCC were immunonegative (0 out of 30) and (0 out of 23) respectively. The difference in the percentages of expression may be due to using different antibodies and different sample sizes.

Sukov *et al*. [21] stated that 21 out of 63 (33%) oncocytomas were immunopositive for cyclin D1 while none of 36 ChRCCs were positive. This supports our study concerning the percentage of oncocytoma positive cases. The difference in percentage of ChRCC may be due to number of cases studied.

Another study which was done by **lin *et al*.** [22] found that Cyclin D1 immunoreactivity was observed in 9 of 18 (50%) oncocytomas, 23 out of 45 (51%) ccRCC, 5 out of 18 (28%) PRCC, and 2 out of 15 (13%) ChRCC. were positive for cyclin D1.

This is consistent with this study as regard oncocytoma, ccRCC and PRCC with some difference in the percentage of ChRCC which may be due to variation in sample size.

CONCLUSION

We conclude that napsin A may be useful in differentiating between ccRCC and PRCC (particularly type 1 which showed more vacuolated or clear cytoplasm). Although both were napsin A positive, but the staining pattern is more diffuse and marked in PRCC. CD82 is useful in differentiating between ChRCC, which is CD82 positive, and oncocytoma, which is CD82 negative. Cyclin D1 has no significant value in the differentiation of different types of renal epithelial tumors.

RECOMMENDATION

We recommend the usage of more studies with large number of cases to evaluate napsin A in differentiating between ChRCC and oncocytoma and to evaluate cyclin D1 value in differentiating between types of renal epithelial tumors. Also, we recommend the use of CD82 in differentiation between ChRCC and oncocytoma in challenging cases.

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