

Expression of Paired Box-2 Gene Product in Renal Tumors as a Diagnostic Immunomarker

Muhammad Shabbir, Mulazim Hussain Bukhari¹, Shahida Niazi²

PGR Pathology, King Edward Medical University, ¹Department of Pathology, University Medical College, UOL, ²King Edward Medical University, Lahore, Pakistan

Abstract

Objective: This study was aimed at evaluating the usefulness of paired box-2 gene (PAX-2) in the diagnosis of renal tumors. **Materials and Methods:** This study included 60 renal tumors. The newly prepared hematoxylin and eosin stained slides of all cases were evaluated and the diagnoses were confirmed or revised for each tumor according to the 2004 World Health Organization classification of renal tumors. Representative and consecutive sections of each tumor were submitted for anti-PAX-2 antibody immunohistochemistry. The pattern of staining (nuclear or cytoplasmic) was also noted. PAX-2 expression in tumors was correlated with low- and high-nuclear grades (Fuhrman nuclear grades). **Results:** The 45/60 (75%) cases showed PAX-2 nuclear immunoreexpression. The frequency of positivity in renal tumors was seen in 29/34 (85.5%) and 12/15 (80%) cases of clear cell RC, papillary RCC, respectively. The PAX-2 was positive in 20/45 cases for score 1+. The 16/45 cases were categorized into score 2+, and 9/45 cases were categorized into score 3+. **Conclusion:** PAX-2 is a diagnostically useful marker for primary renal tumors and is inversely proportion to the grades of the renal tumor.

Keywords: Fuhrman nuclear grades, paired box-2 gene immunomarker, renal cell carcinoma, renal tumor

INTRODUCTION

Renal tumors include benign, malignant, and metastatic tumors. The benign tumors of the kidney are very rare. The most common benign epithelial tumors include papillary adenoma, renal oncocytoma, metanephric adenoma, and metanephric adenofibrom. Papillary adenoma is the most common benign tumor of the tubular epithelium of the kidney.^[1] Renal oncocytoma is a benign tumor comprises about 3%–7% of all renal tumors. It usually appears as mahogany brown with a central scar.^[2] Metanephric adenoma and adenofibroma are also benign renal tumors which develop both in adults and children.^[1,2] The common mesenchymal tumors include leiomyosarcoma, angiomyolipoma, epithelioid angiomyolipoma, leiomyoma, hemangioma, and lymphangioma.^[1]

The most common malignant tumor of the adult kidney is renal cell carcinoma (RCC) accounting for 2%–3% of all malignant tumors.^[3] It is the 3rd most common malignant tumor of genito urinary system.^[4] The incidence rate of RCC vary substantially across the world with a high incidence in Europe and low incidence in Asia.^[5] In Europe, the incidence

is about 14.5/100,000 for men and 6.9/100,000 for women.^[6] In Pakistan, no adequate data is available but a study conducted in Karachi in 2007 shows a frequency of 1.8% of RCC.^[7] The average age at the time of diagnosis is around 55–60 years with a male-to-female ratio of about 2:1.^[2]

The most important predisposing risk factors of renal tumors are hypertension, smoking, obesity, and alcohol consumption.^[5] The patients mostly present with symptoms of hematuria, flank pain and/or palpable abdominal mass.^[8] In suspected cases of renal tumors, computed tomography is the preferred imaging technique for surgical planning.^[9]

The tumors with Fuhrman Nuclear Grade (FNG) I and II are considered as low grade while the tumors with FNG III and IV are considered to be high-grade RCC. Low-grade tumors show strong paired box-2 gene (PAX-2) staining while high-grade tumors show weak staining in clear cell and papillary variants

Address for correspondence: Prof. Mulazim Hussain Bukhari, Department of Pathology, University Medical College, UOL, Lahore, Pakistan.
E-mail: mulazim.hussain@gmail.com

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of RCCs. Its immuno-expression is also high in metastatic renal neoplasms and is strongly correlated with the proliferation index of the tumor.^[10]

The expression of PAX genes is important for PAX proteins to function as nuclear transcription factors which is significantly involved in cellular proliferation, differentiation, and migration and therefore, it constitutes the putative targets of disruption during the tumorigenesis.^[11] The expression of PAX-2 gene in RCCs has been established but the molecular mechanisms of oncogenic activation of PAX-2 have not been fully understood.^[12]

Keeping in mind the importance of its role in renal tumors oncogenesis, the present study was carried out to determine the frequency of PAX-2 expression in renal tumors in our setup and to see the association of PAX-2 nuclear staining with Nuclear Grades which is itself a prognostic factor.

This study of PAX-2 renal tumor immunomarker will also be helpful to improve the diagnostic accuracy in gray zone cases and suggests a novel therapeutic target for renal tumors in future.

MATERIALS AND METHODS

Study design

This was a descriptive and analytical cross-sectional study.

Study setting

This study was carried out at the Department of Pathology, King Edward Medical University, Lahore in collaboration with other tertiary care hospitals and research centers.

Sampling technique

Nonprobability purposive sampling.

Inclusion criteria

All tumors of the kidney, patients of both sexes and patients of all age groups

Exclusion criteria

Posttreated cases of renal malignancy by radiotherapy and chemotherapy.

This study included 60 archived formalin-fixed and paraffin-embedded (FF and PE) tissue blocks of renal tumors. The work was started after the approval of synopsis by ethical board/institutional review board and advanced study and research board. The required data of the patients were entered into patient's history pro forma. The obtained FF and PE tissue blocks were cut by microtome, the slides were prepared and stained with hematoxylin and eosin. The sections were picked on positively charged glass slides (Dako, Saline Coated). The immunohistochemistry stained slides were seen by two consultant histopathologists and evaluated for PAX-2 immunoexpression. The built-in RCC was considered as positive control. In positive cases, pattern of staining (cytoplasmic or nuclear), estimated percentage of stained tumor cells and nuclear staining intensity was

recorded. PAX-2 staining was seen in the nucleus of tumor cells. Less than 10% stained tumor cells were also considered to be negative staining. The PAX-2 positive cases were further categorized as follows:

1. 10%–25% stained tumor cell nuclei
2. 26%–75% stained tumor cell nuclei
3. More than 75% staining in tumor cells nuclei.

The intensity of staining in tumor cells was scored as follows:

No staining = 0
Weak staining = 1
Moderate staining = 2
Strong staining = 3+

The PAX-2 expression, PAX-2 nuclear intensity scores and estimated percentage of PAX-2 stained tumor cells were correlated with FNGs. The data entry and analysis was performed using SPSS version 22 software (IBM Corporation). Quantitative variables were presented using mean \pm standard deviation. Qualitative data were presented using frequency tables and percentages. PAX-2 expression in different types of renal tumors was presented as frequency and percentages. Chi-square test was used to see the association of FNG with PAX-2 expression. The results were stratified for RCC in relation to PAX-2 expression and FNGs. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

There were 34 males (56.7%) and 26 females (43.3%) cases in this study. The male to female gender ratio was 1.3:1. The overall mean age was 48.33 ± 16.88 within range of 2–73 years. Out of 60 cases of renal tumors, 27 cases (45%) were of low nuclear grades, whereas 29 cases (48.3%) were of high nuclear grades. Out of 34 cases of clear cell RCC (ccRCC), 20 cases were of low nuclear grades and 14 were of high nuclear grades. There were 15 cases of papillary RCC (pRCC), out of which 5 cases were of low grades and 10 cases were of high grades. Two cases of chromophobe RCC (chRCC) were of low grades. All 5 cases of sarcomatoid variant of RCC were considered to be high-grade tumors by the conventional classification. There were four cases in this study to which FNG was not applicable. These included one case of urothelial carcinoma, two cases of Wilm's tumor both of which were of favorable histology and one case of metanephric adenoma [Table 1].

A total of 60 cases of renal tumors on which anti-PAX-2 antibody immunohistochemistry (rabbit monoclonal, ab150391, abcam, UK) was performed after confirming the diagnosis of renal tumors.

Forty-five out of 60 cases (75%) showed PAX-2 nuclear immunoexpression. The frequency of positivity in renal tumors was as follows: There were 34 cases of ccRCC of which 29 cases (85.5%) were PAX-2 nuclear immunoreactive. Twelve cases (80%) of pRCC out of 15 cases were PAX-2 positive. One case (20%) of sarcomatoid variant of RCC out of 5 cases was PAX-2 positive. There was one case of metanephric adenoma which was PAX-2 positive (100%). There were

2 cases of Wilm’s tumors both of which were PAX-2 positive (100%). Two cases of chRCC and the single case of urothelial carcinoma were PAX-2 negative [Table 2].

The PAX-2 positive cases were evaluated for nuclear staining intensity and 20 cases out of 45 cases were categorized into score 1+, 16/45 cases were categorized into score 2+, and 9/45 cases were categorized into score 3+.

Table 1: Distribution according to types of renal tumors and Fuhrman Nuclear Grades (n=60)

Type of tumors (n=60)	Male: 34 (56.7%) Female: 26 (43.3%)	Fuhrman Nuclear Grade	
		Low grade	High grade
Clear Cell RCC	34 (57%)	20	14
Papillary RCC	15 (25%)	5	10
Chromophobe RCC	2 (3%)	2	0
Sarcomatoid variant RCC	5 (8%)	0	5
Urothelial Carcinoma	1 (2%)	0	0
Wilm’s Tumor	2 (3%)	0	0
Metanephric adenoma	1 (2%)	0	0
Total	60	27	29

The male to female gender ratio was 1.3:1. The overall mean age was 48.33±16.88 with range of 2-73 years

There were 27 low FNG tumors out of 60 cases which showed staining score 0, 1+, 2+ and 3+ in 3, 9, 8, and 7 cases, respectively. Twenty-nine high Furhman nuclear grades tumors out of 60 cases showed staining scores 0, 1+ and 2+ in 11, 11, and 7 cases, respectively [Table 3 and Figure 1].

DISCUSSION

In our study, the age range was 2–73 years and mean age was 48.33 ± 16.88 years. Majority of the renal tumors were seen at the age of 60 years. The tumors were more common in males (34/60; 56.7%) as compared to females (26/60; 43.3%). The ratio of male to female patients was 1.3:1. The variation in mean age and gender ratio^[2] may be due to our small sample size and unicentric study.

Our study demonstrated the frequency of lesions arising from different sites and poles of the kidney. Seven tumors were found to arise from the upper pole of kidney, 10 tumors arose from the mid of kidney, 9 tumors arose from the lower pole, 31 tumors showed the whole kidney involvement and 3 tumors showed multiple tumor nodules in the kidney. The results of this study were not consistent with the study of Ordóñez and Rosai^[2] which might be due to the small sample size in our study.

Table 2: Breakup of the 45 PAX-2 positive, negative and staining intensity in different renal tumors (n=60)

Type of renal tumors	Total	PAX-2		Intensity of staining			
		Negative (%)	Positive staining (%)	0	1+**	2+**	3+**
Clear cell RCC	34	5 (14)	29 (85)	5	12	10	7
Papillary RCC	15	3 (20)	12 (80)	3	7	5	0
Chromophobe RCC	2	2 (100)	0.00	2	0	0	0
Sarcomatoid variant RCC	5	4 (80)	1 (20)	4	1	0	0
Urothelial carcinoma	1	1 (100)	0.00	1	0	0	0
Wilm’s tumor	2	0	2 (100)	0	0	0	2
Metanephric adenoma	1	0 (100)	1 (100)	0	0	1	0
Total	60	15*	45*	15	20	16	9

*The P=0.003 which shows strong association with PAX-2 positive and negative cases, **P=0.005 which is highly significant. RCC: Renal cell carcinoma

Table 3: A Comprehensive comparison of association of different types of renal tumors with different variables included in this study

Type of tumor	No.of tumors	PAX-2 positivity		PAX-2 staining score			Estimated %age of PAX-2 staining			Low FNG grades	High FNG grades
		Positive	Percentage	1+	2+	3+	10-25%	26-75%	>75%		
Clear cell RCC	34	29	85.30	12	10	7	14	10	5	20	14
Papillary RCC	15	12	80.0	7	5	0	9	3	0	5	10
Chromophobe RCC	02	0	0	-	-	-	-	-	-	2	0
Sarcomatiod variant RCC	05	01	20	1	0	0	1	0	0	0	5
Urothelial carcinoma	01	0	0	-	-	-	-	-	-	FNG not applicable on 4 cases	
Metanephric adenoma	01	01	100	0	1	0	1	0	0		
Wilm’s tumor	02	02	100	0	0	2	0	0	2		
Total	60	45		20	16	9	25	13	7	27	29

The P=0.000, which is significant among the grades of RCC and types. The P=0.003, which shows strong association with PAX-2 staining among different grades of RCC and types

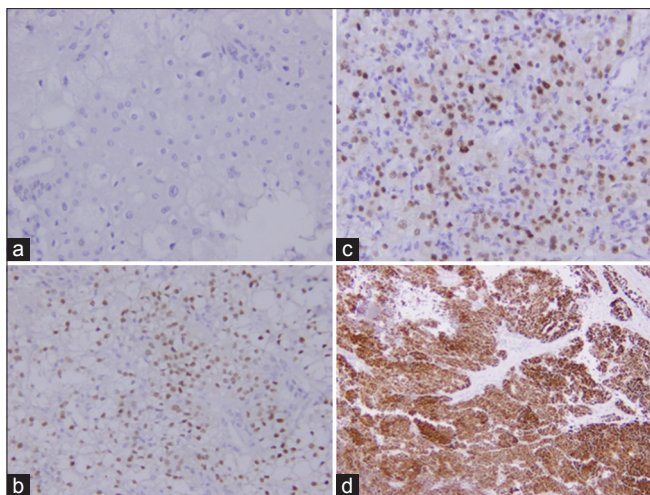


Figure 1: Photomicrograph of histological examination of a, b, c and d of PAX-2 nuclear expression in renal cell carcinoma with intensity negative, score 1+, 2+, and +3, respectively (immunohistochemistry stain, $\times 40$)

There were 60 cases of renal tumors on which anti PAX-2 antibody immunohistochemistry was performed. The overall frequency of PAX-2 positive staining was 75% (45/60). The staining scores 1+, 2+ and 3+ were in 44.44% (20/45), 35.55% (16/45) and 20% (9/45) respectively in these PAX-2 positive cases. The overall mean of staining scores was 33.34% (i.e., score 2+). These findings were somewhat consistent with the results of Ozcan *et al.*^[10,13]

In our study, the frequency of PAX-2 immunoreactivity was 85% (29/34) in ccRCC and 80% (12/15) in pRCC. These findings of our study were almost consistent with different studies mentioned in literature. In the study of Memeo *et al.*, PAX-2 expression was detected in 84% of ccRCC and 85% of pRCC.^[14] The results of Daniel *et al.* showed PAX-2 expression in 93.3% of ccRCC and 100% of pRCC.^[15] Mazal *et al.* studied 202 renal tumors and found PAX-2 expression in 88% of ccRCC and 18% of pRCC.^[16] In the study of Ozcan *et al.*, PAX-2 expression was 95% in ccRCC and 76% in pRCC.^[13] In another study by Sharma *et al.*, the frequency of PAX-2 expression in pRCC was 67%.^[17] The results of Gokden *et al.* showed 61% PAX-2 staining in renal tumor cytopathology.^[18] Thus, the frequency of PAX-2 expression in ccRCC and pRCC in our study was consistent with these studies.

In our study, ccRCC showed staining scores 1+ in 41.37% (12/29) of cases, staining scores 2+ in 34.48% (10/29) of cases and staining scores 3+ in 24.13% (7/29) of cases. The mean of staining scores in ccRCC was 33.34% (i.e., score 2+). pRCC showed staining score 1+ in 58.33% (7/12) and staining score 2+ in 41.66% (5/12). The mean of staining scores in pRCC was 50% (i.e., score 2+). Hence, the results of our study were almost consistent with studies of Ozcan *et al.*^[10,13]

The percentage of PAX-2 stained tumor cells were also evaluated and <10% stained tumor cells were considered as negative cases according to previous studies.^[3,14] The

percentage of stained tumor cells were categorized as 10%–25%, 26%–75% and >75% in positive cases.

In our study, the mean of the percentage of stained tumor cells was 33.33% in ccRCC and 50% in pRCC. These results of our study were not consistent with the study of Ozcan *et al.*^[10,13] which may be due to our small sample size, improper fixation or necrotic cells in the tissues.

In our study, the frequency of PAX-2 expression was 48.5% (27/56) in low FNGs and 51.5% (29/56) in high FNGs. In ccRCC, 59% of cases (20/34) were low grade and 41% of cases (14/34) were high grade. In pRCC, 33.3% cases (5/15) were low grade and 66.7% (10/15) were high grade. The lower grade tumors showed strong staining intensity and higher percentage of stained tumor cells as compared to high grades. The results of our study were consistent with studies of Ozcan *et al.*^[10,13] and Mazal *et al.*^[16] which showed an inverse relation of PAX-2 intensity and staining extent to FNGs.

In our study, there were only 2 cases of chRCC because of less frequency of this tumor and both of 2/2 (100%) cases were negative for PAX-2 staining. Both cases were of low nuclear grade tumor. The result of our study was consistent with the study by Patricio *et al.*^[3] Our data about chRCC has some variations with other studies such as Mazal *et al.*^[16] and Memeo *et al.*^[14] Their results of PAX-2 expression in chRCC was 13% and 9%, respectively, as these studies were having larger sample size. If more number of chRCC were included in this study, the PAX-2 positivity might be seen in some cases.

In our study, there were 05 cases of sarcomatoid variant of RCC. The frequency of PAX-2 staining was 20% (1/5). There was weak staining and estimated percentage of stained tumor cell was also low (10%–25%). As the name indicates, all cases were high grade tumors. The results of our study were consistent with the studies of Ozcan *et al.*^[10,13] and Mazal *et al.*^[16] who demonstrated that PAX-2 immunoreactivity was weak or absent in all poorly differentiated carcinomas or sarcomatoid component of ccRCC.

Metanephric adenoma is a benign lesion which is rare in its frequency. It shows tubular structures lined by small well differentiated cells.^[2] There was one case of metanephric adenoma in our study, which showed PAX-2 immunoreactivity (1/1; 100%) of moderate intensity. The result of our study was not consistent with results of Ozcan *et al.*^[13] which showed strong and diffuse PAX-2 staining in metanephric adenoma in all cases. The variation may be due to poor fixation.

Urothelial carcinoma of the renal pelvis is also a rare variant of kidney tumors. The well differentiated urothelial carcinomas are easily diagnosed, but poorly differentiated carcinomas are difficult to diagnose so in these situations specific immunomarkers are helpful.^[2] In our study, one case of urothelial carcinoma of high grade (according to its own grading system of invasiveness^[2]) was included, which showed negative PAX-2 immunostaining. This result of our study was consistent with the study of Ozcan *et al.*^[13]

In our study, 2 cases of Wilm's tumors (favorable histology) were included and both of these cases showed 100% (2/2) PAX-2 positivity. The nuclear staining was diffuse, homogeneous and strong in almost all of blastemal and epithelial components. The stromal component positivity was not appreciated. In these cases, cytoplasmic staining was also apparent but was not significant. The results of our study were consistent with the study of Davis *et al.*^[19] which showed 97% PAX-2 staining and it was strong and diffuse in epithelial and blastemal components.

So, overall the results of our study were consistent with the studies mentioned above which showed the role of PAX-2 in renal tumorigenesis. Further work needs to be done to evaluate the role of PAX-2 in renal pathogenesis and to consider it a therapeutic target for curative purposes and prevention of renal tumors progression.

CONCLUSION

PAX-2 is a diagnostic immunomarker of renal tumors. It was positive in 75% of the renal tumors in this study. Its frequency was 85% in clear cell RCC, 80% in pRCC and 100% in Wilm's tumor. PAX-2 is strongly associated with FNGs. Its frequency of expression is high in renal tumors and the intensity and percentage of staining is more in well differentiated low grade renal tumors as compared to the poorly differentiated high grade renal tumors.

Recommendations

A panel of immunomarkers can be used in which PAX-2 may help to improve the diagnostic yield and differential diagnosis of renal tumors because kidney is of Mullerian origin which shows almost uniform positivity of PAX-2 in most of the renal tumors.

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Conflicts of interest

There are no conflicts of interest.

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