Immunohistochemical Expression of Carbonic Anhydrase IX and PAX8 in Renal Cell Carcinoma: A Clinicopathological Correlation Study

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ABSTRACT:

BACKGROUND:
Renal cell carcinoma is the third most common cancer of genitourinary tract and account for approximately 2-3% of all cancer death. Conventional clear cell carcinoma comprises 75% of all renal cell carcinoma. Immunohistochemical had greatly improved the diagnostic accuracy of renal cell carcinoma.

AIM OF STUDY:
Evaluation of the expression of PAX-8 & Carbonic anhydrase IX in randomly selected samples from Iraqi patients who have RCC and correlate their expression with the clinicopathological variables.

PATIENTS &METHODS:
This is a retrospective study of 40 patients who were diagnosed to have renal cell carcinoma, CAIX & PAX-8 markers to be applied to the tumor tissue sections.
The results will be evaluated & correlated with the tumor grade, stage & other clinicopathological features.

RESULTS:
The present study showed that twenty five of cases(62.5%) were conventional clear cell RCC while fifteen of cases (37.5%) were non clear cell RCC. Five of cases (12.5%) were grade 1, twenty three of cases (57.5%) were grade 2, ten of cases (25%) were grade 3 and two of cases (5%) were grade 4. Eighteen of cases (45%) were stage I, seven of cases (17.5%) were stage II and fifteen of cases (37.5%) were stage III. The high expression of CAIX was presented in 71.0% of cases while the low expression was presented in 29.0% of cases, While the PAX-8 expression showed 22.5% of cases were score 0, 20% of cases were score 1, 25% of cases were score 2 and 32.5% of cases were score 3.

CONCLUSION:
Higher expression level of CAIX and PAX-8 in clear cell RCC than in non-clear cell type RCC so aid in differential diagnosis of RCC subtypes. PAX-8 expression was decreases with high nuclear grade while decrease CAIX levels are independently associated with advanced stage. There was no significant correlation between pax-8 &CAIX and clinicopathological variables as age and sex.

KEY WORDS: Carbonic Anhydrase IX, PAX8 expression.

INTRODUCTION:

Renal cancer is a heterogeneous disease consisting of various subtypes with diverse genetic, biochemical and morphologic features.[1] It originates from the lining of the proximal convoluted tubule. [2]The classic symptoms& signs are: flank pain, hematuria and palpable flank mass is comparatively uncommon (5-10% of cases).[3,4] Cigarette smoking and obesity are considered as the leading causes and strongest risk factors of RCC.[5] . WHO (2016) classification of renal cell carcinoma recognized several subtypes of renal cell carcinoma , most common subtypes are: clear cell renal cell carcinoma (70%), papillary renal cell carcinoma (15-18%), chromophobe renal cell carcinoma (5%), collecting duct carcinoma (about 1%) and unclassified renal cell carcinoma (4-5%) [6,7]. The current preferred method of assessing the growth potential of renal cell carcinoma is the Fuhrman nuclear grade. This relies on a subjective assessment of the nuclear characteristics of tumor cells and is recognized as being highly subjective. [8]

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CAIX is most important immunohistochemical markers in kidney carcinoma today; its presence is linked to VHL mutation. Its expression is associated with improve response to therapy [IL_2], so consider most powerful diagnostic and predictive marker for renal cell carcinoma [especially conventional type]. [9]PAX8 (paired box gene 8) is a transcription factor expressed by renal cells, its silencing leads to an arrest of cell cycle progression at the G1/S1 phase, so suggests the PAX8 expression confer growth advantage to cancer.[10]

AIM OF THE STUDY:

Evaluation of the expression of PAX-8 & Carbonic anhydrase IX in randomly selected samples from Iraqi patients who have RCC and correlate their expression with the clinicopathological variables.

MATERIALS AND METHODS:

Formalin fixed, paraffin embedded tissue blocks were collected from archived materials from Ghazi Al-Hariri specialized surgical hospital in Baghdad (covering the period from March 2018 to May 2019). Formalin-fixed paraffin tissue blocks represent 40 cases of renal cell carcinoma, all of them were small nephrectomy biopsies. All patients were newly diagnosed and received no prior therapy. Information regarding the patient's age at presentation, gender and type of samples were obtained from patient's request forms. Age of the patients ranges from 17 years to 84 years. Three sections of 5 μm thickness were taken from each block, the first was stained with hematoxylin and eosin stain (H&E) for histological revision, the others sections were stained immunohistochemically for CAIX and PAX8.

Interpretation of the staining results:

The evaluation of the immunohistochemical expression of PAX8: Positive staining for pax8 was defined as a nuclear staining pattern of epithelial tumor cells and scored as the following: no positive cells (score 0), between 1% and 15% reactive cells(score 1), between 16% and 50% positive cells (score 2) and more than 50% positive cells (score 3).[10]

The evaluation of the immunohistochemical expression of CAIX : positive staining was scored based on the percentage of positive cells.[12] specimens in which >85% of tumor cells stained for CAIX were labeled as high CAIX expressing tumors, whereas those in which ≤85% of tumor cells stained for CAIX were labeled as low CAIX expressing tumors[9]

Statistical Analysis:

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test and Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly. Chi square test was used to assess the association between certain categorical variables. A level of P – value less than 0.05 was considered significant.

RESULTS:

The total number of study cases was 40. All of them were diagnosed with renal cell carcinoma.

Age and gender

The distribution of study patients by age and gender is shown in figures (1 and 2). Study patients age was ranging from 17 to 78 years with a mean of 54.07 years and a standard deviation (SD) of ± 12.7 years. The highest proportion of study patients was aged between 40 - 59 years (52.5%).

Regarding gender, proportion of males was higher than females (84% versus 16%) with a male to female ratio of 1.66:1.
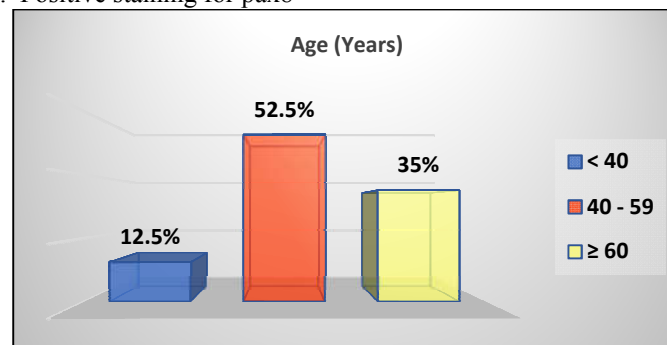

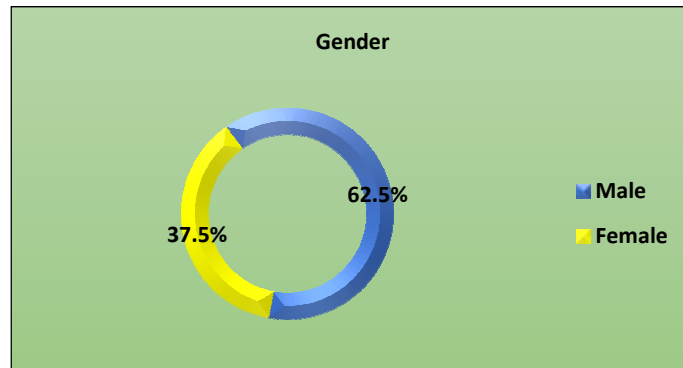**Figure 1: Distribution of study patients by age.**

Figure 2: Distribution of study patients by gender.

Tumor information:

Table 1 shows the distribution of study patients by tumor information. We noticed that clear cell carcinoma was the most common type of tumors (62.5%).

Regarding tumor grade, 57.5% of tumors were diagnosed as grade 2. figure (3) and figure (4) Tumors were staged I in 45% of cases.

Table 1: Distribution of study cases by tumor information.

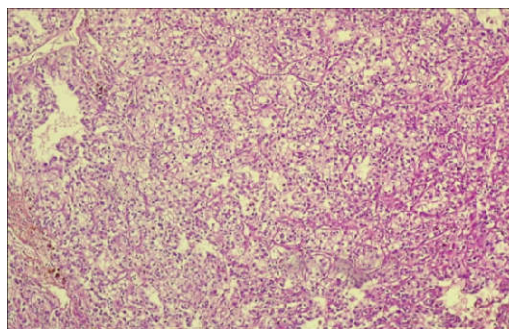| Tumor information	No. (n= 40)	Percentage (%)
Tumor type		
Clear cell carcinoma	25	62.5
Papillary cell carcinoma	8	20.0
Chromophobe	6	15.0
Tubulocystic	1	2.5
Tumor grade		
1	5	12.5
2	23	57.5
3	10	25.0
4	2	5.0
Tumor stage		
I	18	45.0
II	7	17.5
III	15	37.5

Figure 3: Renal cell carcinoma, clear cell type (x10).
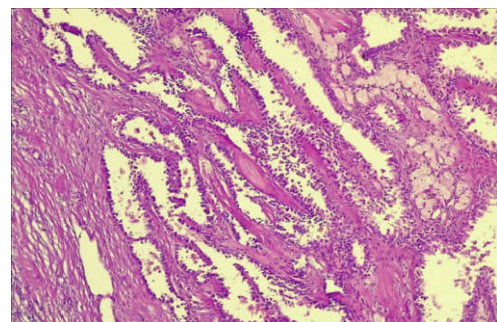
Figure 4: Renal cell carcinoma, papillary type 2 (X10).

CAIX expression

Figure (5) and figure (6) show the CAIX expression. The high expression of CAIX was presented in 62.5% of study patients as shown in figure (7).
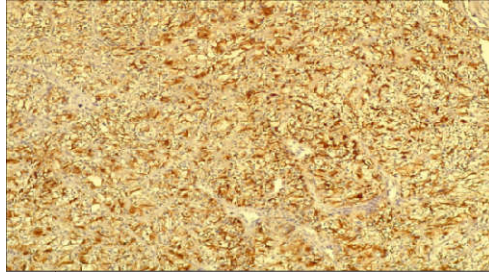

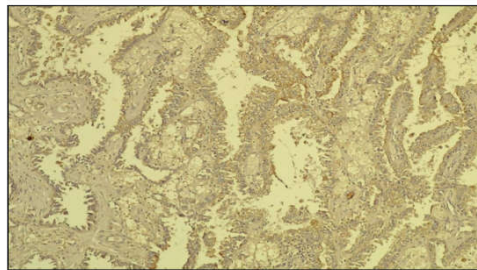**Figurer 5: Renal cell carcinoma, clear cell type immunostained for CAIX. (X10).**

Figurer 6: Renal cell carcinoma, Papillary type 2 showing weak cytoplasmic immunostain for CAIX (X10).
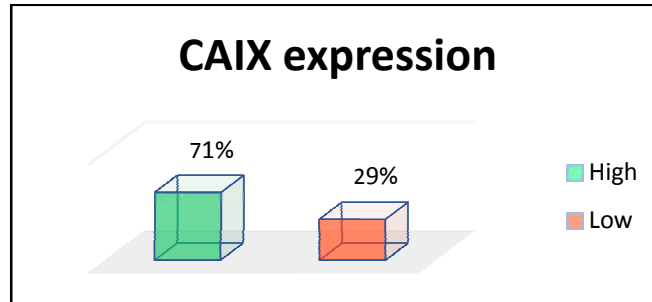

Figure 7: CAIX expression.

Association between CAIX expression and certain characteristics

The association between CAIX expression and certain characteristics is shown in table (2).

No statistical significant associations ($P \geq 0.05$) between PAX-8 expression and all characteristics.

Table 2: Association between CAIX expression and certain characteristics.

Variable	CAIX expression		Total (%) n= 40	P - Value
	High (%) n= 25	Low (%) n= 15		
Age (Year)				
< 40	3 (75.0)	1 (25.0)	4 (13.2)	
40 - 59	10 (66.6)	7 (33.4)	17 (54.8)	0.359
≥ 60	9 (90.0)	1 (10.0)	10 (32.0)	
Gender				
Male	17 (81.0)	4 (19.0)	21 (68)	0.109
Female	5 (50.0)	5 (50.0)	10 (32)	
Tumor type				
Clear RCC	18 (82.0)	4 (18.0)	22 (71.0)	0.673
Non clear RCC	4 (44.0)	5 (56.0)	9 (29.0.)	
Tumor grade				
1	4 (80.0)	1 (20.0)	5 (12.5)	
2	15 (79.9)	4 (20.1)	19 (57.5)	0.676
3	2 (40.0)	3 (60.0)	5 (25.0)	
4	1 (50.0)	1 (50.0)	2 (5.0)	
Tumor stage				
I	12 (92.0)	1 (8.0)	13 (41.9)	
II	3 (60.0)	2 (40.0)	5(16.1)	0.863
III	7 (54.0)	6 (46.0)	13 (41.9)	

PAX-8 expression

Figure (8) and figure (9) show the PAX-8 expression. We noticed that 32.5% of tumors scored (3) regarding PAX-8 expression figure (10).
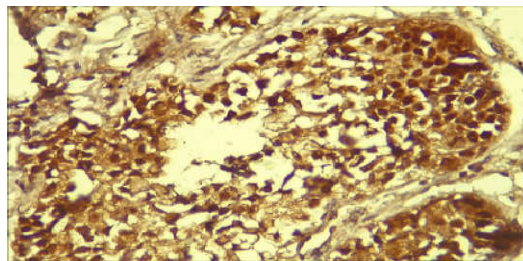

Figure 8: Renal cell carcinoma, clear cell type immunostained for PAX-8. Strong nuclear reactivity is present (X40).
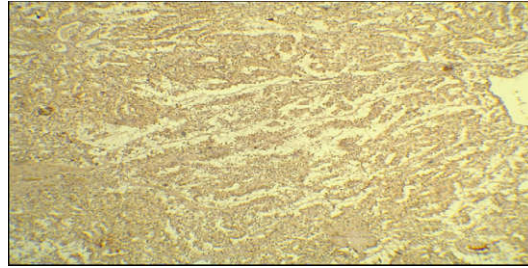
Figure 9: Renal cell carcinoma, papillary type 1 show negative immunoreactivity for PAX-8 (X10).
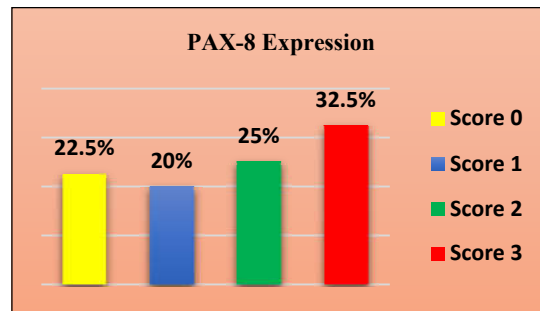

Figure 10: PAX-8 expression.

Comparison in PAX-8 expression according to certain characteristics

The comparison in PAX-8 expression according to certain characteristics is shown in table (3). No statistical significant differences ($P \geq 0.05$) in PAX-8 expression by all characteristics.

Table 3: Comparison in PAX-8 expression according to certain characteristics.

Variable	PAX-8 expression Mean ± SD	P- Value
Age (Year)		
< 40	1.4 ± 1.1	
40 - 59	1.38 ± 1.3	0.097
≥ 60	2.21 ± 0.8	
Gender		
Male	1.76 ± 1.1	0.577
Female	1.53 ± 1.3	
Tumor type		
Clear RCC	1.72 ± 1.1	0.763
Non clear RCC	1.6 ± 1.2	
Tumor grade		
1	1.51 ± 1.5	
2	2.0 ± 1.0	0.13
3	1.0 ± 1.2	
4	1.5 ± 1.4	
Tumor stage		
I	1.3 ± 1.1	
II	1.42 ± 1.1	0.834
III	1.73 ± 1.3	

Panel of CAIX and PAX-8 expression in relation to carcinoma type

We noticed that twenty of cases showed CAIX +/PAX8+ results were clear cell RCC while four of cases that showed CAIX -/PAX8 – were non-clear cell RCC, as shown in table 4.

Table 4: Panel of CAIX and PAX-8 expression in relation to carcinoma type.

CAIX/PAX8	Clear cell RCC	Non-clear cell RCC	total
CAIX +/PAX8 -	2 (66.6%)	1 (33.3%)	3
CAIX -/PAX8 +	1 (33.3%)	2 (66.6%)	3
CAIX -/PAX8 -	2 (33%)	4 (67%)	6
CAIX +/PAX8 +	20 (68%)	8 (32%)	28
total	25	15	40

DISCUSSION:

Association of CAIX expression and clinicopathological variables:

Regarding age, there was no statistical correlation between CAIX expression & Age with p. value (0.097). This is agree with *Gupta, et al*.[11] reported that showed no correlation between PAX-8 and age with p. value (0.355).Regarding sex: there was no statistical correlation between CAIX expression & sex with p. value (0.577). The present study result was relatively similar to *AtKins et al*.[12] who reported that there was no correlation between PAX-8 and sex with p. value (0.983).The study found that clear cell RCC& non-clear cell RCC had some degree of cytoplasmic membrane reactivity for CAIX ;However, clear cell RCC more often &more consistently demonstrated high CAIX expression (>85%) than non- clear cell RCC. There was no statistical correlation between CAIX expression & tumor type with p. value (0.673). This disagreed with *Elizabeth M.Genega,Musie Ghebremichael,* [9]that showed a significant correlation between CAIX expression & tumor type with p. value (<0.001).The study found that CAIX expression decrease with high nuclear grade. There was no statistical correlation between CAIX expression &tumor grade with p. value (0.676). *Kroeger N, Seligson DB, Klatte T et al.* [13] found that decrease CAIX expression occurs in tumors with highest malignant potential.The study found that CAIX expression levels were decreased with advanced stage. There was no statistical association between CAIX expression &tumor stage with p. value (0.863), agreed with *AtKins et al.* [12]

Association of PAX-8 expression and clinic pathological variables:

Regarding age: there was no statistical correlation

between PAX-8 expression & Age with p. value (0.077). This agrees with *Sangio AR, et al.*[14]. There was no statistical correlation between PAX-8 expression & sex with p. value (0.327). This is agree with *Sangio AR, et al.*[14]

Regarding the histological type of tumor: there was no statistical correlation between PAX-8 expression &tumor type with p. value (0.673), this agrees with *Tacha D,ZhouD, chengL*.[15]

Decrease PAX-8 expression with high nuclear grade. There was no statistical correlation between PAX-8 expression &tumor grade with p. value (0.13). similar to *Piao H, et al* .[16] while increase PAX-8 expression with advanced stage. There was no statistical association between CAIX expression & tumor stage with p. value (0.834), agrees with *Meghan L.Barr, Lucia B Jila vennu*.[10]

Panel of CAIX and PAX-8 expression in relation to carcinoma type: an immunoprofile of CAIX +/PAX8 + was (68%) sensitive and specific for clear cell RCC while the reverse profile of CAIX -/PAX8 – supported non-clear cell RCC. However, this study was limited to 40 cases and these findings should be validated in a large series. This agrees with Ashley Cimino-Mathers[17] found 100% sensitivity and specifity of CAIX in metastatic clear cell RCC while disagree with pax8 in the same study, showed that the pax8 was not useful Immunomarker in distinguishing metastatic clear cell RCC, also agrees with Wenjuau Yu[18] who found both CAIX and PAX8 could be used as the reliable markers to determine the renal origin in cases of metastatic RCC

CONCLUSION:

Higher expression level of CAIX and PAX-8 in clear cell RCC than in non clear cell type RCC so helpful in differential diagnosis of RCC subtypes. PAX-8 expression decreases with high nuclear

grade while decreased CAIX levels are independently associated with advanced stage. There was no significant correlation between pax-8 & CAIX and clinicopathological variables as age and sex.

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