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Immunohistochemical expression of carbonic anhydrase IX in renal cell carcinoma subtypes

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Abstract

Background: Renal cell carcinoma comprises 85% of adult kidney tumors, with its incidence rising due to improved abdominal imaging. Diagnosis relies on Hematoxylin-Eosin stained slides, immunohistochemistry, and molecular studies. Subtyping Renal cell carcinoma is crucial for prognosis, and Carbonic anhydrase IX (CAIX) expression aids in subclassification. This study evaluates CAIX expression frequency in RCC subtypes and its association with clinicopathological parameters, including age, gender, laterality, histopathological subtype, grade, and stage.

Methods: A retrospective and prospective case series study included 40 cases of primary renal cell carcinoma diagnosed between June 2021 and June 2023. Samples were collected from Al-Jumhori Teaching Hospital, AL-Rabee Private Hospital, and private laboratories in Nineveh province. The study was conducted from January 1, 2023, to September 1, 2023. Carbonic anhydrase IX protein expression was tested using immunohistochemistry with a rabbit monoclonal primary antibody (isotype IgG, clone EP161) supplied by Bio SB company, USA.

Results: The study included 40 renal cell carcinoma cases, with ages ranging from 21-77 years (mean 55.55±12.46). The sample comprised 70% males and 30% females, with 67.5% being clear cell subtype, 20% papillary, 10% chromophobe, and 2.5% clear cell-papillary variant. Significant CAIX expression was found in clear cell RCC, especially in lower-grade tumors, with no significant association with age, gender, laterality, or stage.

Conclusion: CAIX was mainly positive in clear cell subtype, thus it has important role in the differentiation of renal cell carcinoma subtypes and also for differentiating renal cell carcinoma from its mimickers. CAIX has significant association with grade of the tumor. No association was found between CAIX expression and age, gender, laterality & stage of the tumor.

Keywords: IX, Immunohistochemical, expression, carbonic, anhydrase, renal cell carcinoma, subtypes

Introduction

Renal cell carcinoma (RCC) is a heterogeneous and complex disease with numerous pathophysiological variants [1]. RCC incidence rates have been increasing, and in higher-income settings, this may partially be due to an increase in the incidental detection of renal masses when abdominal imaging is performed for nonspecific musculoskeletal or gastrointestinal complaints [2]. It accounts for more than 85% of all renal malignancies. With a steady increase of 4% in the incidence and 2.3% of the mortality, RCC has ranked thirdly most common urological cancer. Clear cell renal cell carcinoma (CCRCC), which comprises roughly 75%-80% of all RCC cases, is the most prevalent histological subtype. Due to the absence of effective diagnostic methods, along with non-specific symptoms at early stage, nearly one-third of patients present with metastasis at the time of diagnosis [3]. The prognosis of RCC patients with metastasis is unfavorable, with a fact that 5-year survival rate is less than 10% and the median survival time is 1.5 years [3]. While surgical removal is the gold standard treatment for localized kidney cancer, many targeted therapies have been recently introduced for the treatment of metastatic renal cell cancer [4]. In recent years, multiple immunohistochemical markers have been studied and offered as tools to distinguish the various renal neoplasms from each other and from morphologically similar non-renal tumors [5]. No one marker has been found to be entirely specific for RCC in general or for any specific type of RCC. Carbonic anhydrase IX (CAIX) is a marker that shows expression in RCC. CAIX is a hypoxia-induced protein that plays a role in regulating intracellular and extracellular pH [5]. The great majority of RCC cases are clear cell carcinoma, which is characterized by the bi-allelic inactivation of the Von Hippel-Lindau (VHL) gene.

This genetic event leads to the stabilization of the α-subunit of hypoxia inducible factor 1 (HIF-1). HIF-1 is a key transcriptional regulator that promotes cell growth and survival under hypoxic conditions. Its activation subsequently leads to the overexpression of multiple genes responsible for tumor angiogenesis, cell migration, glucose transport, glycolysis and pH control. Among the genes, carbonic anhydrase CAIX is one of the most prominent markers of tumor hypoxia mainly due to the unique localization of its hypoxia response element at the -10/-3 position from the transcription start site of the CAIX gene [6]. CAIX protein, as a member of the carbonic anhydrase family, catalyzes the reversible conversion of carbon dioxide to bicarbonate and protons and is thus involved in ion transport and pH control. CAIX is a transmembrane protein which is expressed in various types of human cancer (e.g., cervical, lung, breast, head and neck). CAIX overexpression in CCRCC appears to be an early event and is associated with better prognosis. The clinical and prognostic significance of CA IX in RCC have been extensively evaluated. CAIX is regarded as one of the most promising biomarkers in clear cell RCC as it has a conclusive diagnostic, prognostic, as well as therapeutic potential [6]. Aim of the study to evaluate the frequency of immunohistochemical expression of carbonic anhydrase IX in different subtypes of renal cell carcinoma and to asses the association of its expression with some clinicopathological parameters: Age, gender, laterality, histopathological subtype, grade and stage of the tumor.

Methods

This retrospective and prospective case series study included 40 cases of primary renal cell carcinoma (RCC) diagnosed between June 2021 and June 2023. Samples were collected from Al-Jumhori Teaching Hospital, AL-Rabee Private Hospital, and private laboratories in Nineveh province. The study was conducted from January 1, 2023, to September 1, 2023. Histological typing was based on WHO classification, and grading followed the WHO/ISUP grading system. Immunohistochemical staining for CAIX was performed using rabbit monoclonal antibody (isotype IgG, clone EP161) supplied by Bio SB company, USA, with membranous localization. Inclusion criteria included patients who underwent nephrectomy for RCC, confirmed by H&E staining. Histological mimickers like renal oncocytomas and angiomyolipomas were excluded. The staining protocol involved cutting and mounting formalin-fixed paraffin-embedded tissues, deparaffinizing, rehydrating, and subjecting tissues to heat-induced epitope retrieval. Antibody incubation and washing steps followed, with immunohistochemical expression of CAIX evaluated using a light microscope. Complete membranous staining was scored manually, and the proportional score was based on the percentage of tumor cells stained, ranging from negative (<5%) to 4+ (>75%). Intensity scores ranged from 0 (negative) to 3 (strong circumferential membranous staining). The CAIX total score, calculated by multiplying the intensity by the proportional score, the cases were scored according to Cleveland scoring system and the total score was ranged from 5 to 300. Tumors with scores >85% were labeled high CAIX expressing, while those ≤85% were low expressing [5,7]. Data were analyzed using SPSS software version 26.0, with qualitative data presented as frequencies and percentages. Chi-square and Fisher exact tests compared qualitative variables between groups, with a P-Value ≤ 0.05 considered statistically significant. The study found significant statistical association between CAIX expression and tumor grade, but no significant association with age, gender, laterality, or stage.

Results

The age range from 21-77 years with mean age ± SD of 55.5±12.4 years.

Gender distribution of the RCC cases

The studied sample consist of 28 (70%) males and 12 (30%) females with male to female ratio 2.33:1. with significant male preponderance (P=0.01).

Lateraliry distribution of the studied cases

Regarding laterality reveal that 24 (60%) were at left kidney and 11 (27.5%) were at right, while the remaining 5 (12.5%) of tumor were undetermined. With significant left laterality preponderance (P=0.04).

Histopathological subtypes of the cases

In this study four histological variants were reported in the following descending frequencies: Clear cell in 27 cases (67.5%), Papillary in 8 cases (20.0%), chromophobe in 4 cases (10.0%) and one case as clear cell-papillary variant (2.5%).

Grading distribution of the cases

Grading of tumor revealed that more than half of tumor was graded as grade 2 (N=23, 57.5%), nine tumors are grade 3 (22.5%), five tumors are graded as grade 4 (12.5%), three graded as grade 1 (7.5%).

Regarding the association of histopathological subtypes with the age, the most common age group was middle aged in the CCRCC, PRCC and CHRCC, with a mean age of (54.6, 59.7 and 58.2) respectively, while the one case of CCPRCC was in a patient at age of 35 years, Table 1.

Table 1: Histopathological subtypes of RCC distribution of the cases by age

Characteristics	Range	Mean±SD	P-Value
Histopathological variants			0.2
Clear cell	21-76	54.67±11.98	
Papillary	39-69	59.75±10.97	
Chromophobe	39-77	58.25±16.99	
Clear cell-papillary	35-35	35.00	

Regarding the association with gender, most of the cases were reported in males in both CCRCC and PRCC, having statistically significant association with the later (P value of 0.03), while the 4 cases of CHRCC were equally distributed between the two genders and the only one case of CPRCC being in male. From 35/40 cases having known laterality, most of CCPRCC 19/27 (70.4%) were in the left side while 7/27 (25.9%) were in the right side (P value statistically significant 0.0001). Grades of 40 cases were reported, with grade 2 being most frequent and having statistical significance in both CCRCC, P-Value: 0.001 and PRCC, (P value: 0.04). Regarding the stages of 35 cases (5 cases with unknoun stage), stage 1 and 3 were most common in CCRCC with 14/23 (51.9%) and 6/23 (22.6%) respectively and P-Value being statistically significant in CCRCC: 0.01, while PRCC was statistically not significant (P-Value: 0.7), as shown in Table 2.

Table 2: Histopathological subtypes of RCC distribution of the cases by gender, laterality, grades and stage

Gender	Clear cell	Papillary	Chromophobe	Clear cell papillary
Male	18 (64.3%)	7 (25.0%)	2 (7.1%)	1 (3.6%)
Female	9 (75.0%)	1 (8.3%)	2 (16.7%)	-
P-Value	0.08	0.03	0.7	-
Laterality	Clear cell	Papillary	Chromophobe	Clearcell Papillary
Undetermined	1 (3.7%)	2 (25.0%)	2 (50.0%)	-
Left	19 (70.4%)	4 (50.0%)	-	1 (100%)
Right	7 (25.9%)	2 (25.0%)	2 (50.0%)	-
P-Value	0.0001	0.7		
Grade	Clear cell	Papillary	Chromophobe	Clearcell Papillary
1	2 (7.4%)	-	-	1 (100%)
2	15 (55.6%)	6 (75.0%)	2 (50.0%)	-
3	7 (25.9%)	1 (12.5%)	1 (25.0%)	-
4	3 (11.1%)	1(12.5)	1 (25.0%)	-
P-Value	0.001	0.04	0.7	-
Stage	Clear cell	Papillary	Chromophobe	Clearcell Papillary
Undetermined	4 (14.8%)	-	-	1 (100%)
1	14 (51.9%)	4 (50.0%)	2 (50.0%)	-
2	2 (7.4%)	2 (25.0%)	-	-
3	6 (22.6%)	2 (25.0%)	2 (50.0%)	-
4	1 (3.7%)	-	-	-
P-Value	0.01	0.7		-

There was no significant association between CAIX expression and the age of the cases (P value=0.9), No significant association (P= 0.2) was detected between CAIX expression in CCRCC and the gender of the cases, No significant association was detected (P=0.3) between CAIX expression and the laterality of the tumor, Significant statistical association was identified between CAIX expression and tumor grade (high expression in lower grades), No significant association was detected (P=0.5) between CAIX expression and the stage of the tumor, as shown in Table 3.

Table 3: CAIX expression in CCRCC in association with the age groups, gender, Laterality, grades and stage of the cases

Age group	CA IX expression -ve No.	CA IX expression -ve %	CA IX expression +ve No.	CA IX expression +ve %
21-30	0	-	1	3.2%
31-40	1	11.1%	3	9.7%
41-50	2	22.2%	10	32.3%
51-60	1	11.1%	6	19.4%
61-70	4	44.4%	9	29%
71-80	1	11.1%	2	6.5%
P-Value	0.9			
Gender	No.	CA IX (>85%)	CA IX (≤85%)	P-Value (within gender)
Male	18	12 (66.7%)	6 (33.3%)	
Female	9	8 (88.9%)	1 (11.1%)	
P-Value (total)	27	20	7	0.2
Laterality	No.	CA IX (>85%)	CA IX (≤85%)	P-Value (within laterality)
Left	19	13 (68.4%)	6 (31.6%)	
Right	7	6 (85.7%)	1 (14.3%)	
P-Value (total)			0.3	
Grade	No.	CA IX (>85%)	CA IX (≤85%)	P-Value (within grade)
Grade 1	2	2 (100.0%)	-	
Grade 2	15	14 (93.3%)	1 (6.7%)	
Grade 3	7	2 (28.6%)	5 (71.4%)	
Grade 4	3	2 (66.7%)	1 (33.3%)	
P-Value (total)			0.01	
Stage	No.	CA IX (>85%)	CA IX (≤85%)	P-Value (within stage)
Undetermined	4	2 (50.0%)	2 (50.0%)	
Stage 1	14	12 (85.7%)	2 (14.3%)	
Stage 2	2	1 (50.0%)	1 (50.0%)	
Stage 3	6	4 (66.7%)	2 (33.3%)	
Stage 4	1	1 (100.0%)	-	
P-Value (total)			0.5	

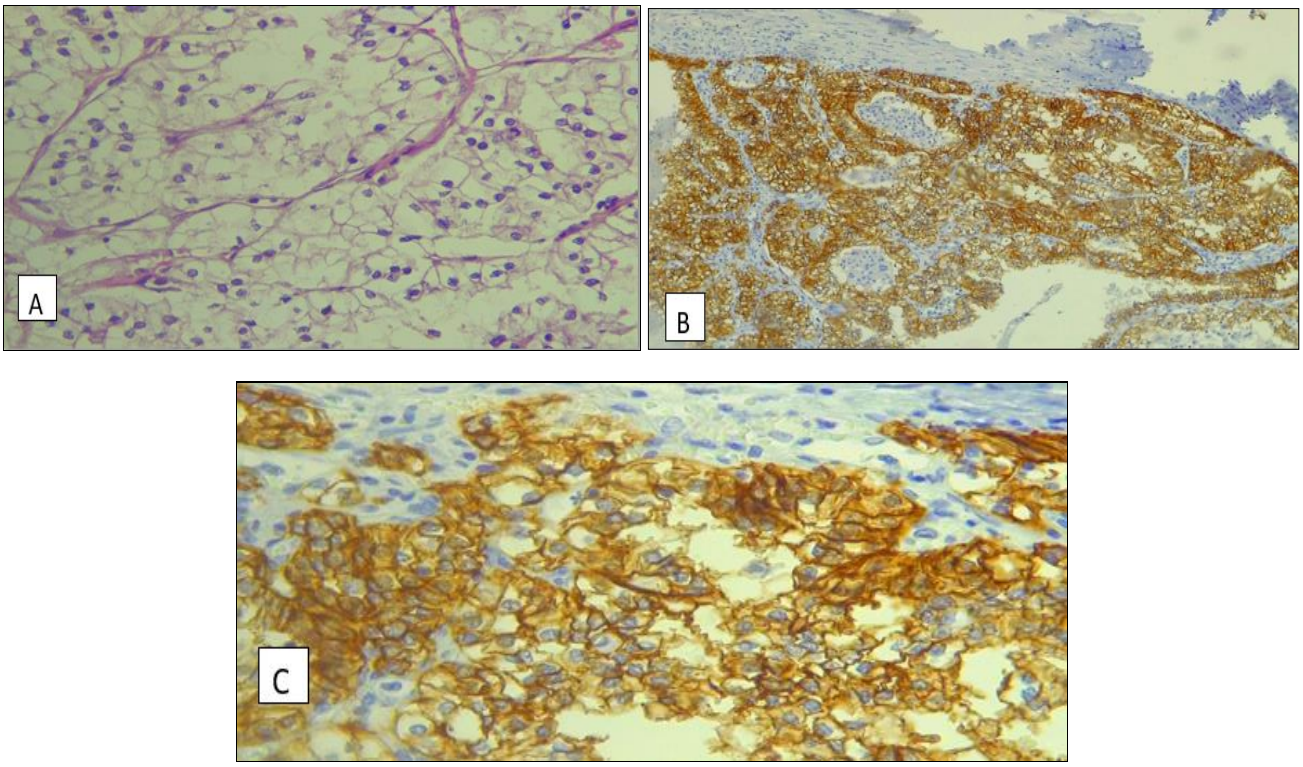

Fig 1: Clear cell RCC (A H&E 400X), B and C immunostains high CAIX expression (more than 85% of the tumor cells), (B100X, C 400X)

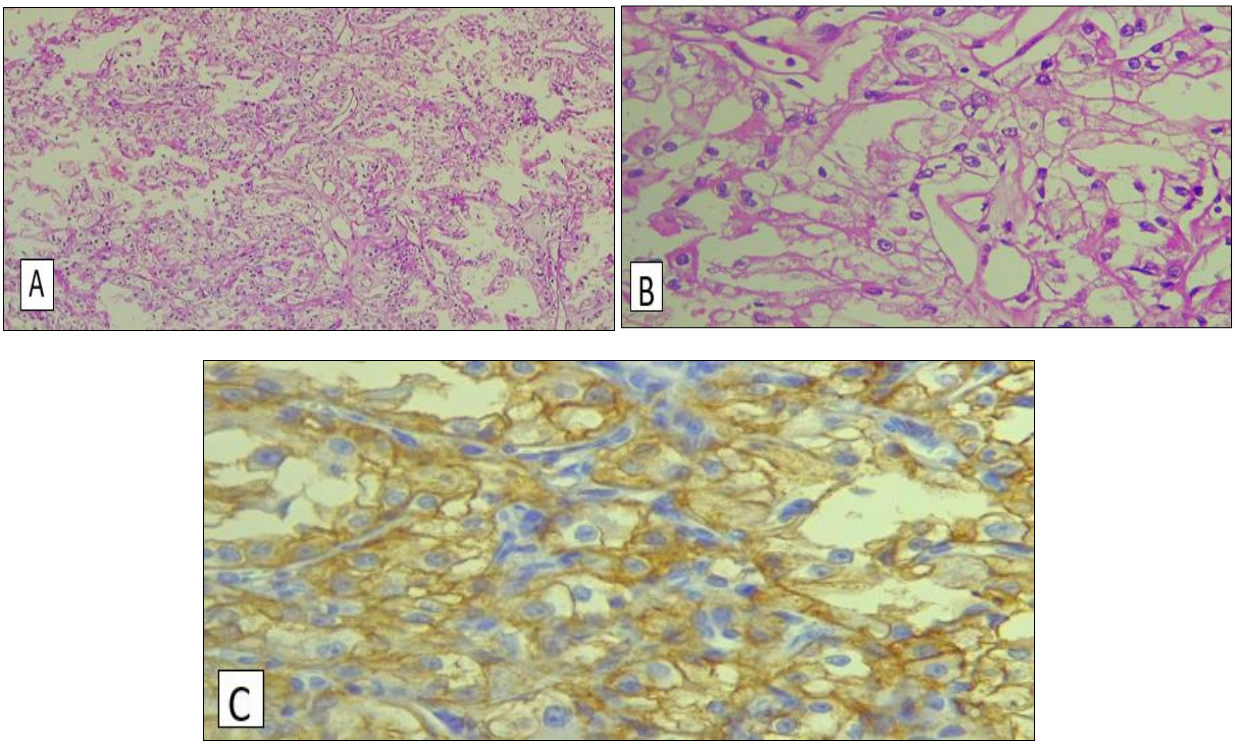

Fig 2: Clear cell RCC (H&E stain at A:100, B:400X) C: CAIX immunostain low CAIX expression: Less than 85% of the tumor cells at 400X)

Discussion:

CAIX, a hypoxia-induced protein, is a marker whose expression is reported in RCC, predominantly in the clear cell type. CAIX is a member of the carbonic anhydrase group of enzymes and has both a transmembrane and cytosolic domain [8]. In this study, 40 cases of primary RCC were included to evaluate CAIX expression using immunohistochemical staining.

The age range of participants was 21-77 years, with a Mean±SD of 55.55±12.46 years. This finding is consistent with studies by Rawa M in Slemani [1], Safaa A in Thi Qar [9], and Du Plessis in South Africa [10]. According to Buford TW, RCC is more prevalent in older individuals, possibly due to risk factors like hypertension and the long-term effects of smoking [11]. The sample comprised 28 males (70%) and 12 females (30%), aligning with studies by Fateh SM in Sulaimani [12], Ansari D in Iran [13], Chen Y in China [14], and Mancini M in Italy [15]. Chen *et al*. [14] found a positive correlation between elevated androgen receptor expression and RCC progression in males, while Mancini M

[15] noted that ER acts as a suppressor in RCC development, explaining the gender differences. This study found clear cell RCC (CCRCC) as the most common subtype (67.5%), followed by papillary RCC (PRCC) (20%), chromophobe RCC (CRCC) (10%), and one case of clear cell-papillary variant (2.5%). These results are comparable with studies by Atif Ali Hashimi *et al*. in Pakistan [16], Padala SA *et al*. in the USA [17], Muglia VF in Brazil [18], and Genega EM *et al*. in the USA [5]. Accurate classification of renal tumors is crucial due to the variable biological behavior of RCC subtypes, which affects patient prognosis and targeted therapy [5]. Tumor grading showed that 57.5% were grade 2, 22.5% grade 3, 12.5% grade 4, and 7.5% grade 1. These findings align with studies by Atif Ali Hashimi, *et al*. in Pakistan [16] and Genega EM in the USA [5]. Staging revealed that 50% of patients were stage 1, 25% stage 3, 10% stage 2, 2.5% stage 4, and 12.5% undetermined, consistent with Karakiewicz PI's study in Canada [19].

Out of 40 cases, 27 were CCRCC, all showing positive CAIX staining, with 74.1% high CAIX and 25.9% low CAIX expression. Significant statistical association was found between CAIX expression and tumor grade, with higher expression in lower grades, aligning with studies by Guorong *et al*. in France [20] and Genega EM in Brigham City, USA [5]. However, no significant association was noted by Grace X Zhao in Ohio, USA [21], and Ramachandran K in India [7], possibly due to the small sample size in this study. No significant associations were found between CAIX expression in CCRCC cases and age, gender, laterality, or stage. The single case of clear cell-papillary RCC showed CAIX reactivity, similar to Zhao J, *et al*. study in Galveston, USA [22] of the 8 PRCC cases, 37.5% showed focal CAIX positivity, while 62.5% were negative, consistent with findings by Genega M, [5] in Brigham, USA, and Gupta R, *et al*. in the USA [23]. This focal positivity is important in small biopsies to avoid diagnostic confusion. All 4 chromophobe RCC cases were negative for CAIX, in agreement with Courcier J in France, [24] and Genega EM in the USA [5].

Conclusion

The study found that RCC is most frequent in the 41-50 age group, with a male predominance. Clear cell RCC is the most common subtype, and most cases were grade 2 and stage 1. CAIX expression was positive in all clear cell RCC and clear cell-papillary RCC, focally positive in some papillary RCC, and negative in chromophobe RCC, aiding in subtype differentiation. A significant association was noted between CAIX expression and tumor grade, but not with age, gender, laterality, or stage.

Conflict of Interest

Not available

Financial Support

Not available

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