GMR
The Original

DIAGNOSTIC UTILITY OF CARBONIC ANHYDRASE IX EXPRESSION IN CLEAR CELL RENAL CELL CARCINOMA: AN IMMUNOHISTOCHEMICAL STUDY

Adhila S[1], Krishnaraj Upadhyaya [2], M H Shariff [3] , Alif Shah S[4]

[1]Senior resident, Department of Pathology.
[2]Professor Department of Pathology,
[3]Professor and Head, Department of Pathology,
[4] Senior resident, Department of Anaesthesiology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangaluru, Karnataka, India]
*Corresponding author: Alif Shah S, Yenepoya Medical college, alifzhah@gmail.com]

ABSTRACT

Background: Clear cell renal cell carcinoma (CCRCC) is the most prevalent subtype of renal cell carcinoma and is characterized by distinct molecular alterations, particularly involving the von Hippel–Lindau (VHL) gene and hypoxia-inducible factor (HIF) pathway. Although routine histopathology remains the cornerstone of diagnosis, morphological overlap with other renal neoplasms can pose diagnostic challenges. Carbonic anhydrase IX (CAIX), a hypoxia-regulated transmembrane enzyme, is consistently overexpressed in CCRCC and has emerged as a valuable immunohistochemical marker for diagnostic confirmation.

Objectives: The present study aimed to evaluate the diagnostic utility of CAIX expression in clear cell renal cell carcinoma and to assess its association with histopathological parameters.

Materials and Methods: This descriptive, observational immunohistochemical study included 42 cases of histologically diagnosed clear cell renal cell carcinoma obtained from nephrectomy specimens over a period of August 2022 to January 2024 at the Department of Pathology, Yenepoya Medical College. Routine hematoxylin and eosin-stained sections were evaluated for tumour grade, stage, and associated pathological features. Representative tumour sections were subjected to immunohistochemical staining using CAIX antibody (rabbit monoclonal, clone EP161) and scored as negative, weak (1+), moderate (2+), or strong (3+). Associations between CAIX expression and clinicopathological variables were tested using the Chi-square test and Fisher's exact test, with $p < 0.05$ considered significant.

Results: The study population ranged from 29 to 80 years (mean 57 years) with a male predominance (2.8:1). Most tumours were confined to the kidney and classified as ISUP grade 2. CAIX expression was observed in 100% of cases: 52.3% showed strong (3+) diffuse membranous "box-like" staining, 33.3% moderate (2+) staining, and 14.2% weak (1+) staining. CAIX expression intensity was significantly associated with age group ($p = 0.020$) and presence of tumour haemorrhage ($p = 0.021$); no significant association was found with gender, tumour size, lymphovascular invasion, sarcomatous differentiation, necrosis, histological grade, or pathological stage.

Conclusion: Uniform and strong expression of CAIX in clear cell renal cell carcinoma underscores its reliability as a diagnostic immunohistochemical marker. CAIX can serve as a valuable adjunct in confirming CCRCC, particularly in cases with overlapping histomorphological features.

KEYWORDS: Clear cell renal cell carcinoma, Carbonic anhydrase IX, Immunohistochemistry, Renal cell carcinoma

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignant neoplasm of the kidney, accounting for approximately 90% of all primary renal malignancies and nearly 2% of all cancers worldwide [1]. Globally, RCC represents a significant cause of cancer-related morbidity and mortality, with a rising incidence over recent decades. In 2020, over 430,000 new cases of kidney cancer were diagnosed worldwide, with a clear male predominance [2]. In India, RCC incidence is comparatively lower but steadily increasing, likely due to improved imaging, early detection, and lifestyle-related risk factors. Renal cell carcinoma encompasses a heterogeneous group of epithelial malignancies arising from renal tubular epithelium. Among its subtypes, clear cell renal cell carcinoma (CCRCC) is the most common, accounting for approximately 60–75% of all RCCs [3]. CCRCC is characterized by cells with clear cytoplasm, rich vascular networks, and distinct molecular alterations. Despite its characteristic morphology, diagnostic difficulty may arise due to overlapping features with other renal tumours such as papillary RCC, chromophobe RCC, and clear cell papillary renal cell tumour [4]. Histopathological examination using hematoxylin and eosin-stained sections remains the gold standard for RCC diagnosis. However, ancillary immunohistochemical markers are frequently employed to support diagnosis in morphologically ambiguous cases or in small biopsy specimens [4]. Accurate classification of renal tumours is crucial, as prognosis, therapeutic decisions, and targeted therapies vary significantly among RCC subtypes.

Molecular studies have elucidated the pivotal role of the von Hippel–Lindau (VHL) tumour suppressor gene in the pathogenesis of CCRCC. Inactivation of the VHL gene leads to stabilization and accumulation of hypoxia-inducible

factors (HIF-1α and HIF-2α), which in turn activate transcription of multiple hypoxia-responsive genes involved in angiogenesis, glucose metabolism, and cell survival [5]. One of the key downstream targets of the HIF pathway is carbonic anhydrase IX (CAIX).

Carbonic anhydrases are zinc-containing metalloenzymes that catalyze the reversible hydration of carbon dioxide, thereby playing a vital role in pH regulation and acid–base homeostasis [5,6]. CAIX is a transmembrane isoenzyme that is overexpressed in hypoxic tumour environments and contributes to tumour cell survival by maintaining intracellular alkalinity and extracellular acidity [7]. This adaptive mechanism promotes tumour growth, invasion, and resistance to apoptosis.

CAIX expression is strongly associated with CCRCC due to constitutive activation of the VHL–HIF pathway, even in normoxic conditions. Immunohistochemically, CAIX demonstrates a characteristic diffuse membranous "box-like" staining pattern in CCRCC, while its expression is absent or only focally present in most non–clear cell renal tumours [7]. This distinct expression pattern makes CAIX a highly sensitive and specific marker for CCRCC.

Since its initial description in renal cell carcinoma in 1986, CAIX has been extensively studied for its diagnostic and prognostic implications [7]. Several studies have demonstrated near-universal expression of CAIX in CCRCC, supporting its use as a reliable diagnostic adjunct. Additionally, variations in staining intensity and extent have been explored in relation to tumour grade, stage, and patient outcomes.

Given the diagnostic challenges posed by morphologic overlap among renal tumours and the importance of precise classification, the present study was undertaken to evaluate CAIX expression in clear cell renal cell carcinoma and to assess its correlation with histopathological features. This study aims to reinforce the role of CAIX as a dependable immunohistochemical marker in routine diagnostic practice.

AIMS AND OBJECTIVES

Aim

To study the diagnostic utility of Carbonic Anhydrase IX (CAIX) expression in clear cell renal cell carcinoma and its implications.

Objectives

- To evaluate the histopathological features of clear cell renal cell carcinoma.
- To assess the intensity and pattern of CAIX immunohistochemical expression.
- To correlate CAIX expression with histopathological and clinicopathological parameters.

MATERIALS AND METHODS

Study Design: A descriptive, observational immunohistochemical study.

Study Period: August 2022 to January 2024.

Study Setting: Department of Pathology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangaluru.

Study Material: A total of 42 nephrectomy specimens histologically diagnosed as clear cell renal cell carcinoma. Sample size was calculated using an anticipated CAIX positivity rate of 94% and a 6% margin of error at 95% confidence, yielding a minimum sample of 42.

Inclusion Criteria

- Specimens from clear cell renal cell carcinoma patients of all age groups and both genders.
- Radical and partial nephrectomy specimens with adequate tumour tissue available for immunohistochemical analysis.

Exclusion Criteria

- Non–clear cell renal tumours.
- Post-chemotherapy or post-radiotherapy specimens, and inadequate or poorly preserved tissue samples.

Histopathological Evaluation

All specimens were fixed in formalin, processed routinely, and stained with hematoxylin and eosin. Tumours were evaluated for size, grade (ISUP grading system), pathological stage (AJCC staging), necrosis, hemorrhage, lymphovascular invasion, and sarcomatous differentiation.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue. Representative tumour blocks were stained using a primary antibody against CAIX (rabbit monoclonal, anti-carbonic anhydrase IX, clone EP161) following the manufacturer's protocol. Antigen retrieval was performed prior to staining, and detection was carried out using a PolyExcel HRP (non-biotin, micro-polymer based) DAB detection system, with appropriate positive and negative controls run alongside each batch. Membranous brown staining of tumour cells was considered positive for CAIX. Slides were independently assessed by two observers, with discrepancies resolved by consensus.

Grading of CAIX Expression

CAIX expression was scored as follows: grade 0 (negative) when no immunoreactivity was seen; grade 1+ (weak) when 1–25% of tumour cells showed membranous immunoreactivity; grade 2+ (moderate) when 26–50% of cells were immunoreactive; and grade 3+ (strong) when more than 50% of cells showed diffuse, box-like membranous immunoreactivity.

Table 1: Scoring Criteria for Carbonic Anhydrase IX Expression

Grade of Expression	Characteristic Features
Grade 0	No immunoreactivity
Grade 1+	1–25% of cells immunoreactive (weak)
Grade 2+	26–50% of cells immunoreactive (moderate)
Grade 3+	>50% of cells immunoreactive (strong, diffuse box-like)

Statistical Analysis

Categorical data were expressed as number and percentage of cases. Associations between CAIX expression intensity and clinicopathological parameters were tested using the Chi-square test and Fisher's exact test, as appropriate, with two-tailed hypotheses tested against the null hypothesis of no difference. A p-value < 0.05 at 95% confidence was considered statistically significant. Statistical analysis was performed using SPSS version 27 and MS Excel.

RESULTS

Table 2: Demographic Characteristics of Patients with Clear Cell Renal Cell Carcinoma (n = 42)

Variable	Category	Number of Cases (n)	Percentage (%)
Age group (years)	< 40	5	11.9
	40–60	20	47.6
	> 60	17	40.4
Mean age	—	57 years	—
Gender	Male	31	73.8
	Female	11	26.2
Male:Female ratio	—	2.8 : 1	—

Table 2 summarizes the demographic profile of the 42 patients diagnosed with clear cell renal cell carcinoma. The majority of patients belonged to the 40–60-year age group (47.6%), followed by those older than 60 years (40.4%). Only 11.9% of cases were observed in patients younger than 40 years. The mean age of the study population was 57 years. There was a marked male predominance, with males constituting 73.8% of cases and females 26.2%, resulting in a male-to-female ratio of 2.8:1.

Table 3: Surgical and Tumour Characteristics of Clear Cell RCC (n = 42)

Parameter	Category	Number of Cases (n)	Percentage (%)
Nature of nephrectomy	Radical nephrectomy	27	64.3
	Partial nephrectomy	15	35.7
Tumour focality	Unifocal	40	95.2
	Multifocal	2	4.8
Tumour laterality	Right kidney	18	42.9
	Left kidney	24	57.1
Tumour size (pT classification)	< 4 cm	12	28.5
	4 to < 7 cm	16	40.4
	7 to < 10 cm	8	19.0
	≥ 10 cm	6	14.2

Table 3 depicts the surgical approach and tumour-related characteristics of the study population. Radical nephrectomy was the most commonly performed procedure (64.3%), while partial nephrectomy was carried out in 35.7% of patients. Most tumours were unifocal in nature (95.2%), with multifocal disease observed in only 4.8% of cases. Tumours were more frequently located in the left kidney (57.1%) compared to the right kidney (42.9%). Regarding tumour size, the largest proportion of tumours measured between 4 and <7 cm (40.4%), followed by tumours <4 cm (28.5%). Larger tumours measuring 7–<10 cm and ≥10 cm accounted for 19.0% and 14.2% of cases, respectively.

Table 4: Histopathological Features of Clear Cell Renal Cell Carcinoma (n = 42)

Histopathological Feature	Category	Number of Cases (n)	Percentage (%)
Histological diagnosis	Clear cell RCC	42	100
WHO ISUP grade	Grade 1	14	33.3
	Grade 2	24	57.1
	Grade 3	3	7.1
	Grade 4	1	2.4
Sarcomatous differentiation	Present	3	7.1
	Absent	39	92.9
Rhabdoid differentiation	Present	0	0
Haemorrhage	Present	17	40.4
	Absent	25	59.6
Tumour necrosis	Present	20	47.6
	Absent	22	52.4
Lymphovascular invasion	Present	4	9.5
	Absent	38	90.5
Perineural invasion	Present	0	0

Table 4 presents the histopathological characteristics of all 42 cases, all of which were confirmed as clear cell renal cell carcinoma. According to the WHO-ISUP grading system, Grade 2 tumours were the most common (57.1%), followed by Grade 1 (33.3%). High-grade tumours (Grades 3 and 4) were relatively uncommon, accounting for 7.1% and 2.4% of cases, respectively. Sarcomatous differentiation was identified in 7.1% of tumours (3 of 42), while rhabdoid differentiation was not observed in any case. Haemorrhage was present in 40.4% of tumours, and tumour necrosis was noted in 47.6% of cases. Lymphovascular invasion was detected in 9.5% of cases, whereas perineural invasion was absent in all specimens.

Table 5: Distribution of Cases According to AJCC Pathological TNM Staging (n = 42)

Pathological Stage (pTNM)	Number of Cases (n)	Percentage (%)
pT1aN0M0	12	28.5
pT1bN0M0	13	30.9
pT2aN0M0	3	7.1
pT2bN0M0	1	2.3
pT3aN0M0	12	28.5
pT3cN0M0	1	2.3
Total	42	100

Table 5 shows the distribution of cases according to the AJCC pathological TNM staging system. Early-stage disease (pT1) accounted for the majority of cases, with pT1aN0M0 and pT1bN0M0 stages comprising 28.5% and 30.9% of cases, respectively. Stage pT2 disease was less common, with pT2aN0M0 and pT2bN0M0 observed in 7.1% and 2.3% of cases, respectively. Advanced local disease (pT3) was seen in 30.8% of patients, predominantly pT3aN0M0 (28.5%), with a single case of pT3cN0M0 (2.3%). All patients were node-negative and had no distant metastasis at diagnosis.

CAIX Immunohistochemical Expression

CAIX immunohistochemistry was performed on all 42 cases of clear cell renal cell carcinoma. No case showed a negative (grade 0) result. Of the 42 cases, 6 (14.2%) showed weak (1+) staining, 14 (33.3%) showed moderate (2+) staining, and 22 (52.3%) showed strong (3+) diffuse, box-like membranous staining. Strong CAIX expression was most frequent among ISUP grade 1 and grade 2 tumours.

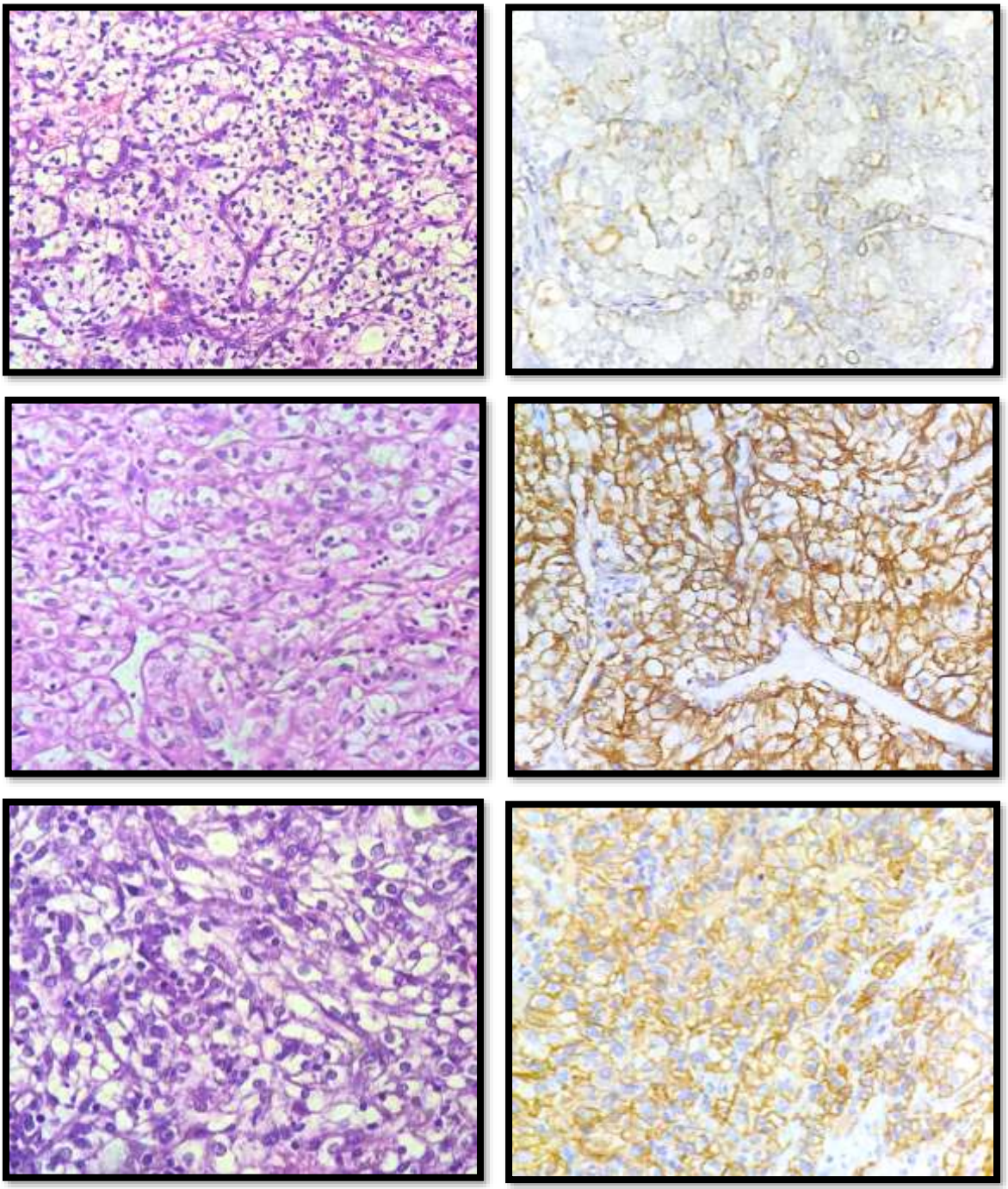

Figure 1: Histomorphology and CAIX immunohistochemical expression in clear cell renal cell carcinoma. (A) Grade 1 tumour with basophilic nuclei and inconspicuous nucleoli (H&E, 40X). (B) Grade 2 tumour with occasional nucleoli (H&E, 40X). (C) Grade 3 tumour with prominent nucleoli (H&E, 100X). (D) Weak (1+) CAIX membranous expression (IHC, 40X). (E) Moderate (2+) CAIX membranous expression (IHC, 40X). (F) Strong (3+) diffuse box-like CAIX membranous expression (IHC, 40X).

Table 6: Distribution of CAIX Expression Intensity in Clear Cell RCC (n = 42)

CAIX Grade	Number of Cases (n)	Percentage (%)
Negative (0)	0	0
Weak (1+)	6	14.2
Moderate (2+)	14	33.3
Strong (3+)	22	52.3
Total	42	100

Correlation of CAIX Expression with Clinicopathological Parameters

Table 7: Correlation of CAIX Expression with Age Group (p = 0.020)

Age Group	Negative	1+	2+	3+	Total
< 40 years	0	0	0	5	5
40–60 years	0	6	6	8	20
> 60 years	0	0	8	9	17
Total	0	6	14	22	42

All 5 cases in the <40-year group showed strong (3+) staining, and the association between age group and CAIX expression was statistically significant.

Table 8: Correlation of CAIX Expression with Gender (p = 1.00)

Gender	Negative	1+	2+	3+	Total
Male	0	5	10	16	31
Female	0	1	4	6	11
Total	0	6	14	22	42

No significant correlation was observed between gender and CAIX expression intensity.

Table 9: Correlation of CAIX Expression with Tumour Size (p = 0.522)

Tumour Size	Negative	1+	2+	3+	Total
< 4 cm	0	1	3	8	12
4 to < 7 cm	0	1	7	8	16
7 to < 10 cm	0	3	2	3	8
≥ 10 cm	0	1	2	3	6
Total	0	6	14	22	42

Tumour size did not show a statistically significant correlation with CAIX expression intensity.

Table 10: Correlation of CAIX Expression with Lymphovascular Invasion (p = 0.446)

Lymphovascular Invasion	Negative	1+	2+	3+	Total
Present	0	1	2	1	4
Absent	0	5	12	21	38
Total	0	6	14	22	42

No statistically significant correlation was found between lymphovascular invasion and CAIX expression.

Table 11: Correlation of CAIX Expression with Sarcomatous Differentiation (p = 0.866)

Sarcomatous Differentiation	Negative	1+	2+	3+	Total
Present	0	0	1	2	3
Absent	0	6	13	20	39
Total	0	6	14	22	42

Sarcomatous differentiation was present in 3 of 42 cases (7.1%) and showed no statistically significant correlation with CAIX expression.

Table 12: Correlation of CAIX Expression with Tumour Necrosis

Necrosis	Negative	1+	2+	3+	Total
Present	0	3	7	10	20
Absent	0	3	7	12	22
Total	0	6	14	22	42

No statistically significant correlation was observed between tumour necrosis and CAIX expression.

Table 13: Correlation of CAIX Expression with Haemorrhage (p = 0.021)

Haemorrhage	Negative	1+	2+	3+	Total
Present	0	4	7	6	17
Absent	0	2	7	16	25
Total	0	6	14	22	42

The presence of tumour haemorrhage showed a statistically significant correlation with CAIX expression intensity.

Table 14: Correlation of CAIX Expression with Histological Grade (p = 0.156)

ISUP Grade	1+	2+	3+	Total
Grade 1	1	2	11	14
Grade 2	5	10	9	24
Grade 3	0	2	1	3
Grade 4	0	0	1	1
Total	6	14	22	42

Histological grade did not show a statistically significant correlation with CAIX expression intensity.

Table 15: Correlation of CAIX Expression with AJCC Pathological Stage (p = 0.146)

CAIX Grade	pT1a	pT1b	pT2a	pT2b	pT3a	pT3c	Total
1+	0	1	1	0	3	1	6
2+	3	7	1	0	3	0	14
3+	9	5	1	1	6	0	22
Total	12	13	3	1	12	1	42

Pathological stage did not show a statistically significant correlation with CAIX expression intensity.

Table 16: Correlation of CAIX Expression with AJCC Prognostic Stage (p = 0.174)

CAIX Grade	Stage I	Stage II	Stage III	Total
1+	1	1	4	6
2+	10	1	3	14
3+	14	2	6	22
Total	25	4	13	42

AJCC prognostic stage did not show a statistically significant correlation with CAIX expression intensity.

Table 17: Summary of Correlations Between CAIX Expression and Clinicopathological Parameters

Parameter	Statistical Test	p-value	Significance
Age group	Chi-square	0.020	Significant
Gender	Fisher's exact	1.00	Not significant
Tumour size	Chi-square	0.522	Not significant
Lymphovascular invasion	Fisher's exact	0.446	Not significant
Sarcomatous differentiation	Fisher's exact	0.866	Not significant
Tumour necrosis	Chi-square	> 0.05	Not significant
Haemorrhage	Chi-square	0.021	Significant
Histological grade	Chi-square	0.156	Not significant
Pathological stage (pT)	Chi-square	0.146	Not significant
AJCC prognostic stage	Chi-square	0.174	Not significant

DISCUSSION

Clear cell renal cell carcinoma (CCRCC) is the most common subtype of renal cell carcinoma, accounting for approximately 70% of all RCC cases [6]. The global burden of kidney cancer continues to rise, with an estimated 81,610 new cases and 14,390 deaths expected in the United States alone in 2024 [8]. CCRCC typically presents as a solitary cortical lesion and affects both kidneys with nearly equal frequency. Accurate histological subtyping of RCC is essential due to its significant therapeutic and prognostic implications [9].

Renal neoplasms exhibit considerable morphological heterogeneity, with overlapping architectural and cytological features among different subtypes, particularly between clear cell RCC and papillary RCC. Although routine hematoxylin and eosin–stained sections are usually sufficient for diagnosis, certain cases pose diagnostic challenges, necessitating the use of immunohistochemistry (IHC) as an adjunct for definitive classification [10]. The present study evaluated the expression of Carbonic Anhydrase IX (CAIX) in CCRCC and correlated it with clinicopathological parameters.

In the current study, 42 cases of clear cell RCC were analyzed. The age of patients ranged from 29 to 80 years, with a mean age of 57 years. This is slightly lower than the mean age of 62 years reported by Verhoest G et al. [11] and Bui MH et al. [12]. However, the findings are comparable to the study by Pallagani L et al. [13] who reported a mean age of 58 years. These variations may reflect demographic and geographic differences in the study populations.

A clear male predominance was observed in the present study, with a male-to-female ratio of 2.8:1. This finding is consistent with multiple studies in the literature, including those by Bui MH et al. [12] and Pallagani L et al. [13] which reported similar ratios. Although Abraham GP et al. [14] reported a much higher male predominance, all studies consistently demonstrate male preponderance in CCRCC, possibly due to hormonal, environmental, or lifestyle-related risk factors.

Radical nephrectomy constituted the majority of specimens in this study (64.3%), followed by partial nephrectomy (35.7%), reflecting standard surgical practices for localized and advanced renal tumours. Tumour laterality showed no significant predilection, consistent with published literature. Tumour size in the present study ranged from 2 cm to 19 cm, comparable to findings by Dagher et al. [15] and Ali RM et al. [16], indicating similar disease presentation patterns.

Most tumours in the current study were unifocal (95.2%), consistent with observations by Gürsoy D et al. [17] and Perrino CM et al. [18]. Lymphovascular invasion (LVI) was identified in only 9.5% of cases, which is lower than rates reported in other series [15]. The absence of perineural invasion in all cases further supports the relatively localized nature of disease in this cohort. Differences in LVI rates across studies may be attributed to variations in sample size and pathological assessment criteria.

Sarcomatous differentiation, a marker of aggressive tumour behavior, was observed in 7.1% of cases in this study (3 of 42), comparable to other reported series [15,16]. Hemorrhage and necrosis were present in 40.4% and 47.6% of cases, respectively. Necrosis has been reported as an adverse prognostic factor and was more frequently observed in grade 2 tumours in the present study, similar to observations by Khor LY et al. [19].

Histological grading revealed that ISUP grade 2 was the most common, aligning with findings from Bui MH et al. [12] and Yoshida T et al. [20]. Unlike several studies where grade 3 was more prevalent than grade 1, the present study demonstrated grade 1 as the second most common, similar to findings by Ali RM et al. [16].

Pathological staging showed that the majority of cases were pT1 (59.4%), followed by pT3 (30.8%). This trend of early-stage detection is consistent with other studies [20]. AJCC staging remains a crucial prognostic indicator guiding patient management.

Immunohistochemical evaluation demonstrated CAIX positivity in 100% of CCRCC cases, with strong (3+) membranous staining in 52.3% of cases, moderate (2+) staining in 33.3%, and weak (1+) staining in 14.2%. This finding is consistent with studies by Al-Ahmadie HA et al. [5,21], reinforcing CAIX as a highly sensitive marker for CCRCC. Although Büscheck F et al. [22] reported occasional CAIX-negative CCRCCs, such discrepancies may be due to interobserver variability or tumour heterogeneity.

Age group and the presence of haemorrhage were shown to be statistically significantly correlated with CAIX expression ($p = 0.020$ and $p = 0.021$, respectively). Gender, tumour size, lymphovascular invasion, sarcomatous differentiation, necrosis, histological grade, and pathological stage did not show a statistically significant correlation with CAIX expression intensity. These results contrast with those of Büscheck et al. [22], who found strong correlations with grade and stage, although they are consistent with the work of Ramachandran K et al. [23]. Such disparities may be related to larger sample sizes in those studies.

Overall, the present investigation supports the diagnostic value of CAIX as a valid immunohistochemical marker for clear cell renal cell carcinoma, particularly in instances with morphological ambiguity.

CONCLUSION

The present immunohistochemical study highlights the diagnostic utility of Carbonic Anhydrase IX (CAIX) in clear cell renal cell carcinoma. All 42 cases of histologically confirmed clear cell RCC demonstrated positive CAIX expression, with the majority showing strong diffuse membranous ("box-like") staining. This consistent expression pattern highlights the great sensitivity of CAIX as a clear cell RCC diagnostic marker and validates its use as a trustworthy supplement to standard histological assessment, especially in patients with difficult morphology. Clinicopathological analysis showed that clear cell RCC often manifested in the fifth or seventh decades of life and primarily affected men. The majority of tumors were categorized as ISUP grade 2, unifocal, and limited to the kidney. Only patient age and the presence of tumor hemorrhage were shown to be statistically significantly correlated with changes in CAIX staining intensity. Gender, tumor size, histological grade, pathological stage, necrosis, lymphovascular invasion, and sarcomatous differentiation did not significantly correlate with CAIX expression.

LIMITATIONS OF THE STUDY

- Small sample size: The study included only 42 cases of clear cell renal cell carcinoma, which may limit the statistical power and generalizability of the findings.
- Single-center study: As the study was conducted at a single tertiary care center, the results may not reflect the full demographic and biological variability of clear cell RCC seen in the broader population.
- Lack of comparison with other RCC subtypes: Only clear cell RCC cases were included; hence, comparative evaluation of CAIX expression with other renal cell carcinoma subtypes was not performed.
- Absence of survival and follow-up data: Prognostic implications of CAIX expression could not be assessed due to lack of long-term follow-up and survival analysis.
- Limited immunohistochemical panel: The study focused solely on CAIX expression, and inclusion of additional immunohistochemical markers could have provided a more comprehensive diagnostic and prognostic assessment.

ETHICAL CONSIDERATIONS

This study was approved by the Institutional Ethics Committee, Yenepoya (Deemed to be University) [YEC-1/2022/114, 31-08-2023]. The study was conducted in accordance with the Declaration of Helsinki.

CONFLICT OF INTEREST: None declared
SOURCE OF FUNDING: NIL

REFERENCES

1. Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, Rawla P, Barsouk A. Epidemiology of renal cell carcinoma. World J Oncol. 2020;11(3):79.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49.
3. Genega EM, Ghebremichael M, Najarian R, Fu Y, Wang Y, Argani P, Grisanzio C, Signoretti S. Carbonic anhydrase IX expression in renal neoplasms: correlation with tumor type and grade. Am J Clin Pathol. 2010;134(6):873-9.
4. Al-Ahmadie HA, Alden D, Qin LX, Olgac S, Fine SW, Gopalan A, Russo P, Motzer RJ, Reuter VE, Tickoo SK. Carbonic anhydrase IX expression in clear cell renal cell carcinoma: an immunohistochemical study comparing 2 antibodies. Am J Surg Pathol. 2008;32(3):377-82.
5. Frew IJ, Moch H. A clearer view of the molecular complexity of clear cell renal cell carcinoma. Annu Rev Pathol. 2015;10:263-89.
6. Robertson N, Potter C, Harris AL. Role of carbonic anhydrase IX in human tumor cell growth, survival, and invasion. Cancer Res. 2004;64(17):6160-5.
7. Little MH, Combes AN. Kidney organoids: accurate models or fortunate accidents. Genes Dev. 2019;33(19-20):1319-45.
8. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12-49.
9. Batai K, Harb-De la Rosa A, Lwin A, Chaus F, Gachupin FC, Price E, Lee BR. Racial and ethnic disparities in renal cell carcinoma: an analysis of clinical characteristics. Clin Genitourin Cancer. 2019;17(1):e195-202.
10. Phuoc NB, Ehara H, Gotoh T, Nakano M, Kamei S, Deguchi T, Hirose Y. Prognostic value of the co-expression of carbonic anhydrase IX and vascular endothelial growth factor in patients with clear cell renal cell carcinoma. Oncol Rep. 2008;20(3):525-30.
11. Verhoest G, Veillard D, Guillé F, De La Taille A, Salomon L, Abbou CC, Valéri A, Lechevallier E, Descotes JL, Lang H, Jacqmin D. Relationship between age at diagnosis and clinicopathologic features of renal cell carcinoma. Eur Urol. 2007;51(5):1298-305.
12. Bui MH, Seligson D, Han KR, Pantuck AJ, Dorey FJ, Huang Y, Horvath S, Leibovich BC, Chopra S, Liao SY, Stanbridge E. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res. 2003;9(2):802-11.
13. Pallagani L, Choudhary GR, Himanshu P, Madduri VK, Singh M, Gupta P, Shrivastava N, Baid G, Meenakshi R, Aasma N, Pareek P. Epidemiology and clinicopathological profile of renal cell carcinoma: a review from tertiary care referral centre. J Kidney Cancer VHL. 2021;8(1):1.
14. Abraham GP, Cherian T, Mahadevan P, Avinash TS, George D, Manuel E. Detailed study of survival of patients with renal cell carcinoma in India. Indian J Cancer. 2016;53(4):572-4.
15. Dagher J, Dugay F, Verhoest G, Cabillic F, Jaillard S, Henry C, Arlot-Bonnemains Y, Bensalah K, Oger E, Vigneau C, Rioux-Leclercq N. Histologic prognostic factors associated with chromosomal imbalances in a contemporary series of 89 clear cell renal cell carcinomas. Hum Pathol. 2013;44(10):2106-15.
16. Ali RM, Muhealdeen DN, Fakhralddin SS, Bapir R, Tahir SH, Rashid RJ, Omer CS, Abdullah HO, Abdalla BA, Mohammed SH, Kakamad FH. Prognostic factors in renal cell carcinoma: a single-center study. Mol Clin Oncol. 2023;19(3):1-7.
17. Gürsoy D, Seçinti İE. Renal cell carcinoma: epidemiological profile and histopathological features.
18. Perrino CM, Grignon DJ, Williamson SR, Idrees MT, Eble JN, Cheng L. Morphological spectrum of renal cell carcinoma, unclassified: an analysis of 136 cases. Histopathology. 2018;72(2):305-19.
19. Khor LY, Dhakal HP, Jia X, Reynolds JP, McKenney JK, Rini BI, Magi-Galluzzi C, Przybycin CG. Tumor necrosis adds prognostically significant information to grade in clear cell renal cell carcinoma: a study of 842 consecutive cases from a single institution. Am J Surg Pathol. 2016;40(9):1224-31.

20. Yoshida T, Ohe C, Ikeda J, Atsumi N, Ohsugi H, Sugi M, Higasa K, Saito R, Tsuta K, Matsuda T, Kinoshita H. Eosinophilic features in clear cell renal cell carcinoma correlate with outcomes of immune checkpoint and angiogenesis blockade. J Immunother Cancer. 2021;9(9).
21. Kuroda N, Tanaka A, Ohe C, Nagashima Y. Recent advances of immunohistochemistry for diagnosis of renal tumors. Pathol Int. 2013;63(8):381-90.
22. Büscheck F, Fraune C, Simon R, Kluth M, Hube-Magg C, Möller-Koop C, Shadanpour N, Bannenberg C, Eichelberg C, Höflmayer D, Clauditz T. Aberrant expression of membranous carbonic anhydrase IX (CAIX) is associated with unfavorable disease course in papillary and clear cell renal cell carcinoma. Urol Oncol. 2018;36(12):531.e19.
23. Ramachandran K, MR B, Jojo A, Pooleri GK, Thomas A. Role of CAIX expression in conventional renal cell carcinomas as a diagnostic marker and its prognostic importance. Indian J Surg Oncol. 2021;12:79-84.