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Research Article

Immunohistochemical Expression of Cyclin D1 in Renal Cell Carcinoma Subtypes

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Abstract

Background: Renal cell carcinoma (RCC) is the second-leading cause of mortality among urological malignancies and accounts for 2–3% of adult malignancies. Approximately 25% of patients experienced recurrence or metastasis subsequent to surgery, accompanied by radiation and chemotherapy failure. Cyclin D1, a critical regulator of the Growth/Synthesis (G1/S) transition, is a subject of significant research. ***Objectives***: To assess the frequency of immunohistochemical expression of cyclin D1 in RCC subtypes and its association with some clinicopathological parameters such as age, gender, size of tumor, histological type, and grade. ***Methods***: During a period of eleven months extending from March 2023 through January 2024, 64 RCC cases were included in this retrospective case series study, obtained from the histopathological departments of private laboratories in Mosul city, Iraq. An immunohistochemistry investigation using Cyclin D1 was performed, and data of the positive cyclin D1 expression were evaluated. ***Results***: The mean age was 54.8 years, with male predominance (53%). Renal cell carcinoma cases included in this study show immunohistochemical expression of Cyclin D1, but with different staining grades. 39% were low expressers, versus 61% were high expressers. Cyclin D1 expression showed a significant association with age, histological type, tumor size, and nuclear grade, respectively. ***Conclusions***: Cyclin D1 is overexpressed in renal cell carcinoma, and targeting it may benefit treatment and prevention. In renal cell cancer, cyclin D1 expression is associated with a favorable prognosis and can aid in predicting patient outcomes.

Keywords: Cyclin D1, Immunohistochemistry, Renal cell carcinoma subtypes.

التعبير المناعي الكيميائي للسيكلين D1 في الأنواع الفرعية لسرطان الخلايا الكلوية

الخلاصة

الخلفية: سرطان الخلايا الكلوية (RCC) هو السبب الرئيسي الثاني للوفيات بين الأورام الخبيثة في المسالك البولية ويمثل 2-3٪ من الأورام الخبيثة لدى البالغين. ما يقرب من 25 ٪ من المرضى يعانون من تكرار أو ورم خبيث بعد الجراحة، يرافقه فشل الإشعاع والعلاج الكيميائي. Cyclin D1 منظم حاسم لانتقال النمو/التوليف (G1/S) ، هو موضوع بحث مهم. **الأهداف:** تقييم تواتر التعبير المناعي الكيميائي للسيكلين D1 في الأنواع الفرعية لسرطان الخلايا الكلوية وارتباطه ببعض المعلمات السريرية المرضية مثل العمر والجنس وحجم الورم والنوع النسيجي والدرجة. **الطريقة**: خلال فترة أحد عشر شهرا امتدت من مارس 2023 حتى يناير 2024 ، تم تضمين 64 حالة RCC في دراسة سلسلة الحالات بأثر رجعي هذه، والتي تم الحصول عليها من أقسام علم الأنسجة المرضية في المختبرات الخاصة في مدينة الموصل، العراق. تم إجراء فحص الكيمياء الهيستولوجية المناعية باستخدام Cyclin D1 ، وتم تقييم بيانات التعبير الأيجابي ل Cyclin D1. **النتائج**: كان متوسط العمر 54.8 سنة، مع غلبة الذكور (53٪). تظهر حالات سرطان الخلايا الكلوية المدرجة في هذه الدراسة تعبيرا كيميائيا مناعيا ل Cyclin D1 ، ولكن بدرجات صبغية مختلفة. 39٪ كانوا معبرين منخفضين، مقابل 61٪ كانوا معبرين عاليين. أظهر تعبير Cyclin D1 ارتباطا كبيرا بالعمر والنوع النسيجي وحجم الورم والدرجة النووية على التوالي. **الاستنتاجات**: يتم التعبير عن Cyclin D1 بشكل مفرط في سرطان الخلايا الكلوية، وقد يفيد استهدافه العلاج والوقاية. في سرطان الخلايا الكلوية، يرتبط تعبير سيكلين D1 بتكهن إيجابي ويمكن أن يساعد في التنبؤ بنتائج المرضى.

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INTRODUCTION

Renal cell carcinoma (RCC) makes up approximately 2–3% of malignant tumors in adults and about 80%–90% of kidney cancer cases [1-3]. It is the second-leading cause of death among urological malignant tumors. 25% of RCC patients eventually have a disease recurrence or metastases after radical surgical resection [4], with a tendency for widespread metastasis and high mortality with 5% 5-year survival in metastatic RCC [5,6]. RCC pathogenesis and development are known to be strongly

correlated with a number of risk factors, such as dietary practices, physical activity, occupational exposure to certain carcinogens and others such as Simian virus 40 (SV40), an oncogenic virus that implicated dysregulation of cyclin dependent kinase (CDK) regulators in RCC [1,7]. Three main subtypes of RCC may be recognized histologically: clear-cell RCC (ccRCC), papillary RCC (pRCC), and chromophobe RCC (chRCC). Of them, the most aggressive subtype is ccRCC, which makes up over 80% of all RCCs [8,9]. Only 11.7% of advanced RCC patients will survive for five years, and the cancer often responds poorly to chemotherapy and conventional radiation [8]. Cyclin D1 (CCND1) is a key regulator of the G1/S transition and is hence of great interest [2]. It is a 36-kilodalton (kDa) protein encoded by the *CCND1* gene, which is located on chromosome 11q13 [10], necessary for cell cycle progression through the G1 phase. CCND1 controls the G1/S phase transition by binding with cyclin-dependent kinase 4 (CDK4) and CDK6. The CCND1-CDK4 complex inhibits retinoblastoma protein (Rb) by phosphorylation and allows transcription factors such as E2F to transcribe genes needed for entry into the S phase [2]. When CCND1 is overexpressed, the G1 phase is shortened and there is less reliance on growth hormones, which causes aberrant cell proliferation and may encourage the development of more genetic defects [11]. CCND1 is an established oncogene that is overexpressed in a significant number of human malignancies, such as RCC [4], breast cancer [12], melanoma [13], endometrioid carcinoma [14], high-grade astrocytic tumors [15], colorectal carcinoma [16], 50 to 55% of gastric cancer [17], 35% to 40% of squamous cell carcinoma of the head and neck [18], carcinoma of the bladder, pituitary tumors, pancreatic cancers, and nonsmall-cell lung cancers [2]. The role of CCND1 as a regulator of cellular invasiveness and aggressiveness has been redefined in light of recent in vitro and in vivo studies. The interactions between cancer cells and the stroma are determined in large part by CCND1 overexpression, which has the effect of "promoting" tumor growth [10]. Overexpression of CCND1 is mostly associated with the early onset of cancer, tumor development, decreased cancer patient survival, and an increase in metastases [19]. The determination of CCND1 expression in tumors may help the use of CDK inhibitors in cancer therapy [10]. Previous studies revealed that higher levels of the CCND1 protein have been linked to improved clinical outcomes in RCC. Furthermore, it appears that various RCC subtypes have distinct patterns of CCND1 expression [20]. This study aimed to assess the frequency of immunohistochemical expression of cyclin D1 in renal cell carcinoma subtypes in addition to assessing the association between cyclin D1 and variable clinicopathological parameters such as age of the patients, gender, size of tumor, histological type, Fuhrman nuclear grade, and stage of renal cell carcinoma.

METHODS

Patients and sample collection

In this retrospective case series study, during a period from March 2023 to January 2024, 64 cases of previously diagnosed renal cell carcinoma [paraffin-embedded tissue blocks] obtained through partial or radical nephrectomy operations were collected from the labs of many private hospitals in Mosul city/North of Iraq. Clinicopathological information (age, gender, laterality, and tumor size) was obtained from the medical record. A 4-micron-thickness section was obtained from paraffin blocks of cases included in this study, and then hematoxylin and eosin (H&E) slides were prepared and examined for histopathological re-evaluation of each case regarding the histological type and Fuhrman nuclear grade [21].

Immunohistochemistry procedure

A block of formalin-fixed paraffin-embedded sufficient non-necrotic tumor tissue was collected, and 4 micron-thick sections were rehydrated after deparaffinization in xylene. The immunohistochemistry method followed the manufacturer's specifications. Heat-induced antigen retrieval techniques were used by boiling tumor tissue sections twice in citrate buffer, pH 6.0, in a microwave for ten minutes, cooling to room temperature for twenty minutes, and then washing in phosphate buffered saline. Non-specific binding was blocked by adding two drops of protein-blocking serum (Cat # AEX080-IFU, ScyTec Laboratories, USA, ready-to-use) for ten minutes. To apply monoclonal rabbit antibody Anti-Cyclin D1 (Dako-USA-clone EP12 Catalog #IR083-USA), tumor tissue slices were incubated overnight in a humid atmosphere at 4 °C. The previous stage resulted in the employment of a ready-to-use universal staining kit (Cat # AEX080-IFU, ScyTec Laboratories, USA) to detect the immune complex. Tumor tissue sections were treated with biotinylated goat anti-polyvalent antibody (Econo Tek, ScyTec laboratories, ready to use), followed by peroxidase-labeled streptavidin (Econo Tek HRP, ScyTec laboratories, ready to use) for thirty minutes at room temperature, followed by a phosphate buffered saline rinse, incubation with a substrate/chromogen diaminobenzidine (DAB) mixture for 5-15 minutes, and a rinse with distilled water. The tissue sections were counterstained with hematoxylin, rinsed in tap water, dehydrated with alcohol, clarified in xylene, and allowed to dry before being mounted with an aqueous-based mounting solution, dibutylphthalate polystyrene xylene (DPX), and the cover slid. Skin (the basal layer of the epidermis) serves as an external control tissue.

Interpretation of CCND1 expression

The immunohistochemical sections stained for CCND1 protein were evaluated under the microscope. The immunohistochemical expression of CCND1 was categorized into 4 scoring groups according to the percentage of positive cells (nuclear cyclin D1 expression) in the tumor section, as shown in Table 1.

Table 1: Scoring system for Cyclin D1 immunohistochemical expression (nuclear staining) [20].

Group	Positive cells (%)	CCND1 protein expression
1	No positive cells	Low
2	30	
3	>30 to 60	High
4	> 60	

Cases with scores 1 and 2 were regarded as having a low expression of CCND1, while cases showing grades 3 and 4 were considered to have a high expression of CCND1 [20].

Ethical consideration

This study was carried out in accordance with the principles of ethics outlined in the Declaration of Helsinki, and it was authorized by the Medical Research Ethics Committee, College of Medicine, University of Mosul, on February 14, 2024, under the reference number (UOM/COM/MREC/23-24/FEB1).

Statistical analysis

The results were statistically analyzed using the chi-square test. A Fisher exact test was used when indicated. A p-value <0.05 is regarded as statistically significant.

RESULTS

This study included 64 cases of RCC, with the majority of cases (46) being of the clear cell subtype, while 18 cases were of the non-clear cell subtype, which included papillary (13), chromophobe (4), and one poorly differentiated subtype. The age of patients ranged from 35 to 82 years (mean 54.8 years), with 67.2% of them being ≤ 60 years of age. Regarding gender, 34 out of 64 (53%) were male and 30 out of 64 (47%) were female, with a male-to-female ratio of 1.13:1. Tumor size ranged from 2 to 17 cm, with an average of 6.21±3.27 cm. 67.2% of the cases were ≤ 7 cm (pathological stage 1), and 32.8% of the cases were > 7 cm (pathological stage ≥ 2). Although in 18.75% of the cases, sides were not specified, most of the cases were on the right side, with a right-to-left ratio of 1.48:1. Figure 1 shows the frequency of CCND1 expression in RCC cases. Figure 2 demonstrates the level of expression in the studied cases, where high expression is detected in 61% of cases. Cyclin D1 Immunohistochemical staining (scoring groups) of cases included in this study is illustrated in Figure 3. The frequency of immunohistochemical expression of cyclin D1 in renal cell carcinoma subtypes and its association with gender, age, side, tumor size, nuclear grade, and tumor stage are summarized in Tables 2 and 3.

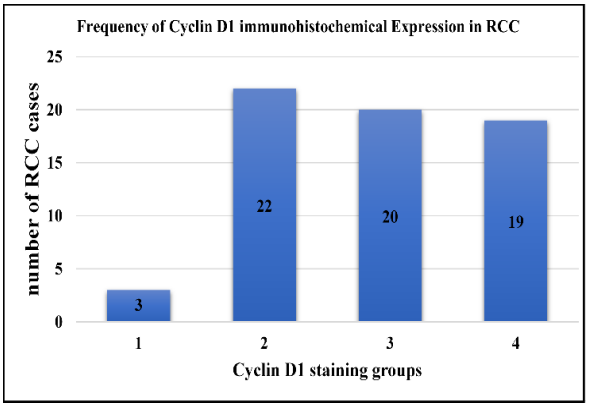

Figure 1: Frequencies of CCND1 expression in RCC cases.

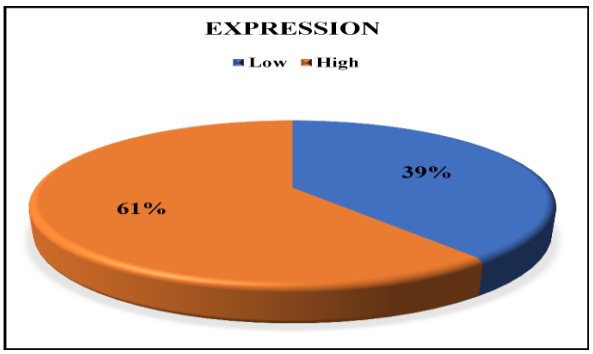

Figure 2: Frequency of Cyclin D1 Immunohistochemical staining of cases included in this study. Low Expression includes groups (1 and 2), High Expression includes (groups 3 and 4).

Cyclin D1 expression showed a significant association with age, histological type, tumor size, and nuclear grade (p= 0.008, <0.00001, 0.008, and 0.006, respectively).

DISCUSSION

Cyclins, discovered in 1982, have been the focus of cancer research due to their position as master regulators of the cell cycle. Cyclins were first thought to be simple switches that permitted the cell cycle to continue. However, several years of research have revealed that they play a significantly more complex role and can affect a variety of cellular functions [22]. This study assesses the prevalence of cyclin D1 immunohistochemistry expression in 64 RCC cases from various subtypes (Figure 4). This study's histological subtypes of RCC align with those found by Latić *et al*. in Serbia, Lima *et al*. in Brazil, Rizwan *et al*. in Lahore, Pakistan, and Xue *et al*. in China. Clear cell RCC was the most common subtype of renal cell carcinoma, accounting for 66.2%, 71.55%, 77.4%, and 84% of cases, respectively [23,20,21,24].

Table 2: The frequency of immunohistochemical expression of cyclin D1 in renal cell carcinoma subtypes

Histologic type		Cyclin D1 staining grade				Total	*p*-value
		1 (Negative)	2 (<30%)	3 (30-60%)	4 (>60%)		
Clear cell RCC		0	10	17	19	46	
Non clear cell	Papillary RCC	3	9	1	0	13	
	Chromophobe RCC	0	2	2	0	4	<0.00001*
	Poorly differentiated RCC	0	1	0	0	1	
RCC	Total	3	22	20	19	64	

Values are presented as frequencies. *Fisher's Test has been used.

Table 3: The frequency of immunohistochemical expression of cyclin D1 in renal cell carcinoma subtypes and its association with gender, age, side, histological type, tumor size, Fuhrman nuclear grade and tumor stage

Clinicopathological parameter		Cyclin D1 Expression n(%)		Total	*p*-value
		Low	High		
Gender	Male	17(26.5)	17(26.5)	34(53)	0.056*
	female	8(12.5)	22(34.5)	30(47)	
	Total	25(39)	39(61)	64(100)	
Age	≤ 60	12(18.7)	31(48.5)	43(67.2)	0.008*
	> 60	13(20.3)	8(12.5)	21(32.8)	
	Total	25(39)	39(61)	64(100)	
Side	Right	13(20.3)	18(28.2)	3(48.5)	0.431*
	Left	6(9.35)	15(23.45)	21(32.8)	
	Not specified	6(9.35)	6(9.35)	12(18.7)	
	Total	2(39)	39(61)	64(100)	
Histologic type	Clear cell RCC	10(15.6)	36(56.2)	46 (71.8)	<0.0001*
	Non-clear cell RCC	15(23.4)	3(4.8)	18(28.2)	
	Total	25(39)	39 (61)	64(100)	
Tumor size	<7	12(18.7)	31(48.5)	43(67.2)	0.008*
	>7	13(20.3)	8(12.5)	21(32.8)	
	Total	25(39)	39(61)	64(100)	
Fuhrman nuclear grade (Clear cell & papillary RCC)	1	0(0)	10(17)	10(17)	0.006†
	2	14(23.7)	23(39)	37(62.7)	
	3	5(8.5)	3(5.1)	8(13.6)	
	4	3(5)	1(1.7)	4(6.7)	
	Total	22(37.2)	38(62.8)	59(100)	
Tumor Stage	T1 and T2	23(35.8)	37(57.8)	60(93.6)	0.643*
	T3 and T4	2(3.2)	2(3.2)	4(6.4)	
	Total	25(39)	39(61)	64(100)	

Values are presented as frequencies and percentages *Chi square test; † Fisher's test.

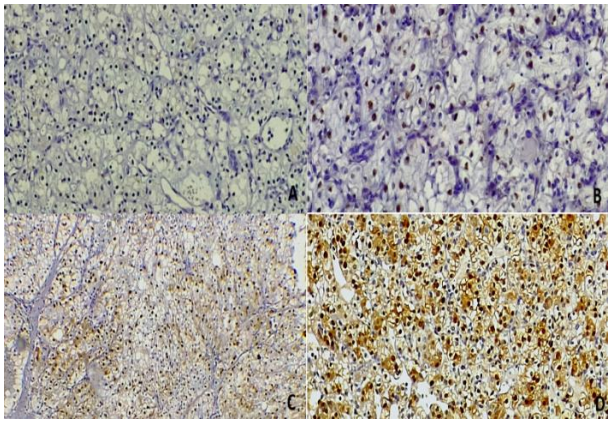

Figure 3: Cyclin D1 Expression in Renal Cell Carcinoma (nuclear expression, brown stain) (IHC, DAB x400). A) group 1 (No positive cells). B) group 2 (30% positive cells). C) group 3 (> 30 and 60% positive cells). D) Group 4 (> 60% positive cells).

Lima *et al*., Latić *et al*., and Ghafour *et al*. studied non-clear cell subtypes in Brazil, Serbia, and Egypt and found comparable results: papillary RCC (pRCC) came in second at 11%, 32%, and chromophobe RCC (chRCC) at 7%, 9.5%, and 16%, respectively [7–11]. Xue *et al*. from China discovered that chromophobe RCC was 5% and papillary RCC was 4% [24]. In the current study, the age range of patients was similar to those seen in Ghafour *et al*. and Latić *et al*. investigations in Egypt and Serbia (53.5 and 59.29 years, respectively) [25,23]. This study found that RCC was more common in males, consistent with previous findings by Rizwan *et al*. (52.7%), Ghafour *et al*. (61.9%), Xue *et al*. (63.3%), Latić *et al*. (64.9%), and Lima *et al*.

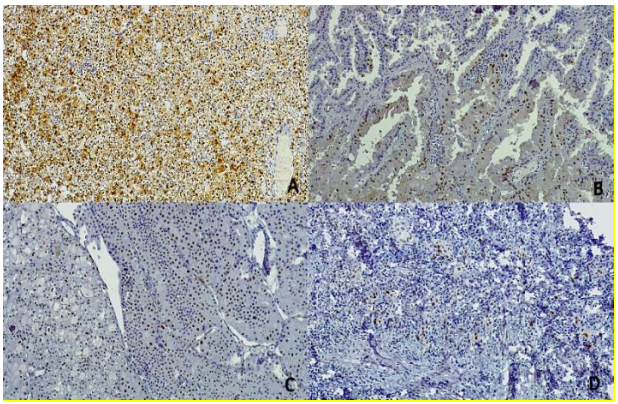

Figure 4: Cyclin D1 Expression in different Renal Cell Carcinoma subtypes (nuclear expression, brown stain) (IHC, DAB x100). A) clear cell RCC . B) papillary RCC. C) Chromophobe RCC. D) poorly differentiated RCC.

This study, as well as those by Wang *et al*. and Mandrekar *et al*., found that right-sided RCC was slightly more prevalent than left-sided RCC [2,26]. In this investigation, the range and average tumor size were consistent with Lima et al.'s study, in which tumor sizes ranged from 1.0 cm to 15.0 cm, with an average of 6.17 cm [20]. This study found a strong correlation between cyclin D1 expression and histological type ($p< 0.00001$). Cyclin D1 immunohistochemical staining of ccRCC patients included in this investigation was carried out concurrently with the Wang *et al*. study, in which 80 out of 101 ccRcc cases (79%) were grade 4 cyclin D1 and 66.7% of clear RCC showed high cyclin D1 expression in the Lima et al. study [2,20]. According to Lima *et al*.'s study, 91.7% of papillary RCC and 85.7% of chromophobe RCC have low expression [20]. The Ghafour *et al*. study found positive cyclin D1 expression in 77.7% of clear cell RCC, 37.5% of papillary RCC, and 50% of chromophobe RCC, which is consistent with the current findings [25]. Despite using the identical cyclin D1 scoring system, the Rizwan *et al*. investigation found that cyclin D1 expression was low in 64.5% and high in 35.5% [21]. These findings differ from those of the current investigation since the latter did not specify histological subtypes. The current study also sought to determine the relationship between cyclin D1 expression and several other clinicopathological characteristics. Cyclin D1 immunohistochemistry staining in male patients was evenly distributed between low and high expression, whereas expression in female patients was primarily high with no statistical significance. The Lima *et al*. study found that cyclin D1 expression was high in 69.38% of males and 65.23% of females, but there was no statistical significance ($p= 0.734$) [20]. Patients under 60 years old had significantly higher levels of cyclin D1 expression ($p= 0.008$). The Lima *et al*. investigation found no variation in cyclin D1 expression across age categories, with 75%, 58.7%, and 81.25% of cases <50 years, 50-69 years, and >70 years, respectively, showing high expression [20]. This study found a significant relationship between cyclin D1 and tumor size ($p= 0.008$). Although the Lima *et al*. study found a significant correlation, 90% of tumors > 4 cm and 57% of tumors > 4 cm expressed high levels of cyclin D1 [20]. The variation in percentage could be attributed to the cut-off size of the tumor (4 cm) used in their investigation. Cyclin D1 also had a significant correlation with the Fuhrman nuclear grade of clear and papillary RCC ($p= 0.006$). Lima *et al*. and Rizwan *et al*. found a significant correlation ($p< 0.0001$ and <0.05), with 83% and 88.37% of low nuclear grade cancers having high cyclin D1 expression, and 60% and 56% of high nuclear grade tumors having low cyclin D1 expression [20,21]. Wang *et al*. also observed an adverse relationship, as CCND1 expression is low with high ccRCC grades [2]. In terms of stage, this study found no statistical significance. The Lima *et a*l. study likewise found a majority of high cyclin D1 expression in low-stage RCC (73.47%), while high-stage RCC exhibited 44.83% low vs. 55.17% high, which is consistent with the findings of the current investigation [20].

Study limitation

This study was limited by the low number of non-clear cell RCC cases and the few cases with high nuclear grades.

Conclusion

There is an overexpression of cyclin D1 in renal cell carcinoma with variable immunohistochemical staining patterns in the tumor cells, depending mainly on the histological subtype in addition to other clinicopathological parameters. So therapeutic targeting of cyclin D1 may help in the treatment and prevention of RCC. High expression of cyclin D1 in RCC is associated with good prognostic factors. Therefore, it can be used in addition to standard histomorphologic prognostic factors to predict patient outcomes.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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