Egyptian Journal of Cell and Tissue Research
Print ISSN: 2812-5436 / Online ISSN: 2812-5444

Immunohistochemical Expression of Heat Stable Antigen (CD24) in Renal Cell Carcinoma

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Abstract :

Renal cell carcinoma (RCC) is the most common type of urogenital cancer. It has a mortality rate of 30- 40% and is more commonly seen in men than women. Cluster of differentiation (CD)24 is a small, highly glycosylated cell adhesion protein that is normally expressed by immune cells as well as epithelial, neural, and muscle cells. Tumor CD24 expression had been linked with alterations in several oncogenic signaling pathways. The aim of our work to evaluate the immunohistochemical expression of CD24 in RCC and detect its relationship with other clinicopathological parameters. Thirty-nine (39) cases (97.5%) showed positive CD24 expression while single case (2.5%) was negative. Score 0 was seen in this single case (2.5%), score +1 was seen in 18 cases (45%), score +2 in 16 cases (40%) and score +3 was seen in 5 cases (12.5%). High CD24 expression had been presented in (52.5%) of cases. There was no significant correlation between CD24 expression and other clinicopathological parameters such as age, gender, histological subtype, tumor grade, pathological tumor stage, lymph node status, capsular invasion, tumor necrosis and lymphovascular emboli.

Keywords:

Renal cell carcinoma; urogenital cancer, CD24 expression.

1. Introduction:

RCC is regarded as the most prevalent type of renal malignancy, accounting for 2% to 3% of entirely adult cancers globally each year **[1].** The main histological subtypes in RCC are clear cell renal cell carcinoma (CCRCC), papillary renal cell carcinoma (PRCC) and chromophobe renal cell carcinoma (ChRCC), these three subtypes form more than 90% of all RCCs, and among them, CCRCC accounts for the vast majority **[2].** In Egypt, the male to female ratio is roughly 2:1, and the incidence of cancer is almost 1.53% in men, ranking it as

the 10th most common cancer in men, and about 0.97% in women, considering it as the 17th most common disease in women **[3].** Unfortunately, prognosis of RCC is poor, as the 5-year overall survival rate of patients in early stages is around 60%, while patients with advanced and metastatic stages exhibit less than 10% five-year overall survival. Additionally, almost 30% to 40% of cases diagnosed in the early stages develop recurrence or metastasize **[4].** Cigarette smoking, obesity and hypertension are the most well-established risk factors for sporadic RCC worldwide. Acquired cystic kidney disease is also a considerable risk factor, particularly in dialysis patients **[5].** Cluster of differentiation (CD) 24 is a glycosyl-phosphatidyl-inositol (GPI) anchored glycoprotein composed of a short 31 to 34 amino acid core protein **[6].** CD24 is broadly overexpressed on many types of tumor tissues, including B-cell lymphomas, gliomas, small cell lung cancer, esophageal squamous cell carcinoma, hepatocellular carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, urothelial carcinoma, ovarian cancer, breast cancer, primary neuroendocrine carcinomas, and prostate carcinomas **[7].** The objective of our study was to determine the immunohistochemical expression of CD24 in renal cell carcinoma tumor cells to detect any special clinicopathologic features among Egyptian patients.

2. Materials and Methods:

Tumor tissue sections of RCC specimens were collected from 40 patients who underwent radical, simple, and partial nephrectomy. Ethical approval was sought from university Ethical Committee (approval No.: FMBSUREC\01112021\ Ali). For the sake of data confidentiality and to ensure that it will be used only for scientific research, the patient's identity was taken from each case and replaced with a number. Also, these samples were studied immunohistochemically for CD24 using monoclonal CD24 antibody. The intensity of positively stained tumor cells was evaluated.

2.1 Histopathological evaluation:

Paraffin blocks of the tumors were sectioned at 4µm thickness. Then, they were stained with routine Hematoxylin and Eosin stain for pathological examination and morphologic classification of the renal cell carcinoma according to the recommendations of the World Health Organization (WHO) including histological subtype, tumor grade, renal vein invasion, lymphovascular emboli, tumor necrosis, and capsular invasion while staging was performed using TNM staging system of AJCC -8th edition.

2.2Immunohistochemical examination:

Section from each case was mounted on positively charged slide and stained by immunohistochemical stain (anti-CD24 antibody), a rabbit monoclonal antibody, 1 ml.

concentrated, pH 7.4 (CD24 Antibody, QUARTETT, clone SN3 CD107-13 Schichauweg, Berlin, Germany ready to use for immunohistochemistry).

2.3 Interpretation of CD24 positivity:

CD24 staining was detected as brownish stain mainly in the cytoplasm and cell membranes. The intensity of CD24 staining was semiquantitatively

Cases in which the percentage of positive cells is less than 10% were regarded as negative (score 0). In sections containing >10% of the area staining positive, the sections were scored by intensity (score +1, +2, and +3);

-Score (+1): Mild staining intensity

- Score (+2): Moderate staining intensity

-Score (+3): Dense staining intensity

The final evaluation of cases were subdivided into a CD24 low expression group (score 0 and +1) and CD24 high expression group (score +2 and +3)

2.4 Slide examination and imaging:

Slides were examined by Olympus BX53 light microscope and images were captured using Leica digital pathology slide scanners (Aperio LV1 IVD).

Statistical methodology

The statistical software for the social sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was used to code and enter the data. The following metrics were used to summarize the data: frequency (count) and relative frequency (%) for categorical data; mean, standard deviation, median, minimum, and maximum for quantitative data. The non-parametric Mann-Whitney test was used to compare quantitative variables.

An analysis using the Chi square (2) test was done to compare categorical data.

Statistics were considered significant for p-values under 0.05.

3. Results:

Our research conducted on a random sample of RCC cases that were collected during the period between January 2017 and December 2021, from the Pathology laboratory at Specialized Medical Centre, Faculty of Medicine Beni-Suef University. After exclusion and inclusion criteria, 40 cases have been included in this study.

Demographic and histopathological data of the participants in our study are demonstrated in table (1). The studied cases were predominantly males (62.5%). The age incidence ranged from 24 years to 80 years with a mean age of 56.9±11.9 SD. About 37.5% of our cases showed tumor size > 7cm.

Regarding tumor grade, most of the studied cases (80.6%) were low grade while (19.4%) were high grade. In that study, CCRCC was the most frequent histological type representing (62.5%) of the total studied cases followed by PRCC (20%) and ChRCC (17.5%).

According to pathological (pT) stage; pT1 represented (62.5%), pT2 (20%) and pT3 represented (17.5%) of the total studied cases.

Lymph node metastases were detected in (5%) of cases. Moreover, tumor necrosis was detected in (55%) of studied cases. Only (5%) showed renal vein invasion in the examined specimens. Also, there were capsular invasion and lymphovascular emboli in (12.5%) and (10%) of the studied cases, respectively.

It was noticed that positive expression of CD24 was seen in 97.5% of cases, and only one case (2.5%) negative. Regarding CD24 scoring 2.5% of cases scored as 0, 45 % of cases had score +1, 16 40% of cases had score +2, and 12.5% of cases had score +3**.**

According to our thesis, the correlation between CD24 expression and other clinicopathological parameters such as age, gender, tumor grade, pathological tumor stage, lymph node status, tumor necrosis, lymphovascular invasion, and capsular invasion were statistically non-significant as illustrated in **table (2).**

Table (1) Demographic and histopathological data of the participants (N =40):

		Frequency(no)	Percent
Gender; N (%)	**Male**	25	62.5%
	Female	15	37.5%
Age group	**≥ 60y**	22	55%
	<60y	18	45%
Tumor size	**≤7cm**	25	62.5%
	>7cm	15	37.5%
Histopathological type	**Clear RCC**	25	62.5%
	Papillary RCC	8	20%
	Chromophobe RCC	7	17.5%
Tumor grade	**I**	16	48.5%
	II	11	33.3%
	III	6	18.2%
	IV	0	0
pT	**T1a**	8	20%
	T1b	17	42.5%
	T2a	5	12.5%
	T2b	3	7.5%
	T3a	7	17.5%
pN	**Nx**	34	85%
	N0	4	10%
	N1	2	5%
Tumor Necrosis	**Positive**	22	55%
	Negative	18	45%
Lympho-vascular Emboli	**Positive**	4	10%
	Negative	36	90%
Capsular invasion	**Positive**	5	12.5%
	Negative	35	87.5%

Table (2): Correlation between CD24 expression among RCC cases with clinicopathological parameters (N=40):

		Low expression n	High expression N	*P* value
Gender	**Male**	9	16	0.06
	Female	10	5	
Age	**<60**	11	7	0.119
	≥60	8	14	
Tumor size	**≤7cm**	10	15	0.22
	>7cm	9	6	
Histological subtype	**CCRCC**	11	14	0.444
	PRCC	3	5	
	ChRCC	5	2	
Grade of CCRCC & PRCC (n=33)	**I**	6	10	0.643
	II	6	5	
	III	2	4	
	IV	0	0	
pT stage	**T1a**	4	4	0.127
	T1b	6	11	
	T2a	4	1	
	T2b	3	0	
	T3a	2	5	
pN stage	**Nx**	17	17	0.799
	N0	1	3	
	N1	1	1	
Tumor necrosis	**Positive**	9	13	0.356
	Negative	10	8	
Lympho-vascular Emboli	**Positive**	1	3	0.607
	Negative	18	18	
Capsular invasion	**Positive**	1	4	0.345
	Negative	18	17	

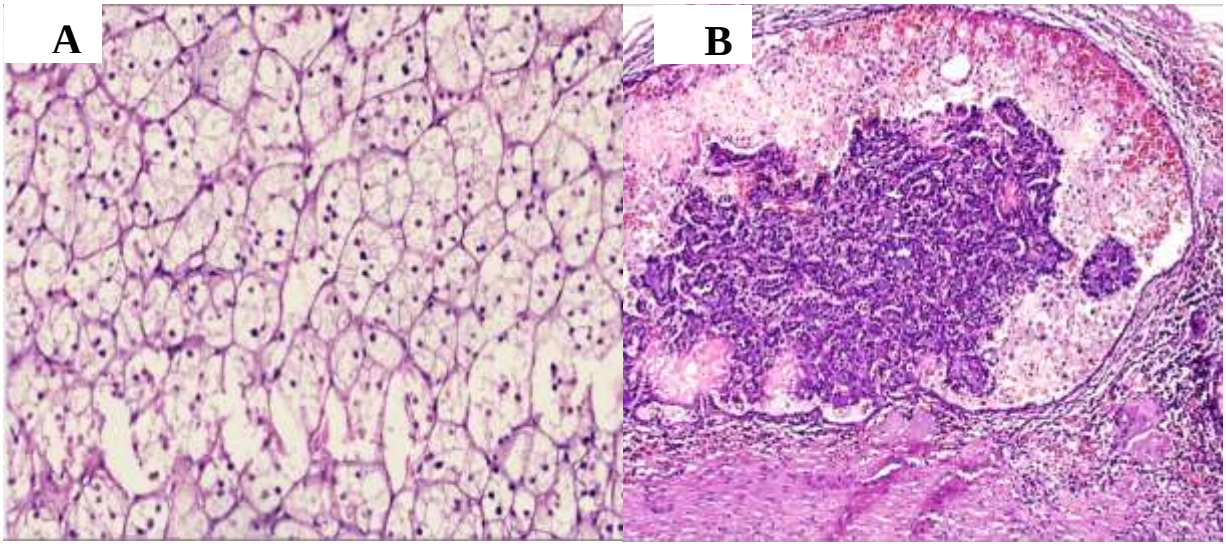
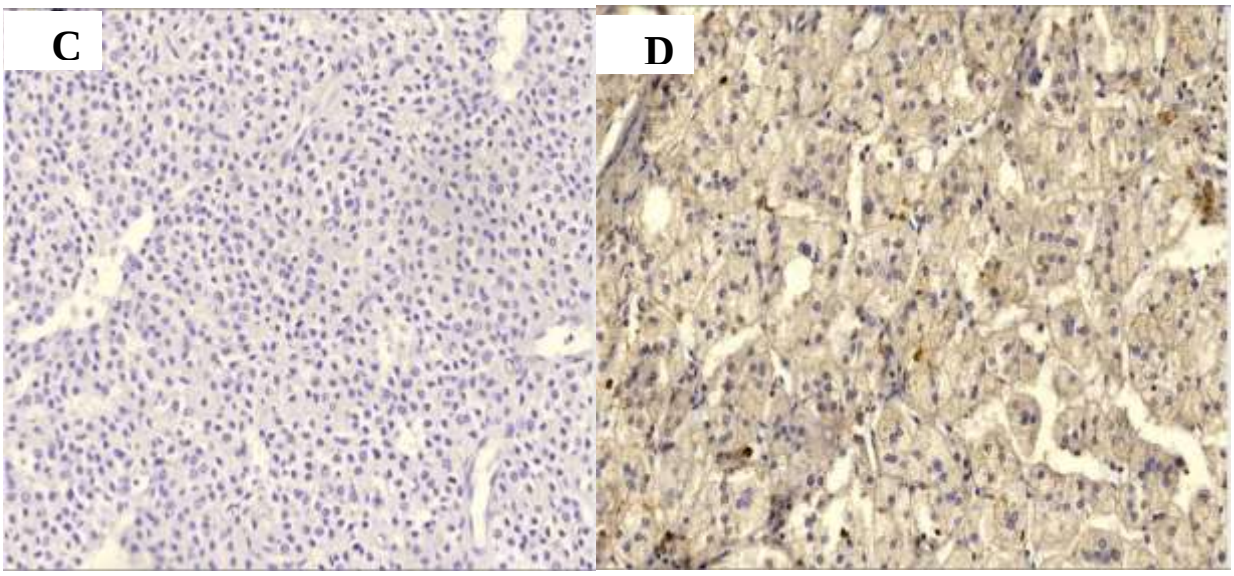


Figure (A) **Clear cell renal cell carcinoma GI (H&E)** *original magnification x100*

Figure (B) **Papillary renal cell carcinoma with positive lymphovascular emboli (H&E)** *original magnification x100*



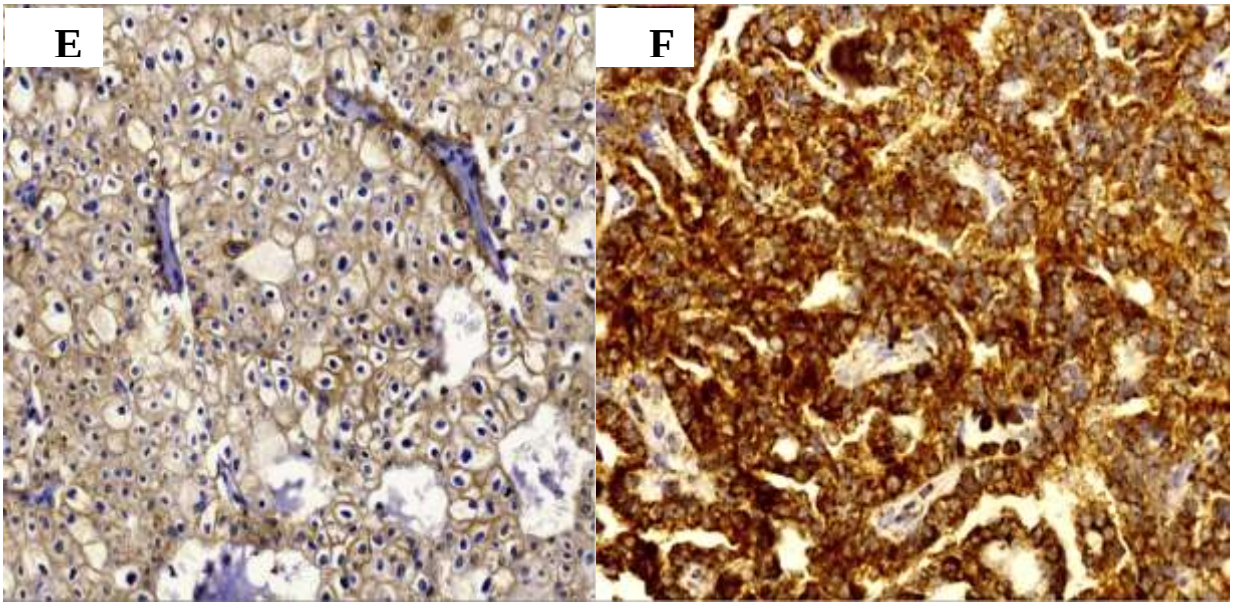


Figure (C) **Chromophobe RCC showing negative CD24 expression (score 0),** *original magnification x200.*

Figure (D) **CCRCC, tumor cells showing positive CD24 expression mild cytoplasmic staining intensity (score+1)-** *original magnification x200.*

Figure (E) **Chromophobe RCC with positive CD24 expression, moderate membranous staining intensity (score +2)** *-original magnification x200.*

Figure (F) Papillary RCC showing, positive CD24 expression, intense membranous staining intensity (score+3)- *original magnification x400.*

4. Discussion:

In the current study, The mean age of the collected cases was 56.9±11.9SD years (ranging between 24-80 years). That was consistent with the data of WHO classification, where the mean age of RCC patients was to be in the sixth decade **[8],** and our results were in line with studies done by **[9]** and **[10]** who stated that the mean age at presentation for RCC was 56 and 58 years, respectively.

As regards the gender, the current study showed male predilection (62.5%), with male to female ratio of 1.7:1, this was coordinated with the results reported by **[11]** which showed that males were (62.9%).

According to tumor size, (37.5%) of specimens had tumors >7 cm in size, that was nearly reliable to **[11]** study as less than (30%) of cases had tumors larger than 7cm. Regarding the incidence of tumor necrosis, it was presented in (55%) cases. These results were approximating those found by **[12]** who noted tumor necrosis in (41%) of total cases, while **[9]** detected tumor necrosis only in (24%) of cases.

Regarding the histological types of collected cases, CCRCC (62.5%), PRCC (20%) and

ChRCC (17.5%). Our results were close to the WHO classification which reported that CCRCC ranging between (65-70%) and PRCC accounts for (18.5%), However, ChRCC ranges between (5- 7%) **[8].**

Regarding Fuhrman nuclear grade, low grade cases accounted for (82%) of CCRCC and PRCC cases, compared to high grade cases (18%). These findings were roughly in line with those provided by **[13]** they pointed that (73%) of cases were low grade, although study by **[14]** indicated greater results of high-grade cases (38.7%).

According to the pathological tumor stage, (62.5%) of cases in this work presented at pT1 stage, (20%) of cases presented at pT2 stage and (17.5%) of cases presented at pT3 stage. Our findings were almost close to the study done by **[12]** which showed (47%) pT1, (26%) pT2, (25%) pT3, while only (2%) pT4.

In this study, the nodal status of majority of cases can't be assessed (pNx: 85%), the remaining cases (15%) were divided as negative nodal metastasis (pN0: 10%) and positive nodal metastasis (pN1: 5%). Those findings were different from the results reported by **[15]** which reported (pN0: 90%) of their cases were negative for tumor metastasis, (pN1:6.3%) of cases had positive lymph node involvement and only (pNx:3.4%) of cases could not be identified.

As regard CD24 expression in this study, only single ChRCC case showed negative CD24 expression (2.5%) while most cases showed diffuse positive CD24 expression. That was almost like what was informed by **[16]** who showed diffuse CD24 staining in RCC.

We noted in our study that there was no statistically significant association between CD24 expression and patient age as well as gender (p-values 0.119 and 0.06, respectively). Our findings agreed with studies done by **[9]** and **[12],** which concluded that there was no statistical relationship between CD24 expression and age or gender.

There was no statistical significant correlation between expression of CD24 and tumor size groups (p-values 0.22). These results agreed with those from **[12].** Our results, however, contrasted with those obtained by **[9],** who observed a significant connection (p-value 0.04) between CD24 expression and tumor size group.

According to our results, no statistically significance between the expression of CD24 and the grade, (p- value 0.643), also there was no statistically significant relationship between CD24 expression and the grade (p- value 1 each). Our findings contrasted with the studies performed by **[9]** and **[12]** who reported that there was a significant association between CD24 expression and the tumor grade (p-value 0.005 and 0.023 respectively), this contrast could be explained as majority of our cases were low grade. In our research there was no statistically significant relationship between CD24 expression and pT stage (p-value

0.127). That result was compatible with **[9]** and **[12]** who founded that there was no statistically significance in CD24 expression on tumor cells and pT stage. Our results showed that no statistically significant relation between CD24 expression and pN stage in the studied cases (P-value 0.799)**,** That agreed with **[9]** who reported that no significant association was found between CD24 expression and pN stage. Our current study found no statistically significant correlation between expression of CD24 and the associated tumor necrosis and which was consistent with findings of **[12]** that displayed no correlation between CD24 expression and the aforementioned parameters.

5. Conclusion and Recommendations:

The results of our study proposed that CD24 expression was higher in age group ≥60 years, and tumor necrosis, making it a potential bad prognostic marker.

Further studies with larger samples are recommended to establish the prognostic significance of CD24 and its role as cancer marker in tumor metastasis in order to provide targeted therapy.

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