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Immunohistochemical Expression of Chemokine Receptor CXCR4 as a Valuable Prognostic Marker in Renal Cell Carcinoma

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Abstract:

Background and aim: Chemokine receptor (CXCR)4 is a G-protein coupled receptor involved in many biological processes as inflammation, angiogenesis and immune responses. Previous researches illustrated that CXCR4 expression has been detected in many carcinomas of various origins. The aim of this study was to evaluate the possible prognostic value of CXCR4 in RCCs by correlating immunohistochemical expression of CXCR4 with different patients' clinical and pathological criteria.

Methods: Formalin-fixed paraffin embedded tissue blocks of 49 specimens of RCCs were evaluated for CXCR4 expression by immunohistochemistry (IHC). Correlation of CXCR4 expression with different clinical and pathological data was measured statistically.

Results: Nuclear expression of CXCR4 was correlated to International society of urological pathology (ISUP) grading system that is applied for ccRCCs and papillary RCCs *(p=0.024).* Both cytoplasmic and membranous expression of CXCR4 were associated with histological subtypes of the studied RCC cases *(p<0.0001)* and Fuhrman nuclear grading system *(p=0.008 &p<0.0001*). Membranous CXCR4 was inversely correlated to pathological T stage of the studied RCCs *(p=0.035).*

Conclusions: Expression of CXCR4 decreases in advanced stages of RCC. CXCR4 is a valuable prognostic biomarker in RCCs and should be evaluated in each subcellular localization.

Abbreviations: CXCR4: Chemokine receptor 4, **RCC:** Renal cell carcinoma, **ccRCC:** Clear cell renal cell carcinoma, **IHC:** Immunohistochemistry, Immunohistochemical. **CSC:** Cancer stem cells, **ISUP:** International society of urological pathology.

Key Words: Renal Cell Carcinoma, CXCR4, Cancer Stem Cells, ISUP, Fuhrman grading system.

Introduction:

Renal cell carcinoma (RCC) is the sixth leading cause of cancer-related deaths in the western world and comprises 2-3% of all newly diagnosed malignancies in adults. It represents about 85% of all renal neoplasms. Peak incidence is in the 6th decade of life with male to female ratio about 2:1. Incidence of bilaterality about 1% [1]. Nearly about 30 % of patients with RCC come with metastatic disease when diagnosed for first time and about 60% of those patients die from aggressive disease and metastasis. So metastatic dissemination of RCC seems to be the most important prognostic factor [2]. Recent studies

showed the existence of small populations of cancer stem cells (CSCs) that reside among the tumor cells. These CSCs have been identified in many tumors as melanoma [3] and prostatic carcinoma [4].

Like normal stem cells, these CSCs share common properties as having the ability to renew themselves in addition to the ability to introduce transplantable tumors in immunodeficient mice. On the basis of different protein expression on their surfaces; many CSCs were identified as CXCR4+cells [5].

CXCR4 belongs to the large superfamily of G protein-coupled receptors, and it is directly involved in a number of biological processes including organogenesis, hematopoiesis, and immune response. The expression of CXCR4 has been detected in many different cancers of various origins and is the most common chemokine receptor expressed on cancer cells [6]. Therefore, we evaluated the expression of CXCR4 in RCC specimens and correlated these results with patients' clinical and pathological criteria.

Patients and methods:

1) Tissue samples:

Approval to perform this prospective study was obtained from the Institutional Research Ethical Committee. Forty-nine patients with clinical and radiological findings of renal neoplasms admitted to Urology Department of Sohag University hospital from January 2018 to June 2019. Nephrectomy was done for each patient and the specimen labeled with patient's name, age, sex and side of nephrectomy specimen was sent to the Pathology Laboratory of the same hospital. All cases were primary RCCs. Cases with extensive necrosis or those with history of pre-operative anticancer therapy were excluded. For each specimen, tumor size was recorded as the longest diameter of the tumor, capsular and/or peri-nephric fat invasions were documented from the pathological reports. Multiple tissue samples from the tumor with its overlying capsule and peri-nephric fat were obtained. The morphological classification of the submitted renal neoplasms was conducted according to World Health Organization (WHO) specifications in 2016.

Tumors were divided into four groups according to their size: T1≤7cm, T2 >7 cm in greatest dimension but still confined to the kidney, T3; tumor extend to the major veins or perirenal fatty tissue but not extending to the ipsilateral adrenal gland or beyond Gerota fascia and T4 when the tumor extend into the ipsilateral adrenal gland or beyond Gerota fascia according to what was adopted from AJCC staging system, 2010.

Tumor size in addition to peri-nephric fat invasion were used to broadly determine pT- stage of the resected tumor.

2) Immunohistochemical staining of Anti-CXCR4:

Formalin-fixed paraffin-embedded RCC tissue blocks were sectioned into 4µm thick tissue sections. Deparaffinization in Xylene and hydration in descending grades of alcohol were done. Sections were incubated in 3% H2O2 for 30 minutes at room temperature in order to block the endogenous peroxidase activity. Then heated in 0.01 mmol/L citrate buffer fluid at 92ºC as an antigen retrieval solution, for only seven minutes. Sections were incubated with primary antibody overnight at 25ºC. The primary antibody used was anti CXCR4, a mouse monoclonal antibody against human (in a concentrated form 0.1 ml, Catalog number; Cat # sc-53534, Clone 4G10, Santa Cruz Biotechnology Corporation, California, USA). The sections then were incub-

ated with goat serum secondary antibody followed by streptavidin biotin for ten minutes each separated by washing in PBS for five minutes after each step. The reaction products were visualized by immersing the sections in diaminobenzidine (DAB) for fifteen minutes at room temperature. Sections were counterstained by immersion in Hematoxylin stain for few seconds and rapid wash in tap water to remove extra dye. Dehydration, clearance and cover mounting were performed.

Each staining run included positive and negative control sections to confirm that both staining systems were working properly and positive signals were specific. The positive control slides were prepared from normal renal tissue (**Figure 1**). Negative control sections were from renal tumor, but with PBS instead of primary antibody.

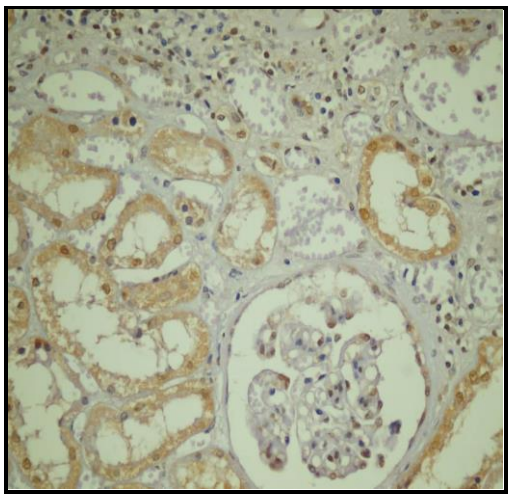

Figure 1: CXCR4 expression in tubules of normal renal tissues, X200.

3) Evaluation of immunostaining:

Nuclear CXCR4 Staining: it was divided into three categories; Negative nuclear staining if less than 15% of tumor cell nuclei were positive for Anti CXCR4. Partial nuclear expression is considered when 15-50% of tumor cell showed nuclear positive immunostaining. If more than 50% of nuclei were positive for anti-CXCR4; diffuse nuclear expression is assigned [7].

Cytoplasmic CXCR4 staining:

We used both the overall histochemical score (H-Score) and the immune-reactive score (IRS). H-Score was scored on a scale of 0-3, with a score of 0= no visible staining, 1= weak staining, 2= moderate staining and 3= strong staining. Percentage of tumor cells with positive staining was graded as <25, 25-50, 50-75 and >75%. The overall histochemical score was assigned for each case by multiplying intensity score by percentage of stained cells. A final score of 0-300 was obtained. A cutoff point of 200 was chosen based on median H score to categorize samples as high or low CXCR4 expression [8].

IRS was determined by multiplying an estimate of the percentage of the immuneoreactive cells (quantity score; QS) with an estimate of the staining intensity (intensity score; IS) according to Cregger, et al., 2006. Staining quantity is scored as follows; No staining = 0, 1-10% of cells stained = 1, 11-50% of cells stained = 2, 51-80% of cells stained = 3 and 81-100% of cells stained = 4. Staining intensity is scored on a scale of 0-3 where No staining = 0, Weak = 1, Moderate = 2 and Strong = 3. The intensity score and quantity score were multiplied to give the IRS. An IRS of 0-4 was considered weak, 6-8 was moderate, and 9-12 was considered strong [9].

Membranous CXCR4 Staining: was recorded as either positive or negative [10].

4) Statistical analysis: Data was analyzed using SPSS version 20 (Statistical Software package versi-on 20). Quantitative data was repres-ented as mean, standard deviation, median and range. Data was analy-zed using student t-test to compare means of two

groups and ANOVA for comparison of the means of three groups or more. Chi-square and Fisher's exact tests were used to compare between groups. P value was considered significant if it was less than 0.05.

Results:

Patients' clinical characteristics:

The current study included 49 patients with RCCs. Their clinical and pathological characteristics were summarized in **(Table 1).** Their ages ranged from 28 to 75 years old. The study included 30 male patients and 19 females. In the studied patients, the tumors were confined to the left kidney in 23/49 of cases, while in the remaining 26 patients, tumors were right sided.

Histopathological findings:

Histopathological examination of the studied 49 cases of RCCs revealed that 33/49 cases were ccRCCs including nine cases showed focal sarcomatoid changes, 11/49 cases were chromophobe RCCs and 4/49 cases were papillary RCCs and only one case was collecting duct carcinoma. Capsular invasion was detected in 36/49 of RCCs. Tumors in 10/49 cases didn't show capsular invasion within the limits of the examined sections. In the remaining 3/49 cases, capsular invasion couldn't be assessed as the available blocks contain only the tumor tissues. Peri-nephric fat invasion was detected in 29/49 patients. Absence of peri-nephric fat invasion was present in 14/49 cases. Peri-nephric fat invasion couldn't be assessed in 6/49 cases. A histologically-confirmed coagulative necrosis was detected in 14/49 patients, while it was absent in 35/49 cases. Applying Fuhrman's nuclear grading system on the 49 cases of RCCs, 15/49 cases were Fuhrman's grade 1, 18/49 were grade 2, 6/49 were grades 3 and 10/49 were grade 4 tumors. The ISUP grading system recommended by the WHO in 2016 is applicable only for cases of ccRCCs and papillary RCCs which represent 37/49 of the studied cases. Of those cases 20/37 were ISUP grade 1, 5/37 cases were ISUP grade 2, 1/37 cases were ISUP grades 3, and 11/37 cases were ISUP grade 4 **(Table 2).** None of the resected nephrectomy specimens contained adrenal tissues, lymph nodes or definite vascular structures and there is no available data in their submitted reports about their status whether involved or not. So, the largest tumor diameter in addition to peri-nephric fat invasion were used to broadly determine pT- stage of the tumor. Only one case of the 49 cases was staged as pT1a, 12/49 cases were staged as pT1b, 7/49 cases were staged as pT2a, and 29/49 cases were staged as pT3a.

Variable	Incidence & Percentage
Age range/year	28:75
Gender	
Female	19 (38.8%)
Male	30 (61.2%)
Side involved	
Left	23 (46.9%)
Right	26 (53.1%)
Size (cm.)	4:15 cm.
Histologic subtype	
Clear cell RCCs	24 (49%)
Chromophobe	11 (22.4%)
Clear cell RCCs with Sarcomatoid change	9 (18.4%)
Papillary	4 (8.2%)
Carcinoma of collecting duct	1 (2%)
Capsular invasion	
Negative	10 (20.4 %)
Positive	36 (73.5%)
Can't be assessed	3 (6.1%)
Perinephric fat invasion	
Negative	14 (28.6%)
Positive	29 (59.2%)
Can't be assessed	6 (12.2%)
Associated necrosis	
Negative	35 (71.4%)
Positive	14 (28.6%)
Fuhrman's grading system	
Grade 1	15 (30.6%)
Grade 2	18 (36.7%)
Grade 3	6 (12.3%)
Grade 4	10 (20.4 %)
T staging of studied cases	
Ia	1 (2%)
Ib	12 (24.5%)
IIa	7 (14.3%)
IIIa	29 (59.2%)

Table1: linicopathological data of the studied cases.

Variable	Incidence & Percentage
ISUP grading system	
Grade 1	20 (54.1%)
Grade 2	5 (13.5%)
Grade 3	1 (2.7%)
Grade 4	11 (29.7%)

Table 2: ISUP grading system of the studied clear cell and papillary RCCs.

Immunohistochemical Findings:

IHC detection of CXCR4:

CXCR4 was evaluated in nuclei, cytoplasm and membranes of cells of RCCs All cases of RCCs in the current study showed nuclear expression of CXCR4; 7/49 cases showed focal nuclear expression **(Figure 2),** while 42/49 cases showed diffuse nuclear expre-ssion **(Figure 3).**

There was a statis-tically significant relationship between nuclear expression of CXCR4 and ISUP grading system which is applied for ccRCCs and papillary RCCs *(p=0.024).* We found that 17/20 cases of ISUP grade 1 and all cases of ISUP grade 2 showed diffuse nuclear expression of CXCR4.

in addition to its expression in the cytoplasm of normal renal tubules.

Nuclear expression of CXCR4:

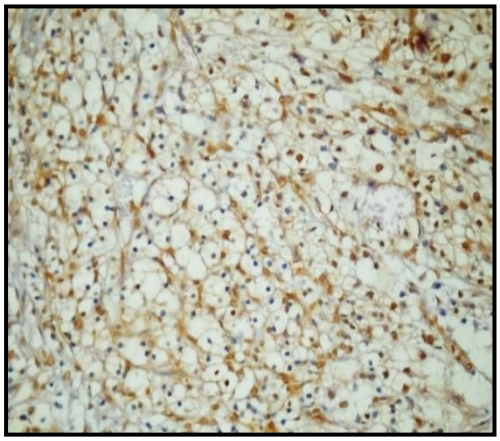

Figure 2: Focal nuclear expression of CXCR4 of ccRCC, X200.

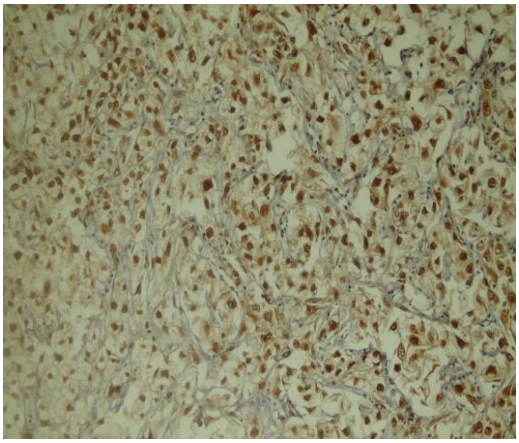

Figure 3: CXCR4 expression in ccRCC with sarcomatoid change showing diffuse nuclear staining, X200.

Cytoplasmic expression of CXCR4:

IHC expression of CXCR4 was evaluated by using two different scoring systems; IRS and H score. On applying IRS; cytoplasmic expression of CXCR4 was detected in 30/49 cases of RCCs **(Figure 4).** The remaining 19 cases didn't show cytoplasmic localization of CXCR4 (**Figure 5).**

A statistically significant association was detected between cytoplasmic expression of CXCR4 and the histological subtype of the studied RCCs *(p <0.0001).* 18/19 cases which didn't show cytoplasmic expression of CXCR4 were diagnosed as ccRCCs. All cases of chromophobe and papillary RCCs showed positive cytoplasmic CXCR4 with variable staining intensities **(Table 3).**

As regard to capsular and perinephric fat invasions; loss of cytoplasmic CXCR4 expression in RCCs associated with capsular invasion (*p= 0.038*) and peri-nephric fat invasion *(p= 0.037).*

Variable	Negative N=19	Mild N=12	Moderate N=15	Strong N=6	P value
Size					
Mean ± SD	7.45±2	6.33±1.23	7.04±1.98	8.7±3.7	0.18 (NS)
Histologic subtype					
Clear cell RCCs	12	8	3	1	
Chromophobe	0	2	8	1	<0.0001**
Clear cell RCCs with Sarcomatoid change	6	1	2	0	
Papillary	0	1	0	3	
Carcinoma of the collecting duct	1	0	0	0	
Capsular invasion					
Negative	2	5	2	1	0.038*
Positive	17	7	8	4	
Can't be assessed	0	0	3	0	
Perinephric fat invasion					
Negative	5	7	2	0	0.037*
Positive	13	5	7	4	
Can't be assessed	1	0	4	1	
Associated necrosis					
Negative	12	9	9	5	
Positive	7	3	4	0	0.43 (NS)
Fuhrman Grading					
Grade I	4	5	5	1	0.36(NS)
Grade II	6	5	5	2	
Grade III	2	1	1	2	
Grade IV	7	1	2	0	
T staging					
Ia	1	0	0	0	0.18(NS)
Ib	1	6	5	0	
IIa	4	1	1	1	
IIIa	13	5	7	4	

Table 3: Comparison cytoplasmic expression of CXCR4 as regard characteristics of the tumor, (IRS).

One-way ANOVA test was used for parametric continuous data (size)
Chi-square test was used for other categorical data
NS= non-significant, *= significant, **=for highly significant.

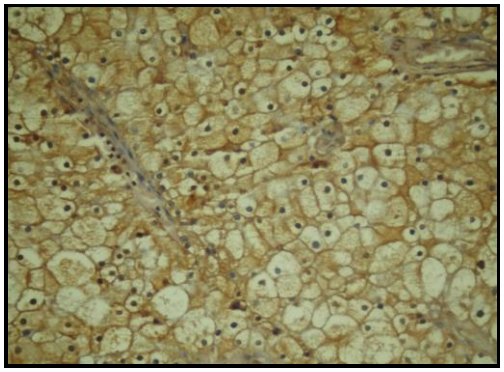

Figure 4: Positive cytoplasmic and membranous expression of CXCR4, X200.

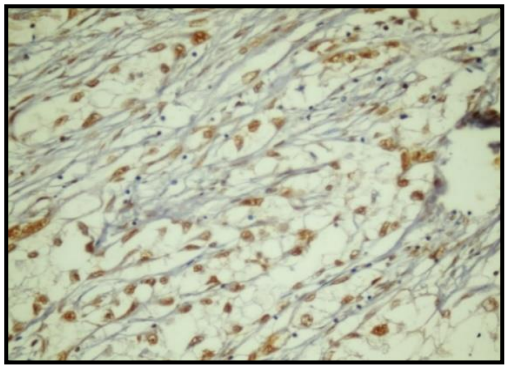

Figure 5: Negative cytoplasmic and membranous expression of CXCR4 in ccRCCs, X200.

On applying the H Score, 43/49 of the studied RCC cases showed low cytoplasmic expression. The remaining six cases showed high cytoplasmic expression of CXCR4.

There was a significant association between cytoplasmic CXCR4 and the histological subtype *(p= 0.052).* 32/33 cases of ccRCCs showed low cytoplasmic expression of CXCR4.

Applying the H Score aided in detecting a significant relationship between cytoplasmic CXCR4 and Fuhrman Grading system *(p= 0.008).* all Fuhrman Grades I, III, IV showed low cytoplasmic expression, while all the reported cases of high cytoplasmic expression were graded as Fuhrman Grade II **(Table 4).**

No significant association detected between cytoplasmic CXCR4 scored by H Score and pT stage.

Variable	Low intensity N=43	High intensity N=6	P value
Size/ cm.			
Mean ± SD	7.24±2.21	6.8±1.2	0.66 (NS)
Histologic subtype			
Clear cell RCCs	23	1	
Chromophobe	7	4	0.052*
Clear cell RCCs with Sarcomatoid change	9	0	
Papillary	3	1	
Carcinoma of the collecting duct	1	0	
Capsular invasion			
Negative	8	2	0.6 (NS)
Positive	32	4	
Can't be assessed	3	0	
Peri-nephric fat invasion			
Negative	13		0.77 (NS)
Positive	25	1	
Can't be assessed	5	4	
		1	
Associated necrosis			
Negative	32	3	0.33 (NS)
Positive	11	3	
Fuhrman Grading			
I	15	0	0.008 *
II	12	6	
III	6	0	
IV	10	0	
T staging			
Ia	1	0	0.94 (NS)
Ib	11	1	
IIa	6	1	
IIIa	25	4	

Table 4: Comparison cytoplasmic expression of CXCR4 as regard **Table 4:** Comparison cytoplasmic expression of CXCR4 as regard characteristics of the tumor, (H Score).

Independent t- test was used for parametric continuous data (size). Fisher's exact and Chi-square tests were used for other categorical data. NS= non-significant, *= for significant.

Membranous expression of CXCR4:

Positive membranous expression of CXCR4 was found in 33/49 cases of RCCs **(Figures 4, 6),** while the remaining16/49 cases of RCCs were negative for membranous CXCR4 expression **(Figure 5).** The relationship between membranous expression of CXCR4 and different tumor characteristics were summarized in **(Table 5).** As regard to the histological subtypes, all studied cases of chromophobe RCCs showed positive membranous expression of CXCR4, while all cases of ccRCCs with sarcomatoid change did not show membranous expression of CXCR4 and this difference was statistically significant *(p<0.0001).*

Membranous expression of CXCR4 appeared to be strongly correlated with Fuhrman's nuclear grading of RCCs *(p<0.0001).* Membranous expression of CXCR4 decreases with increasing Fuhrman nuclear grading of RCCs; membranous expression of CXCR4 was lost in all cases with Fuhrman grade 4, while 29/33 cases of RCCs that showed low Fuhrman grades (Grades 1&2) retained the positivity of CXCR4 on their cell membranes.

There was a significant association between membranous expression of CXCR4 and pT stage of the studied cases *(p= 0.035).* We found that 17/20 cases of low pT stages (p T1 & p T 2a) showed positive membranous expression of CXCR4, whereas membranous expression of CXCR4 was lost in 13/16 cases of RCCs staged as pT3a.

Variable	Negative N=19	Positive N=33	P value
Size/ cm.			
Mean ± SD	8.06±2.10	6.8±1.99	0.043*
Histologic subtype			
Clear cell RCCs	5	19	
Chromophobe	0	11	<0.0001**
cc RCCs with sarcomatoid change	9	0	
Papillary	1	3	
Carcinoma of the collecting duct	1	0	
Capsular invasion			
Negative	1	9	0.08(NS)
Positive	15	21	
Can't be assessed	0	3	
Perinephric fat invasion			
Negative	3	11	
Positive	13	16	0.059
Can't be assessed	0	6	(NS)
Associated necrosis			
Negative	9	26	0.18 (NS)
Positive	7	7	
Fuhrman Grading			
Grade 1	2	13	
Grade 2	2	16	<0.0001**
Grade 3	2	4	
Grade 4	10	0	
pT staging			
1 & 2a	3	17	0.035*
3a	13	16	

Table 5: Comparison of membranous expression of CXCR4 as regard to the characteristics of the resected renal tumors.

Independent t- test was used for parametric continuous data (size). Fisher's exact and Chi-square tests were used for other categorical data. NS= non-significant, *= for significant, **= highly significant.

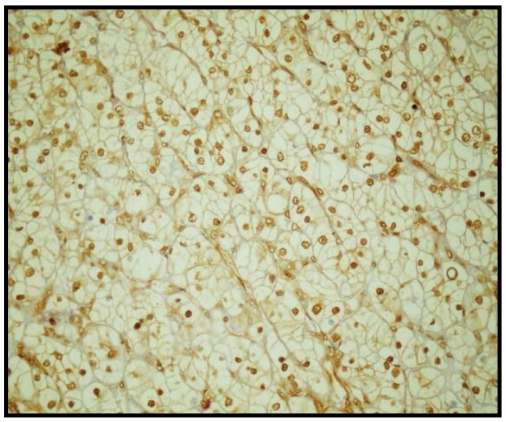

Figure 6: Positive membranous and nuclear expression of CXCR4 in ccRCCs, X 200.

Discussion:

RCC is the 6th leading cause of cancer-related mortality and it is the most lethal and aggressive urological cancer. The 5-year survival rate is about 65% [1].

CXCR4 is a G-protein coupled receptor that was initially described to mediate inflammatory response. However, it has shifted into focus as it is the most chemokine receptor expressed on cancer cells [11]. As regard to CXCR4 expression; many studies applied the expression of CXCR4 to the whole examined tissue sections without subce-llular localization as that published by *Wehler, et al.*, (2008) [11]., while others report CXCR4 expression in only one subcellular location neglecting other locations as what was done by *Rasti and colleagues* (2017); who evaluated CXCR4 expression in the cytoplasm of RCC cells [8].

In 2005, Zagzag and colleagues studied CXCR4 expression in cases of ccRCCs resulting from loss of VHL tumor suppressor gene. They described nuclear and/or cytoplasmic expression of CXCR4 in their studied cases. They also described some cases in which CXCR4 immunoreactivity was shown to highlight the cellular contour in a pattern consistent with membranous expression [10].

In the present study, the nuclear, cytoplasmic and membranous expression of CXCR4 were assessed in the studied 49 cases of RCCs in order to detect the best prognostic factor in RCCs. CXCR4 expression was detected in the nuclei, cytoplasm and membranes of the studied cases with variable proportions. Firstly, we evaluated the association between cytoplasmic expression of CXCR4 and different clinicopatho-logical parameters in RCCs. There was a significant association between cytoplasmic expression of CXCR4 and histological subtypes of RCCs *(p <0.0001)*. To the best of our know-ledge, there is no any previous study mentioned such association. This may be explained as most of previous studies were performed on ccRCCs, however, in the current study, we added other variants; papillary and chromophobe RCCs.

The signaling pathway of CXCR4 and its ligand SDF-1 has been emerged as a potential therapeutic target for human tumors. This signaling pathway plays a critical role in tumor initiation and progression by activating multiple signaling pathways that enhance tumor cell invasion and distant metastasis. Recently, SDF-1/CXCR4 antagonists have been produced which have shown encouraging results in anticancer therapy [12]. So, evaluating the expression of CXCR4 in different histopathological types of RCCs may lead to promising results in treatment of RCCs.

We also found a statistically significant association between cytoplasmic expression of CXCR4 and both capsular and peri-nephric fat invasion *(p=0.038 & p=0.037)*. None of previously publicshed data evaluate these parameters as they didn't include capsular and perinephric fat invasion as separate items into the tested parameters. We belief that small sized tumors with low pT stage may undergo capsular or perinephric fat invasion through lymphatic or vascular emboli. Actually, not only tumor size, but also capsular and fat

invasion should be taken into consider-ation as predictors of prognosis in RCCs.

A significant association was found between cytoplasmic expression of CXCR4 and Fuhrman's nuclear grading *(p= 0.008).* this was keeping with what observed by *Wehler, et al.,* (2008) and *Rasti, et al.,* (2017) [8, 11].

We didn't find any association between cytoplasmic expression of CXCR4 and patients' age, tumor size or necrosis. This was keeping with what was observed by *Li, et al., (2011)* and *Rasti, et al., (2017)* [8& 13].

No association was found between cytoplasmic expression of CXCR4 and pT stage of the studied RCC cases. This was keeping with what was observed by Li and colleagues who didn't find any significant relationship between CXC-R4 and tumor stage [13]. How-ever, *Rasti's* study which was performed on a relatively large number of patients; 173 RCCs, (102 cases of ccRCCs, 35 cases of papillary RCCs and 32 cases of chro-mophobe RCCs) showed that CXCR4 expression is positively associated with tumor stage [8]. We think that the large study sample size is responsible for such statistical differences. Also, most of the studied cases in the current study were in pT3a stage (29 out of 49 patients) with minimal present-ation of other stages.

Membranous expression of CXCR4 was investigated in RCCs included in the current study. We detected associa-tions between membranous expression of CXCR4 and histological subtypes *(p <0.0001),* Fuhrman grade *(p <0.0001)* and pT stage *(p =0,035).*

In the current study, we observed that membranous expression of CXCR4 decreased with high Fuhrman nuclear grading. CXCR4 expression was also lost in high tumor stages.

Wang and colleagues studied the subce-llular localization of CXCR4 in cases of RCCs. They established that CXCR4 is located mainly in cytoplasm/membrane region in primary RCCs. In metastatic RCCs and in higher stages, CXCR4 is internalized into the cytoplasmic and nuclear regions [14]. This finding was also described by Bao and colleagues when they detected nuclear transloc-ation of CXCR4 in all metastatic RCC tissues included in their study [7].

As regard to nuclear expression of CXCR4, there was a statistically signi-ficant association between nuclear CX-CR4 and ISUP Grading for ccRCCs and papillary RCCs. Most of the low ISUP grades (Grades1&2) showed diffuse nuclear localization of CXCR4 *(p= 0.024).* None of previously public-shed studies investigated the association bet-ween nuclear CXCR4 and ISUP grading system. This may be explained as this grading system is applicable only to ccRCCs and papillary RCCs.

No association was observed between nuclear CXCR4 and tumor size, necro-sis, Fuhrman grading or tumor stage. In contrast, *An, et al.,* (2014), found correl-ations between nuclear CXCR4 and both tumor size and necrosis. They also found highly significant relationships between nuclear CXCR4 at one hand and Fuhrman grade and TNM stage at the other hand. [15]. It is important to mention that their study was performed on a relatively large sample size (225 patients) and all cases were exclusively ccRCCs. These factors may contribute to dissimilarities between our results and their study.

Conclusions:

CXCR4 immunostaining in RCC shou-ld be detected in different cellular local-izations; nuclear, cytoplasmic and me-mbranous localizations. Both cytopl-asmic and membranous expression of CXCR4 were significantly associated with both histological subtypes and Fu-hrman grading system. However, loss of cytoplasmic CXCR4 is associated

with capsular and perinephric fat invasion. Additionally, loss of membranous expression of CXCR4 is accompanied by increasing grade or stage of RCCs.

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