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# Immunohistochemical Study of Cancer Stem Cells and Angiogenic Markers in Renal Cell Carcinoma, Clinicopathologic Correlation

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## ABSTRACT

**Background:** Despite advanced therapy of renal cell carcinoma (RCC), up to 40% RCC develop recurrence with high metastatic rate and continues to be one of the fatal forms of cancer. Therefore, detecting new biomolecular markers for prognosis of RCC is important and a major need. Relevant markers of CSCs and angiogenic may serve as prognostic biomarkers of RCC. However, their actual prognostic significance remains inconclusive. **Aim of the work**: we planned this study to evaluate CD133 and VEGF immunohistochemical expression in renal cell carcinoma cases and its correlation with clinicopathologic data to evaluate their clinical significance and prognostic value.

**Methods:** this study was carried out on 50 cases of radical nephrectomy specimens. Hematoxylin and eosin-stained sections from all cases were re-evaluated and further stained immunohistochemically stained sections were done by using antibodies against CD133 and VEGF.

**Results:** expression of CD133 was down -regulated with the level of malignancy of the RCC and was tightly correlated with tumor grade (p=<0.001), capsular invasion (p=<0.001) and grade of lymphocytic infiltrate (p=<0.001), while, there were no significant associations between CD133 expression and tumor stage, the type, size, TNM stage grouping and tumor laterality. Expression of VEGF was associated with high grade (p<0.001) and clinical stage (p=0.026), large size (p=0.008), capsular invasion (p=<0.001), nodal invasion (p=0.011) and grade of lymphocytic infiltrate (p=0.002) of RCC. There was a statistically significant correlation between CD133 and VEGF with adverse relation between the two markers.

**Conclusions:** our study demonstrated that the expression of CD133 was down -regulated with the level of malignancy of the RCC and was tightly correlated with tumor grade, capsular invasion and grade of lymphocytic infiltrate. These facts demonstrated that CD133 play an important role in the development and progression of RCC. Elevated expression of VEGF is a characteristic feature of high grade and stage, large size and capsular invasion of RCC. There was a statistically significant correlation between CD133 and VEGF with adverse relation between the two markers.

**Keywords**: Renal cell carcinoma, CD133, VEGF.

## INTRODUCTION

Renal cell carcinoma (RCC) is a significant health problem with a wide variation in prognosis usually associated with a high metastatic index at the diagnosis. Up to 40% of patients experience recurrence following surgery for clinically localized disease because of the resistance to radiations and chemotherapies [(1)]. Finding better prognostic markers are the goal in order to optimize patient selection for specific therapeutic approaches. Accumulated evidence showed that cancer can be considered as a stem cell disease [(2)]. The concept of contribution of CSCs to proliferate, self-renewing, and multi-differentiation, as well as tumor initiation, progression, metastasis and resistance to treatments was widely accepted [(3)], so it is better to understand the characteristic and prognostic role of CSCs in RCC to create therapeutic strategies for treatment of tumors. CD133 has been investigated as a marker for identification of CSCs in renal carcinomas and it is a possible marker related to tumor progression and invasion that has been extensively studied in different tumors in the body [(4)].

Some research results reported that over-expression of CD133 CSC marker in cancer patients including gastric cancer [(5)], ovarian cancer [(6)] and hepatic cancer [(7)] correlates with a poor prognosis. However, its prognostic significance in RCC is not yet clear. The function of VEGF is not limited to angiogenesis and vascular permeability. Autocrine and paracrine VEGF signaling occurs in some tumor cells, contributing to induction of CSCs, independent of any role that VEGF may have in angiogenesis. Recently, VEGF-targeted therapies have been identified as a promising therapeutic approach in treatment of RCC [(8)]. In



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our study, we planned to evaluate CD133 and VEGF immunohistochemical expression in renal cell carcinoma cases and their correlation with clinicopathologic data to evaluate their clinical significance and prognostic value.

## MATERIALS AND METHODS

This study was carried out on 50 cases of RCC retrieved from the archives of the National Institute of Urology and Nephrology laboratory from the period between 2009 and 2013 after obtaining an informed consent and approval of local ethical committee. The clinicopathological data were obtained from the available histopathological charts. Multiple sections were obtained from the paraffin blocks of the specimens; one was stained by Hematoxylin and Eosin for the histopathological re-evaluation and other 2 sections were mounted on positive charged slides and immunostained by rabbit Monoclonal antibody against CD133 (Dilution 1:50.

Dako, California USA and rabbit Monoclonal antibody against VEGF (Dilution 1:30, Lab Vision / Neomarker, USA) were used according to the manufacturer's protocol. Hematoxylin and eosin stained paraffin sections were examined microscopically to re-evaluate the histological types**,** grade according to Fuhrman nuclear grading system [(9)]. Pathologic staging was evaluated according to Xx **Edge *et al.* [(10)].** Anatomic Staging Groups according to **NCCN [(11)].** The amount of tumor infiltrating lymphocytes (TILs) was scored according to **Rao *et al.* [(12)].** Immunohistochemical reactions were carried out using Labeled Streptavidin-Biotin2 System-Horseradish Peroxidase (LSAB2 System-HRP). The LSAB2 System-HRP is based on a modified labeled Avidin-Biotin (LAB) technique in which a biotinylated secondary antibody forms a complex with peroxidase-congugated streptavidin molecules. For CD133 immunostaining, a section of normal renal cortical tissue was used as an internal positive control showing strong staining of the epithelial lining of renal proximal convoluted tubules according to **da Costa *et al.* [(13)]**.

For VEGF immunostaining, a section of positive VEGF hydronephrotic kidney was used as external positive control showing strong staining of the glomerular endothelium according to **Magers *et al.* [(14)].** Negative controls for the wo markers, processed through replacement of primary antibodies with the buffer solution.

### Interpretation and Evaluation of Immunostaining

Positive staining of CD133 was indicated as brown color in the cell membrane of the tumor cells, while positive staining for VEGF was indicated as brown staining of the cytoplasm and cell membrane of tumor cells. Immunohistochemical staining intensity for CD133 was scored according to **Zeng *et al.* [(15)]** as follows: score 0: staining was observed in less than 25% of the tumor cells, score 1: staining is observed in 25%-50% of the tumor cells, score 2: staining was observed in 50%- 75%- of the tumor cells and score 3: staining was observed in more than 75%- of the tumor cells. Scores 0 and 1 were considered as low expression, whereas scores 2 and 3 were considered as high expression**.**

**Immunohistochemical staining intensity for** VEGF was scored according to **Song *et al.* [(16)]**, on a score of 0 to 3 into:

Score 0-1: weak expression level and score 2-3: strong expression level.

### *Statistical analysis*

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Chi-square (X2) test of significance was used in order to compare proportions between two qualitative parameters.

## RESULTS

### I- Clinicopathological findings

The overall clinicopathological characteristics of the 50 cases of RCC were summarized in **Table 1.**

### II- Results of immunohistochemical expression of CD133 of RCC cases

Immunoreactivity to CD133 showed brown color in the cell membrane of the positive tumor cells, but usually with cytoplasmic staining in high grade tumors. Twenty nine of our cases (58%) showed low membranous expression, while 21 cases (42%) showed high positive membranous expression. Different types of RCC showed different expression as showed in **graph 1 and figure 1.** There was no statistically significant difference between low and high CD133 expression according to the type (P-value 0.161).

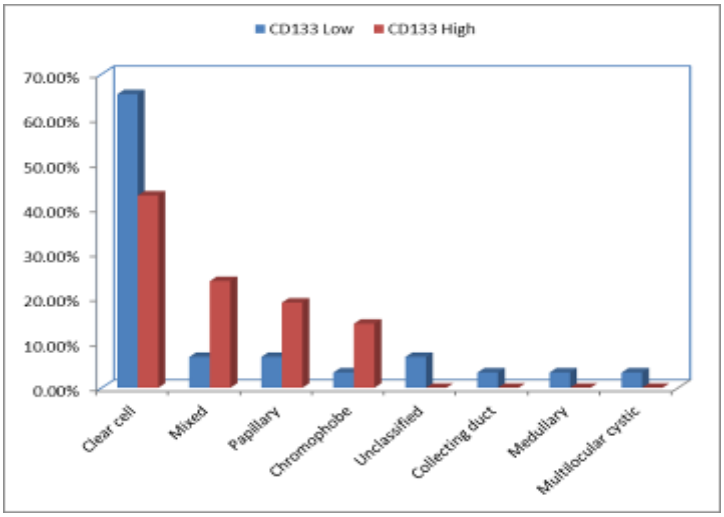
| **Table 1: clinico- pathologic characters of primary renal cell carcinoma** | | |
|---|---|---|
| Variable | Case number | % |
| No of cases | 50 | 100% |
| Gender | | |
| Male | 32 | 64% |
| Female) | 18 | 36% |
| Age | | |
| >60 years | 21 | 42% |
| ≤60 years | 29 | 58% |
| Medium and range: 40-77 (58.3±8.37 | | |
| Histopathological types | | |
| Clear cell | 2 | 56% |
| Mixed | 8 | 14% |
| Papillary | 6 | 12% |
| Chromophobe | 4 | 8% |
| Unclassified | 2 | 4% |
| Collecting duct | 1 | 2% |
| Medullary | 1 | 2% |
| Multilocular cystic | 1 | 2% |
| Tumor size | | |
| >7 | 17 | 34% |
| ≤7 | 33 | 66% |
| Laterality of the tumor | | |
| Right | 25 | 50% |
| left | 25 | 50% |
| Capsular invasion | | |
| Positive | 19 | 38 % |
| negative | 31 | 62 % |
| Pathologic stage | | |
| T1 | 16 | 32% |
| T2 | 19 | 38% |
| T3 | 14 | 28% |
| T4 | 1 | 2% |
| Nodal metastasis | | |
| N0 | 40 | 80% |
| N1 | 10 | 20% |
| Distant metastasis | | |
| MX | 42 | 84% |
| M1 | 8 | 8% |
| TNM stage | | |
| I | 15 | 30% |
| II | 14 | 28% |
| III | 13 | 26% |
| IV | 8 | 16% |
| Grade | | |
| I | 13 | 26% |
| II | 16 | 32% |
| III | 20 | 40% |
| IV | 1 | 2% |

**Relation between CD133 expression and clinicopathologic parameters of RCC**

There was a significantly negative correlation between the level CD133 expression and grade, lymph node status, capsular invasion and tumor infiltrating lymphocytes (TILs). High level of expression of CD133 in the histologic grade I and II was higher than in grade III and IV as well as in the lymph node metastatic group was also higher than in the non-metastatic group. Negative capsular invasion was associated with 95.2% high expression and lymphocytic infiltration increased with low CD133 expression cases and decreased with the high CD133 expression cases ($p < 0.05$). While, CD133 expression was not correlated with the size, site of the tumor, distant metastasis, TNM and T stage ($P > 0.05$) (**Table 2).**

**Table 2 : relation between CD133 expression and clinicopathologic parameters of RCC**

| | CD133 | | | | Chi-square test | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | No. | % | No. | % | x2 | p-value |
| **Grade** | | | | | 21.233 | <0.001 |
| I | 3 | 10.3% | 10 | 47.6% | | |
| II | 6 | 20.7% | 10 | 47.6% | | |
| III | 19 | 65.5% | 1 | 4.8% | | |
| IV | 1 | 3.4% | 0 | 0.0% | | |
| **T stage** | | | | | 4.728 | 0.193 |
| T1 | 6 | 20.7% | 10 | 47.6% | | |
| T2 | 12 | 41.4% | 7 | 33.3% | | |
| T3 | 10 | 34.5% | 4 | 19.0% | | |
| T4 | 1 | 3.4% | 0 | 0.0% | | |
| **Nodal metastasis** | | | | | 5.255 | 0.022 |
| N0 | 20 | 69.0% | 20 | 95.2% | | |
| N1 | 9 | 31.0% | 1 | 4.8% | | |
| **Size** | | | | | 2.993 | 0.084 |
| <7 cm | 7 | 24.1% | 10 | 47.6% | | |
| ≥7cm | 22 | 75.9% | 11 | 52.4% | | |
| **Cap.Inv** | | | | | 16.978 | <0.001 |
| Positive | 18 | 62.1% | 1 | 4.8% | | |
| Negative | 11 | 37.9% | 20 | 95.2% | | |
| **Side** | | | | | 0.739 | 0.390 |
| Rt. | 16 | 55.2% | 9 | 42.9% | | |
| Lt. | 13 | 44.8% | 12 | 57.1% | | |
| **TNM** | | | | | 7.282 | 0.063 |
| I | 5 | 17.2% | 10 | 47.6% | | |
| II | 8 | 27.6% | 6 | 28.6% | | |
| III | 9 | 31.0% | 4 | 19.0% | | |
| IV | 7 | 24.1% | 1 | 4.8% | | |
| **TILs** | | | | | 15.244 | <0.001 |
| Grade I | 4 | 13.7% | 11 | 52.3% | | |
| Grade II | 7 | 24.1% | 8 | 38.0% | | |
| Grade III | 18 | 62.0% | 2 | 9.5% | | |
| **Distant metastasis** | | | | | 3.402 | 0.065 |
| M0 | 22 | 75.9% | 20 | 95.2% | | |
| M1 | 7 | 24.1% | 1 | 4.8% | | |




**Graph. 1:** low and high CD133 expression according to the type

**III- Results of immunohistochemical expression of VEGF in RCC cases**

Immunoreactivity to VEGF was cytoplasmic and membranous brown color expression of the positive tumor cells. Thirty three of our cases (66%) showed strong cytoplasmic and membranous expression, while 17 cases (34%) showed weak expression. As regard to histopathological types of RCC there was a significant correlation between the level of VEGF expression and different types (P <0.001)(**Table 3).**

**Table 3 : comparison between low and high VEGF expression according to type**

| **Type** | **VEGF** | | | | **Chi-square test** | |
|---|---|---|---|---|---|---|
| | **Weak** | | **Strong** | | | |
| | **No.** | **%** | **No.** | **%** | **x2** | **p-value** |
| Clear cell | 24 | 72.7% | 4 | 23.5% | 25.437 | <0.001 |
| Mixed | 0 | 0.0% | 7 | 41.2% | | |
| Papillary | 4 | 12.1% | 2 | 11.8% | | |
| Chromophobe | 1 | 3.0% | 3 | 17.6% | | |
| Unclassified | 2 | 6.1% | 0 | 0.0% | | |
| Collecting duct | 1 | 3.0% | 0 | 0.0% | | |
| Medullary | 1 | 3.0% | 0 | 0.0% | | |
| Multilocular cystic | 0 | 0.0% | 1 | 5.9% | | |
| **Total** | **33** | **100** | **17** | **100** | | |

**Relation between VEGF expression and clinicopathologic parameters of RCC**

There were significantly correlation between the VEGF expression and grade, pathologic stage, lymph node status, size, capsular invasion and TILs of the tumor. While VEGF expression was not correlated with the s site of the tumor, TNM stage and distant metastasis (P> 0.05).

Strong expression was revealed in all cases of grade III and IV (60.6% and 3.0% respectively), while only in 4 cases (12.1%) of grade I tumors and 8 cases (24.2%) of grade II tumors. According to tumor T stage the strong expression showed in cases with high tumor stage. All weak expression rate was notice of N0 (100.0%) and in no cases (00.0%) of N1, while strong expression were revealed in 23 cases (69.7%) of N0 and in all cases of N1 (30.3%). According to tumor size, the strong expression were revealed in 7 (21.2%) cases whom size less 7 cm and in 26 (78.8%) cases of tumor equal or more 7 cm. As regard TILs**, t**here was significant relationship between VEGF expression and grade of lymphocytic infiltrate as the lymphocytic infiltration increases with strong VEGF expression cases and decreases with the weak VEGF expression cases (**Table 4).**

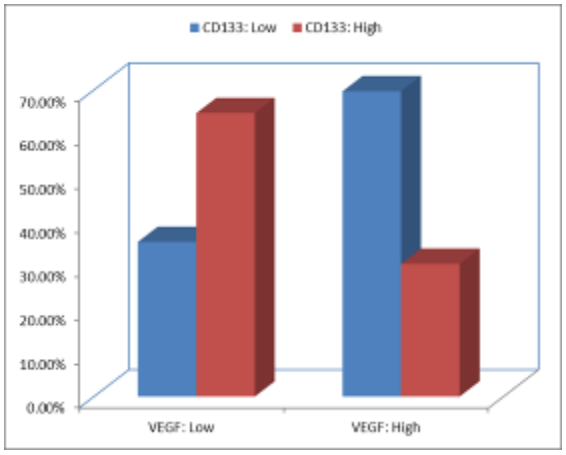
| Table 4 : relation between VEGF expression and clinicopathologic parameters of RCC | | | | | | |
|---|---|---|---|---|---|---|
| | VEGF | | | | Chi-square test | |
| | weak | | strong | | | |
| | No. | % | No. | % | x2 | p-value |
| **Grade** | | | | | 19.834 | <0.001 |
| I | 9 | 52.9% | 4 | 12.1% | | |
| II | 8 | 47.1% | 8 | 24.2% | | |
| III | 0 | 0.0% | 20 | 60.6% | | |
| IV | 0 | 0.0% | 1 | 3.0% | | |
| **T stage** | | | | | 9.231 | 0.026 |
| T1 | 10 | 58.8% | 6 | 18.2% | | |
| T2 | 5 | 29.4% | 14 | 42.4% | | |
| T3 | 2 | 11.8% | 12 | 36.4% | | |
| T4 | 0 | 0.0% | 1 | 3.0% | | |
| **Nodal metastasis** | | | | | 6.439 | 0.011 |
| N0 | 17 | 100.0% | 23 | 69.7% | | |
| N1 | 0 | 0.0% | 10 | 30.3% | | |
| **Size** | | | | | 7.073 | 0.008 |
| <7 cm | 10 | 58.8% | 7 | 21.2% | | |
| ≥7cm | 7 | 41.2% | 26 | 78.8% | | |
| **Cap.Inv** | | | | | 15.787 | <0.001 |
| Positive | 0 | 0.0% | 19 | 57.6% | | |
| Negative | 17 | 100.0% | 14 | 42.4% | | |
| **Side** | | | | | 0.089 | 0.765 |
| Rt. | 9 | 52.9% | 16 | 48.5% | | |
| Lt. | 8 | 47.1% | 17 | 51.5% | | |
| **TNM** | | | | | 7.282 | 0.063 |
| I | 9 | 52.9% | 6 | 18.2% | | |
| II | 2 | 11.8% | 12 | 36.4% | | |
| III | 3 | 17.6% | 10 | 30.3% | | |
| IV | 3 | 17.6% | 5 | 15.2% | | |
| **TILs** | | | | | 14.869 | 0.002 |
| Grade I | 10 | 58.8% | 5 | 15.2% | | |
| GradeII | 6 | 35.2% | 9 | 27.3% | | |
| Grade III | 1 | 5.9% | 19 | 57.5% | | |
| **Distant metastasis** | | | | | 0.052 | 0.820 |
| M0 | 14 | 82.4% | 28 | 84.8% | | |
| M1 | 3 | 17.6% | 5 | 15.2% | | |

**Correlation between CD133 and VEGF of studied cases**

The majority of cases with low CD133 expression (29 cases) showed (23 cases) strong VEGF expression. Both CD133 and VEGF were concurrently strongly expressed in 10 cases .There is adverse relation between the two markers in total 50 cases with high VEGF in association with bad prognostic factors such as grade, stage and type, and high expression of CD133 associated mostly with good prognostic factors (P value < 0.05) as showed in **table 5 and graph 2.**

**Table 5:** relation between CD133 and VEGF expression of the studied cases

| CD133 | VEGF | | | | Total | | Chi-square test | |
|---|---|---|---|---|---|---|---|---|
| | Weak | | strong | | | | | |
| | No. | % | No. | % | No. | % | x2 | p-value |
| Low | 6 | 35.3% | 23 | 69.7% | 29 | 58.0% | 5.451 | 0.020 |
| High | 11 | 64.7% | 10 | 30.3% | 21 | 42.0% | | |
| Total | 17 | 100.0 % | 33 | 100.0 % | 50 | 100.0 % | | |




**Graph 2:** relation between CD133 and VEGF expression of the studied cases

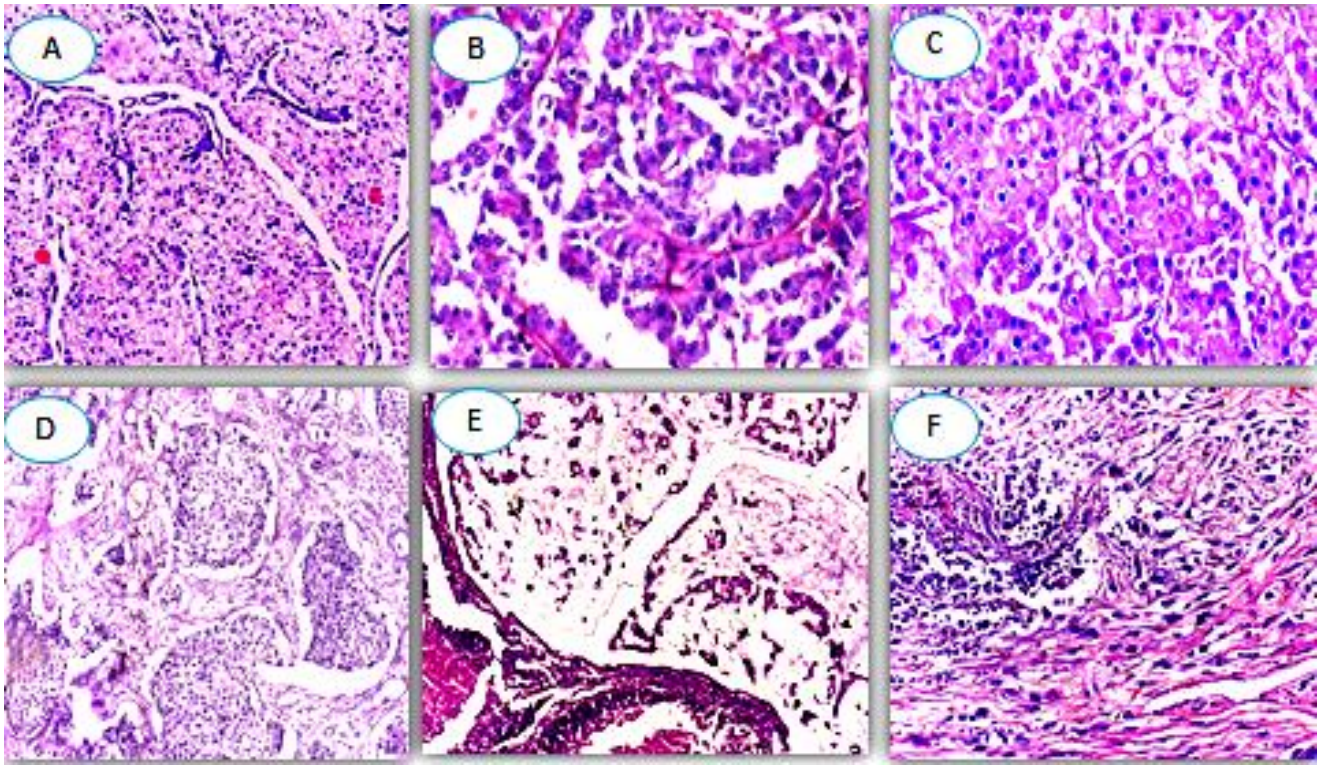



Fig. (1): H& E stain. (a):clear RCC grade I. (b):papillary RCC type II. (c): chromophobe RCC. (d): collecting duct RCC. (e): medullary RCC. (f): unclassified RCC.

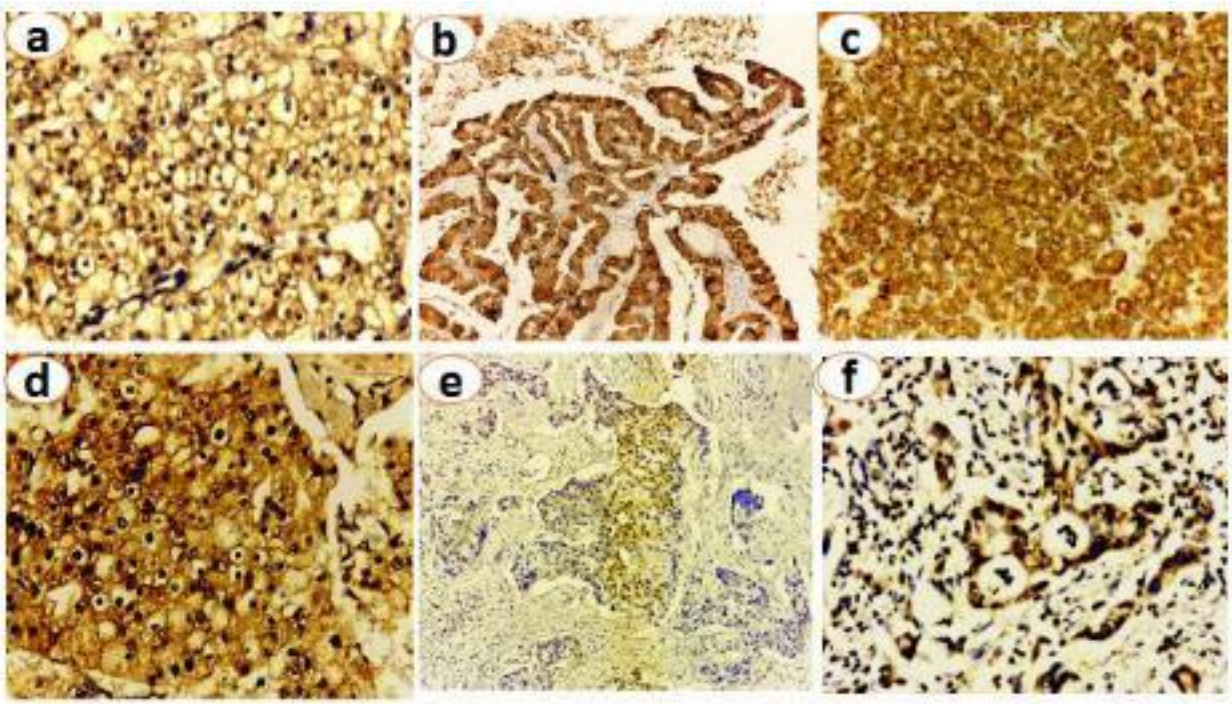



**Fig. 1:** CD133 immunoactivity (a)clear cell RCC showing strong diffused membranous immunoreactivity (x100), (b)papillary RCC type II showing diffused membranous immunoreactivity (x300),(c) papillary RCC type I showing diffused membranous immunoreactivity (x200), (d)chromophobe RCC showing diffused membranous and focal cytoplasmic immunoreactivity (x200), (e)collecting ducts RCC showing focal membranous and cytoplasmic immunostaining (x100), (f)medullary RCC showing diffused membranous and cytoplasmic expression (x200).

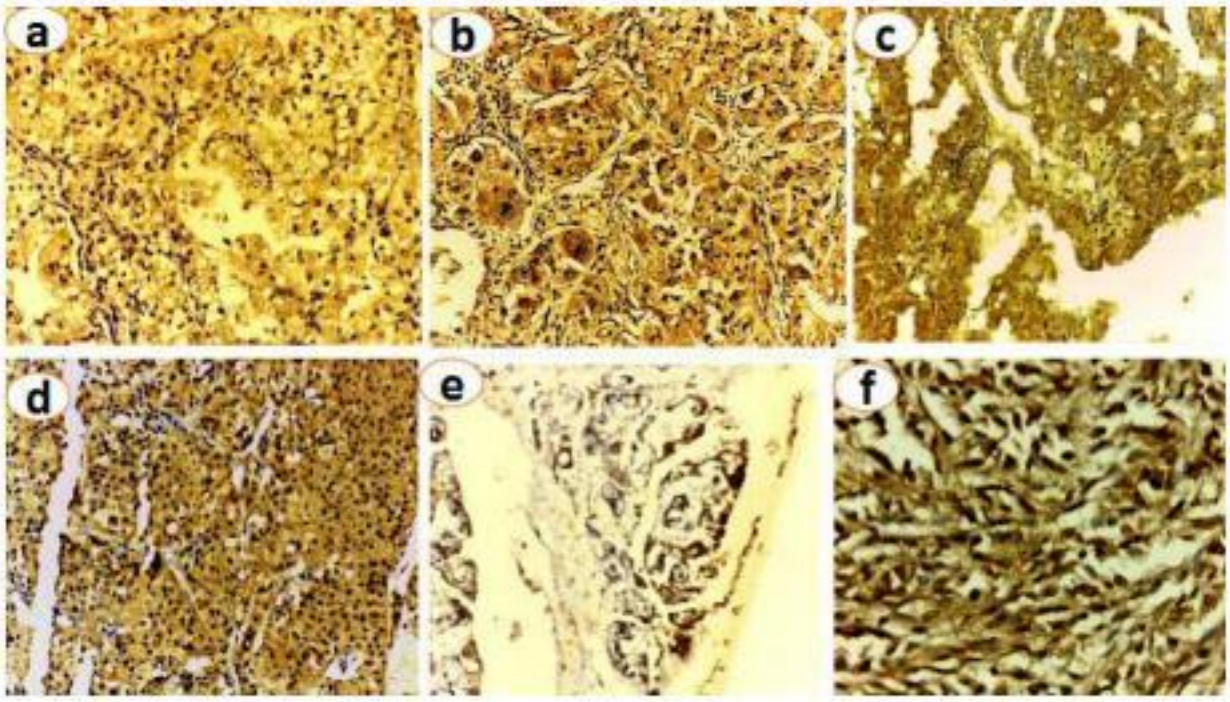



**Fig. 2:** VEGF immunoactivity (a)clear cell RCC grade I showing strong diffused cytoplasmic and membranous immunoreactivity (x200), (b)clear cell RCC grade IV showing strong diffused cytoplasmic and membranous immunoreactivity (x300),(c)papillary RCC type I showing diffused cytoplasmic and membranous immunoreactivity (x300), (d)chromophobe RCC showing diffused cytoplasmic and membranous immunoreactivity (x200), (e)medullary RCC showing strong diffused cytoplasmic and membranous immunostaining (x200), (f)unclassified RCC showing diffused strong cytoplasmic and membranus expression (x300).

## DISCUSSION

Although targeted therapy of metastatic renal cell carcinoma (RCC) has rapidly developed in the past few years, RCC continues to be one of the fatal forms of cancer. Therefore, detecting new biomolecular markers for prognosis of RCC is important and a major need [17]**.** Cancer stem cells are a small sub-population of cells within tumors with the ability of self-renewal, tumorigenicity, as well as keeping the tumor bulk, invasion, metastasis and recurrence were characterized in diverse human solid tumors [18]. Multiple cell surface markers have been suggested for isolation and enrichment of CSCs. One of them, CD133 (Prominin-1) that is the primary during a category of novel pentaspan membrane proteins to be known in both humans and animals. **Li *et al.*** identified that CD133 expression was related to progenitor cells, normal stem cells, cancer stem cells, regeneration, differentiation and metabolism [19].In our study, we planned to evaluate CD133 and VEGF immunohistochemical expression in renal cell carcinoma cases and its correlation with clinicopathologic data to evaluate their clinical significance and prognostic value.

In the current study, the age of our patients ranged from 40 to 77 years with mean age of 58.3±8.37 which was in accordance with a study done by **Alkhateeb *et al.*** [20] with a mean age of 57.8 , 55.15±13.34 years at diagnosis of patients with RCC respectively. Regarding to gender, our study showed male predominance with a 64% of cases , this is typical to a population based analysis done by **Bianchi *et al.*** [21] revealed a male percent of 64% too. Our study revealed that 66% of cases, the tumor size were ≥ 7 cm.Our data revealed that the same percentage (50% right side and 50% left side) regarding the laterality of the tumors**.** While, most of other studies showed right side predominance such as **Zhang *et al.*** [22] and **Ingimarsson *et al.*** [23] who showed that right kidney tumors were 69%, 54.5% , 59% of cases respectively. Clinical significance of CD133 expression in human RCC was inconsistent and varies greatly between studies. **da Costa *et al.*** [13] found that patients in the CD133 low-expression group had a higher probability of death from RCC and disease progression **.** Conversely, **D'Alterio *et al.*** [14] did not see any correlation with the clinic-pathological features or patient prognosis**.** However, another study done by **Zhang *et al.*** [22] observed that CD133 expression was correlated with tumor grade, stage, histological type and tumor location. Our results showed that 58% of cases demonstrated low membranous expression, while 42% of cases demonstrated high membranous expression. Close to our study, that done by **da Costa *et al.*** [13] on 142 RCC cases showed 54% of cases with CD133 low expression and 46% of cases with CD133 high expression. In a study on 119 RCC cases done by **Kim *et al.*** [25] only 17.8% of cases showed CD133 high expression. In addition, CD133 expression was significantly correlated with tumor grade, with increase low expression in high grade tumor and increase high expression in low grade tumors. Besides, there was a significant correlation between CD133 expression and capsular invasion with high expression mostly associated with negative capsular invasion and grade of lymphocytic infiltrate as the lymphocytic infiltration decrease with high CD133 expression While, there were no significant associations between low and high CD133 expression according to, the type, tumor stage, tumor size, TNM stage grouping and tumor laterality.

Despite CD133 was detected only in the proximal tubules of normal renal parenchyma, CD133 was not useful as a possible marker of RCC-specific histologic types (P = 0.59, Indeed, it was observed that 55% of clear cell RCC tumors, which are derived from proximal tubules, showed low expression of CD133 ,this may be due to lose several features of their proximal tube origin.

**Saeednejad *et al.*** [26] found that cytoplasmic CD133 expression was positively associated with the advanced histological grade and stage of clear cell RCC whereas the study failed to find an association between the membranous expression of CD133 with the tumor grade and stage. **Kim *et al.*** [25] observed a correlation between high level expression of membranous CD133 in clear cell RCC and favorable clinicopathological characteristics. **D'Alterio *et al.*** [14] evaluated cytoplasmic and membranous CD133 expression and found that CD133 had no prognostic role in RCC.

**Zhang *et al.*** [22] concluded that CD133 expression was significantly associated with tumor grade, TNM stage and histological type and was found to be more frequent in the left kidney. An article reported similar results in other tumor types, such as glioma, colon and hepatocellular cancer [27] . Thus, it seems that the prognostic significance of CD133 expression may differ according to the tumor site [25].The positive prognosis of CD133 expression may be clarified in three ways. First, CD133 is a cell-membrane protein normally expressed in the plasma membrane, including microvilli [28]. As long as

microvilli are plentiful in the proximal tubules of the kidney, this might justify the reduced levels of CD133 in the undifferentiated sarcomatoid region and fewer differentiated alveolar regions. But, this observation does not absolutely explain the high expression levels of CD133 within the macro-/microcystic regions of clear cell RCC since not of these regions expressed the marker. Second, since CD133 is a glycoprotein, its expression may reflect a variation in glycosylation status according to the degree of tumor differentiation. It has been well established that the glycosylation of CD133 varies with cellular differentiation and malignant transformation [(29)]. Third, CD133+ cells may represent a heterogeneous population of tumor cells that contains a small number of CSCs and many differentiated non-CSC cells.

The precise mechanism by which CD133 could contribute to tumor progression or recurrence in RCC continues to be unclear. It is reported that the expression of CD133 is controlled by HIF1 alpha during hypoxia [(30)]. The changes in its function could confer a more aggressive phenotype to the tumor and favor the occurrence of metastatic spread. However, that is only an assumption and further studies are needed to confirm this hypothesis.

There were previous reports of the existence of CD133 multipotent progenitor cells in the proximal tubules and Bowman's capsule in kidneys of adults. **Bruno *et al.*** [(31)] described the presence of CD133 progenitor cells in the renal parenchyma capable of differentiation into vascular structures in the presence of tumor-derived growth factors. It was observed that such cells lost the expression of CD133 antigen and acquired expression of endothelial-specific origin markers favoring vascularization and tumor growth. Therefore, such description is appropriate to lower expression of CD133 in RCC compared to non-neoplastic tissue.Regarding VEGF expression, our findings showed that 33 (66%) of cases showed high cytoplasmic and membranous expression, while 17 cases (34%) showed low expression. As regards to the staining patterns of VEGF in the different histopathological types of RCC, there were different expressions in the different types. High statistically significant difference was noted between low and high VEGF expression according to the type, grade, stage, size, and capsular invasion. In the present study, there was a significant relationship between VEGF expression and grade of lymphocytic infiltrate as the lymphocytic infiltration increased with high VEGF expression cases and decreased with the low VEGF expression cases. Different stages showed different expressions with no statistically significant difference between VEGF expression and TNM stage grouping. There was a statistically significant correlation between CD133 and VEGF expression in total 50 cases in the picture of the adverse relation between the two markers with high VEGF in association with bad prognostic factors such as grade, stage and type, and high expression of CD133 associated mostly with good prognostic factors (P value < 0.05).Our study had some limitations. This is a single-center study with a small group of patients; it is subject to criticism due to the immunohistochemical procedure itself, such as problems posed by inadequate technique of fixation in formalin material.

**In conclusion,** our study demonstrated that expression of CD133 was down -regulated with the level of malignancy of the RCC and it was tightly correlated with tumor grade, capsular invasion and grade of lymphocytic infiltrate. These facts demonstrated that CD133 play an important role in the development and progression of RCC. Elevated expression of VEGF is a characteristic feature of high grade and stage, large size and capsular invasion of RCC. There was a statistically significant correlation between CD133 and VEGF with adverse relation between the two markers.

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