ABSTRACT

Objective: Certain subtypes of Renal cell carcinomas (RCCs) are diagnostically challenging owing to their overlapping histopathological features. Recently, c-KIT (CD117) has come into focus as a potential diagnostic marker of some renal tumors. c-KIT also provides a potentially suitable for targeted tumor therapy. The present study was designed to investigate the expression of c-KIT in RCCs in order to evaluate its diagnostic usefulness as a phenotypic marker and to establish the basis for a new possible therapeutic modality.

Material and Methods: The present work was conducted on 49 patients with RCC: Clear cell RCC (CRCC): 30 cases (61.22%); chromophobe RCC (ChRCC): 9 cases (18.37%); papillary RCC (PRCC), type I: 5 cases (10.20%); and carcinoma of the collecting ducts of Bellini (CdRCC): 5 cases (10.20%). The expression of c-KIT was assessed using immunohistochemistry. The diagnostic performance of c-KIT expression was evaluated using ROC curve analysis.

Results: Overall, 11 (22.5%) cases of RCC showed c-KIT expression: 2 (6.7%) CRCC, 7 (77.8%) ChRCC, and 2 (40.0%) CdRCC. Among the study group, ChRCC had the highest frequency \( (p=.001) \) and staining score \( (p=.001) \) for c-KIT. In addition, only ChRCC showed membranous pattern of c-KIT staining, while other tumors showed cytoplasmic staining \( (p=.013) \). c-KIT showed a sensitivity of 77.78% and a specificity of 95% for the diagnosis of ChRCC. The relation between c-KIT expression and clinicopathological parameters was not significant. High grade tumors had a statistically significant higher total score of c-KIT expression \( (p=.023) \).

Conclusions: c-KIT is frequently and significantly expressed in chromophobe RCC suggesting that it might play a role in its pathogenesis. Immunohistochemical detection of c-KIT expression could be used to aid histological diagnosis of chromophobe RCC with a high sensitivity and specificity. The substantial c-KIT immunoreactivity in chromophobe RCC may be of clinical importance especially in the field of targeted therapy.

Key Words: Renal cell carcinoma – c-KIT (CD117) – ROC curve analysis.

INTRODUCTION

Renal cell carcinomas (RCCs) account for over 90% of all adult primary renal neoplasms and 2-3% of all adult malignant tumors [1]. In Egypt, National Cancer Institute reported that RCCs represent 6% of the newly diagnosed genitourinary cancers and 0.8% of all newly diagnosed cancers [2].

RCCs represent a heterogeneous group of tumors with potential prognostic and therapeutic differences. Reaching a final definite diagnosis is possible in the majority of cases by examining the tumor’s gross and light microscopic morphologic features; however, there is a sufficient overlap between several entities [3]. In addition, needle biopsies from renal masses are being increasingly performed. In these small biopsies, the entire range of cytoarchitectural features that are generally necessary to make a diagnosis may not be fully appreciated. In such situations, ancillary techniques may be necessary to narrow the differential diagnosis or to arrive at a definitive diagnosis [3].

In diagnostic surgical pathology practice of renal tumors, a common diagnostic dilemma is the distinction between chromophobe RCC (ChRCC) and the clear cell RCC (CRCC) predominantly composed of granular cells (also known as the granular cell variant of conventional RCC) [1,4]. Accurate diagnosis is important not only for its correlation with the cytogenetic findings, but also for its prognostic implications. Generally, the outcome for patients with ChRCC is more favorable than for those with conventional RCC [5,6].
Traditionally, the diagnosis of ChRCC was supported by the strong and diffusely positive reticular staining pattern of the Hale’s colloidal iron stain [7] and the intracytoplasmic microvesicles observed under the electron microscope [8]. However, these techniques are not always feasible for ordinary pathology laboratories [7,8]. Therefore, identifying commercially available immunohistochemical markers that could assist in the differential diagnosis of renal tumors would be invaluable.

Several previous studies have addressed this issue with different immunohistochemical markers being investigated. Different expression patterns of cytokeratins (CK7, CK8, CK18, CK19 and CK20), RCC, CD10, and vimentin have been shown to be potentially useful in the differential diagnosis of renal epithelial neoplasms [9-12]. Recently, c-KIT (CD117) has come into focus as a potential diagnostic marker of some renal tumors [13].

The growth factor receptor protein c-KIT (CD117) is a transmembrane receptor belonging to the class III receptor tyrosine kinase family, which includes the receptor for colony-stimulating factor 1, and the platelet-derived growth factor receptors type A and B [14]. c-KIT (CD117); also known as KIT, stem cell factor receptor, mast cell growth factor receptor, steel factor receptor, and p145 (c-kit); is encoded by the proto-oncogene c-kit that maps to chromosome 4 (4q11-12) [15].

Stem cell factor (SCF) has been identified as the natural ligand of c-KIT. The c-KIT/SCF signaling pathway is involved in the development of several lineages of stem cells, such as germ cells, neural crest-derived melanocytes and hematopoietic precursor cells [16-20].

In addition, it has been shown that c-KIT is constitutionally expressed in various normal tissues: Such as breast epithelium, skin basal cells (seemingly melanocytes), tissue mast cells, interstitial cells of Cajal, fibroblast-like bone lining cells and certain regions of the central nervous system [21-23].

In human neoplasia, the c-KIT tyrosine kinase receptor pathway has been implicated in tumor development and progression. The c-KIT/SCF axis is thought to promote cell transformation and tumorigenicity via auto-/paracrine stimulation in a variety of human tumors, either with or without activating c-kit mutations [24]. Expression of c-KIT has been observed in a spectrum of human neoplasms, chiefly gastrointestinal stromal tumor (GIST), myeloproliferative disorders, mast cell neoplasms, melanoma, and seminoma [21,22,25-29].

Nowadays, immunohistochemical expression of c-KIT is routinely used in pathology laboratories as a reliable diagnostic marker for certain tumors: e.g. GIST and seminoma [5].

In addition to its diagnostic value, c-KIT also provides a potentially suitable target for tumor therapy [30]. Recently, imatinib (STI-571), a relatively nontoxic tyrosine kinase inhibitor, has been used effectively for treating chronic myelogenous leukaemias [31] and unresectable and/or metastatic GISTs [32] raising the hope that other malignancies with c-KIT expression could be treated similarly [29,33].

There are only few reports in the literature regarding c-KIT expression in renal tumors, however, the results are conflicting [4,27,33,34].

The present study was designed to investigate the immunohistochemical expression of c-KIT (CD117) in RCCs in order to evaluate its diagnostic usefulness as a phenotypic marker and to establish the basis for a new possible therapeutic modality.

**MATERIAL AND METHODS**

**Patients and tissue samples:**

The present work included 49 cases of RCCs that were retrieved from the surgical pathology archives at the Pathology Department, Faculty of Medicine, Alexandria University. The preliminary clinical diagnosis was made according to clinical presentation and imaging studies. All the patients underwent open radical nephrectomy procedure at the Urology Department, Faculty of Medicine, Alexandria University. The surgical approach differed according to the size and the location of the tumor. Pedicle control before dissection of the Gerota’s fascia was performed in all cases. Hilar lymph node sampling was performed for staging purposes. Only tumors affecting the upper pole of the kidney were taken out with the ipsilateral adrenal gland. Complete clinical data were available for the 49 patients.
Pathological examination:

Routinely processed paraffin-embedded tissues were cut into 5µm-thick sections and stained with the conventional H&E stain. Each case was carefully reviewed and classified according to the WHO classification criteria [1].

Grading of the CRCC and papillary RCC was done according to the Fuhrman grading system [35]. Tumors with Fuhrman grades I and II were categorized as low grade tumors. Fuhrman grades III and IV were categorized as high grade tumors [36].

Staging was performed according to the criteria of the American Joint Committee on Cancer [37].

Immunohistochemistry for c-KIT:

Immunohistochemical staining for c-KIT was performed. The staining condition was adjusted using GIST samples known to express c-KIT as positive controls. Negative controls included omitting the primary antibody. Both positive and negative controls were included in all runs.

The deparaffinized tissue sections were rehydrated in graded alcohols. The endogenous peroxidase was quenched by using hydrogen peroxide 3% for 10 min. For antigen retrieval, sections were microwaved in 10 mM citrate buffer (pH 6.0).

The primary antibody (CD117/c-KIT/SCF-Receptor clone: Ab-6; Ready-to-Use; rabbit polyclonal antibody, NeoMarkers, labvision, USA) was then applied and the sections were incubated overnight at 4°C. The bound primary antibody was visualized by using the UltraVision detection system (Neomarkers, labvision, USA) which utilizes the streptavidin-biotin method. The final reaction product was developed in diaminobenzidine tetra-hydrochloride (DAB) mixture for 10 minutes. Finally, the slides were counter stained with hematoxylin.

Evaluation of c-KIT immunostaining:

The evaluation system for c-KIT immunostaining proposed by Miliaras, et al. [36] was followed. It included evaluation of the intensity as well as the extent of staining. The intensity of staining was graded as either absent, weak, moderate, or strong (0 to 3 scale). Tissue mast cells, which stain 3+, served as a positive control and were used as an internal scoring guide [27,36] (Fig. 1A). The extent of staining was evaluated semiquantitatively and categorized as 0%, less than 10%, between 10% and 50%, between 50% and 80%, and more than 80% (0 to 4 scale). Aggregate total scores of both intensity and extent of staining were then calculated and recorded for each case (range: 0-7).

The cellular localization of positive reaction was also recorded in an effort to assess the membranous or cytoplasmic nature of immunoreactivity.

Statistical analysis:

Data were analyzed using Minitab (ver.15). Quantitative data were described using measures of central tendency (median) and dispersion (SD, minimum and maximum). Qualitative variables were described using frequencies and percentages. Comparisons between two or more independent quantitative samples were conducted using Kruskal-Wallis test. The associations between two qualitative variables were tested using chi-square statistic. If more than 20% of the cells had have expected count less than 5, Fisher’s exact correction for 2 X 2 Tables and Yates’ chi-square for Tables with more than two levels for each variables were performed. A $p$-value of <0.05 was considered to be statistically significant.

The diagnostic performance of c-KIT expression in RCC was evaluated using Receiver Operating Characteristic (ROC) curve analysis. The following statistics were calculated: Sensitivity; specificity; positive likelihood ratio; negative likelihood ratio; positive predictive value; and negative predictive value.

In a ROC curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (1-Specificity) for different cutoff points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. The cutoff point at which the highest diagnostic accuracy is reached was estimated using Youden Index.

RESULTS

The present study included 49 cases of RCCs. 27 patients (55.1%) were males and 22 patients (44.9%) were females with a M:F sex ratio of 1.23:1. The age of the patients ranged from 20 to 80 years (mean: 53.14, median: 50).
The studied cases were classified according to the WHO classification [1] into clear cell RCC (CRCC): 30 cases (61.22%); chromophobe RCC (ChRCC): 9 cases (18.37%); papillary RCC (PRCC), type I:5 cases (10.20%); and carcinoma of the collecting ducts of Bellini (CdRCC): 5 cases (10.20%).

CRCC showed variable tinctorial cytoplasmic features ranging from clear cell (16.67%) to predominant granular cell (30%) to mixed clear and granular cell morphology (53.33%). Three cases (10%) of CRCC showed sarcomatoid change in the form of pleomorphic sarcomatous areas.

CRCC and PRCC were graded according to the Fuhrman’s grading system [35]. Nineteen cases (14 cases of CRCC and all the five cases of PRCC) were categorized as low grade tumors (Fuhrman’s grades I and II) whereas 16 cases of CRCC were categorized as high grade tumors (Fuhrman’s grades III and IV including cases with sarcomatoid change).

According to the criteria of the American Joint Committee on Cancer [37], the 49 studied patients with RCCs were classified as follows: 15 patients (30.61%) were stage I,10 patients (20.41%) were stage II,8 patients (16.33%) were stage III and 16  patients (32.65%) were stage IV.

Immunohistochemical expression of c-KIT:

Out of the 49 cases of RCCs, 14 cases (28.57%) showed immunostaining for c-KIT. The immunoreactive cases were distributed as follows: 4 cases of CRCC, 7 cases of ChRCC, and 3 cases of CdRCC (Figs. 1,C-F).

As regards CRCC, the four immunoreactive cases had granular cell morphology with sarcomatoid change seen in two (50%) of the cases. All the immunoreactive cases were of high grade (grade IV) and stage (stage IV). The immunoreactive cases showed cytoplasmic staining of weak or moderate intensity. The staining pattern was focal in two cases and diffuse in the other two cases. The mean total staining score was 0.5±1.3 (Median=0).

Seven cases of ChRCC showed diffuse membranous immunoreactivity for c-KIT. Focal fine granular cytoplasmic reactivity was noted in four (57.14%) of the immunoreactive cases. The staining intensity ranged from weak to strong. The mean total staining score was 4.7±2.7 (Median=6). The immunoreactive cases were of stage I (4 cases, 57.14%) and II (3 cases, 42.86%).

Concerning CdRCC, the three immunoreactive cases showed cytoplasmic immunostaining for c-KIT of weak or moderate intensity. The staining pattern was focal in one case and diffuse in the other two cases. The mean total staining score was 2.4±2.6 (Median=2). The immunoreactive cases were of stage I (1 case, 33.33%) and IV (2 cases, 66.67%).

As regards PRCC, All the studied cases lacked c-KIT immunoreactivity.

Non-neoplastic renal tissues adjacent to tumors showed cytoplasmic staining of c-KIT. Membranous immunoreactivity was lacking. Immunostaining was noted in the proximal and distal renal tubules. Renal corpuscles and collecting tubules were negative for c-KIT immunostaining (Fig. 1B).

Comparison between c-KIT expression and histopathological subtypes of RCCs:

Among the study group of RCCs, ChRCC had the highest staining score for c-KIT (p=.001). In addition, only ChRCC showed membranous pattern of c-KIT staining, while other immunoreactive tumors showed cytoplasmic staining. This result was found to be statistically significant (p=.013) (Table 1).

In order to determine a cutoff point for c-KIT staining score at which the highest diagnostic accuracy was reached, ROC curve analysis was conducted (Fig. 2, Table 2), and a cutoff point was determined (using Youden index) at score 4. Accordingly, c-KIT immunoreactive cases of scores ≥4 were considered positive, whereas, those below score 4 were considered negative. Based on this, ChRCC had a statistically significant higher frequency of c-KIT expression compared to other RCC subtypes (p=.001) (Table 1).

At the cutoff point of score 4, c-KIT showed a sensitivity of 77.78% and a specificity of 95% for the diagnosis of ChRCC. The positive likelihood ratio was 15.56 and the negative likeli-
hood ratio was 0.23. Based on a disease prevalence of 5% for ChRCC [1], predictive values were calculated with a positive predictive value of 45% and a negative predictive value of 98.8%.

**Relationship between c-KIT expression and clinicopathological parameters:**

The relationship between frequency and total score of c-KIT expression and the different clinicopathological parameters was evaluated (Table 3).

No statistically significant correlation was found between c-KIT expression as regards frequency and total score on one hand and age, sex, and stage of the patients on the other hand.

As regards tumor’s grade, high grade tumors had a statistically significant higher total score of c-KIT expression ($p=.023$).

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Fig. (1): Immunohistochemistry of c-KIT. (A) Mast cell showing positive staining for c-KIT (x400). (B) Nonneoplastic renal tissue showing positive c-KIT expression in the proximal and distal tubular cells(x40). (C) CRCC showing weak cytoplasmic reactivity for c-KIT (x400) (D) Sarcomatoid area in CRCC showing cytoplasmic c-KIT immunostaining of moderate intensity (x200). (E) ChRCC showing strong membranous expression of c-KIT accompanied by focal granular cytoplasmic staining (x400). (F) CdRCC showing cytoplasmic staining for c-KIT of moderate intensity (x100).
Table (1): Comparison between c-KIT expression and histopathological subtypes of RCCs.

<table>
<thead>
<tr>
<th>Tumor histology</th>
<th>No. of cases</th>
<th>c-KIT positive cases (≥ score 4) no. (%)</th>
<th>Test p value</th>
<th>Total staining score Mean±SD Median (min.-max)</th>
<th>Test p value</th>
<th>Pattern of c-KIT staining</th>
<th>Test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRCC</td>
<td>30</td>
<td>2 (6.7%)</td>
<td>.001*</td>
<td>0.5±1.3 0 (0-5)</td>
<td>21.76 .001</td>
<td>Not Membranous</td>
<td>8.726 .013*</td>
</tr>
<tr>
<td>ChRCC</td>
<td>9</td>
<td>7 (77.8%)</td>
<td></td>
<td>4.7±2.7 6 (0-7)</td>
<td></td>
<td>Membranous</td>
<td></td>
</tr>
<tr>
<td>PRCC</td>
<td>5</td>
<td>0 (0.0%)</td>
<td></td>
<td>0.0±0.0 0 (0-0)</td>
<td></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>CdRCC</td>
<td>5</td>
<td>2 (40.0%)</td>
<td></td>
<td>2.4±2.6 2 (0-6)</td>
<td></td>
<td>Not Membranous</td>
<td></td>
</tr>
</tbody>
</table>

*Yates’ corrected Chi-Square. **Kruskal-Wallis test.

Table (2): Criterion values and coordinates of the ROC curve.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Sensitivity 95% CI</th>
<th>Specificity 95% CI</th>
<th>+LR 95% CI</th>
<th>-LR 95% CI</th>
<th>+PV 95% CI</th>
<th>-PV 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1</td>
<td>100.00</td>
<td>0.00</td>
<td>1.00</td>
<td>5.0</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>66.2-100.0</td>
<td>0.0-8.9</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>77.78</td>
<td>82.50</td>
<td>4.44</td>
<td>0.27</td>
<td>19.0</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>40.1-96.5</td>
<td>67.2-92.6</td>
<td>3.0-6.5</td>
<td>0.07-1.1</td>
<td>2.8-54.5</td>
<td>88.1-99.2</td>
</tr>
<tr>
<td>&gt;2</td>
<td>77.78</td>
<td>87.50</td>
<td>6.22</td>
<td>0.25</td>
<td>24.7</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>40.1-96.5</td>
<td>73.2-95.8</td>
<td>4.3-9.0</td>
<td>0.06-1.1</td>
<td>3.5-67.5</td>
<td>88.9-99.3</td>
</tr>
<tr>
<td>&gt;3</td>
<td>77.78</td>
<td>90.00</td>
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<td>29.0</td>
<td>98.7</td>
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<td>40.1-96.5</td>
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<td>&gt;4*</td>
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<td>95.00</td>
<td>15.56</td>
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<td>45.0</td>
<td>98.8</td>
</tr>
<tr>
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<td>83.0-99.2</td>
<td>10.9-22.2</td>
<td>0.04-1.4</td>
<td>6.7-89.9</td>
<td>89.7-99.3</td>
</tr>
<tr>
<td>&gt;5</td>
<td>55.56</td>
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<td>97.7</td>
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<tr>
<td></td>
<td>21.4-86.0</td>
<td>86.8-99.6</td>
<td>12.4-39.9</td>
<td>0.06-3.6</td>
<td>8.1-91.6</td>
<td>88.2-99.6</td>
</tr>
<tr>
<td>&gt;6</td>
<td>22.22</td>
<td>100.00</td>
<td>0.78</td>
<td>100.0</td>
<td>80.1-100.0</td>
<td>96.1</td>
</tr>
<tr>
<td></td>
<td>3.5-59.9</td>
<td>91.1-100.0</td>
<td></td>
<td></td>
<td></td>
<td>86.1-99.4</td>
</tr>
<tr>
<td>&gt;7</td>
<td>0.00</td>
<td>100.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0 - 33.8</td>
<td>91.1-100.0</td>
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</tr>
</tbody>
</table>

DISCUSSION

RCCs are recognized as comprising morphologically and genetically distinct entities with different prognoses [1,38,39]. In most instances, differentiation between these entities is possible by careful examination of H&E stained sections combined with gross features of tumors. However, some renal tumors may show overlapping morphologic features, requiring the use of ancillary methods to reach a definitive diagnosis [40].

c-KIT belongs to the large family of transmembrane receptors with tyrosine kinase activity, and is important for certain areas of tissue development and maintenance. c-KIT expression has been reported in various normal and neoplastic tissues [20-26,41]. However, there are few reports regarding c-KIT expression in normal and neoplastic renal tissues in the literature [4,27,33,34].

In the present study, c-KIT was expressed in 28.57% of the study group (14 out of 49 cases) with total staining scores ranging from 2 to 7. When the cutoff point of ≥4 was applied, only 11 cases (22.45%) were considered c-KIT positive.

In the present work, 77.78% of ChRCC were positive for c-KIT immunostaining. The mean total staining score was 4.7±2.7 (Median= 6). The present results are in line with Pan, et al. [27] who reported that c-KIT was expressed in 83% of chromophobe RCC. Miliaras, et al. [36] observed reactivity of c-KIT in four of seven (57%) chromophobe RCC with a total staining score ranging from 3-5.

More frequent c-KIT expression in chromophobe RCC was reported by other investigators. Huo, et al. [13] found that 96% of chromophobe RCC showed expression of c-KIT protein. Other reports mentioned that c-KIT was found in 100% of chromophobe RCCs by means of immunohistochemistry [4,33,42,43].

On the other hand, Zigeuner, et al. [44] detected c-KIT immunoreactivity in only 9% of chromophobe RCCs.

In the present study, the pattern of c-KIT expression in chromophobe RCC was membranous with focal fine granular cytoplasmic staining in some (57.14%) of the positive cases. The present result is in agreement with Krüger, et al. [42] who observed membrane-bound c-KIT positivity in chromophobe RCCs with cytoplas-
mic reactivity in about three-quarters of cases. Similar results were reported by Wang, et al. [4] who remarked that c-KIT staining pattern was fine granular cytoplasmic with membrane accentuation. Membranous c-KIT immunoreactivity in chromophobe RCCs was also reported by other studies [27,43,45].

In the current study, 6.67% of clear RCC showed cytoplasmic immunoreactivity for c-KIT. Membranous reactivity was lacking. The mean total staining score was 0.5±1.3 (Median= 0). Low frequencies of c-KIT expression in clear RCC were reported by Huo, et al. [13] who reported c-KIT positivity in 3% of clear RCC and Miliaras, et al. [36] who found that two of 13 cases (15.4%) of clear RCC showed cytoplasmic c-KIT expression with total staining scores of 3 and 5. On the other hand other studies showed lack of c-KIT expression in clear RCCs [4,33,44].

In the present work, the positive cases of CRCC had granular cell morphology with sarcomatoid areas. Positivity was noted in both epithelial and sarcomatoid components. None of the low grade CRCC showed c-KIT expression, whereas 12.5% of high grade CRCC (also comprised 66.67% of CRCC with sarcomatoid change) showed c-KIT positivity. The positive cases were also of high stage.

The results are in general agreement with Miliaras, et al. [36] who reported that none of low grade RCC of conventional type was positive for c-KIT expression, whereas 25% of high grade conventional RCC was positive.

In the study conducted by Castillo, et al. [46], sarcomatoid component of RCC was positive for c-KIT in 94.7% of the cases. On the other hand, Sengupta, et al. [47] remarked that c-KIT expression was identified in less than 5% of high grade RCCs with or without sarcomatoid differentiation.

In the current study, papillary RCC did not show c-KIT immunoreactivity. Similar results were reported by previous studies [27,33,42,44]. On the other hand, Huo, et al. [13] reported immunohistochemical expression of c-KIT in 5% of PRCC. Higher frequencies of c-KIT immunoreactivity in PRCC were reported by Miliaras, et al. [36] and Lin, et al. [43] who observed a moderate to strong cytoplasmic c-KIT positivity in 28.6% and 100% of PRCC respectively.

In the present study, two cases (40%) of collecting duct RCC showed positive cytoplasmic immunostaining for c-KIT. The mean total staining score was 2.4±2.6 (Median= 2). The present result contrasts with the report of Pan, et al. [27] who found that c-KIT expression was lacking in collecting duct carcinoma.

In the current study, nonneoplastic renal tissue showed positive staining of c-KIT in the convoluted renal tubules. The staining was cytoplasmic and of weak to moderate intensity. No membranous immunoreactivity was observed in any of the normal renal tissues. Renal corpuscles and collecting tubules were negative for c-KIT staining. The results are in agreement with other investigators who reported cytoplasmic c-KIT staining of weak to moderate intensity in proximal and distal tubules not accompanied by membranous accentuation. Renal corpuscles and collecting tubules were negative [22,27,33,34,36]. On the other hand, some investigators reported that normal renal parenchyma constantly lacked c-KIT immunoreactivity [44].

An interesting observation noted in the present study is that, the collecting renal tubules of nonneoplastic renal tissue were c-KIT negative, whereas c-KIT was frequently expressed in chromophobe RCC. Since chromophobe RCC are thought to originate from the intercalated cells of renal collecting tubules, [48] it is suggested that c-KIT expression in ChRCC occurs during and might be involved in the process of tumorigenesis. Miliaras, et al. [36], reported similar observation and postulated that a mechanism of c-kit activation may be involved in the development of chromophobe RCC.

Among the studied RCCs, chromophobe RCC had statistically significant higher frequency and total staining score of c-KIT expression. The present results are in line with the findings noted by Miliaras, et al. [36], where frequent c-KIT expression was seen in chromophobe RCC compared to other types of RCCs studied. Huo, et al. [13] remarked that the average c-KIT immunoreactivity was stronger in chromophobe RCC than in other subtypes of RCCs tested.

In the present work, the highest diagnostic accuracy for c-KIT expression in ChRCC was
reached at score ≥4 with a sensitivity of 77.78% and a specificity of 95%. The positive likelihood ratio was 15.56 and the negative likelihood ratio was 0.23. Predictive values were calculated according to a disease prevalence of 5% for ChRCC [1]. Positive predictive value was 45% and negative predictive value was 98.8%.

In the current work, membranous staining for c-KIT was noted only in chromophobe RCC whereas other types of RCCs showed cytoplasmic staining. This result was found to be statistically significant.

Previous reports have shown that among other tumor types that positively stain for c-KIT, the staining pattern is typically membranous in mast cell and malignant germ cell tumors, and cytoplasmic with membrane accentuation in GISTs. In all other tumors, c-KIT immunoreactivity is cytoplasmic with weak to moderate intensity [22,36].

Some investigators reported that the cytoplasmic staining for c-KIT probably represents a nonspecific staining, and it varies greatly with different antibody sources and dilutions and with the immunohistochemical techniques [49]. One study suggested that cytoplasmic staining for c-KIT may be related to antigen retrieval method [50]. When citric acid was used, a minor proportion of papillary renal cell carcinomas revealed a very faint cytoplasmic reactivity, which was considered as being negative. The reaction became more intense when ethylenediaminetetraacetic acid (EDTA) was used, with around half of papillary RCCs exhibited obvious granular cytoplasmic positivity. On the other hand, membranous immunoreactivity in chromophobe RCCs was not altered [50]. The authors postulated that the antibody cross-reacts with another unknown cytoplasmic epitope that bears structural similarity with c-KIT. The cytoplasmic epitope, which is probably expressed at a lower level compared with the true membranous expression of c-KIT, is unmasked by EDTA [50]. Pan, et al. [27] suggested that it is judicious to regard only membranous reactivity as a genuine expression of c-KIT.

Based on the results of the present study, it is suggested that immunohistochemical c-KIT expression could be used as an additional diagnostic criterion to distinguish chromophobe RCC from other types of RCC with a high sensitivity and specificity. In order to increase its diagnostic usefulness, cases should be considered positive only when membranous staining is detected and when total staining score is ≥4.

The results of the present work are supported by previous reports. Pan, et al. [27] and Krüger, et al. [42] suggested that c-KIT should be considered as a useful adjunct for discriminating chromophobe RCC from other subtypes of RCC. Wang, et al. [4] reported that c-KIT is a sensitive marker for chromophobe RCC and they concluded that c-KIT is useful in distinguishing chromophobe RCC from the granular variant of clear cell RCC. Tickoo, et al. [51] remarked that the distinctive membranous pattern of c-KIT immunoreactivity in chromophobe RCC is rather straightforward to interpret in comparison with previously proposed immunohistochemical markers for chromophobe RCC. In addition, c-KIT immunostain is technically easier to use than the Hale colloidal iron stain and electron microscopy. Thus, immunohistochemical stain for c-KIT could be performed instead as a substitute method to support the initial histologic impression of chromophobe RCC [27,51].

In the present study, no statistically significant relation was found between age, sex and stage of the patients on one hand and c-KIT expression on the other hand. On the contrary, c-KIT was found to be significantly expressed in high grade CRCC including those with sarcomatoid change. However, the expression pattern was cytoplasmic rather than membranous. It remains to be determined if such a tendency would carry prognostic or therapeutic implications.

The recent development of targeted therapy, designed to target specific receptors or pathways for controlling cell growth has resulted in the additional use of immunohistochemistry beyond assisting in histopathological diagnosis [30,52,53].

Targeted treatment to inhibit the kinase activity of c-KIT is a rational approach to the treatment of c-KIT positive malignancies [30,52,53]. Currently, the best candidate diseases for treatment with c-KIT inhibitors are GISTs, mastocytosis, seminomas, and possibly some cases of acute myelogenous leukaemia [25].

The frequent expression of c-KIT in ChRCC demonstrated in this study together with previ-
ous studies [27,33,36] suggest that c-KIT could be a possible therapeutic target in chromophobe RCC. However, the underlying mechanism for the overexpression of c-KIT in chromophobe RCC have not been fully elucidated. In the series conducted by Pan, et al. [27], the common c-kit genomic mutations that lead to the constitutive activation of the receptor [25] were not present in chromophobe RCCs. Similar findings were remarked by Krüger, et al. [42]. Nevertheless, it has been suggested that c-KIT inhibitors may play an adjunctive role in malignant tumors where c-KIT activation is secondary to ligand binding rather than an acquired mutation. Thus, it is plausible to speculate that c-KIT positive ChRCC are also candidates for such targeted treatments [36].

Further studies are needed to clarify whether cytoplasmic c-KIT expression noted in other RCC subtypes (especially high grade CRCC with sarcomatoid change) could justify the trial of c-KIT inhibitors in these tumors.

In summary, the substantial c-KIT immunoreactivity in chromophobe RCC noted in the present work provides a rationale to investigate c-KIT inhibitor therapy in clinical trials. In the future, immunohistochemical evaluation of c-KIT expression in ChRCC could serve as a screening test for identifying patients who might benefit from c-KIT inhibitor treatment.

From the present work it can be concluded that, c-KIT is frequently and significantly expressed in ChRCC suggesting that it may be involved in the process of tumorigenesis and raising hope that it could serve as a potentially useful therapeutic target in ChRCC. Immunohistochemical c-KIT expression could be used as a reliable diagnostic marker to distinguish chromophobe RCC from other types of RCC with a high sensitivity and specificity. This work emphasizes the importance of combining the degree and the pattern of c-KIT immunostaining in order to increase its diagnostic usefulness.

REFERENCES


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