Morphological spectrum of Xp11. Translocation-Associated Renal Cell Carcinoma in a Developing Country
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ABSTRACT

Objective: to determine unusual morphological features and a panel of immunohistochemical markers to diagnose Xp11 translocation carcinoma.

Study Design: Retrospective longitudinal study.

Place and Duration of Study: Shaukat Khanum Memorial Cancer Hospital, Lahore Pakistan, from Jun 2015 to Feb 2020.

Methodology: We analyzed clinicopathological features, and evaluated intensity and extent of TFE 3 immunoreactivity of 18 cases with suggested diagnosis of xpi1 translocation associated renal cell carcinoma from 2015-2020.

Results: Different morphological pattern includes papillary (8/18, 44%), nested (2/18, 11.1%), alveolar (3/18, 16.7%), nested and papillary (3/18, 16.7%), solid and nested (1/18, 5.6%), cystic and nested (1/18, 5.6%). Four cases show papillary architecture with a linear array of nuclei away from the basement membrane, a pattern seen in SFPQ-TFE3 renal cell carcinoma and NONO-TFE3 renal cell carcinoma. Strong nuclear TFE3 expression was seen in 9/18 (50%) cases. Cathepsin K expression was seen in 6/11 (54%) cases, Ck7 was focal weak positive in 4/12 (25%) cases, PAX8 was positive in 8/8 (100%) cases, and CA IX was focal weak positive in 1/5 (20%) case. According to follow-up data, disease progression was seen in only one case with the low-stage disease. No death was reported due to renal cell carcinoma to date of follow-up.

Conclusion: We have suggested that young patient age, unusual morphological features and an immunohistochemical panel may help reach the diagnosis in countries with limited resources.

Keywords: Morphological features, TFE3, Xp11 translocation associated renal cell carcinoma.


INTRODUCTION

Xp11 Translocation-associated renal cell carcinoma is a rare tumour characterized by recurrent gene rearrangement involving the TFE3 gene. It was first introduced in the World Health Organization WHO classification of the renal tumour as a distinct entity in 2004.1,2 2016 World Health Organization WHO classification was grouped with t (6:11) renal cell carcinoma as MiT family translocation carcinomas.3-6 Xp11 translocation renal cell carcinoma shows wide variation in the morphological spectrum. It displays overlapping features with other renal cell carcinomas.5,6 Diversity of the morphological spectrum poses diagnostic difficulty in reaching a definitive diagnosis. Immunohistochemically these tumours are immunoreactive for PAX 8, cathepsin K and TFE3 and are non-immunoreactive for cytokeratin and epithelial membrane antigen.6 However, genetic confirmation is obligatory for a conclusive diagnosis of Xp11 Translocation renal cell carcinoma. Strong nuclear labelling with TFE3 is an important surrogate marker.6,7 This is particularly helpful in developing countries like Pakistan, where molecular diagnostic services are not widely available. This study is designed to evaluate the morphological characteristics of renal cell carcinoma with the likely diagnosis of translocation-associated renal cell carcinoma based on morphology and nuclear immunoreactivity of tumour cells for TFE3.

METHODOLOGY

It was a retrospective longitudinal study carried out at the Histopathology Department of Shaukat Khanum Cancer Memorial Hospital, Lahore Pakistan and Research Centre. The study was approved by the Institutional Ethical Review Board (EX 08-09-19-03). A total of 18 cases with suggested Xp11 translocation associated with RCC were obtained from Shaukat Khanum Memorial Cancer Hospital and Research Center archives. Two pathologists reviewed Hema-toxylin and Eosin and immunohistochemical slides.

Inclusion Criteria: The cases with suggested Xp11 translocation associated with RCC were selected irrespective of age, gender and site of the tumour.

Exclusion Criteria: Autolyzed, scanty, poorly preserved cases were excluded from the study.
For each case, clinicopathological data were analyzed. Clinical details such as presenting complaints, age, gender, previous history of malignancy, and history of chemotherapy were recorded. In every specimen, we noted laterality, tumour size, the status of resection margin, involvement of renal capsule, perinephric fat and renal sinus fat, nodal status and direct or metastatic spread into the adrenal gland (specimens in which adrenalectomy was also done). Pathological staging was done according to TNM staging as per AJCC 8th edition.9

For each tumour, various morphological features were evaluated, including architectural pattern (papillary, nested, solid, and alveolar), cell shape, cytoplasmic vacuolization, nuclear location, nuclear pseudo inclusion, nucleoli, psammoma bodies, foamy histiocytes, sarcomatoid features, microscopic necrosis, oncocytic features, Fuhrman grade and mitotic count.

A panel of supporting immunohistochemical stains included cytokeratin, PAX8, CK7, carbonic anhydrase IX, CD10, AMACR, cathepsin K, HMB45, Melan A, Kidney specific cadherin and SDHβ had been performed according to differential diagnosis.

*TFE3* immunostain was already performed in all these cases. The clone used for *TFE3* was anti-*TFE3* (MRQ-37) rabbit monoclonal primary antibody. The staining results of *TFE3* were interpreted by using two variables, intensity and percentage of tumour cells with positive staining. Tumours with mild nuclear staining in less than 20% of cells were scored as (1+). Tumours with moderate nuclear staining in 20-50% of cells were scored as (2+). Finally, tumours with strong nuclear staining in >50% of cells were scored as (3+).

Follow-up data were collected regarding any further treatment, treatment effect, disease stability and disease progression. Statistical Package for Social Sciences (SPSS) version 20.0 was used for the data analysis.

**RESULTS**

A total of 18 cases were enrolled in this study, and all were nephrectomy specimens. Sixteen specimens were received in our centre, and one was fragmented in nature. Two cases were received for a second opinion. Fifteen (83%) patients presented with an abdominal mass. Two (11.1%) patients had symptoms of flank pain, and two had complaints of hematuria. Both patients with hematuria belonged to the pediatric age group. We received incomplete information regarding clinical signs and symptoms in two cases received in our centre for a second opinion. In our series, the left kidney was involved in 8 (44%) patients, and right kidney involvement was seen in 9 (50%) patients. Encompassing 6 (33%) males and 12 (66%) females (M: F, 1:2). The mean age was 24 years, with a standard deviation (SD) of 14.4 and an age range of 2-60 years. Six patients (33.3%) were less than 18 years of age. Only one child had a previous history of Wilms tumour with nodal metastasis. In one case, splenic and lung metastasis were present at the diagnosis time (Table).

Morphological examination revealed that the mean tumour size was 7.2±4.28 cm (range 3 cm-19 cm), and 88% measured less than 10 cm. All tumours were unifocal. Eight (44.4%) out of 18 cases involved more than one pole of the kidney, and 6 (22.2%) involved the entire kidney. Capsule and perinephric fat were uninvolved in all the specimens. Renal sinus fat involvement was seen in four cases. Ureteric and vascular resection margins were free of tumours in all cases. The adrenal gland was received in three cases, and there was no direct extension or metastasis to the adrenal gland in these cases. Lymph node sampling was done in five cases, with extensive sampling in one case in which we received 18 lymph nodes (all were involved by the tumour). Amongst these five cases, 4 (22%) cases showed lymph node metastasis. 6 (33.3%) cases were staged as PT1, 5 (27.7%) as PT2 and 4 (22.2%) as PT3 (Table).

One out of two cases showed focal expression for kidney-specific cadherin. EMA, cyclin D1, Melan A, CD163 and TTF1 were negative. The Morphological examination exhibited papillary architecture (44%) as the predominant architecture. Others are nested and alveolar at 16%, nested at 11%, solid and nested at 5.6%, and cystic and nested at 5.6% (Figure-I). Polyhedral cell shape was observed in 14 (77.8%) and tall columnar 4 (22.2%). Voluminous cytoplasm was present in 14 (77.8%) cases, while 4 (22.2%) showed less abundant cytoplasm. The cytoplasmic clearing was present in 5 (27.8%) cases, and dual cytoplasmic tone was seen in 6 (33.3%) cases. 4 (22.2%) cases demonstrated apical nuclei (linear array) with papillary architecture, cytoplasmic clearing and subnuclear vacuoles (Figure II). In 12 (66.7%) cases, distinct nucleoli were noted. Focal nuclear pseudo inclusion was seen in 2 (11.1%) cases. Psammomatous calcification was present in 14 (77.8%) cases. Three (16.7%) cases exhibited oncocytic features. Necrosis was present in 5 (27.8%) cases, and sarcomatoid features were seen in only
Morphological Spectrum of Xp11

1(5.6%) case. A focal area with foamy histiocytes was present in 2(11.1%) cases.

demonstrated moderate to weak staining in 20-50.0% of cells.

Table: Characteristics of patients with suggested diagnosis of translocation associated Renal Cell Carcinoma (Xp11)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>Clinical Presentation</th>
<th>Location</th>
<th>Tumor Size, cm</th>
<th>Pathological Stage</th>
<th>Fuhrman Grade</th>
<th>TFE3 Immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>Female</td>
<td>Abdominal mass</td>
<td>Right kidney</td>
<td>4.0</td>
<td>pT1aNxMx</td>
<td>2</td>
<td>3+</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>Female</td>
<td>Abdominal mass</td>
<td>Left kidney</td>
<td>10.0</td>
<td>pT3aN1Mo</td>
<td>3</td>
<td>3+</td>
</tr>
<tr>
<td>3</td>
<td>04</td>
<td>Male</td>
<td>Abdominal mass and hematuria</td>
<td>Right kidney</td>
<td>3.0</td>
<td>pT1aN1Mx</td>
<td>1</td>
<td>3+</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>Female</td>
<td>Abdominal mass</td>
<td>Right kidney</td>
<td>8.0</td>
<td>pT2aNxMx</td>
<td>2</td>
<td>3+</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>Female</td>
<td>Abdominal mass</td>
<td>Right kidney</td>
<td>4.8</td>
<td>pT1bNxMx</td>
<td>1</td>
<td>3+</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>Male</td>
<td>Hematuria, flank pain</td>
<td>Left kidney</td>
<td>4.2</td>
<td>pT3NxMx</td>
<td>1</td>
<td>2+</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>Female</td>
<td>Flank pain, abdominal mass</td>
<td>Left kidney</td>
<td>Fragmented</td>
<td>Fragmented</td>
<td>2</td>
<td>1+</td>
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<tr>
<td>8</td>
<td>34</td>
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<td>Abdominal mass</td>
<td>Left kidney</td>
<td>8.0</td>
<td>pT2aNxMx</td>
<td>1</td>
<td>3+</td>
</tr>
<tr>
<td>9</td>
<td>09</td>
<td>Male</td>
<td>Abdominal mass</td>
<td>Right kidney</td>
<td>3.5</td>
<td>pT1aN0Mx</td>
<td>2</td>
<td>2+</td>
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<tr>
<td>10</td>
<td>15</td>
<td>Male</td>
<td>Received for second opinion with incomplete information</td>
<td>Left kidney</td>
<td>5.0</td>
<td>Received for second opinion with incomplete information</td>
<td>1</td>
<td>1+</td>
</tr>
<tr>
<td>11</td>
<td>02</td>
<td>Male</td>
<td>Abdominal mass</td>
<td>Right kidney</td>
<td>8.5</td>
<td>pT2bN1M1</td>
<td>2</td>
<td>1+</td>
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<tr>
<td>12</td>
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<td>Male</td>
<td>Abdominal mass</td>
<td>Right kidney</td>
<td>19.0</td>
<td>pT3aNxMx</td>
<td>3</td>
<td>2+</td>
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<tr>
<td>13</td>
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<td>Female</td>
<td>Abdominal mass</td>
<td>Left kidney</td>
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<td>1</td>
<td>2+</td>
</tr>
<tr>
<td>18</td>
<td>45</td>
<td>Female</td>
<td>Abdominal Mass</td>
<td>Left kidney</td>
<td>14.0</td>
<td>pT1bNxMx</td>
<td>2</td>
<td>2+</td>
</tr>
</tbody>
</table>

A panel of immunohistochemical stains was applied based on the morphological differential diagnosis. PAX-8 immunoreactivity was seen in all the cases in which it was applied. CK was weak focal positive in 1 out of 7 cases. CK7 was applied in 12 cases and was focally positive in four cases. Six cases were immunoreactive for cathepsin k amongst 11 cases. One case showed focal positivity for carbonic anhydrase, while four cases were negative. AMACR showed weak focal expression in three cases and was negative in 4 cases. CD10 positivity was observed in 3 out of 4 cases. HMB45 was positive in 1 out of 4 cases. SDHb expression was intact in 2 out of 2 cases.

The intensity and extent of TFE3 immunoreactivity were estimated in all these cases. Eight (44.0%) out of 18 cases showed strong nuclear staining in more than 50.0% of cells, whereas in one (5.0%) case, strong staining was observed in less than 50% of cells (20-50%). Moderate staining in more than 50.0% of tumour cells was seen in 6 cases (33.0%). Three (16.0%) cases

Follow-up data were available in 13 of 18 cases. Follow-up periods ranged from a minimum of 9 months to a maximum of 48 months. Disease progression was observed in 1 (7.6%) patient with the appearance of regional lymph node metastasis 22 months after primary surgery. The patient was a young female, and the primary stage was PT2aNx. The disease was stable in the remaining 12 (92.3%) cases. No distant metastasis or death due to renal cell carcinoma was documented to date of follow-up.

**DISCUSSION**

Translocation carcinoma (Xp11) is a rare renal carcinoma characterized by recurrent gene arrangement involving the TFE3 gene leading to TFE3 overexpression.

Different TFE 3 fusion partners have been described, including ASPSCR1, CLTC, DVL2, LUC7L3, KHSRP, PRCC, PARP14, NONO, SFPI1, MED-15 and RBM10.2,10 Genetic testing is the gold standard for diagnosis. However, TFE3 overexpression can also be
detected by IHC. The prognosis of these tumours is similar to clear cell renal cell carcinoma and more dismal than papillary renal cell carcinoma.\textsuperscript{1,2} Considering the clinical presentation, different symptoms were described in the literature, including palpable mass, flank pain, hematuria and symptoms related to metastasis. Most studies documented incidental discovery with small tumour size.\textsuperscript{11} In this study, fifteen (83\%) patients were presented with abdominal mass, which was the most common presentation in our patients. This may account for the lack of awareness and medical facilities.

Predominant right-sided involvement had been reported by Hirobe et al.\textsuperscript{12} However, in our study, almost equal involvement was observed. No prognostic significance of laterality has been described in the literature. Xp11 translocation-associated carcinoma frequently involves the pediatric population. It accounts for 40\% of pediatric and 1.4-6\% of adult renal cell carcinoma.\textsuperscript{1,2,4} In this study, the patient age range was 2 years-60 years, with mean patient age of 24 years. Six (33\%) patients were under 18 years. M: F ratio was 1:2. Female predominance was earlier reported by Skala et al. and Zhong et al.\textsuperscript{1,13} The results of the current study were concordant with these previous studies.

Chemotherapy is an established risk factor for translocation-associated renal cell carcinoma. In this study, only one patient had a previous diagnosis of Wilms tumour with a history of chemotherapy.

In this study, tumour size ranges from 3cm-19cm, with a mean tumour size of 7.2cm. Most of the studies reported a smaller tumour size, such as Yang et al.\textsuperscript{14} reported a mean tumour size of 4.3cm in their study. However, few studies have also demonstrated a mean tumour size of 12.5cm.\textsuperscript{13}

In this study, 11(61\%) patients presented with the low stage (PT1 and PT2), and 4(22\%) with advanced stage (PT3), which was compatible with other studies. Nodal metastasis was present in 4 cases; two patients were adults, and two were from the pediatric age group. According to studies by Ning et al.\textsuperscript{15} and Geller et al.\textsuperscript{16} pediatric tumour with lymph node metastasis was not a poor prognostic factor in every case. Unfortunately, in this study, follow-up data was not available.

The most common morphological patterns in the literature and WHO blue book were papillary architecture, clear cells, voluminous cytoplasm and abundant psammoma bodies.\textsuperscript{1,2,5} In this study, we observed various morphological patterns. The majority (44\%) exhibited papillary architecture others were alveolar (16.7\%), nested (11.1\%), nested and papillary (16.7\%), solid and nested (5.6\%) and cystic and nested (5.6\%) (Figure-I).

Four cases demonstrated papillary architecture with a linear array of nuclei away from the basement membrane and subnuclear clearing, a pattern similar to clear cell papillary renal cell carcinoma. Previous studies also observed this pattern in SFPQ-TFE3 renal cell carcinoma and Nono-TFE3 renal cell carcinoma.\textsuperscript{2} The cytoplasmic clearing was observed in 5(27.8\%) cases. Majority of cases, 38.9\% exhibited eosinophilic cytoplasm (Figure-II).

Both clear and eosinophilic cytoplasm was seen in 6(33.3\%) cases. Fourteen (77.8\%) cases showed voluminous cytoplasm, and the remaining 4(22.2\%) demonstrated less abundant cytoplasm. Psammomatous calcification was present in 14(77.8\%) cases. Psammomatous calcification is one of the important morphological diagnostic features but is not essentially present in all cases.\textsuperscript{10}
Nuclear pseudo inclusion was first described by Skala et al.\textsuperscript{1} as a feature that helps identify renal cell carcinoma with MITF aberration.\textsuperscript{1} In our study, focal nuclear pseudo inclusion was present in two cases. In our opinion, this finding needs to compare with other subtypes of renal cell carcinoma before considering it a diagnostic feature (Figure-III).

![Figure-3: Linear Arrays of Nuclei away From Basement](image)

Microscopic tumour necrosis was seen in five cases, 3 with advanced stage pT3a, 1 with pT1b and one with pT2b. Microscopic tumour necrosis was seen in all cases of translocation associated with carcinoma with the pT3a stage in a study by Yang et al.\textsuperscript{14} and they considered it a potential factor for the pT3a stage. Our study did not find any association between tumour necrosis and pathological stage. High-grade features were seen only in one case presented with advanced-stage disease.

Based on morphology, the differential diagnosis includes clear cell renal cell carcinoma, perivascular epithelioid cell tumour (PEComa), clear cell papillary renal cell carcinoma and papillary renal cell carcinoma.\textsuperscript{15,17} Considering these differences, a panel of immunohistochemistry was used in different cases. There were cases in which our differential diagnosis also included PEComa. Both PEComa and translocation-associated renal cell carcinoma show expression for TFE3. We applied PAX-8 to differentiate between them, and all cases turned out to be PAX-8 positive.

Cytokeratin 7 was performed in 12 cases with a differential diagnosis of papillary renal cell carcinoma. Weak focal positivity was reported in three cases. None of the cases showed diffuse strong membranous or cytoplasmic expression for CK7, excluding the possibility of papillary renal cell carcinoma. Four out of these 12 cases also had a differential diagnosis of clear cell papillary renal cell carcinoma. Cytokeratin 7 was negative in all these cases, and carbonic anhydrase IX was focal weak positive in one case only.

Diffuse complete membranous staining for Carbonic anhydrase IX is considered highly specific for clear cell renal cell carcinoma, and this staining pattern is not seen in any other renal tumour. In addition, three of our cases had a histological differential diagnosis of clear cell renal cell carcinoma, and all were negative for carbonic anhydrase IX.

Three out of four cases in our study showed expression for CD10. Positivity for AMACR was observed in 3 out of 7 cases. Focal to diffuse CD10 and AMACR positivity was documented in translocation-associated renal cell carcinoma.\textsuperscript{18} However, the negative expression for carbonic anhydrase IX and CK7 ruled out the possibility of clear cell renal cell carcinoma and papillary renal cell carcinoma, respectively.

Cathepsin K is an important immunomarker for translocation-associated renal cell carcinoma. In our study, Cathepsin k expression was seen in 6 (54%) of 11 cases. Furthermore, Cathepsin k positivity is specifically seen in PRCC-TFE3 renal cell carcinoma.\textsuperscript{18,19}

TFE3 immunostaining is a useful screening test for translocation-associated renal cell carcinoma.\textsuperscript{7,8} In this study, strong (3+) nuclear expression of TFE3 immunostaining was observed in 9 (50%) cases, moderate (2+) staining in 6 (33%) cases and moderate to weak (1+) in 3 (16%). An earlier study showed TFE3 positivity in 28 out of 30 cases of Xp11 translocation associated with renal cell carcinoma confirmed by FISH.\textsuperscript{18} Furthermore, 99.6% specificity and 97.5% sensitivity of TFE3 immunostaining were reported in a recent study by Kuthi et al.\textsuperscript{20} Another study confirmed that Xp11 translocation-associated renal cell carcinoma cases showed strong to moderate staining with TFE3 IHC, and all cases with weak expression of TFE3 immunostaining turned out to be FISH negative.\textsuperscript{21} Therefore, TFE3 was described as an important sensitive and specific marker and recommended using FISH only for cases with equivocal TFE3 immunostain results.\textsuperscript{13}

According to literature, patients with advanced stage disease showed poor prognosis regarding disease progression, metastatic disease and death.\textsuperscript{15,20-22} In this study, disease stability was seen in 12 (92.3%) cases, including three patients with advanced stage (PT3). Disease progression was seen in one patient only (PT2). In contrast to published literature, our study showed disease progression in low-stage
diseases and stability even in high-stage diseases. This was probably due to a short follow-up period and a small sample due to disease rarity.

Genetic testing is obligatory for confirmatory diagnosis of Xp11 translocation-associated carcinoma. Different modalities are available for genetic testing. These include karyotypic analysis, reverse transcriptase–polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization. FISH is a realistic option owing to certain limitations in other techniques. However, in developing countries, this facility is not widely available.

CONCLUSION

We conclude from our study that young patient age, specific morphological features and TFE3 IHC in combination with other immune-markers Pax8, CK7, CA IX and Cathepsin K can be used to diagnose Xp11 translocation-associated carcinoma in developing countries with limited resources. However, TFE3 expression may be observed in older individuals in high-grade tumours and is considered a bad prognostic sign. Therefore, caution must be taken in such cases, and FISH studies should be performed if available.

Conflict of Interest: None.

Author’s Contribution:

Following authors have made substantial contributions to the manuscript as under:

IK: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

SGN & SM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

NA & UH: Drafting the manuscript, data interpretation, approval of the final version to be published.

MH: Study design, data analysis, critical review, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES


