CASE REPORT

Need for Panel of Immunohistochemical Markers in Primary Intraosseous Squamous Cell Carcinoma Ex Odontogenic Keratocyst

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ABSTRACT

Aim: A unique case of Primary Intraosseous Squamous cell carcinoma (PIOSCC) arising from the lining of OKC in a 49-year-old female patient, quite aggressive in nature is presented here.

Background: The occurrence of (PIOSCC) from an odontogenic cyst is rare with the incidence being 0.3–3%.

Case description: In the present case histopathology was deceptive owing to the presence of spindle-shaped cells. This prompted us to use a panel of immunohistochemical markers such as CD34, Pancytokeratin, CK5/6, SMA, S100, and p63 to differentiate from sarcoma.

Conclusion: The probable pathogenesis for such a transformation into malignancy could be attributed to the hypothesis of chronic inflammation. According to the English literature review, 34 cases have been reported until now, with the present case being the 35th case.

Clinical significance: This report emphasizes the use of immunohistochemistry to arrive at a definitive diagnosis in scenarios of overlapping histological features.

Keywords: Immunohistochemistry, Malignant transformation, Odontogenic cyst, Odontogenic keratocyst, Oral squamous cell carcinoma.

World Journal of Dentistry (2022); 10.5005/jp-journals-10015-2055

INTRODUCTION

Neoplastic transformation of odontogenic cyst is a definitive entity that could be encountered in clinical practice despite its rarity in occurrence. The lining of odontogenic cysts has the potential to transform into various tumors such as odontoma, ameloblastoma, ameloblastic fibroma, adenomatoid odontogenic tumor, calcifying epithelial odontogenic tumor, squamous cell carcinoma (SCC), and mucoepidermoid carcinoma.1 Among these, the lining of Odontogenic keratocyst (OKC) and dentigerous cyst have an increased tendency for neoplastic transformation. OKC has been widely known for its aggressiveness and increased rate of recurrence. Studies at the molecular level have validated this unique nature owing to the increased expression of proliferative and anti-apoptotic markers such as Ki67, p53, PCNA, and Bcl2 in the keratocystic lining.2

Primary Intraosseous carcinoma (PIOSCC) was defined by WHO (2005) as “Squamous cell carcinoma arising within the jaws having no initial connection with the oral mucosa, and presumably developing from residues of odontogenic epithelium.3 PIOSCC is further classified into three subtypes:

- Solid tumor invading marrow spaces and inducing osseous resorption,
- Squamous cell carcinoma arising from the lining of an odontogenic cyst
- Squamous cell carcinoma in association with other benign epithelial odontogenic tumors

PIOSCC ex OKC falls in the second subtype. This report puts forward a rare case of PIOSCC ex OKC in a 49-year-old female patient with unique histologic features consistent with its aggressive behavior. This report also aims at updating the literature review with an added note on pathogenesis in regard to the same.

CASE DESCRIPTION

A 49-year-old female patient reported to RUAS institute, with a chief complaint of a rapidly growing swelling in the front teeth region of the lower jaw that was clinically apparent for about two months. The patient gave a past dental history of pain and swelling 11 months back in the lower left jaw. The previous radiograph showed a multilocular radiolucency in the lower left mandible with perforation of the lower cortical bone (Fig. 1A). Histopathological biopsy report revealed a cystic lining composed of parakeratinized stratified squamous...
Extraoral examination showed facial asymmetry with swelling on the lower left side of the face in association with the previous surgery (Fig. 2A). Intraoral examination revealed an ovoid-shaped swelling obliterating the lower vestibule of the mandibular anterior teeth region with respect to 43, 42, 41, 31, 32, and 33 (Fig. 2B). Surgically, Hemi-mandibulectomy of the left side was done (Fig. 1C).

The patient had a recurrence after a period of 2 months with similar symptoms in the lower anterior region of the mandible. Epithelium of 6–8 layer cell thickness with surface corrugations and palisading arrangement of basal cell layer along with the transition of cystic lining into malignancy. Tumor cells were arranged in islands showing dysplastic features like altered nuclear-cytoplasmic ratio, cellular and nuclear pleomorphism, hyperchromatism, and 1–2 mitotic figures per high power field (Fig. 1B). Surgically, mucosa overlying the swelling appeared normal. On palpation, the swelling was soft in consistency. Radiographic investigations showed an ill-defined radiolucency extending from the distal part of 43 continuing toward the resected part of the mandible along with the epithelium of 6–8 layer cell thickness with surface corrugations and palisading arrangement of basal cell layer along with the transition of cystic lining into malignancy.
Panel of Immunohistochemical Markers

with partial loss of interdental bone. There was no evidence of tooth resorption.

An incisional biopsy was performed, the gross specimen received was reddish-brown in color, irregular in shape, and measured about 1 x 0.7 x 0.4 cm. Histopathologically, the biopsied specimen showed sheets of squamous cells in the connective tissue. The squamous cells showed dysplastic features like cellular and nuclear pleomorphism, hyperchromatism, increased nuclear/cytoplasmic ratio, loss of cohesion, numerous bizarre cells, and 4–5 abnormal mitotic figures per high power field. The stroma also consisted of sparse chronic inflammatory infiltrate comprising of lymphocytes, a few endothelial lined blood vessels engorged with RBCs, and a few hemorrhagic areas (Fig. 3). A diagnosis of recurrent intra- osseous carcinoma of the poorly differentiated grade was given.

Central arch resection of the mandible was done and the margins of the tumor were ensured to be free from the tumor.
Absence of carcinomatous changes in the overlying oral epithelium.
Absence of any source of carcinoma from the adjacent structures.
Any possibility that the lesion represents a metastasis from a distant tumor must be ruled out by physical and radiological examination and the subsequent clinical course (added by Slootweg and Muller).

A literature search was conducted by Acharya et al. where-in 25 cases of OOC/OKC transforming into malignancy were reported in English literature. After which, 10 more cases have been reported till 2016 including the present case (Table 1).

The mean age of the 34 cases reported along with the present case, was 45 years (range 18–81 years), and the male:female ratio was 2:1. The most commonly involved site was the mandible as was seen in the present case. It has been suggested that this increased incidence of the OKC in the mandible could be attributed to the presence of dental lamina of high activity, which is more often seen in the posterior mandible. Pain and swelling are the most common clinical symptoms presented by the affected patients. Orthopantamograph most commonly reveals a multilocular radiolucency with the expansion of buccal and lingual cortices. Perforation of the cortices was observed in 25 cases including the present case.

Histopathologically, for a lesion to be diagnosed as PIOSCC ex Odontogenic Keratocyst, the above-mentioned criteria must be fulfilled. In the present case, there was evidence of OKC lining transforming into squamous cell carcinoma. Clinically, the overlying mucosa appeared normal and did not show any malignant changes thus suggesting that the lesion was a separate entity without any connection to the overlying epithelium. Since the PET scan revealed the absence of any primary tumor elsewhere in the body, the diagnosis of PIOSCC was favored. Similar to most of the other cases that have been reported in the literature, the present case showed a parakeratinized

Histopathology of the excisional biopsy specimen showed sheets of noncohesive bizarre cells with cellular and nuclear pleomorphism, hyperchromatism and increased nuclear/cytoplasmic ratio, and presence of numerous mitotic figures, along with a foci of pleomorphic spindle-shaped cells with vesicular nuclei and multiple nucleoli. The connective tissue stroma showed diffuse inflammatory component predominantly consisting of lymphocytes and plasma cells with occasional giant cells (Fig. 4). Decalcified section of teeth also showed tumor tissue associated with the adjacent attached soft tissue. However, the presence of pleomorphic spindle-shaped tumor cells posed a diagnostic dilemma of sarcoma or carcinosarcoma that had to be ruled out. Hence, the representative tumor tissue was later subjected to a panel of immunohistochemical markers to determine the cell of origin. The panel of markers included CD34, Pancytokeratin [AE1/AE3], CK5/6, SMA, S100, and p63. Immunohistochemistry showed negativity for CD34, S100 and strong positivity for pan cytokeratin, and weakly positive for p63 and SMA (Fig. 5). These results suggested that the tumor was of squamous origin of the poorly differentiated variant.

**Discussion**

The incidence of PIOSCC arising from an odontogenic cyst is 0.3–3%. The incidence and prevalence of PIOSCC ex OKC are unavailable due to less number of reported cases. It has been suggested that there is an increased risk for an OKC to transform into malignancy but evidence supporting the same seems to be lacking. The other probable reason for this could be the lack of definitive criteria which defines these group of lesions.

However, the following criteria were proposed by Gardner in the year 1975 in order to diagnose PIOSCC ex odontogenic cyst:

- Microscopic evidence shows a transitional area from benign cystic epithelial lining to invasive PIOSCC.

Figures 5A–F: Photomicrographs showing immunohistochemical findings (10x). (A) CD34. (B) AE1/AE3. (C) CK5/6. (D) p63. (E) S100. (F) H&E stained section showing sheets of tumor cells
Panel of Immunohistochemical Markers

Table 1: Cases of PIOSCC ex OKC reported in English literature from 2013 to 2016

<table>
<thead>
<tr>
<th>Sl.</th>
<th>Author(s)</th>
<th>Year</th>
<th>Age/ Sex</th>
<th>Site</th>
<th>Symptoms</th>
<th>Radiographic features</th>
<th>Lining</th>
<th>Grade of SCC</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tan B et al.</td>
<td>2013</td>
<td>45/Male</td>
<td>Maxilla</td>
<td>Swelling</td>
<td>Radiolucency</td>
<td>- WDSCC</td>
<td>Maxillectomy, Radiotherapy</td>
<td>Not stated</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Tan B et al.</td>
<td>2013</td>
<td>49/Male</td>
<td>Mandible</td>
<td>Pain, Swelling, Fever</td>
<td>Radiolucency</td>
<td>- MDSCC</td>
<td>Bloc-resection, Chemotherapy, Radiotherapy</td>
<td>Not stated</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Tamgadge et al.</td>
<td>2013</td>
<td>20/Female</td>
<td>Mandible</td>
<td>Pain, Swelling</td>
<td>Multi-locular Radiolucency with Radioluency with Sclerotic margins</td>
<td>- WDSCC</td>
<td>Hemi-mandibulectomy, Neck Dissection</td>
<td>Not stated</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Jain et al.</td>
<td>2013</td>
<td>70/Male</td>
<td>Mandible</td>
<td>Pain, Swelling</td>
<td>Unilocular Radiolucency</td>
<td>Parakeratinized</td>
<td>WDSCE</td>
<td>Resection of Mandible</td>
<td>3 years, Free of disease</td>
</tr>
<tr>
<td>5.</td>
<td>Park et al.</td>
<td>2015</td>
<td>36/Male</td>
<td>Mandible</td>
<td>Pain, Limited mouth opening</td>
<td>Multi-locular Radiolucency</td>
<td>-</td>
<td>SCC</td>
<td>Segmental Mandibulectomy, Neck Dissection</td>
<td>24 months</td>
</tr>
<tr>
<td>6.</td>
<td>Lukanda et al.</td>
<td>2015</td>
<td>32/Female</td>
<td>Mandible</td>
<td>Pain, Swelling</td>
<td>Multi-locular radiolucency</td>
<td>Parakeratinized and Orthokeratinized</td>
<td>Infiltrative SCC</td>
<td>Hemi-mandibulectomy</td>
<td>Not stated</td>
</tr>
<tr>
<td>7.</td>
<td>Bai et al.</td>
<td>2015</td>
<td>59/Male</td>
<td>Mandible</td>
<td>Swelling</td>
<td>Multi-locular radiolucency</td>
<td>-</td>
<td>WDSCE</td>
<td>Mandibular excision</td>
<td>Not stated</td>
</tr>
<tr>
<td>8.</td>
<td>Saxena et al.</td>
<td>2015</td>
<td>60/Male</td>
<td>Mandible</td>
<td>Pain, Swelling, Paraesthesia</td>
<td>Multi-locular radiolucency</td>
<td>Parakeratinized</td>
<td>MDSCC to PDSCC</td>
<td>-</td>
<td>Not stated</td>
</tr>
<tr>
<td>9.</td>
<td>Ramya et al.</td>
<td>2015</td>
<td>35/Male</td>
<td>Mandible</td>
<td>Swelling</td>
<td>Multi-locular radiolucency</td>
<td>Parakeratinized</td>
<td>SCC</td>
<td>Hemi-mandibulectomy</td>
<td>Not stated</td>
</tr>
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<td>10.</td>
<td>Present case</td>
<td>2016</td>
<td>40/Female</td>
<td>Mandible</td>
<td>Pain, Swelling</td>
<td>Multi-locular radiolucency</td>
<td>Parakeratinized</td>
<td>PDSCC</td>
<td>Hemi-mandibulectomy</td>
<td>Follow up being continued</td>
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OKC lining. Squamous cell carcinoma seen in most of the cases belonged to the well-differentiated type, but in the present case, the poorly differentiated variant was observed, which could explain the aggressiveness of the lesion and the reason for its recurrence.

In the present case, the presence of spindle-shaped tumor cells leads to the addition of spindle cells lesions in the list of histopathologic differential diagnoses. Sarcomas, as well as carcinomas, were ruled by a panel of Immunohistochemical markers which included a few epithelial markers such as pan cytokeratin and CK5/6 along with a few mesenchymal markers such as CD34, SMA, and S100. p63 was also included to confirm the poorly differentiated grade as most poorly differentiated squamous cell carcinomas stain positive for it. As the tumor cells exhibited negativity for mesenchymal markers and strong positivity for pan cytokeratin, its origin as a squamous cell was thereby confirmed. Negative staining for CK5/6 and weak positivity for p63 favored the poorly differentiated grade. This case scenario also signifies the importance of immunohistochemistry that could be utilized for a definitive diagnosis in the presence of such overlapping histopathological features.

Fig. 6: Pathogenesis of malignant transformation of odontogenic cysts
The exact molecular pathogenesis of PIOUSC ex OKC is not clear and still needs further exploration. Whosoever, hypotheses have been put forward by Bodner et al. that could give a possible explanation. There has been a known co-relation between chronic inflammation and carcinogenesis. It has been observed that the presence of inflammation in connective tissue is frequently associated with the malignant transformation of cystic epithelium. Chronic inflammation-induced carcinogenesis has been accepted even in other cancers such as oral squamous cell carcinoma. Though the underlying molecular mechanisms are not clearly understood, three main hypotheses by which inflammation can cause cancer have been proposed (Fig. 6):

- Chronic inflammation is often accompanied by the formation of reactive oxygen and nitrogen species by phagocytes. These have the potential to damage DNA, proteins, and cell membranes, modulate enzyme activities and gene expression, and thereby favor carcinogenesis. Chronic inflammation appears to promote apoptosis of normal cells that leads to a compensatory proliferative response by the remaining cells. This process increases the number of cells that are dividing and therefore are subject to DNA damage and promotes the growth of malignant cells.

- Infectious agents may directly transform cells by inserting active oncogenes into the host genome, inhibiting tumor suppressors, or stimulating mitoses. Infectious agents may also induce immunosuppression with consequently reduced immunosurveillance.

- Genetic factors of the host may also be involved.

In the present case, the most probable reason for such transformation of OKC lining into malignancy could be the presence of chronic inflammation. Hence, patients presenting with odontogenic cysts must be monitored thoroughly from both clinical and pathological points of view.

**CONCLUSION**

Though the incidence of Primary Intra-osseous Carcinoma ex OKC is rare, its possibility of occurrence should not be neglected in clinical scenarios. As these cases can present as a highly aggressive lesion, all cases of OKC must be thoroughly scrutinized for any malignant changes. Moreover, in cases posing a diagnostic dilemma, immunohistochemistry must be utilized to rule out the presence of any factors that could affect the treatment outcome. In case of any evidence of malignant changes, appropriate surgical treatment and follow-up must be carried out for improving the prognosis of such patients.

**REFERENCES**


