Abstract. Hyalinizing clear cell carcinoma (HCCC) is very rare in the oral cavity, arising more frequently in the minor salivary glands. We present the case of a 57-year-old woman with a swelling on the anterior palatoglossal arch of 2x1 cm size. An incisional biopsy was taken and histological examination revealed typical clear cells arranged in anastomosing trabeculae, cords, nests, and solid sheets with a hyalinizing stroma. These clear cells were strongly positive to periodic acid-Schiff stain (PAS) but were negative for mucicarmine. Immunohistochemically, the neoplastic cells were immunoreactive to pancytokeratin, focally positive to EMA, but negative for smooth muscle actin (SMA), vimentin and S-100 protein, HMB45, CD68, carcinoembryonic antigen (CEA) and glial fibrillary acid protein (GFAP). These findings allowed us to define this tumor as a clear cell tumor of the anterior palatoglossal arch. The tumor was subjected to radical excision and the patient is doing well at twelve months after surgery. This report focuses on the heterogeneous group of clear cell neoplasms with the intent of pointing out some aspects that may contribute to forming a diagnosis of HCCC, and which, above all, enable us to distinguish this neoplasm from other very similar forms occurring in the oral cavity.

In the oral cavity, HCCC generally arises in the minor salivary glands, with the most frequent site of occurrence being the tongue, followed by the palate, floor of the mouth, buccal mucosa, retromolar trigone (2-8) and jaws (9). Other sites are the parotid glands (10), the hypopharynx and the nasopharynx (11, 12).

HCCC generally develops in women in the fifth to seventh decades; it presents as a slow-growing and painless submucosal mass without surface ulceration, unless it has been secondly traumatized (2, 3). Numbness, pain and even limitation of movement have been noted if the lesion involves the tongue (2, 5, 13-16). Bone destruction and movement of teeth have been reported when the lesion affects the jaws (9).

A rare case of clear cell carcinoma of the anterior palatoglossal arch is reported here, which, to our knowledge is the first such report. The histopathological diagnosis and immunohistochemical profile are also discussed.

Case Report

A 57-year-old woman sought treatment at the Department of Oral Pathology of the Milan Stomatological Institute, for a swelling measuring 2 cm x 1 cm on the anterior palatine pillar that had appeared approximately two months earlier (Figure 1). Her medical history was unremarkable. Given the cyst-like aspect of the lesion, a cytological needle biopsy was performed and the cytology of the specimen was reported to be compatible with the diagnosis of mucosal cyst. An excisional biopsy was performed via the intra-oral approach with Nd: YAG laser therapy (125 W, 15 Hz, 60 s for 5 applications, fiber 329 μm) (Figure 2) and the specimen was sent to the Institute of Pathological Anatomy for histological examination. The pathological diagnosis was of HCCC with positive margins. Based on the histopathological diagnosis, the patient was hospitalized and an extensive surgical excision with marginal resection of the left palatoglossal arch was performed. The patient underwent thoracic and abdominal computed tomography to exclude the possibility of metastases from a clear cell kidney tumor. Twelve months after surgery, the patients is tumor-free and in a good condition.
Figure 1. Clinical photograph shows a mass of 2x1 cm in the left palatoglossal arch; the overlying mucosal surface is normal in colour.

Figure 2. Clinical photograph of the area during excisional biopsy via intra-oral approach with Nd: YAG laser.
Figure 3. Tumor comprises two cell populations: a population of predominantly clear cells (displaying water-clear cytoplasm) and another population of smaller cells with eosinophilic cytoplasm (hematoxylin and eosin staining; original magnification x200).

Figure 4. Clear cells and some eosinophilic cells arranged in anastomosing thick trabeculae with a hyalinizing and myxoid stroma (hematoxylin and eosin staining; original magnification x150).
Figure 5. A strong positive immunoreactivity for cytokeratins (original magnification x150).

Figure 6. Focal positive immunoreactivity for EMA expression (original magnification x150).
Materials and Methods

The excised biopsy specimens were fixed in 10% buffered-formalin and paraffin-embedded. They were cut into 4-μm-thick sections, mounted on glass slides and stained with hematoxylin and eosin, periodic acid Schiff (PAS) and mucicarmine. For the immunohistochemical study, the standard streptavidin biotin immunoperoxidase method was employed. Primary antibodies used for immunohistochemistry and a summary of the results are given in Table I.

The immunohistochemical reactivity was determined and graded as follows: negative, no staining; positive, focally positive for a limited number of cells; and intensely positive, focally or diffusely positive for numerous cells.

Results

On microscopy, the tumor showed epithelial cells and clear cells (displaying water-clear cytoplasm), with a subpopulation of smaller cells showing eosinophilic cytoplasm immersed in a myxoid stroma (Figure 3). In some areas, aggregates of clear cells were visible, interspersed with hyalinized areas (Figure 4). Tumor cells were positive for PAS but negative for mucicarmine. Immunohistochemically, all tumor cells displayed the expected pattern of immunoreactivity, with positive results for pancytokeratin (Figure 5), focally positive for EMA (Figure 6), and negative results for staining with antibodies against MIB-1, S-100 protein, HMB45, smooth muscle actin, CD68 and CEA. Tumor cells were also negative for vimentin and glial fibrillary acidic protein immunostaining.

Discussion

The presence of clear cells may be observed in a wide variety of salivary glands tumors, including mixed tumors, myoepitheliomas, oncocytes, mucoepidermoid tumors, acinic cells, adenocarcinomas and adenoid cystic carcinomas, as well as in clear cell tumors metastizing from the kidney. The presence of clear cells in most of these tumors generally comprises a lesser component; however, they may also be more abundant, which raises the problem of differential diagnosis. It is also true that the afore-mentioned tumors sometimes present typical morphological features that aid diagnosis, but in other cases they may be a diagnostic challenge for the oral pathologist, especially, for epithelial-myoeplithelial tumors with a large component of clear cells, and for a clear cell kidney tumor metastized to the oral cavity.

The epithelial-myoeplithelial tumor is a biphasic neoplasm comprising ductal and large myoepithelial cells, in which large clear cells may sometimes be predominant. There is also less overall cellular uniformity compared to the clear cell carcinoma. The clear cells contain glycogen that can be demonstrated with PAS stain, while mucicarmine staining is negative and the immunohistochemical profile indicates that the tumor cells are of epithelial origin without myoepithelial differentiation.

There is uncertainty with mucoepidermoid carcinomas, which generally exhibit 10% of clear cells but occasionally this component may be larger. These carcinomas stain positive with mucicarmine, whereas clear cell tumors do not. Similarly, acinic cell adenocarcinomas are infiltrative and well-circumscribed tumors in which, though rarely, clear cells can sometimes predominate. They may be distinguished by serous acinar differentiation and also stain positively with mucicarmine.

Clear cell oncocytes and nodular oncocytic hyperplasias (usually circumscribed and non-infiltrative tumors) are frequently multifocal, and here, too, clear cells can sometimes dominate, but both react positively to PAS stain and mucicarmine.

The sebaceous adenoma and sebaceous adenocarcinoma are very rare tumors, exhibiting cytoplasm that is optically

Table I. Immunohistochemical findings of the presented case.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Supplier</th>
<th>Dilution</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mib-1</td>
<td>Dako (North America, Inc, Carpinteria, CA, USA)</td>
<td>1:200</td>
<td>–</td>
</tr>
<tr>
<td>Desmin</td>
<td>Dako</td>
<td>1:200</td>
<td>–</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Ventana (Medical System Inc, Arizona, USA)</td>
<td>Prediluted</td>
<td>–</td>
</tr>
<tr>
<td>S-100</td>
<td>Dako</td>
<td>1:200</td>
<td>–</td>
</tr>
<tr>
<td>Pancytokeratin (AE1/AE3)</td>
<td>Ventana</td>
<td>Prediluted</td>
<td>+ +</td>
</tr>
<tr>
<td>CD68</td>
<td>Dako</td>
<td>1:500</td>
<td>–</td>
</tr>
<tr>
<td>HMB45</td>
<td>Ventana</td>
<td>Prediluted</td>
<td>–</td>
</tr>
<tr>
<td>SMA</td>
<td>Dako</td>
<td>1:200</td>
<td>–</td>
</tr>
<tr>
<td>GFAP</td>
<td>Dako</td>
<td>1:1000</td>
<td>–</td>
</tr>
<tr>
<td>CEA</td>
<td>Dako</td>
<td>1:100</td>
<td>–</td>
</tr>
<tr>
<td>EMA</td>
<td>Dako</td>
<td>1:100</td>
<td>+</td>
</tr>
</tbody>
</table>

– No staining; + focally positive for a limited number of cells; ++ (intensely positive), focally or diffusely positive for numerous cells; SMA: Alpha smooth muscle Actin; GFAP: Glial fibrillary acid protein.
empty since it is full of liquids that are lost in the course of routine histological procedures. They contain lipid droplets, giving the cytoplasm a foamy appearance resembling the clear cell tumor. However, they develop more frequently close to the maxillary or mandibular alveolar ridges, and enter into differential diagnosis with the clear cell odontogenic carcinoma (9), a rare tumor of the odontogenic tissue, visible radiologically as a centralized destructive osseous lesion, this feature being indicative of odontogenic lesions.

Lastly, metastases from a renal cell tumor or from a clinical standpoint a Sarcoidosis must be ruled out. Sarcoidosis, may affect any oral region, including lips, hard and soft palate, tongue, tonsil, major and minor salivary glands, and gingival. Generally it has been described as a swelling non-tender, well-circumscribed, brownish red or lilaceous nodules or papules with feature likewise the present case, but the clinical and radiographic findings should be supported by histological evidence of a widespread non-caseating epithelioid cell granulomas in more than 1 organ (17). While the renal cell tumor may be excluded through clinical and immunohistochemical observation, in the present case we employed radiography and abdominal computed tomography, since this lesion could not be excluded with certainty through routine histological stains, and immunohistochemistry helps since renal cell carcinomas characteristically express vimentin, unlike clear cell tumors (2).

A significant feature that should be considered is hyalinization; this is part of the histopathological spectrum of clear cell carcinoma, and hyalinizing stroma may range from abundant to scarce and may even be entirely absent. Hyalinization is a distinctive morphological feature that allows HCCC, to be distinguished from other salivary gland neoplasms with a clear cell phenotype (2, 7, 8, 18, 19). Microscopically, the hyalinized form shows clear cells arranged in anastomosing thick trabeculae, cords, nests, or solid sheets within a hyalinizing stroma, and our case was consistent with these characteristic features of HCCC.

From the immunohistochemical investigations useful to distinguish among the various forms, we obtained the following results: positive staining for cytokeratins; negative for S-100, SMA, CEA and vimentin (which on the contrary was not found in epithelial myoepithelial tumors) (2, 8, 16,18-21). We also detected focal positivity of the tumor cells to epithelial membrane antigen which is in accordance with previously published data (2). We also tested for HMB45, another marker that might be of value for specifying tumor origin, to exclude clear cell melanoma; the absence of staining thus excluded this possibility.

Regarding treatment, local wide-margin excision seems to be the treatment of choice (2, 9, 12, 13, 22-24). Postoperative radiotherapy has been employed in some cases previously reported (2, 19). In our case, we ensured that the tumor was removed with a wide margin of surgical resection. Recurrence of HCCC after surgical resection has rarely been documented. Tang et al. (18) and Milchgrub et al. (2) reported two cases of recurrence after surgery, due to incomplete resection.

Based on what is reported in the English language literature, and maintaining the distinction between hyalinized and non-hyalinized forms, only 34 cases of hyalinizing clear cell carcinoma originating from the minor salivary glands have been reported in the past (25). With regard to non-hyalinized forms, 18 cases have been reported to date (26). The distinction appears to us entirely arbitrary, in agreement with Ellis et al. (1), since this feature does not appear to condition either the biological behavior of the tumor or its prognosis.

Given the limited experience regarding these tumors, opinions differ also with regard to prognosis. Some authors consider HCCC as a low-grade malignancy, whereas others view it as a more aggressive and invasive one (2, 16, 23). Previous cases have been reported of local regional lymph node metastases (2, 12, 16, 19, 23) and of distant metastasis; Ereno et al. (12) reported one HCCC in the hypopharynx metastasizing to the lung; O’Regan et al. (23) reported a case widely metastasizing to the vertebral bodies, mandible and left femur, in which the patient died within 10 months of the initial presentation. Our patient is doing well 12 months after surgery.

To conclude, the picture that emerges is of a rare tumor, with low malignancy in general. Of the 34 cases reported, only two gave rise to metastases (long-term evaluation is still lacking for our case). Histopathological diagnosis is difficult, because many tumors of the minor salivary glands may present similar features, and differential diagnosis must be especially careful; immunohistochemical investigations are necessary to distinguish between the various forms.

References


Revised August 1, 2007

Received May 28, 2007

Accepted August 14, 2007