CASE REPORT

Sebaceous adenoma of oral cavity: report of case and comparative proliferation study with sebaceous gland hyperplasia and Fordyce’s granules

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Sebaceous adenoma (SA) is a rare solitary tumour with a predilection for the forehead and scalp. In the English literature, less than 10 cases of SA have been described in the oral cavity. The objective of this study was to examine the clinicopathologic features and evaluate the expression of epidermal growth factor and its receptor, estrogen receptor and androgen receptor in SA and in its differential diagnoses including sebaceous gland hyperplasia (SGH) and Fordyce’s granules (FG). Additionally, we analysed the proliferative potential of sebaceous cells from SA, SGH and FG by measuring proliferating cell nuclear antigen (PCNA) expression and quantification of argyrophilic nuclear organizer regions (AgNORs). The SA showed many clinicopathologic similarities to cases previously reported including the biphasic population of cells, in the periphery of lobules undifferentiated basaloid cells whereas the central area was formed by mature sebocytes. SA was composed of 198 lobules of sebaceous cells, whereas SGH and FG showed a mean of 21 ± 7.81 and 5.84 ± 2.83, respectively. The AgNOR and PCNA indices were similar in SA, SGH and FG. These data suggest that lobule counts may be used as additional criteria in distinguishing SA of the oral cavity from other intraoral sebaceous gland lesions.

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Introduction

Sebaceous adenoma (SA) and sebaceous gland hyperplasia (SGH) are rarely diagnosed intraorally (Dent, Hunter and Svirsky, 1995; Iezzi et al, 2002), being more frequent on the face of elderly patients with a predilection for the forehead and scalp (Lipani et al, 1983; Daley, 1992; Koutlas and Yaholnitsky, 1994; Massry et al, 1995). However, intraoral sebaceous glands (Fordyce’s granules, FG) are extremely common and usually found on the vermilion of the upper lip and on the buccal mucosa (Daley, 1993). FG occurs in both sexes and in all age groups, although less often in young children (Daley, 1992). Histologically, SA is a sharply demarcated tumour composed of incompletely differentiated sebaceous lobules containing different amounts of sebocytes and undifferentiated basaloïd germinative cells (Lever and Schaumberg-Lever, 1975). SGH is a single enlarged sebaceous gland composed of numerous lobules grouped from a centrally located wide sebaceous duct (Dent et al, 1995). Intraoral sebaceous glands are identical to their cutaneous counterpart but not associated with hair follicles (Koutlas and Yaholnitsky, 1994; Dent et al, 1995). Interestingly, a patient with a follicle and hair shaft associated with sebaceous gland within the mouth has been described (Baughman and Heidrich, 1980).

Approximately 18 cases of intraoral SGH (Daley, 1992; Koutlas and Yaholnitsky, 1994; Dent et al, 1995) and less than 10 cases of intraoral SA (Iezzi et al, 2002) have been reported in the English literature. The aim of present study was to describe the clinicopathologic and histomorphometric features of one new case of SA affecting the retromolar pad, and to compare the proliferative potential and the immunohistochemical features of SA of the oral cavity and its differential diagnosis, including the SGH and FG, with a panel of antibodies against epidermal growth factor (EGF), epidermal growth factor receptor (EGFr), estrogen receptor (ER), and androgen receptor (AR). Proliferative activity was determined by quantitative analysis of argyrophilic nuclear organizer regions (AgNORs) and of proliferating cell nuclear antigen (PCNA) expression.

Case report and methods

A 71-year-old Caucasian female was referred to the Orocentro – Center for the Study of Oral Diseases – at
the University of Campinas Dental School, Piracicaba-SP, because of a nodular lesion that caused a slight discomfort on mastication. Clinical examination revealed a nodular gray-yellowish lesion covered by normal mucosa with $1 \times 1$ cm in size, involving the left retro-molar pad (Figure 1). The systemic exploration and laboratory data were normal. The clinical diagnosis was fibroma and an excisional biopsy was performed. The tissue removed during biopsy was fixed in formalin and embedded in paraffin. Microscopically, the lesion presented as a well-delimited but not encapsulated nodule, separated from the neighbouring tissue by a thin layer of fibrous connective tissue (Figure 2). It was composed of lobules with undifferentiated basaloid germinative cells in the periphery, while the central area was formed by mature sebocytes (Figure 3). No cellular atypia was observed, and mitotic figures were uncommon. Many of the lobules exhibited central cystic spaces. It was composed of 198 lobules being diagnosed as SA. After 1 year of follow-up, no recurrence was observed.

The study samples consisted of one oral SA, three cases of oral SGH and 13 cases of FG. All samples were retrieved from the files of the University of Campinas Dental School, Department of Oral Pathology. New sections were cut from the paraffin blocks and stained with haematoxylin and eosin (H & E) and periodic acid-Schiff (PAS). FG and SGH were diagnosed according to the criteria described by Daley (1992). The study protocol was approved by the Ethical Committee in Research at the University of Campinas Dental School.

Immunohistochemical analyses were performed using the streptavidin-biotin peroxidase complex method. Paraffin sections (3-μm thick) were cut and mounted on silane-coated glass slides. Following deparaffinization and hydration in graded alcohol solutions, the sections were treated with 3%H$_2$O$_2$ followed by antigen retrieval with microwave (Coletta et al., 2001) for PCNA, ER and AR or with 0.1% trypsin (Sigma Chemical Co., St Louis, MO, USA) in phosphate buffered saline (PBS) at $37^\circ C$ for 50 min for the antibody against EGF and EGFr. After washing with PBS, the sections were treated with 1% bovine serum albumin (BSA, Sigma) in PBS for 1 h, and then incubated with primary antibodies for 16 h at 4°C. The primary antibodies were used as follows: anti-PCNA diluted 1:16,000; anti-ER diluted 1:100; anti-AR diluted 1:100; anti-EGF diluted 1:50; and anti-EGFr diluted 1:50. All antibodies were purchased from Dako Co. (Carpenteria, CA, USA), with exception of antibodies anti-AR and anti-EGF, which were purchased from Upstate® biotechnology (Lake Placid, NY, USA) and Oncogene Research Products (Boston, MA, USA), respectively. Subsequent incubations were with biotinylated IgG followed by streptavidin-biotin peroxidase complex (StrepABC Complex/HRP Duet kit, Dako). Reactions were developed by incubating the sections with 0.6 mg ml$^{-1}$ 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) containing 0.01% H$_2$O$_2$ and counterstained with Mayer’s haematoxylin.

Positive and negative controls were included in all reactions. The PCNA index, expressed as the percentage of cells with labelled nuclei, was determined by counting

![Figure 1]( Clinical photograph of sebaceous adenoma shows a sessile, round, raised nodule in retromolar pad)

![Figure 2]( Sebaceous adenoma presented as a well delimited mass separated from the neighbouring tissue by a thin layer of fibrous connective tissue, cystic areas, squamous ductal metaplasia and minor salivary glands around the sebaceous adenoma (H & E, ×25)

![Figure 3]( Photomicrograph of sebaceous adenoma consisting of numerous lobules of well-differentiated sebaceous cells centrally and a basaloid germinative layer (H & E, ×200)
500 cells in three independent reactions for each sample using an image analysis system KONTRON 400 (Zeiss, Munich, Germany).

The AgNOR technique was adapted from Chatterjee et al (1997). Four-micrometre sections of 10% formalin-fixed, paraffin-embedded tissues were dewaxed in xylene and rehydrated through graded alcohol. The sections were then incubated for 30 min at 37°C in a fresh solution of one part of 1% gelatin (Sigma Chemical Co., St Louis, MO, USA) in 1% formic acid (Reagen, Rio de Janeiro, Brazil) with two parts of 50% aqueous silver nitrate (Merck, Darmstadt, Germany). The AgNORs were visualized as intranuclear black dots under light microscopy. For each sample, the number of AgNOR dots on 500 cells was determined using an image analysis system.

All reactions were performed at least three times. Student’s t-test (two-tailed) was used for statistical analysis. In our comparisons, \( P \leq 0.05 \) was considered to indicate statistical significance.

Results

Clinical and histopathological findings of SGH and FG

The mean age of SGH patients was 29 ± 6 years with a range from 23 to 35 years and all cases affected women. Two cases occurred on the retromolar trigone and one on the buccal mucosa. The clinical diagnoses were FG in two cases and one lipoma. All cases of SGH showed a well-circumscribed but not encapsulated lesion, covered by parakeratinized stratified squamous epithelium. The connective tissue exhibited several sebaceous gland units with dilated ducts interwoven by dense fibrous stroma. The number of lobules ranged between 16 and 30 lobules, with a mean of 21 ± 7.81 (Figure 4).

The age of patients with FG ranged between 18 and 83 years, with a mean of 41 ± 17.06 years. Four cases occurred in women and nine in men. Nine cases affected the buccal mucosa, three the upper lip and one the alveolar process. All FGs were clinically diagnosed as FG, except three cases, which were diagnosed as fibroma and haemangioma. The FG was composed of mature sebaceous lobules with a common duct and was surrounded by a connective tissue stroma. The number of lobules ranged between 3 and 12, with a mean of 5.84 ± 2.82 lobules (Figure 5).

Immunohistochemical findings

In SA, SGH and FG samples staining for EGF was detected throughout the cytoplasm and membrane of sebaceous cells, whereas EGFr was only visualized in the membrane. Compared with SGH and FG, the SA showed a higher expression of EGF and EGFr. In all lesions, the immunostaining for EGF and EGFr was more often positive in the germinative cells than in the differentiated sebocytes. SA, SGH and FG showed strong nuclear immunoreactivity for AR, whereas ER expression was not found in the sebaceous cells.

Proliferative potential

The proliferative rates of SA, SGH and FG sebaceous cells were assessed by measuring PCNA expression and quantification of AgNORs. In all samples, AgNORs were strictly located within the cell nucleus and were clearly visible as black dots (Figure 6). Nuclear
immunoreactivity for PCNA was clearly and easily identified (Figure 7). Nuclei with a clear brown colour, regardless of the intensity of staining, were interpreted as positive. The data showing the AgNOR and PCNA indices are listed in Table 1. The mean number of AgNORs per nucleus and PCNA index were similar comparing SA with SGH and FG.

Discussion

The solitary SA is particularly rare, and it represents about 0.5–0.7% of all monomorphic adenomas (Iezzi et al, 2002). Sebaceous neoplasms of the cheek have been reported in Torre’s syndrome, a rare disease characterized by the presence of sebaceous tumours associated with multiple visceral neoplasms such as adenocarcinomas of the gastrointestinal and genitourinary tract (Ferguson, Geary and Macalister, 1987; Daley, 1992; Massry et al, 1995; Iezzi et al, 2002). Our patient displayed no manifestations of Torre’s syndrome and the SA showed no recurrence after 12 months of surgical excision. A large study of cutaneous sebaceous neoplasms reported that isolated SA occurs mainly on the nose and scalp of men older than 50 years (Rulon and Helwig, 1974). A review of 21 cases of sebaceous neoplasms from salivary glands showed five cases of SA with an age distribution ranging between 42 and 74 years (Gnepp and Brannon, 1984). Additionally, this study demonstrated that SA has a strong predilection for the parotid gland (Gnepp and Brannon, 1984), but may affect other sites such as buccal mucosa and floor of the mouth (Ribeiro et al, 1996).

In the present study, the findings related to our case of SA involving the retromolar pad support the observations initially described in detail by Lipani et al (1983) and retrospectively substantiated by Daley (1992). They demonstrated that the major clinicopathologic features of this tumour are: a sharply circumscribed lesion with an organoid pattern; irregularity of size and shape of the sebaceous lobule; appearance of both mature sebaceous cells and small germinative cells which may be arranged in an irregular pattern; lack of a dilated excretory duct or a common excretory duct, and in some areas great lobules presented centrally cystic spaces because of disintegration of mature sebocytes.

Sebaceous gland hyperplasia consists of numerous well-differentiated lobules associated with a dilated duct and the periphery of these lobules contains one or more layers of germinative cells (Daley, 1992). Ferguson et al (1987) described in SGH pseudostratified and ciliated columnar epithelium with goblet cells resembling intestinal crypts. However, this metaplasia occurred only in the covering mucosal epithelium. Koutlas and Yaholnitsky (1994) depicted SGH with ductal cells exhibiting mucous-producing and metaplasia with occasional ciliae. We detected one case with squamous metaplasia in the ductal cells. In the present paper, SGHs followed the criteria proposed by Daley (1992). They appeared as a clinically distinct lesion that was sufficiently abnormal to require a biopsy for definitive diagnosis and histologically more than 15 well-differentiated sebaceous lobules per gland were present. Fewer than 15 lobules are interpreted as FG (Daley, 1992). In this study FG were removed either because of their abnormal appearance or by patient request.

Androgenic hormones stimulate the growth of sebaceous glands and sebum production (Whitaker, Vigneswaran and Singh, 1997; Günes and Fetil, 2000), while estrogens have the opposite effect (Günes and Fetil, 2000). The size and secretory activity of the sebaceous glands are influenced not only by androgens and other steroids, but also by non-steroidal factors including EGF (Matias and Orentreich, 1983). EGF belongs to a group of naturally occurring peptides that exerts its variety of effects through binding to its transmembranic receptor (EGFr), which shows an intrinsic protein tyrosine kinase activity (Assoian, 1997). In the sebaceous gland, the biological response of EGFr triggered after EGF binding is the stimulation of sebaceous cell proliferation (Nanney et al, 1984). EGF and EGFr expression in sebaceous glands is more intense at the periphery of the germinative cells compared with the central portion, where the mature sebocytes are located. EGF and EGFr were also found

Table 1 Mean number of argyrophilic nuclear organizer region (AgNOR) dots per nucleus and proliferating cell nuclear antigen (PCNA) index in sebaceous adenoma (SA), sebaceous gland hyperplasia (SGH) and Fordyce’s granules (FG) germinative and central sebaceous cells

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<tr>
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<th>Number of NORs per nucleus</th>
<th>PCNA (%)</th>
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<tr>
<td></td>
<td>Germinative</td>
<td>Central</td>
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<tr>
<td>SA</td>
<td>1.55</td>
<td>1.90</td>
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<tr>
<td>SGH</td>
<td>2.47 ± 0.46</td>
<td>2.71 ± 0.12</td>
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<tr>
<td>FG</td>
<td>1.98 ± 0.43</td>
<td>1.78 ± 0.19</td>
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in normal human epidermis and its appendages that do not undergo rapid proliferation, suggesting that EGF may have a more complex regulatory role in this tissue than cellular division and proliferation (Nanney et al., 1984). Experimental study showed that when testosterone and EGF are injected together in hamster sebaceous glands, a synergistic effect on the sebaceous cell proliferation is observed, resulting in an increase in the number of cells per sebaceous gland (Matias and Orentreich, 1983). In this study all samples showed similar immunopositivity for AR, whereas EGF and EGFR expression was more intense in SA than in SGH and FG. In addition, no immunopositivity for ER was observed in any case.

Distribution and quantification of AgNORs and immunostaining techniques using monoclonal antibodies against PCNA antigen have been used as parameters for evaluation of cell proliferative activity of tumours and it may reflect the aggressive potential of the tumour (Ansai et al., 1995). Ansai et al. (1995) reported significant differences in AgNOR count and PCNA labelling index between sebaceous carcinoma and basal cell carcinoma with sebaceous differentiation (BCSD). Additionally, the authors demonstrated that the differences were positively related to the tumour being aggressive or non-aggressive. In our study, AgNOR and PCNA demonstrated that the proliferative potential of sebocytes from SA, SGH and FG is similar.

In summary, sebaceous neoplasms of the oral cavity are extremely rare and we report here one new case of intraoral SA and three new cases of intraoral SGH. There were no proliferative differences among SA, SGH and FG in the AgNOR and PCNA indexes. The lobule counts may be used as additional criteria in distinguishing intraoral SA from other intraoral sebaceous gland lesions. Further studies are necessary to obtain more insights about these entities.

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References


