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In the present study, the immunoprofile of chronic sclerosing sialadenitis, also known as Kuttner tumor, was analyzed. Two cases that occurred in the submandibular gland of male patients were submitted to immunohistochemical reactions to different antibodies. Histological examinations showed a submandibular gland exhibiting various degrees of atrophy with destruction of acini, infiltration by inflammatory cells, and periductal fibrosis. Reactions to cytokeratins (CKs) showed acini and duct remnants positive to CKs 7, 8, 19, and 13. CK14 stained myoepithelial cells around preserved acini and intercalated duct, and also basal cell of excretory ducts, but was negative in proliferating and branching ducts. Smooth muscle actin (SMA) was expressed by myofibroblasts in periductal fibrosis, and an intense expression of extracellular components was also seen. Lymphocyte markers showed, besides mature follicles, a higher presence of CD45RO positive cells. Thus, the immunoprofile of Kuttner is much more in keeping with an inflammatory-induced degenerative disease than with a preneoplastic lesion.

Keywords: Kuttner tumor; chronic sclerosing sialadenitis; submandibular gland; immunohistochemistry

Introduction

Chronic sclerosing sialadenitis (also known as the Kuttner tumor) is a chronic inflammatory condition of the salivary glands, first described by Kuttner in 1896. The lesion was described as a tumor-like lesion of the submandibular gland expressed clinically by hardness and swelling of the gland.[1] It occurs almost exclusively in the submandibular gland; actually, it is the most common disease at this site.[2] Clinically, it cannot be distinguished from a true neoplasm.

Sialolithiasis is the most common etiologic or pathogenetic factor associated with this disease, sialoliths being found in 29% to 83% of the cases.[3,4] However, it is impossible to establish if the sialolith is the cause or the result of the inflammatory process.[4] Obstructive electrolyte sialadenitis secondary to a secretion disorder obstructing small ducts has been pointed out as another possible pathogenetic mechanism.[5]

According to Seifert and Donath,[3] a Kuttner tumor goes through different histological stages, from a mild focal chronic inflammation with periductal fibrosis and ductal ectasia to extensive fibrosis with marked acinar atrophy and ductal dilatation. The lobular architecture of the gland is usually preserved. The inflammatory infiltration in the prefibrotic stage may be intense, with lymphoid follicle formation. The degree of fibrosis and inflammation varies from lobule to lobule within the same gland.

Although the Kuttner tumor was described more than a century ago, and it is not a rare condition, it remains a poorly recognized entity. In the present study, we analyzed immunohistochemically 2 cases diagnosed as Kuttner tumor, focusing on the remaining

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glandular parenchyma, the quality of inflammatory infiltrate, and the extracellular matrix.

Material and Methods

Two cases diagnosed as Kuttner tumor (chronic sclerosing sialadenitis) were retrieved, one from the files of the Oral Pathology Laboratory at the University of São Paulo and the other from the Oral Pathology Department at the Federal University of Uberlândia. The first case was from a 35-year-old man who reported a history of a painful swelling in the right submandibular gland that had been present for 6 months. Extraoral examination revealed a 4 × 3 cm swelling in the right submandibular gland covered by normal skin. The lesion was painful to light pressure. No other significant findings were noted and the past medical history of the patient was unremarkable. A computed tomography scan of the right submandibular gland revealed a well-defined swelling. The lesion was clinically diagnosed as adenoma, and under this diagnosis, it was surgically excised. A diagnosis of Kuttner tumor (chronic sclerosing sialadenitis) of the submandibular gland was rendered.

The second case was from a 43-year-old white man who presented with a nodule in the submandibular region. Extraoral examination revealed a mobile, painless swelling measuring 4.0 cm. It was covered by normal overlying skin, had an oval form, and an elastic consistency. The patient's general health was otherwise good. A sialogram showed foreign bodies in the duct of Wharton. Clinical impression was that of submandibular sialadenitis or benign tumor of the submandibular gland, and an excisional biopsy was made. The excised gland demonstrated an irregular surface with salivary calculi (Figure 1A). A histological diagnosis of Kuttner tumor (chronic sclerosing sialadenitis) of the submandibular gland was established.

Sections stained with hematoxylin and eosin were reviewed, and the lesions were classified according to the histological stages of the Kuttner tumor defined by Seifert and Donath[3] as follows: *Stage 1* is when chronic inflammation with nests of lymphocytes around moderately dilated salivary ducts is present; *stage 2* is when there is a more marked lymphocytic infiltration and more severe periductal fibrosis, and the ductal system shows inspissated secretion and focal metaplasia with proliferation of ductal epithelium; periductal lymphoid follicles are well developed, and also, atrophy of acini could be present; *stage 3* may be defined as a chronic sclerosing sialadenitis where there is an even more prominent lymphocytic infiltration, with lymphoid follicle formation, parenchymal atrophy, periductal hyalinization, and sclerosis; and *stage 4* is the end stage, presenting a cirrhosis-like aspect with marked parenchymal loss and sclerosis.

For the immunohistochemical study, 3 μm sections from formalin-fixed, paraffin-embedded tissue of both cases were obtained. To check on structural changes in the cytoskeleton of the glandular parenchyma, cytokeratins (CK) and smooth muscle actin (SMA) present in cells of the normal mature gland and/or in the developing gland were tested (CKs 7, 8, 13, 14, and 19). For the alterations in extracellular matrix proteins, components of the matrix such as collagens I, III, and IV, laminin, fibronectin, and tenascin were studied, and finally, to characterize the lymphocytic infiltrate, markers to B- and T-cell lineage (CD3 and CD20) were also studied. The primary monoclonal antibodies, dilution, incubation time, pretreatments, and source used are listed in Table 1. Horseradish peroxide–labeled polymer conjugated with secondary antibody was incubated in a 1-step technique using the EnVision System (Dako Corp, Glostrup, Denmark) for 30 minutes. Diaminobenzidine (Sigma Chemical Co, St. Louis, MO) was used as chromogen. Samples were counterstained with Mayer's hematoxylin. Omission of the primary antibody was performed for negative controls. Normal oral mucosa and normal salivary gland were used as positive controls.

Results

Histological Findings

According to the staging of Kuttner tumors,[3] both lesions mainly exhibited characteristics of stages 1 and 2, but some lobules also showed aspects of stage 3. The glandular parenchyma was represented by a few acini and ductal structures, some branching ducts (Figure 1B), and some ducts exhibiting severe fibrosis or periductal sclerosis (Figure 1C). An inflammatory infiltration, especially lymphocytic, was present, sometimes accompanied by lymphoid follicle formation. Occasionally neutrophils were observed among duct cells reaching the lumen. In case 2, mineralized material was present within the excretory duct lumen (Figure 1D).

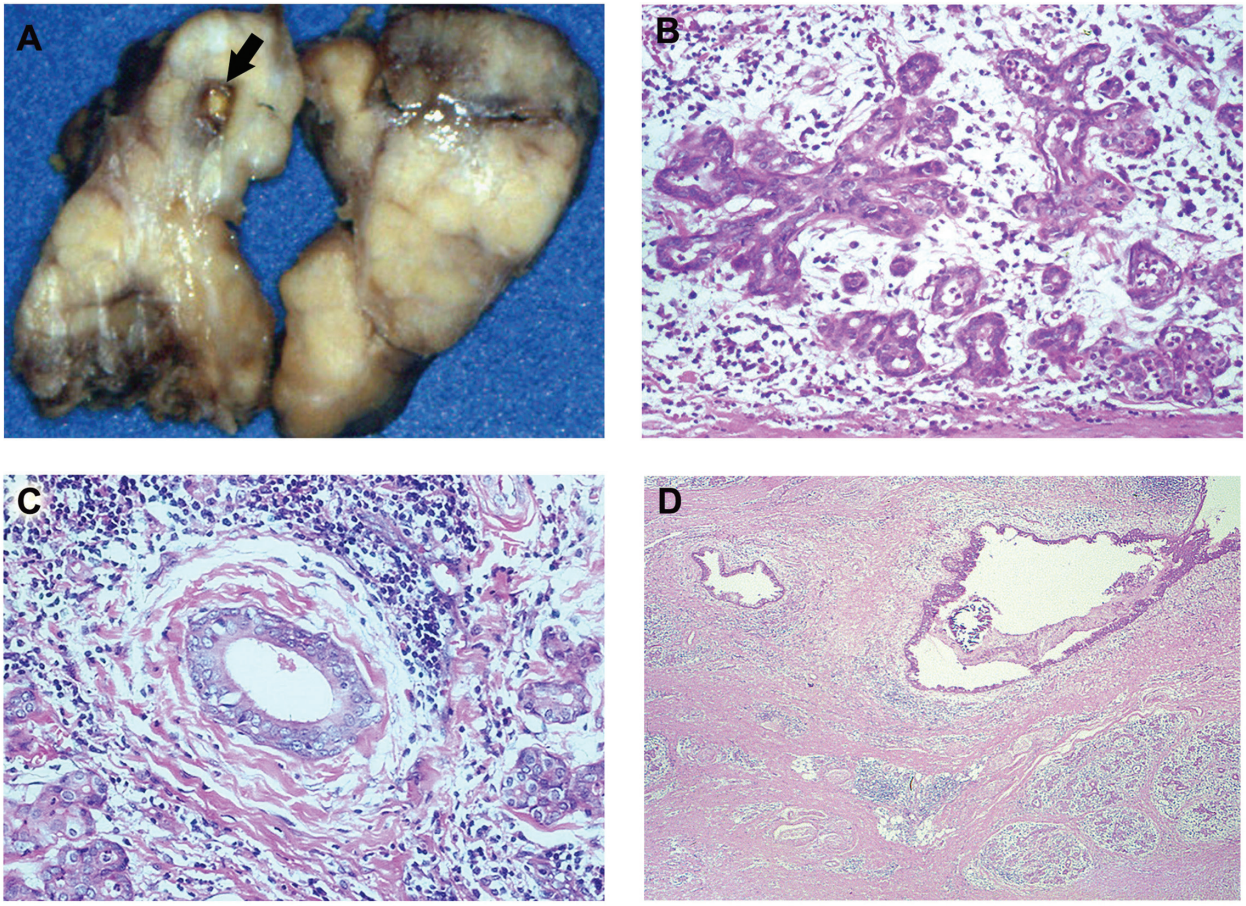

Figure 1. (A) Macroscopic aspect of case 2 showing in the upper portion a salivary calculi (arrow) occupying the excretory duct; (B) branching ducts are seen in one of the affected glands, similar to lumenization stage of developing glands (hematoxylin and eosin [HE]); (C) chronic sclerosing sialadenitis graded as stage 2, exhibiting marked periductal sclerosis (HE); (D) panoramic view of case 1 showing mineralized material within the excretory duct, and atrophy of the gland, which is replaced by fibrous tissue, graded in this area as stage 3.

Immunohistochemical Findings

Acinic and luminal cells of ductal structures and branching ducts exhibited the CKs usually found in normal glandular tissue, that is, CKs 7, 8, and 19 (Figure 2A). CK13 stained cells of the excretory duct, whereas CK14 and SMA were expressed by myoepithelial cells. CK14 was also expressed in basal cells of the excretory ducts (Figure 2B). However, some small ductal structures showing proliferative aspects did not express CK14 and SMA. Cells surrounding ducts exhibiting sclerosis also expressed SMA (Figure 2C). These cells were interpreted as myofibroblasts. Vimentin stained components of the stroma. The inflammatory infiltrate was characterized in some areas by the presence of a massive amount of plasma cells (positive to plasma cell antibody). Most of the lymphocytes present were CD45RO positive (Figure 2D), pointing to a T-cell lineage. CD20-positive lymphocytes were seen more frequently in the germinative centers of the follicles (Figure 2E).

Among the antigens to the extracellular matrix that were studied, type III collagen and fibronectin were strongly expressed, especially around duct structures (Figures 2F and 2G). Type I collagen was weakly expressed in the interlobular areas. Laminin and collagen IV (Figure 2H) were prominently expressed around glandular structures and less expressively in periductal sclerosis. Tenascin slightly stained around the excretory ducts (Figure 2I).

Discussion

The 2 cases of unilateral chronic sclerosing sialadenitis of the submandibular gland (Kuttner tumors) here reported appeared as an enlargement of the gland,

Table 1. Monoclonal Antibodies Used[a]

Antibody	Dilution	Incubation Time in Minutes	Clone
CK7[a]	1:200	30	OV-TL 12/30 (BioGenex)
CK8[a]	1:50	60	C51 (BioGenex)
CK13[a]	1:100	40	AE8 (BioGenex)
CK14[a]	1:1000	40	LL002 (BioGenex)
CK19[a]	1:100	40	BA 17 (DAKO)
CD20[a]	1:100	60	L26 (DAKO)
CD45RO[a]	1:100	60	UCLH1 (DAKO)
Laminin[b]	1:500	60	LAM 89 (Sigma)
Type I collagen[a]	1:20	60	COLL IP/(Novocastra)
Type III collagen[b]	1:300	30	HwDl.l/(BioGenex)
Type IV collagen[b]	1:50	30	CIV-22/DakoS/(DAKO)
Fibronectin[b]	1:600	60	FR1/Dako S/(DAKO)
Tenascin[c]	1:50	ON	BC24/(DAKO)
MSA[a]	1:150	60	1A4 (BioGenex)

NOTE: MSA = muscle-specific actin; ON = overnight; RT = room temperature.
[a] Pretreatment with microwave at 700 W, 10mM in citric acid, 3 cycles of 5 min each. For CD20 was used 2 cycles of 5 min each.
[b] Pretreatment with 1% pepsin solution in 0,1N chloridic acid, pH 1,8 for 60 min at 37°C.
[c] Pretreatment with tripsin solution (0,025%) for 30 min at 37oC.
Dako NS, Glostrup, DENMARK; BioGenex Laboratories Inc, San Ramon, CA, USA; Sigma, St. Louis, MO, USA; Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK.

measuring approximately 4.0 cm. Both lesions occurred in men, aged 35 and 43 years. The literature shows a mean age of 42 to 44 years, and no sex predilection has been established.[4,6]

In most cases of chronic sialadenitis of the submandibular gland, the main complaint of the patient is that of intermittent and/or persistent pain or swelling in the submandibular region, which worsens during mealtimes. However, some lesions are asymptomatic except for a firm swelling, which is frequently mistaken for a neoplasm. The lesion can be unilateral, as in the cases presented, or involve both submandibular glands.[7,8] Involvement of glands other than the submandibular is rare but may occur.[9] According to Chan,[10] the duration of symptoms is highly variable, ranging from less than 1 year to several decades.

According to the pertinent literature, the Kuttner tumor has been associated in many instances to the presence of sialoliths,[3,4] which are considered the most common etiologic or pathogenic factor. In one of the present cases, a sialolith was found on a histological examination. However, even when a sialolith is not found, it does not mean that it could not be the cause, because some calculi can be seen only in ultrastructural studies owing to their small size. Disorders of secretion and immune reactions are the other possible etiologic or pathogenic factors.[1]

Histopathologically, the cases presented here fulfill the diagnostic criteria proposed by Seifert and Donath,[3] which includes variable degrees of atrophy, destruction of acini by infiltration of inflammatory cells, periductal fibrosis, and dilatation of ducts, some of which contain secretion or microliths. Lymphoid follicles may also be present. The lobular architecture is preserved, and the degree of sclerosis is variable. Usually, it begins in the periductal region and extends into the interlobular septa, as described by Chan.[10]

Some features of the Kuttner tumor, such as the abundant presence of inflammatory cells, are shared with so-called benign lymphoepithelial lesions. However, the lack or paucity of epimyoepithelial islands and the prominent sclerosis around duct structures[10] seen in the former help in distinguishing the 2 entities. In the present cases, no typical epimyoepithelial islands were seen. Instead, branching ducts similar to those seen in developing glands[11] evidenced by the CK expression were conspicuous. Besides the branching ducts, gland remnants also expressed CKs 7 and 8 in their luminal cells, whereas CK14 was positive in focal areas of

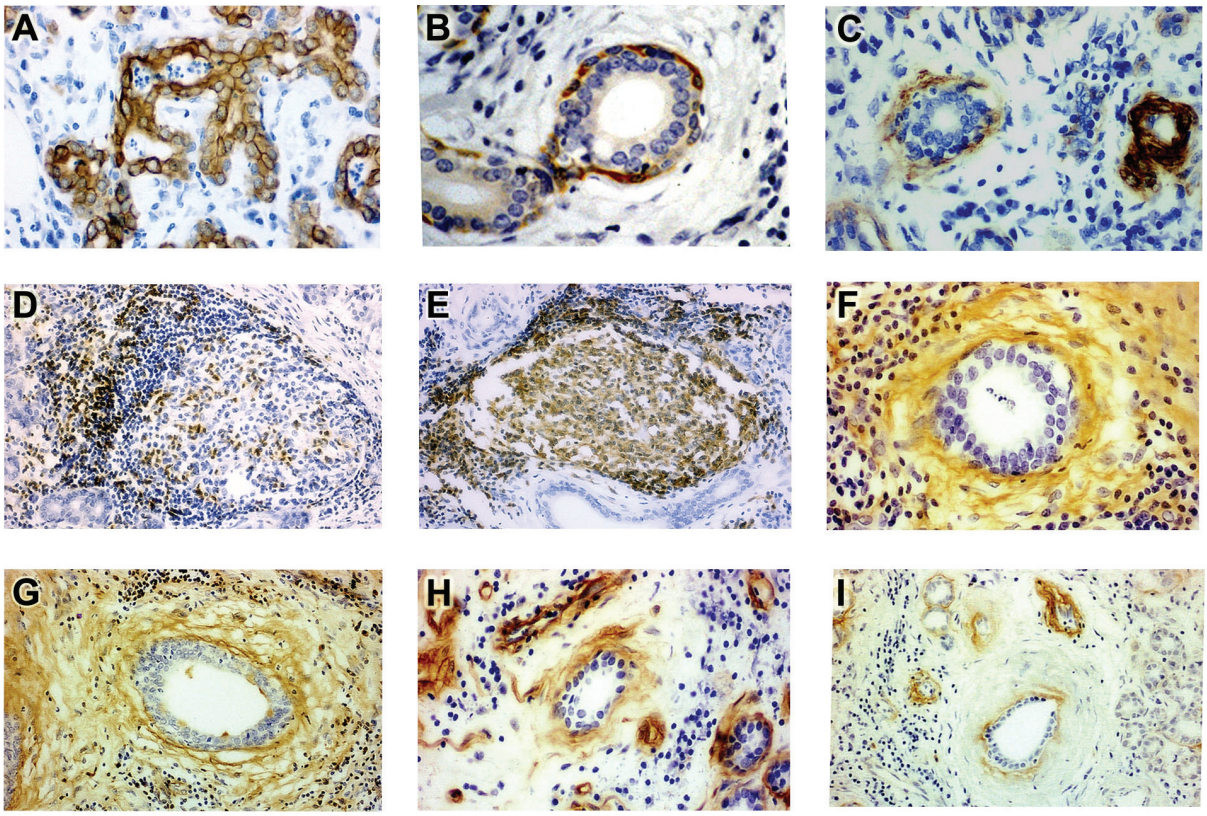

Figure 2. (A) Expression of CK 7 in the branching ducts (EnVision System, Dako Corp Glostrup, Denmark); (B) expression of CK14 in basal cells of ducts showing also periductal sclerosis (EnVision); (C) an early phase of periductal sclerosis showing SMA expressed by myofibroblasts (EnVision). Note the positively stained vascular wall. (D) CD45RO expressed by lymphocytes in germinative center and some proliferating (EnVision); (E) CD20 expressed by lymphocytes in germinative centers (EnVision); (F) strong expression of collagen III in areas of periductal sclerosis (EnVision); (G) fibronectin is also highly expressed in periductal sclerosis, in a fibrilar aspect (EnVision); (H) expression of Collagen IV in areas of ductal sclerosis, and around salivary ducts and acini (EnVision); (I) tenascin is lightly expressed in areas of periductal sclerosis, and more intensely around ducts and vascular walls (EnVision).

basal and myoepithelial cells as seen in the normal gland.[12,13] The proliferating and branching duct systems did not express CK14 or SMA, demonstrating an absence of mature myoepithelial cells as seen in ducts of developing glands.[11] CK13 was detected only in excretory ducts similar to normal glands.[12,13]

SMA stained cells in the areas of periductal fibrosis, interpreted as myofibroblasts, suggesting a possible source of collagen deposition. It is well known that myofibroblast-like cells are the principal source of extracellular matrix in some diseased tissue. In pulmonary fibrosis, myofibroblasts are the main producer of collagen.[14]

Fibronectin was present both in fibrous septae among glandular lobules and in periductal fibrosis and sclerosis—an expected finding because fibronectin is a stromal, widely distributed glycoprotein and also plays a role in helping cells attach to the matrix. The interactions between fibroblasts, their secreted products, and the surrounding matrix are important in tissue remodeling for the development of scar formation and fibrosis.[15] It could be a response to injury of the gland. Type III collagen also showed an expression similar to that of fibronectin. It seems that fibronectin and type III collagen are important in the formative phase of the fibrous component of this lesion. This strong affinity of both has been demonstrated in cultured fibroblasts. Type III collagen and fibronectin synthesis is an early event in tissue repair.[16]

Tenascin, an extracellular matrix glycoprotein, was slightly expressed around excretory ducts. In the normal salivary gland, tenascin is also seen around

the excretory duct wall. Thus, it appears that the production of tenascin, probably in response to mechanical stress,[17] is not altered in the Kuttner tumor.

Laminin and type IV collagen were prominently expressed around glandular parenchyma, acini, and ducts. However, they were less expressed in periductal sclerosing areas showing a fragmentation or disruption of the basement membrane. Studies in developing glands have shown that laminin is seen in all stages of glandular formation,[18] being required for salivary branching and other functions.[17]

The inflammatory component in the initial phases of chronic inflammatory sialadenitis (stage 2) was formed by an abundant accumulation of plasma cells. Later on, there was a strong infiltration by lymphocytes and increasing formation of lymphoid follicles, suggesting the autoimmune character of the reaction.[3] Particularly, activated B cells CD20+ were predominantly observed in germinative centers of lymphoid follicles. T cells CD45RO+ were distributed in the mantle zone and scattered within the gland parenchyma. The distribution pattern of these cells seemed to be similar to antigenically stimulated lymphonodes and was interpreted as a site of exceedingly active local immune cellular responses.[19] According to Tiemann et al,[20] chronic sclerosing sialadenitis may result from an immune process triggered by intraductal agents because there is an intimate relationship between the predominant T-cell infiltrate and acinar and duct cells.

The histopathological aspect of the Kuttner tumor must be differentiated from lymphomas, especially a B-cell lymphoma of mucosa associated lymphoid tissue. However, the inflammatory infiltrate in this type of lymphoma is made up of B cells, whereas the infiltrate in a Kuttner tumor is predominantly composed of T cells,[20,21] as shown in the present study. Although lymphoid follicles are present in both, the lymphoid infiltrate in lymphomas is often much denser and extends through lobular septae, and cytologic atypia is present. The similarity between Kuttner tumors and the sclerosing variant of follicular lymphoma arising from the submandibular gland has also been discussed.[22] A predominant B-cell population is present in the latter, but sometimes, a polymerase chain reaction might be necessary to document the B-cell monoclonality.

The Kuttner tumor also has common histopathological features with Kimura disease, but prominent eosinophilic infiltration (often with the formation of eosinophilic abscesses), hyperplastic lymphoid follicles disclosing vascularization, and necrosis are not present in the Kuttner tumor.[10]

In conclusion, all the evidence leads to the hypothesis that the Kuttner tumor is much more a degenerative disease following an immunological reaction than a preneoplastic lesion.

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