Submandibular Gland Sialolithiasis
—Sialographic and Pathologic Findings with Evaluation Using SEM and EPMA Analysis—

by

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Introduction

Submandibular gland sialolithiasis is a common disease treated by oral surgery, and many case reports have been presented in the Japanese medical literature[1-31]. However, the observation of salivary stones by scanning electron microscopy and electron probe X-ray microanalysis has not been widely reported[25-31].

Recently, we treated four cases of submandibular gland sialolithiasis by removal of the submandibular gland, and examined these stones with a scanning electron microscope (SEM) and electron probe X-ray microanalyzer (EPMA), as well as performing standard clinical sialographic and histopathologic examinations of the extirpated glands.

In the present report, we compare our findings from these various methods of examination.

Report of cases

The clinical aspects of the four cases are summarized in Table 1.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age</th>
<th>Chief Complaint</th>
<th>History</th>
<th>Local status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>36</td>
<td>Swelling of left submandibular region</td>
<td>Swelling of left submandibular region since 23 years previously intra-ductal stones (removed twice)</td>
<td>Swelling of left submandibular region and mouth floor and pus discharge</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>30</td>
<td>Swelling and pain in right submandibular region</td>
<td>Swelling and pain in right submandibular region at mealtimes since 4 years previously</td>
<td>Swelling of right submandibular region and salivary colic</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>36</td>
<td>Swelling and pain in right submandibular region</td>
<td>Swelling and pain in right submandibular region at mealtimes since 3 months previously</td>
<td>Salivary flow rate subnormal and delayed</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>52</td>
<td>Swelling of left submandibular region</td>
<td>Swelling of left submandibular region since 1.5 months previously</td>
<td>Swelling of right submandibular region and little salivary flow Salivary flow rate subnormal and delayed</td>
</tr>
</tbody>
</table>

In all cases, the chief complaint was swelling of the submandibular region. Palpation revealed that case 1 (with the longest history of complaint) showed re-
markable hardness of the submandibular gland in comparison with the other three. Although salivary flow from Wharton's duct could not be confirmed in case 1, swelling of the mouth floor and pus discharge were observed. In cases 2 and 3, salivary flow was confirmed upon stimulation with acetic acid, but the quantity and rate of flow seemed to be less than that on the normal side. These two patients complained of swelling of the submandibular region and so-called salivary colic during or after meals. In case 4 there was scarcely any salivary flow on the affected side, and no complaint of swelling or meal-associated pain. In cases 1 and 3, orthopantomograms revealed calculus level with the inferior border of the mandible, while in the other cases the calculus was below the mandibular border (Figs. 1 and 2).

In all four of these cases the salivary stones could not be palpated bi-manually from either the intraoral or extraoral side. Surgical removal of the submandibular gland was therefore chosen as the method of treatment.

_Sialographic findings_

Urografin (60%) was used as a contrast medium, and examination was conducted radiologically at "the ductal phase"[7,34] in all cases.

Case 1: Interruption and irregular outline of the main duct and extensive disappearance of peripheral ducts were observed, so that the gland itself was not apparent (Fig. 3).

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Fig. 1 Orthopantomogram (Case 1)  
The calculus is on the inferior border of the mandible.  

Fig. 2 Orthopantomogram (Case 4)  
The calculus is under the border of the mandible.  

Fig. 3 Sialographic lateral view (Case 1)  

Fig. 4 Sialographic lateral view (Case 2)
Case 2: Poor ductal filling by the contrast medium was observed at the site of calculus, and the main duct inside the gland was slightly dilated. However, the dendriform peripheral ducts were demonstrated clearly (Fig. 4).

Case 3: The contrast medium did not reach as far as the posterior site of the calculus, so that the duct inside the gland was not apparent (Fig. 5).

Case 4: A radiolucent shadow of the main duct was seen at a portion of the calculus, and the main duct inside the gland was dilated moderately. The peripheral ducts clearly demonstrated a dendric pattern (Fig. 6).

Histopathologic findings

In case 1, remarkably thick and fibrous connective tissues were seen around the excretory duct, together with severe inflammatory cell infiltration composed of lymphocytes and plasma cells. Dilation of the intralobular duct and atrophy or disappearance of acini were marked, while these pathologic alterations were not so obvious in the other cases (Figs. 7 and 8). The excretory duct containing the sialolith was dilated. Squamous cell metaplasia and erosion and ulcer formation were occasionally observed in the covering epithelium (Fig. 9).
Gross sialolith specimens

The size of the stone in case 1 was 6 × 3.5 × 3.5 mm; case 2 had 2 pieces measuring 5 × 5 × 3 mm and 6 × 5 × 3 mm; in case 3 the stone measured 10 × 6 × 6 mm and in case 4 it was 2.5 × 5 × 5 mm. The surface of the stone in case 1 was rough in appearance, but in the other 3 cases the stones were smooth. In every case the stone was located in the submandibular duct within the gland parenchyma near the junction of the duct and gland. A lamellar pattern with one core was seen in the cross-sectioned specimens in all cases.

Observation of calculus with SEM

Outer surface structure: Small granular structures were scattered over the surface of the calculus in every case. The surface of the stone in case 1 was relatively rough with abundant tunnel-like fissures (Fig. 10). Relatively homogeneous structures were demonstrated in the other three cases (Fig. 11).

Cross-sectioned structure (Low magnification): In case 1 the stone exhibited abundant fissures and had a pumice-like appearance, with a fairly small core at its center and an irregularly diffuse lamellar structure at the periphery. In cases 2 and 3, the stones had a single, round and relatively large core in the center and a tight lamellar pattern at the periphery (Fig. 12). In case 4, the stone exhibited no lamellar pattern throughout most of the peripheral area despite the presence of an obvious central core, so that it seemed to be homogeneous and structureless (Fig. 13).

High magnification of the lamellar structure: Aspects of the lamellar structures were diverse in each case. Case 1 was rich in crevices among the lamellar areas (Fig. 14). Case 2 showed different structures consisting of alternate layers of closely homogeneous and roughly diffuse areas (Fig. 15). The stone in case 3 was relatively homogeneous but rough in appearance (Fig. 16), while that in case 4 appeared amorphous and structureless (Fig. 17).

Analysis of calculus with EPMA

Line X-ray energy pulse analysis was performed with an electron probe X-ray microanalyzer (Type JXA-5, Nihon Denshi Co.) with a 15-kV acceleration voltage (Mg-10^3 cps, Ca-3 × 10^3 cps, P-3 × 10^4 cps) to determine the distribution of P, Ca
Fig. 10 SEM view Outer surface (Case 1), showing abundant tunnel-like fissures

Fig. 11 SEM view Outer surface (Case 3) showing small scattered granular structures

Fig. 12 SEM view Cross-section (Case 2, low magnification), showing a lamellar pattern

Fig. 13 SEM view Cross-section (Case 4, low magnification)
Fig. 14 SEM view Cross-section (Case 1, high magnification) Crevices are abundant among the lamellar parts.

Fig. 15 SEM view Cross-section (Case 2, high magnification)

Fig. 16 SEM view Cross-section (Case 3, high magnification)

Fig. 17 SEM view Cross-section (Case 4, high magnification)
Fig. 18  EPMA findings (Cases 1 and 2)

Fig. 19  EPMA findings (Cases 3 and 4)
and Mg. P and Ca distributions almost paralleled each other, but their quantities of distribution were uneven in different regions of the stones. However, the distributions of P and Ca in cases 3 and 4 were more uniform compared with those of cases 1 and 2. The levels of Mg in the 4 cases were uneven in each region, and the line distribution of Mg did not always parallel that of either P or Ca (Figs. 18 and 19).

Discussion

Clinical findings

Sialolithiasis is a disease characterized by the formation of calculus inside a salivary duct. Although this condition may arise in any of the salivary glands, there is a tendency for stones to form in Wharton's duct of the submandibular gland[12, 18, 21, 33].

It is claimed that submandibular gland sialolithiasis is more commonly found in males[3, 8, 21], but this view has also been contested[14, 18], and no definitive conclusion has yet been reached.

With regard to age as a factor in sialolithiasis, many reports agree that the greatest incidence occurs between the ages of 20 and 50 years. As to this aspect[8, 14, 18, 21], OTSUKI and UEDA[3] reported that stones lying within the submandibular gland were found in younger age groups. In our present cases, the sex and age incidence corresponded to these general opinions: three of the four patients were in their thirties.

Many reports have indicated that swelling and pain in the submandibular region are the most common symptoms[14, 18, 21], with salivary colic and swelling of the gland at mealtimes also being characteristic of this condition. A decrease of salivary flow is also commonly reported[14, 18].

Among our cases, pain and swelling at mealtime were observed in cases 2 and 3. In these two cases, salivary flow from the diseased side was observed with a delay after stimulation, and the flow rate was less than that on the normal side. We considered these findings to be characteristic of this condition.

The generally accepted forms of treatment are removal of an intra-ductal stone by the intraoral approach, or removal of the submandibular gland for an intra-glandular stone[14, 18, 21]. There are differences of opinion concerning the methods of treatment for stones which lie at the boundary of the duct and the gland. HARANT[14] recommended removal of the submandibular gland if functional recovery of the gland was not expected or if recurrence of inflammation was feared after sialographic examination. On the other hand, SELDIN[32] emphasized that the intra-oral approach for removal of a stone was the treatment of choice in most cases of sialolith, including stones at the duct/gland boundary. SUZA[18] also recommended intra-oral conservative surgery unless dysfunctional salivary flow or recurrent symptoms indicated otherwise.

We have been trying to remove stones at the duct/gland boundary using the intra-oral approach if they are relatively large and palpable from the mouth floor side, irrespective of whether or not functional disorders are indicated. However, if a stone is not palpable from the mouth floor, such as in the four cases reported
herein, we usually perform extra-oral removal of the submandibular gland. Our rationale is that the intra-oral approach does not provide a sufficiently wide operating field in the most posterior region of the mouth floor, so that there is the possibility of injury to the lingual nerve, artery or vein. Furthermore, we have found that roentgenological evidence showing whether or not the stone is level with the mandibular border has not been useful for determination of our operative approach. **Sialographic findings**

Sialography is valuable for the diagnosis and treatment of this condition. However, it has been pointed out that sialograms are influenced not only by the softness and quantity of the contrast medium but also by the stage at which the X-ray is taken. FUKUDA [34] and ARISUE [20], who carried out continuous X-ray imaging during the infusion of 1.2 ml of 76% urografin, reported that sialoliths appeared as ductal radiolucent shadows within excessively dilated ducts. In addition, such findings as structure, interruption and coarseness of the duct as well as sialostasis and homogeneity of the gland were observed. For our cases (except case 3), we used 0.8–1.2 ml of 60% urografin and took the X-rays at the ductal phase. Our finding in case 1 of interruption of peripheral ducts differed from that in cases 2 and 4. From a comparison of the clinical and sialographic findings, we suspect that the long history of the condition in case 1 had caused severe changes in the peripheral ducts. **Histopathologic findings**

Many reports have been published on histopathological studies of the submandibular gland under conditions of sialolithiasis [1, 19, 20]. Pathological changes in the ductal system such as dilation of the duct, and flattening or abrasion of the epithelium, especially at the site of calculus, were remarkable. Changes noted in the glandular system were degeneration and atrophy of the acini, periductal lymphocytic infiltration, fibrosis and fatty degeneration of the stroma. Of our cases showing inflammatory cell infiltration with periductal fibrosis, marked dilation of the intra-lobular duct and acinar atrophy were observed in case 1. However, these changes were not so obvious in the other three cases. We therefore considered that the degree of pathologic change was correlated with the sialographic findings. **SEM findings**

KURACHI et al. [29] categorized the cross-sectional structure of a stone into three parts: a central core, a lamellar portion and an outer surface. He reported that each of the lamellae had almost the same thickness, but that the structure was not uniform in that the layers were composed of alternately close and roughly fused granules. He also stated that the outer surface presented a granular appearance. MORINAGA [28], on the other hand, categorized the cross-sectional structure as follows: (A) a calculus having a relatively small single core and an apparently lamellar structure of concentric layers throughout; (B) a calculus having a relatively large single core and lamellar structure mainly at the periphery; (C) a calculus having multiple cores and otherwise appearing to be an aggregate of calculi; (D) a calculus having an almost uniform structure with an only slightly apparent lamellar pattern, otherwise appearing homogeneous. MORINAGA [28] also reported lamellar structures in which each lamella was not even in thickness, and other lamellar structures in
which lamellae were closely piled upon each other, or roughly piled up showing crevices. In the closely lamellated structures he also observed board-shaped, needle-shaped or globular crystals.

In our present study, the outer surface in case 1 had abundant crevices, while in the other three cases the outer surface was uniform with small granules. The cross-sectional appearance in every case showed some variation in structure and lamellar thickness, so that we were unable to draw any particular conclusions from these observations.

Results of EPMA

A sialolith is composed of about 80% inorganic and about 20% organic substances. The inorganic substances are mainly calcium phosphate (about 70%) and calcium carbonate (about 20%), with very small amounts of metals accounting for the remainder. AZUMA analyzed a sialolith with EPMA and reported that face X-ray energy pulse analysis revealed Ca and P mainly distributed in the peripheral and core regions, while Mg was distributed uniformly throughout the specimen. He also reported that line X-ray energy pulse analysis revealed a parallel distribution of P and Ca, and a correlation of Mg with Ca, even though the quantity was small. HISANO et al. also reported that the distribution of Ca and P was not uniform, but parallel.

Line X-ray energy pulse analysis of the specimens from our cases revealed an almost parallel distribution of Ca and P in all four. However, the distribution of Mg was not always parallel to that of Ca and P. Comparing the results of SEM and EPMA, the stone from case 1 with a rough and lamellar structure abundant with crevices, and that from case 2 in which the lamellar structure had very pronounced layers, both exhibited remarkable variations in the quantity of Ca, P and Mg in each part of the calculus, relative to cases 3 and 4, which exhibited more uniform and homogeneous structures.

Conclusion

Recently, we treated four cases of submandibular gland sialolithiasis by removal of the submandibular gland, and examined these stones by scanning electron microscopy (SEM) and electron probe X-ray microanalysis (EPMA), in order to supplement the clinical sialographic findings and histopathologic findings of the extirpated glands.

The results were as follows:
1. Clinical findings:
   Two 36-year-old males, one 30-year-old male and a 52-year-old female, all complaining chiefly of swelling of the submandibular region.
2. Sialographic findings:
   Sialography with 0.8 to 1.2 ml of 60% urografin revealed stones hindering the flow in the ductal phase, and one case with the longest history of complaint exhibited severe alteration of the peripheral duct.
3. Histopathologic findings:
   Inflammatory cell infiltration with periductal fibrosis, dilation of intra-lobular duct and acinar atrophy were observed, corresponding to the sialographic find-
ings.

4. SEM findings:
   All cases exhibited abundant small granules on the outer surface of the stones.
   Three cases exhibited a lamellar pattern in the cross-sectional structure.

5. EPMA findings:
   Line X-ray energy pulse analysis revealed an almost parallel distribution of Ca and P. Distribution of Mg was not always parallel with that of Ca and P.

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