Histologic features of sublingual gland herniation through the mylohyoid muscle

Hong Chan Kim1*, Hyung Chae Yang1*, Hye Jung Cho2, Kwang Il Nam2

1Department of Otolaryngology-Head and Neck Surgery, Chonnam National University Medical School and Chonnam National University Hospital, Gwangju, South Korea; 2Department of Anatomy, Chonnam National University Medical School, Gwangju, South Korea. *Equal contributors.

Received September 30, 2019; Accepted November 25, 2019; Epub December 1, 2019; Published December 15, 2019

Abstract: Objectives/Hypothesis: The purpose of this study was to document histologic features of the herniated sublingual gland (SLG) and investigate the histologic correlation between herniated SLG and plunging ranula. Methods: One hundred half-heads from 50 adult cadavers (21 females and 29 males) were included in this study. The presence of SLG herniation and the histologic features SLG were analyzed. The histologic features were analyzed according to the part: intraoral, junctional, and herniated parts. Hematoxylin and eosin (H&E), periodic acid Schiff reaction (PAS), and Alcian Blue (pH 2.5) staining were performed. Results: SLG herniation was found in 42 of 100 half-heads. Non-herniated SLG and the intraoral part of the herniated SLG were mainly composed of mucous acini and a few mixed acini. Junctional and herniated parts were mainly composed of serous acini and showed fatty change. PAS and Alcian blue staining showed that both acidic and neutral mucinous acini of junctional and herniated parts were decreased. However, there was no pseudo-epithelium at any site of herniation. Conclusions: The histologic features of herniated SLG are different according the portions. The herniated part showed fatty degeneration and the remaining acini were mainly serous. We cannot confer any correlation between plunging ranula and the herniated part of SLGs.

Keywords: Sublingual gland herniation, ranula, acini

Introduction

The sublingual space is bound by the genioglossus and geniohyoid muscles medially and the mylohyoid muscle inferolaterally. The mylohyoid muscle is a crucial structure forming the floor of the mouth. It separates the sublingual space from the submandibular space [1]. Frequently, there is a dehiscence in the mylohyoid muscle, and the sublingual gland (SLG) may pass through this defect to reach the submandibular space. This is called a sublingual herniation [2].

Salivary glands are made up of secretory acini, forming round secretory units. The acini can be of serous, mucous, or mixed types [3]. The composition of acini is different according to the type of gland. Acini of parotid glands are mainly of the serous type, producing a thin, watery, and amylase-rich fluid. As compared to the parotid gland, the SLGs are of mucous type, producing thick, viscous, and mucin-rich saliva [4]. However, there is no study that analyzed the histologic features or secretory characteristics of the acini in a herniated SLG.

Previously, the authors have reported the prevalence of sublingual herniation in Korean cadavers. However, we did not also study its histologic features [5]. Therefore, in this study, we tried to analyze the histologic features of SLG. The histologic, morphologic, and secretory functions of the herniated SLG were analyzed. In addition, we investigated the presence of a pseudocyst in the herniated part, which is the critical histologic feature of a ranula.

Materials and methods

Donors

The study was performed following the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). Specimens ac-
Histology of sublingual gland herniation

A total of 100 half-heads from 50 adult cadavers (21 females and 29 males) were included in the study. Herniation was found in 29 (58%) of the 50 cadavers or 42 of the 100 half-heads. The mean age of the donors was 71.9 ± 15.7 years. These cadavers had been donated to the Chonnam National University Medical School for research and education in human anatomy, and the university ethics committee had approved of their use for research.

**Histologic analysis**

The SLGs were divided into two groups: non-herniated SLGs (Group A, Figure 1) and herniated SLGs (Group B, Figure 2). In addition, the SLGs in Group B were divided into three parts: intraoral part (Panel C and D of Figure 2), junctional part (Panel E of Figure 2), and herniated part (Panel F of Figure 2).

The donated cadavers had been fixed by arterial perfusion in 10% v/v formalin solution. From each of the cadaveric heads, we prepared tissue blocks of the SLGs. After performing the routine procedures involved in paraffin-embedded histology, serial sections were prepared and stained with hematoxylin and eosin (H&E) to identify whether they were composed of mucinous acini or serous acini. In addition, sections were stained with periodic acid Schiff reaction (PAS), and Alcian Blue to identify the characteristics of mucin.

For microscopic analysis, a digital image-capture computer system (Aeperio CS2, Leica, Wetzlar, Germany) and a binocular microscope (DM3000, Leica, Wetzlar, Germany) with a 20× objective were used. The images were analyzed using appropriate software (iSolution FL/Auto, IMT i-solution Inc, Daejon, Korea).

**Statistics**

Continuous variables such as age and the ratio of the dyed area are summarized as mean ± standard deviation. Categorical variables such as sex are summarized as proportions. The independent sample t-test was used to compare continuous variables, and Chi-squared tests were used to analyze categorical variables. Statistical analysis was performed using SPSS ver. 20.0 (IBM Corp, Armonk, NY, USA). Two-sided p values < 0.05 were considered significant.
Results

H&E stain

Staining patterns of acini were different according to the type of acini. Mucous acini had abundant cytoplasm with clear mucin and well-rounded basal nuclei, and they were arranged around empty lumina. In mixed acini, serous demilunes were formed around mucous acini. Serous acini contained distinctly eosinophilic zymogen granules in their cytoplasm, and their nuclei were spherical in appearance [4].

In non-herniated SLG, a large number of mucous acini and a small number of mixed acini were detected, whereas serous acini were rarely found. In addition, there were no histologic differences in the H&E stained sections of the non-herniated SLG according to parts (Figure 1). However, herniated SLGs demonstrated different cytologic features according to the part. The intraoral part was mainly composed of mucous acini and mixed acini. On the other hand, the junctional and herniated parts showed fatty degeneration of mucous acini. They were composed of mainly serous acini (Figure 2).

Characteristics of mucin

In addition to H&E stain, we performed Alcian blue and PAS staining to evaluate the characteristics of mucin. The ratio of stained area in non-herniated SLG was 64.0 ± 18.0% by PAS (Figure 3; Table 1), and 49.3 ± 16.5% by Alcian blue (Figure 4; Table 1). The characteristics of mucin were similar in the non-herniated SLG and intraoral part of herniated SLG. However, the staining pattern of the junctional and herniated parts of herniated SLG were statistically different from that of non-herniated SLG (Figures 3, 4; P = 0.003, 0.018 and P < 0.001, P < 0.001 respectively). Both acidic and neutral mucin containing acini were decreased in the herniated part.

Discussion

The SLGs are the smallest, the most diffuse, and the only unencapsulated major salivary gland. They are located beneath the membrane of the floor of the mouth, anterior to the submandibular gland [6]. They are not covered by a capsule and are therefore more dispersed throughout the surrounding tissue. They produce only 3–5% of the total salivary volume. These glands secrete more viscous saliva that contains mainly mucus [7].
Histology of sublingual gland herniation

Figure 3. Periodic Acid Schiff (PAS) reaction of the herniated SLG. PAS staining of the sublingual gland (SLG) was performed: the non-herniated SLG (A) and the herniated SLG (B: intraoral, C: junction, D: herniation). PAS staining showed a strong magenta color reaction with neutral mucin present in the mucous cells in the non-herniated SLG and the intraoral part of the herniated SLG. However, a gradual decrease in the junctional and herniated parts of the SLG was revealed. Magnification, 20×.

SLG herniation is a common condition. In a European cadaveric study, mylohyoid herniation was found in 45 out of 100 adult cadavers, and 25 of these herniations were palpable [8]. In a Korean cadaveric study, the incidence of herniations was similar to those reported in European studies. Forty-two out of 100 SLGs were herniated and showed a female predominance. Of the total herniations, 63% were found in the anterior portion of the mylohyoid muscle. In addition, 86% of the herniations were of the marginal type that slip between the mandible and the mylohyoid muscle [5]. In addition, the previous study found that the gross features of herniated SLGs included smaller size and lesser weight compared with the non-herniated SLGs [5]. However, there were no reports on the histologic features of herniated SLGs.

In this study, we analyzed the histologic features of herniated SLGs. The herniated SLGs show histologic features that differ according to the part. The intraoral part of herniated SLGs was histologically similar to that of the non-herniated SLG. However, the junctional and herniated parts of herniated SLGs were different from that of the non-herniated SLG. On H&E staining, the ratio of mucous acini was decreased and the ratio of serous acini was markedly increased in the junction and herniated parts. This was due to fatty degeneration in mucous glands.

We supposed that a degenerative change of mucous acini resulted from the pressure exerted by the mylohyoid muscle. The mylohyoid muscle pressed on the herniated part of the SLG. Subsequently, it may have caused damage to the duct and nerve supply of the herniated portion of the SLG. Several studies have indirectly shown the possibility of this phenomenon. Harrison et al. reported that salivary gland atrophy was induced by ligation of the excretory duct and the nerve in the excretory duct, together [9, 10]. Takashi et al. also demonstrated that chorda tympani resection induced atrophy of the acinar cells with a reduction in cell size [11].

Additional PAS and Alcian blue staining were performed to identify the cause of the decrease in mucous acini. Both stains can identify the nature of mucin. PAS and Alcian blue staining can differentiate acidic mucin from neutral mucin. Acidic mucin was stained with the Alcian blue technique, and neutral mucin was stained by the PAS reaction [12]. Special stains showed that the junctional and herniated parts were significantly different in terms of acid and neutral mucin secretion, when compared to those of the non-herniated SLG and the intraoral part of the herniated SLG. Special staining showed that degenerative changes were present in both acidic and neutral mucin-secreting acini.

Some authors have reported a relationship between SLG herniation and plunging ranula [13]. A plunging ranula is a mucous extravasation pseudocyst arising from the SLG that
Histology of sublingual gland herniation

Table 1. Comparison of PAS and Alcian blue stained areas according to the part of sublingual gland

<table>
<thead>
<tr>
<th></th>
<th>Non-herniated SLG</th>
<th>Herniated SLG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intraoral part</td>
<td>Junctional part</td>
</tr>
<tr>
<td>PAS (%)</td>
<td>64.0 ± 18.0</td>
<td>56.8 ± 18.9</td>
</tr>
<tr>
<td>(p = 0.194)†</td>
<td>(p = 0.003)†</td>
<td>(p &lt; 0.001)†</td>
</tr>
<tr>
<td>Alcian blue (%)</td>
<td>49.3 ± 16.5</td>
<td>49.4 ± 20.7</td>
</tr>
<tr>
<td>(p = 0.982)†</td>
<td>(p = 0.018)†</td>
<td>(p &lt; 0.001)†</td>
</tr>
</tbody>
</table>

*SLG: sublingual gland; ±: standard deviations; PAS: Periodic acid-Schiff. †Student’s t-test was used to analyze the histologic difference between the parts.

Figure 4. Alcian blue staining of the herniated sublingual gland (SLG). Alcian blue staining was performed on the non-herniated SLG (A, SLG) and herniated SLG (B: intraoral, C: junction, D: herniation). Alcian blue staining showed a blue color reaction with acidic mucin present in the mucous cells. The ratio of the stained areas was similar in the non-herniated SLG and intraoral part of herniated SLG. However, the staining patterns of the junctional part and the herniated part of herniated SLG were statistically different from those of the non-herniated SLG. Magnification, 20×.

Conclusion

The main histologic feature of the herniated part of the SLG was degenerative change of mucous acini. The degenerative change affected both acidic and neutral acini similarly. However, no pseudo-epithelium was found in the herniated part. We cannot infer a correlation between the plunging ranula and herniated SLG.

Acknowledgements

HC Kim was supported by the Bio & Medical Technology Development Program of National Research Foundation of Korea (NRF) funded by the Korean government MSIT (NRF-2019M3E5D1A02-067955). HC Yang was supported by the Bio & Medical Technology Development Program of National Research Foundation of Korea (NRF) funded by the Korean government MSIT (NRF-2017M3A9E8023021) and a grant (BCRI-18020) of Chonnam National University Hospital Biomedical Research Institute.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kwang Il Nam, Department of Anatomy, Chonnam National University Medical School, Gwangju 61469, South Korea. Tel: +82-61-379-2704; Fax: +82-61-375-5834; E-mail: atlas@jnu.ac.kr
References


