

# Efficacy of Minor Salivary Glands as Indicators of Depth of Resection for Superficial Hypopharyngeal Carcinoma

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## Abstract

**Objective.** This study aimed to investigate the utility of minor salivary glands in the hypopharynx as novel indicators for safe resection of superficial hypopharyngeal carcinomas with fewer complications.

**Study Design.** Cadaveric study.

**Setting.** Cadavers were stored in the pathology laboratory at Kansai Medical University.

**Methods.** Twenty-three cadaveric specimens were examined for minor salivary glands in the pyriform sinus, posterior wall, and postcricoid regions of the hypopharynx. Their count, size, and depth were assessed. Resected specimens from 5 consecutive patients with superficial hypopharyngeal carcinomas were pathologically analyzed to determine the positional relationship between cancer and minor salivary glands.

**Results.** Minor salivary glands were present in more than 70% of patients in each region during autopsy, with the postcricoid region having a larger count and size. The glands were universally present, regardless of sex, height, or body mass index. Minor salivary glands in the pyriform sinus and postcricoid region were present at a depth of 30% from the bottom of the submucosal layer, whereas those in the posterior wall were present in the shallow muscularis. During surgery, endoscopic findings revealed minor salivary glands as small white nodules in the submucosal layer. Pathological examination of the resected specimen confirmed that the white nodule was a minor salivary gland. In addition, tumor position in relation to minor salivary glands provided an adequate margin for resection.

**Conclusion.** Minor salivary glands may serve as reliable indicators for determining adequate deep safety margins during surgery for superficial hypopharyngeal carcinoma.

## Keywords

autopsy, hypopharynx, margins of excision, salivary glands, minor, squamous cell carcinoma of head and neck

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Minimally invasive surgical procedures, such as endoscopic laryngopharyngeal surgery (ELPS), transoral robotic surgery, and transoral video surgery, have been used to treat superficial pharyngeal cancer. These surgeries are less invasive than lateral pharyngeal incisions or radiotherapy and have been recognized as valuable methods.<sup>1</sup> However, complications associated with surgical resection include suffocation due to laryngeal edema, hemorrhage due to vascular injury, superior laryngeal nerve injury, subcutaneous emphysema, neck abscess due to pharyngeal perforation, stenosis, and vocal cord movement disorders due to scarring.<sup>2,3</sup>

In a previous study, areas with less intense staining with Lugol's solution were resected<sup>4</sup> to provide a wide safety zone for resection of superficial pharyngeal carcinoma. Preoperative narrowband imaging has been used to predict invasion,<sup>5,6</sup> and artificial intelligence has been used to evaluate invasion.<sup>7</sup> However, no established method has been proposed to ensure adequate depth of resection margins during surgery.

Even when grossly resected lesions appear adequately resected, positive margins may sometimes require additional resection or radiation therapy. Therefore, ensuring

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adequate depth of resection of superficial carcinoma is essential, confirming that the submucosal layer has been adequately resected and promoting improvement in minimally invasive surgeries.

Based on our experience with surgery and histological examination of superficial carcinomas of the hypopharynx, we noticed the presence of minor salivary glands within the mucosa. We also found that these glands were located just above the muscularis muscle and were preserved during surgery.

Based on our findings, we postulated that the minor salivary glands in the hypopharyngeal mucosa might indicate an adequate depth of resection of superficial cancers. The primary aim of this study was to investigate the distribution of minor salivary glands in the hypopharyngeal mucosa using autopsy specimens and examine individual differences. The secondary aim was to determine whether minor salivary glands were valuable as novel indicators of the depth of safety margin during surgery in patients with superficial hypopharyngeal carcinomas.

## Methods

### Ethical Approval

This study was approved by the Institutional Review Board of the Kansai Medical University Ethics Commission (No. 2023034). All procedures involving human participants in this study were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any animal studies conducted by any authors. Informed consent was obtained from all participants included in the study.

### Autopsy Study

Among the 38 autopsy specimens subjected to autopsy at our clinic between January 2018 and March 2022, we included 23 autopsy specimens with no concomitant

head and neck cancers and a preserved hypopharynx (Supplemental Figure S1, available online).

The cadavers were stored in 20% neutral buffered formalin. The hypopharynx was excised from all specimens and sectioned into the following subsections: pyriform sinus (PS), posterior wall (PW), and postcricoid (PC). The specimens were cut at 3 to 4-mm intervals, embedded in paraffin, cut into 4- $\mu$ m-thick sections, and stained with hematoxylin and eosin.

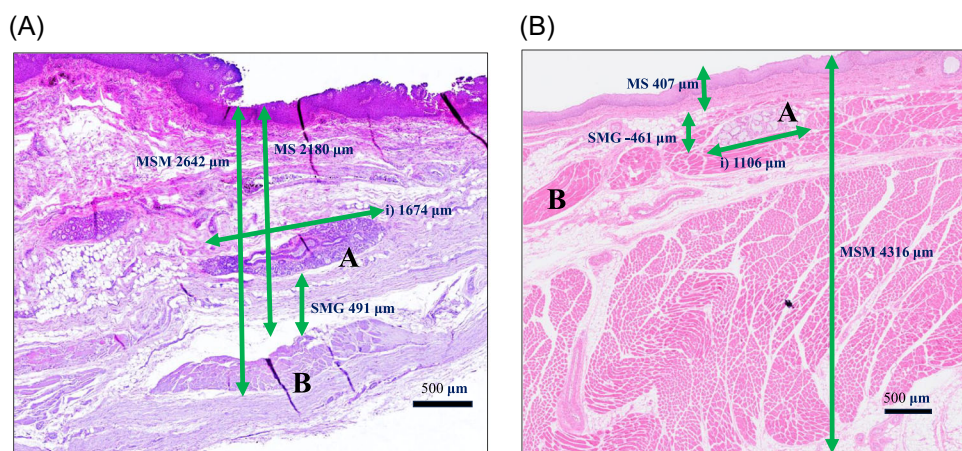
The number and width of minor salivary glands were measured for each subregion. A singular mass in the minor salivary glands was measured as a single unit. The thickness of the mucosa, submucosa (MS), and entire mucosa, consisting of the mucosa, submucosa, and muscularis (MSM), and the distance from the submucosa to the salivary glands (SMG) were measured (**Figure 1**).

Differences in the counts and size of glands according to sex and body size (height and body mass index [BMI]) were assessed to measure individual differences in minor salivary glands. The patients were divided into 2 groups according to their mean height and BMI, 1.62 m and 22.7 kg/m<sup>2</sup>, respectively.

### Patient Study

ELPS was performed in 5 consecutive patients (with 5 lesions) from January to August 2023 after the autopsy study. All patients were diagnosed with superficial carcinoma of the hypopharynx through endoscopic examination and radiological assessment before the procedure.

ELPS was performed with the patient lying supine under general anesthesia with oral or nasal endotracheal intubation. The larynx was elevated using a SATOU'S curved laryngopharyngeal scope (Nagashima Medical Instrument Corp.) to ensure adequate view and working space in the pharynx. Upon visualization of the lesion, the tumor edge was marked by chromoendoscopy with 1% Lugol staining, using a single-channel upper gastrointestinal endoscope (GIF-EZ1500; Olympus Medical Systems



**Figure 1.** (A and B) Show histopathological image of the hypopharyngeal mucosa in PS and PW. A, minor salivary gland; B, muscularis; MS, mucosa and submucosa; MSM, mucosa, submucosa, and muscularis; PS, pyriform sinus; PW, posterior wall; SMG, from the submucosa to the salivary glands.

Corp.). The procedure up to this point was similar to that for endoscopic submucosal dissection of the esophagus and stomach.

The endoscopist delineated the width of the lesion and marked the area to be resected using a DualKnife (KD-655L; Olympus Co.). The tumor margin was then resected, and the submucosal layer was trimmed and dissected using a high-frequency generator (VIO 200D or VIO-3; ERBE Elektromedizin GmbH) until the lesion could be grasped using forceps. Subsequently, the submucosal layer was dissected using an electric needle knife (KD-600; Olympus Medical Systems) and traction forceps. A mixture of normal saline and hyaluronic acid-containing indigo carmine was used for the submucosal epinephrine injection. After resection, polyglycolic acid sheets were placed over the ulcer and fixed using fibrin glue containing thrombin.

All patients were classified according to the TNM classification of Malignant Tumours (American Joint Committee on Cancer/International Union Against Cancer 8th).

### Statistical Analysis

The OlyVia (EVIDENT) software was used for microscopic examination, enabling the inspection and measurement of tissue sections. Student's *t* test was used to compare between 2 groups, and the analysis of variance method was used to compare between 3 groups. The Bonferroni method was used to adjust for the significance level of the multiple comparisons. EZR software version 1.54 (Saitama Medical Center, Jichi Medical University) was used for statistical analysis. The threshold for statistical significance was set at  $P < .05$ .

## Results

### Anatomical Distribution of the Salivary Glands in the Pharynx

There were 23 cadavers (16 male and 7 female cadavers) with a median age of 70 years (**Table 1**). Minor salivary glands were found in the PS in 16 (70%), in the PW in 19 (83%), and in the PC in 18 (78%) cadavers. The mean values were 1.7 (standard deviation [SD], 1.5) units in the PS, 1.7 (SD, 1.4) units in the PW, and 2.6 (SD, 1.6) units in the PC for each subsites. The average size of the minor salivary glands was 1694  $\mu\text{m}$  (SD, 603  $\mu\text{m}$ ) in the PS, 1351  $\mu\text{m}$  (SD, 469  $\mu\text{m}$ ) in the PW, and 4765  $\mu\text{m}$  (SD, 1892  $\mu\text{m}$ ) in the PC. The minor salivary glands in the PC were larger ( $P < .001$ ) than those in the PS and PW (**Figure 2**).

Upon examination of men and women, no significant differences were found in the number of units or sizes. Height and BMI were also analyzed; however, the differences were not significant except for that in the number of units in the PW (**Figure 3**).

We assessed mucosal thickness. The average thicknesses across all layers, including the MSM, were 3579  $\mu\text{m}$  (SD, 844  $\mu\text{m}$ ) in the PS, 4959  $\mu\text{m}$  (SD, 1139  $\mu\text{m}$ ) in the PW, and 3751  $\mu\text{m}$  (SD, 961  $\mu\text{m}$ ) in the PC. The PW was thicker than the PS and PC ( $P < .001$ ) (**Figure 4A**). The average thickness in the MS was 2435  $\mu\text{m}$  (SD, 941  $\mu\text{m}$ ) in the PS, 1215  $\mu\text{m}$  (SD, 588  $\mu\text{m}$ ) in the PW, and 3465  $\mu\text{m}$  (SD, 1117  $\mu\text{m}$ ) in the PC, with the PC being the thickest ( $P < .001$ ) and the PW, the thinnest ( $P < .001$ ) (**Figure 4B**).

The average SMG distance was 686  $\mu\text{m}$  (SD, 377  $\mu\text{m}$ ) in the PS, -66  $\mu\text{m}$  (SD, 517  $\mu\text{m}$ ) in the PW, and 1019  $\mu\text{m}$  (SD, 554  $\mu\text{m}$ ) in the PC. That is, in the PW, the minor salivary glands were located within the muscularis, deeper than the submucosal layer (hence labeled negatively) and significantly deeper than the PC and PS ( $P < .001$ ) (**Figure 4C**). Dividing the SMG by the MS provides the percentage of minor salivary gland depth in the MS. The average percentage was 30% (SD, 20.9) in the PS, -20% (SD, 59.9) in the PW, and 30% (SD, 11.2) in the PC. Subtracting the MS from the MSM provides an approximate thickness of the muscularis, whereas the PW has a thicker muscle layer (Supplemental Figure S2, available online).

Upon examining the mucosa separately based on sex, height, and BMI, although most groups did not show significant differences, the PW was thicker ( $P < .05$ ) in the taller group. Additionally, the mucosa of the PC was thicker ( $P < .05$ ) in the group with a higher BMI (**Figure 5**).

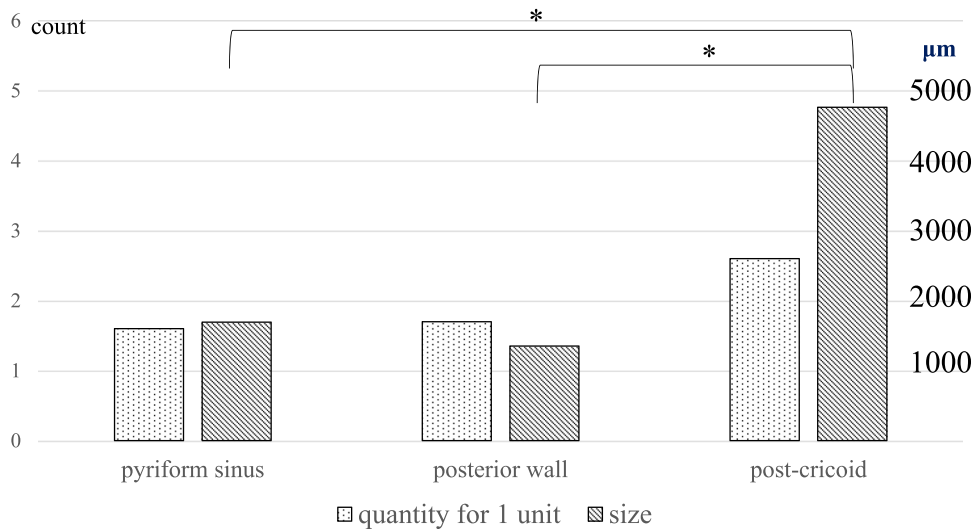
### ELPS Findings and the Safety of the Depth of Resection Margin in the Pharynx

In the hypopharynx, the minor salivary gland was located in the submucosal layer of the superficial carcinoma (**Figure 6**). During ELPS, small white

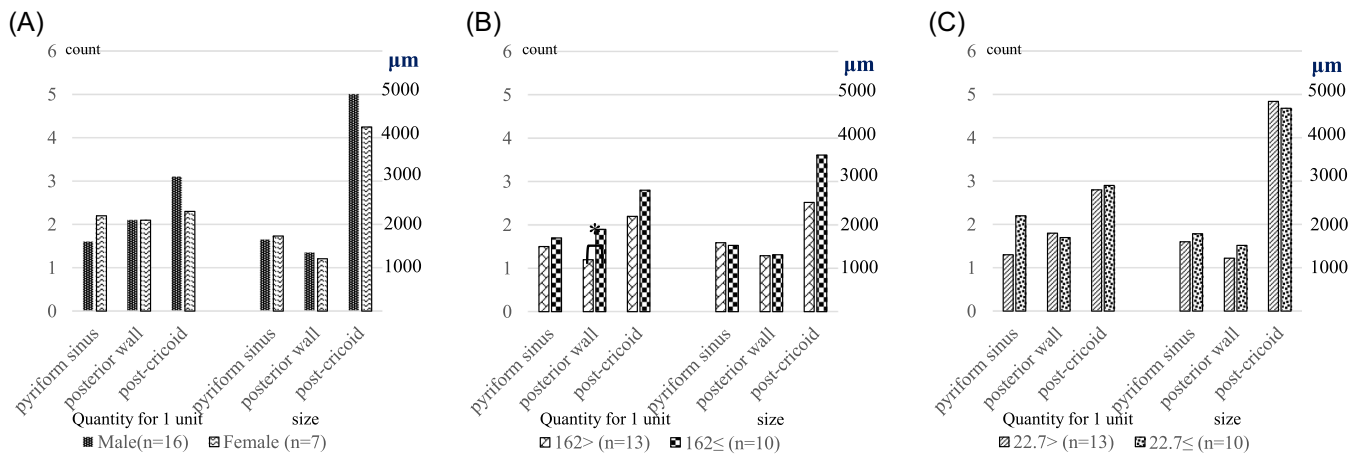
**Table 1.** Characteristics of the Autopsy Patients

Characteristics of the autopsy patients	
Number	23
Age	70 years (SD, 13.0)
Sex	
Male	16
Female	7
Height	
Male	1.65 m (SD, 0.63)
Female	1.55 m (SD, 0.87)
Body weight	
Male	60 kg (SD, 13.3)
Female	63 kg (SD, 16.4)
BMI	
Male	21.3 kg/m <sup>2</sup> (SD, 4.8)
Female	25.2 kg/m <sup>2</sup> (SD, 5.9)

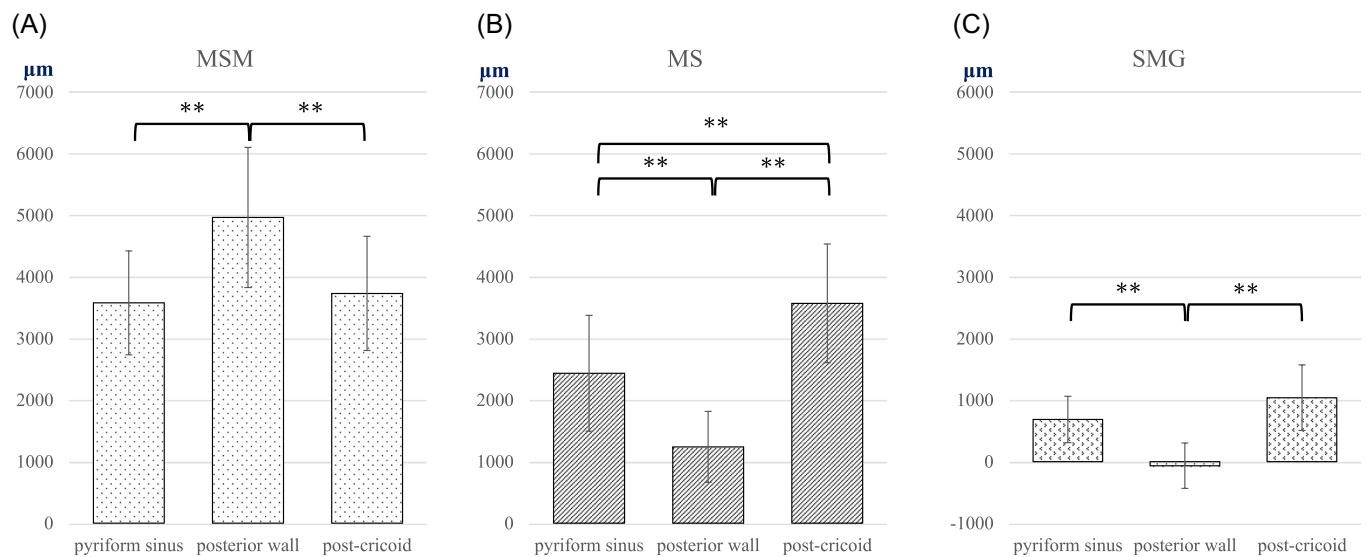
Abbreviations: BMI, body mass index; SD, standard deviation.



**Figure 2.** Quantity and size of minor salivary glands in each subregion of the hypopharynx. **\*\*P < .001.**

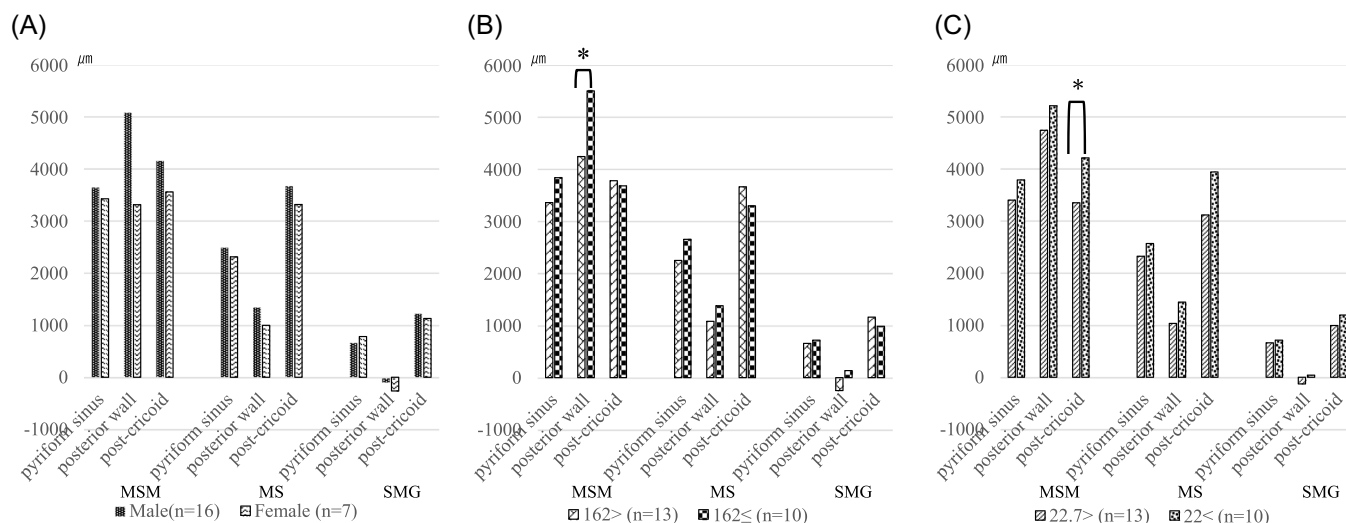


**Figure 3.** (A–C) Show the comparison of the number and size of minor salivary glands in male and female, short and tall and people with low and high BMI. BMI, body mass index, **\*P < .05.**

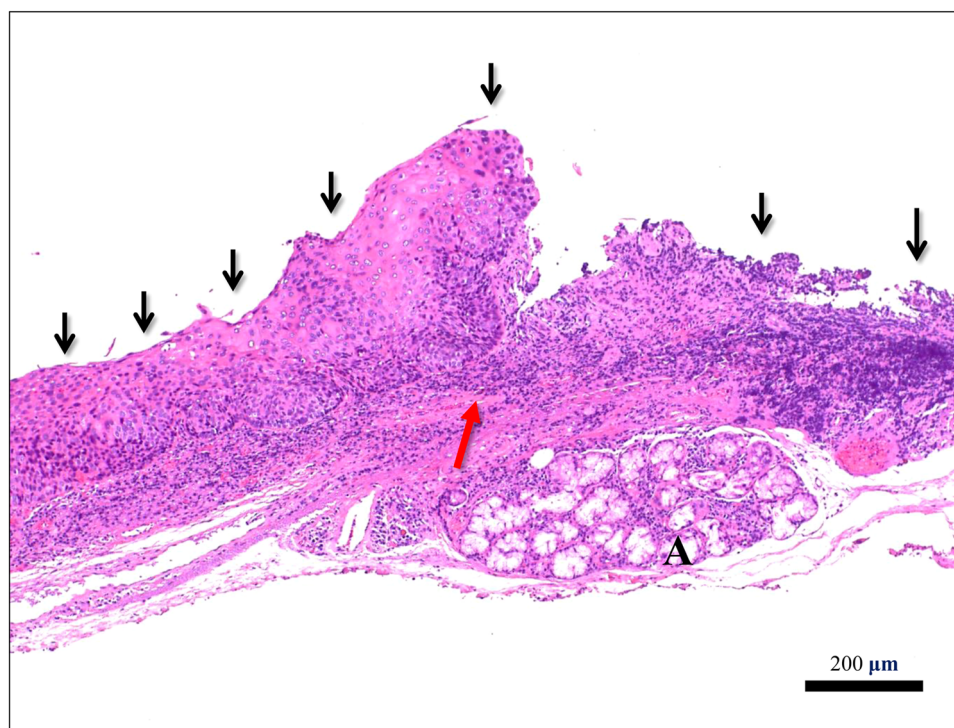


**Figure 4.** (A–C) Show the thickness of the hypopharyngeal mucosa in MSM, MS, and SMG. MS, mucosa and submucosa; MSM, mucosa, submucosa, and muscularis; SMG, from the submucosa to the salivary glands, **\*\*P < .001.**





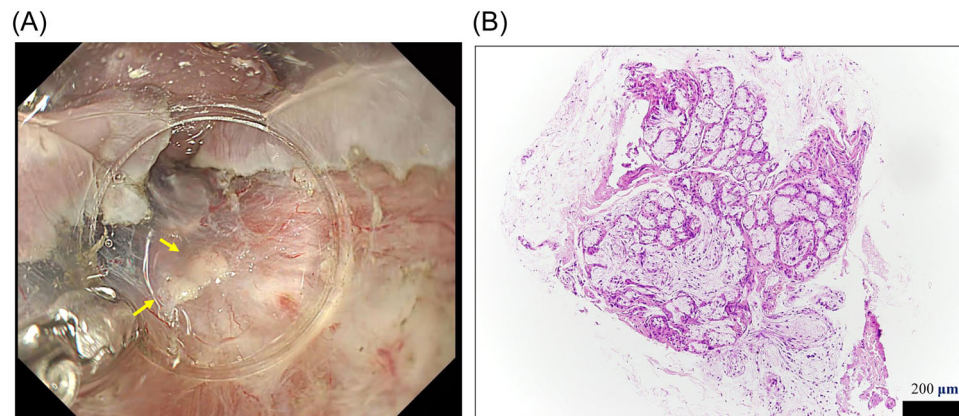
**Figure 5.** (A–C) Show the location of the mucosa and minor salivary glands in male and female, short and tall and people with low and high BMI. BMI, body mass index; MS, mucosa and submucosa; MSM, mucosa, submucosa, and muscularis; SMG, from the submucosa to the salivary glands, \* $P < .05$ .



**Figure 6.** Pathological findings of the hypopharynx carcinoma. Arrows indicate carcinomas. Red arrow shows an extension of the submucosal layer, but the carcinoma was adequately resected along with minor salivary gland (A).

nodules were observed in the submucosal layer overlying the muscularis (**Figure 7**). Histological examination revealed that these small white nodules were minor salivary glands. When we performed operation in 5 consecutive patients (with 5 lesions) diagnosed with superficial hypopharyngeal carcinoma (**Table 2**), the tumor was resected while carefully observing the minor salivary glands. During surgery, glands were identified in the resection area in 4 of the 5 patients. Histological examination of these 4 patients confirmed

the presence of minor salivary gland tissue, matching the white structure recognized during surgery. Notably, minor salivary glands were present in all 4 patients at a point superficial to the depth of resection margin, and transection could be secured by excision of the minor salivary glands (**Figure 8**). All 5 patients had no history of radiotherapy or local recurrence or metastasis over a follow-up period of at least 10 months after surgery. None of the patients required additional postoperative treatment.



**Figure 7.** Minor salivary glands during resection of superficial carcinoma of the hypopharynx. (A) White structure in the submucosa (yellow arrows). Histological examination of the white structure is shown in (B).

**Table 2.** Patients Who Underwent Endoscopic Laryngopharyngeal Surgery

Patients	
Age	75 (SD, 5.2)
Sex	
Male	5
Female	0
Subsite	
PS	2
PW	2
PC	1
Pathological findings	
Pathological T classification	
p T1	5
Depth of resection margin	
Negative	5
Positive	0
Minor salivary gland	
Present	4
Absent	1

Abbreviations: PC, postcricoid; PS, pyriform sinus; PW, posterior wall; SD, standard deviation.

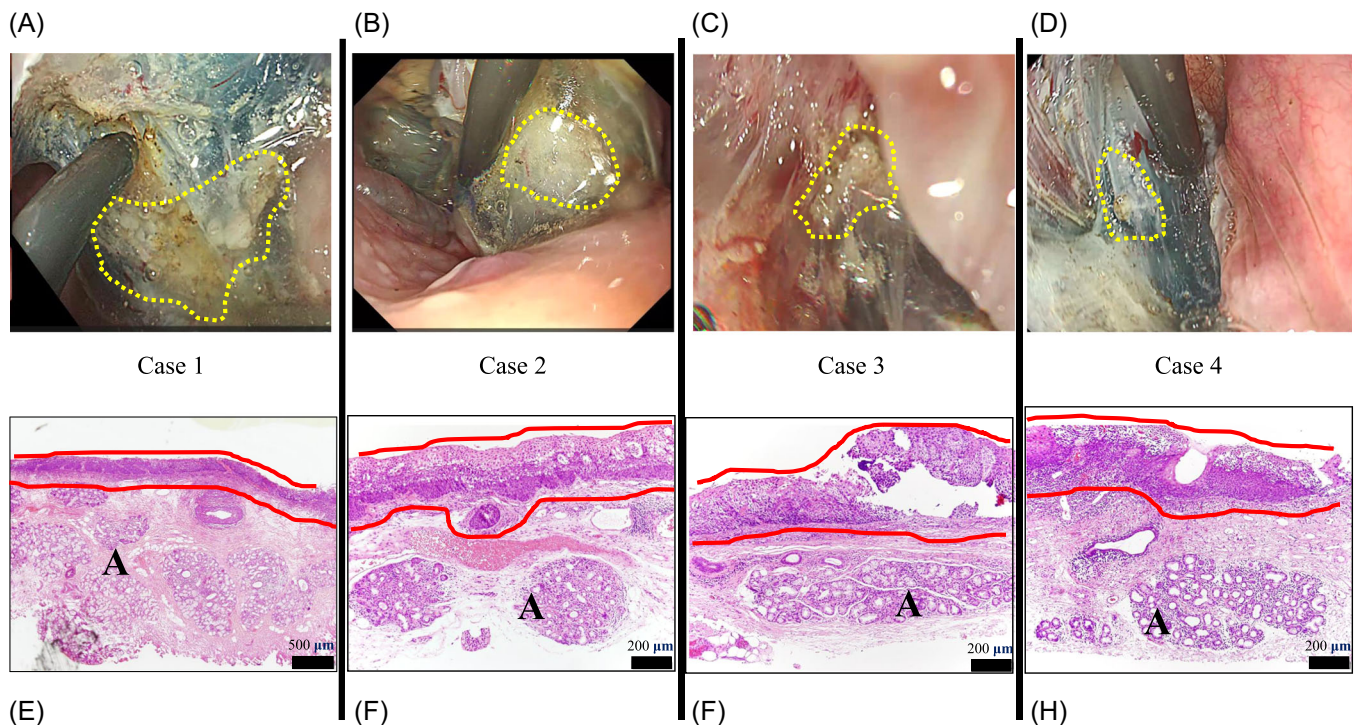
## Discussion

The presence of minor salivary glands was examined across different regions of the hypopharynx, revealing their universal presence in >70% of patients in each region, regardless of sex, height, or BMI. In particular, the PC region had a larger number and larger size of minor salivary glands. A previous study showed a positive correlation between height, weight, and buccal mucosa thickness.<sup>8</sup> However, the present study showed only some significant differences between the PW and the PC in the hypopharynx, indicating that the hypopharyngeal mucosal structure is less likely to be affected by sex, height, or BMI.

In the PS and PC, minor salivary glands were present at a depth of approximately 30% from the depth of the MS. This finding indicates that simultaneous resection, including the minor salivary glands, is an indicator of preservation of the muscularis and resection of most of the submucosa. Conversely, resection of the minor salivary gland in the PW involves resection of the MS and a small portion of the muscularis. Given the thickness of the muscularis layer in the PW, preserving this depth of resection would preserve most of the muscle layer. The PW MSM was the thickest among the components of the hypopharyngeal mucosa and the thinnest in the MS, reflecting the thickness of the PW muscularis, which included the contractile muscles of the hypopharynx. A previous examination of serial whole-organ sections of the hypopharynx and larynx in 3 patients showed relatively large salivary glands in the PC.<sup>9</sup> These large salivary glands may have differentiated the PC from the other 2 regions (PS and PW), potentially reflecting the thickness of the MS.

Finally, ELPS and histological examination revealed that the small white nodules in the submucosal layer were minor salivary glands, which might be valuable novel depth indicators for determining the surgical depth of resection margin in the hypopharynx. Detecting malignancies at an early stage, making appropriate pretreatment diagnoses, and performing endoscopic submucosal resection (ESD) are crucial.<sup>10</sup> It has been emphasized that, for resection of superficial carcinomas, ensuring a secure resection of the submucosal layer is essential, and controlling the depth of the resection is vital to reduce the risk of complications.<sup>11</sup> The surgical approach for superficial hypopharynx carcinomas involves ESD techniques and management of the depth of resection. Regarding ELPS, the submucosal layer was targeted for excision during dissection. As minor salivary glands were found on the resection surface, we considered that the depth of resection could be adjusted. Resection that included the minor salivary glands revealed that the mucosal and





**Figure 8.** (A–D) A white nodule surrounded by a yellow dotted line. Pathological findings are shown from (E–H) (The red lines show areas of carcinoma). A: minor salivary gland.

submucosal layers could be resected. In 4 out of 5 patients (80%), minor salivary glands were identified intraoperatively with the endoscope and in the resected specimen. All patients had negative depth of resection margins that coincided with the inferior surfaces of minor salivary glands.

The findings of the current study open new avenues for improving surgical outcomes and reducing complications associated with superficial carcinoma of the hypopharynx.

This study has some limitations. First, although 23 autopsy specimens were considered sufficient, the number of surgical cases was only 5. The data presented by the small number of cases were limited; further accumulation of cases is required. Second, the study was conducted at a single institution; therefore, further evaluation using surgical techniques other than ELPS (eg, transoral robotic surgery or transoral video surgery) is required. This study highlights the potential of using minor salivary glands as depth markers during the surgical resection of superficial hypopharyngeal carcinomas, enhancing the precision of resection margins and reducing complications. These findings can be integrated into surgical education and training, improving clinical outcomes and standard care practices.

In conclusion, submucosal resection, including resection of the minor salivary glands, allowed for resection of most of the submucosal layer. This procedure might be a valuable indicator of adequate resection of the

submucosal layer during endoscopic surgery for superficial hypopharyngeal carcinoma.

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### Author Contributions

**Tomofumi Sakagami**, conceptualization, methodology, investigation, writing—original draft; **Yuri Noda**, writing—review and editing; **Naohiro Nakamura**, investigation; **Kensuke Suzuki**, Investigation; **Takuo Fujisawa**, investigation; **Masao Yagi**, data curation; **Hiroto Kawasaki**, support for specimen preparation; **Hiromasa Egawa**, support for specimen preparation; **Wataru Sumita**, support for specimen preparation; **Koji Tsuta**, supervision; **Hiroshi Iwai**, project administration.

### Disclosure

**Competing interests:** Dr Tsuta has received research funding from Ono Pharmaceutical Co., Ltd. and personal fees from MSD, Roche, Novartis, Janssen Pharmaceutical K.K., and AstraZeneca. The other authors declare no conflicts of interest.

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### Data Availability Statement

All data relevant to the study have been included in the article or uploaded as Supplementary Information.

## Supplemental Material

Additional supporting information is available in the online version of the article.

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