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Title: Observation of Arytenoid Movement during Laryngeal Elevation Using Videoendoscopic Evaluation of Swallowing

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Keywords: deglutition; arytenoid movement; aspiration; swallowing reflex; videoendoscopic evaluation of swallowing; deglutition disorders.

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List of responses to the comments of reviewer #1

1. We rewrote the purpose of the present study in the section of the introduction as well as abstract. The sentence mentioned aspiration was deleted in accordance with your advice.

2. We deleted the sentence mentioned aspiration in the conclusion, and rewrote the conclusion in accordance with your advice.

3. We added some literatures about airway protection using VF including the paper written by Kendall et al, and comparison of VF and VE.

4. We added methods for selection of participants.

5. The methods section was reorganized, and some unclear sentences were revised in accordance with your advice. We indicated the total number of swallows and number of pictures per swallow.

6. In the results section, we made clear the number of subjects that did not provide successful pictures. Since Figure 3 was unclear, we redrew it. Means and the standard deviations were written in the figure.

7. Information about R, Pr, and Po was described in the figure legend of the figure 3.

8. The conclusion section was completely rewritten in line with your suggestion.

9. The entire manuscript was corrected by a native English speaker, Professor David H. Waterbury, who is an English teacher in Kawasaki Medical School.

10. We gave page numbers in the manuscript.
List of responses to the comments of reviewer #2

1. We added the sentence “It should be kept in mind that the presence of endoscope in the pharynx undoubtedly interferes with normal swallowing.” in the discussion section. Additionally, the conclusion section was rewritten in moderate expression.

2. As the limitation of the study, we added the sentence “It must be noted that the variable distance between the tip of the endoscope and the structure to be examined may influence the measurement value.” in the discussion section.
Arytenoid movement during swallowing

Title of paper: Observation of Arytenoid Movement during Laryngeal Elevation Using Videoendoscopic Evaluation of Swallowing

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**Abstract.** The purpose of this study was to confirm that the arytenoid regions dynamically adduct and extend above toward the epiglottis during laryngeal elevation. While 14 healthy volunteers aged 19 to 32 years old swallowed 5ml of white soft yogurt in one gulp without chewing, the movement of the arytenoid regions was observed for videoendoscopic evaluation of swallowing (VE). Each moving image was stored simultaneously on videotape. A cross-sectional area surrounded by the epiglottis and the bilateral arytenoid regions (S) and the length of a straight line passing through the anterior borders of the left and right arytenoid regions (L) were measured. The relative area of the entrance in the laryngeal vestibule was calculated as value \[ S/L^2 \] before the swallowing reflex (resting condition), just before laryngeal closure, and just after laryngeal closure. Value \[ S/L^2 \] was smaller just before epiglottal descent than resting condition, and became smallest just after the epiglottis started to ascend. The mean area narrowed to 30.4 % of the resting area just before epiglottal descent and in the most extreme case to 7.8 % of the resting area. It was demonstrated that the arytenoid regions adducted and extended above toward the epiglottis during laryngeal elevation. The technique used to measure the cross-sectional area of the entrance in the laryngeal vestibule employing VE was an effective analytical procedure.

**Keyword:** deglutition; arytenoid movement; aspiration; swallowing reflex; videoendoscopic evaluation of swallowing; deglutition disorders.
Introduction

The number of patients with dysphagia visiting physiatrists to receive diagnosis and swallowing rehabilitation training has been increasing yearly as Japan’s population ages. The initial diagnostic tests of choice for swallowing function in clinical practice are history taking, physical examination, and imaging modalities. The representative imaging modalities are videofluoroscopic evaluation of swallowing (VF) [1] and videoendoscopic evaluation of swallowing (VE) [2].

VF is particularly useful for confirmation of aspiration during laryngeal closure and for observation of several swallowing dysfunctions continuously from the oral preparative stage to the esophageal stage. Consequently, it has been preferred as the initial diagnostic test of choice and has been regarded as a gold standard for swallowing evaluations. However, it does have some disadvantages: 1) It can only be carried out in X-ray fluoroscopy rooms; 2) It cannot be performed during the acute phase of diseases; 3) Special equipment is required for changing the postures of patients; 4) The examiners are exposed to high-level radiation during frequent repeated VF trials; 5) The X-ray moving images in VF are virtual images, not images observed directly with the naked eye [3]. VE, however, can be carried out anywhere, can be performed during the acute phase of diseases, and involves direct observation with the naked eye [4]. Nevertheless, VE also has some disadvantages: 1) Findings at the instant of laryngeal closure cannot be observed; 2) The laryngoscope that is used during
the examination, in itself, may have some adverse effects on physiological swallowing [5]. Findings such as laryngeal penetration and aspiration are extremely important for patients with dysphagia, because they pose potentially fatal risks. Therefore, in clinical assessments, VE is carried out in combination with VF.

Recently, endoscopic observation of the larynx at the moment of the laryngeal closure has been made possible by progress in the frame step function of video recorders [3, 6]. Furthermore, the pharynx and larynx can now be exposed under direct vision using VE. As a result, the arytenoid regions have been found to move more dynamically than previously thought. Kendall et al., in their VF study, reported that the arytenoid cartilage approximated the epiglottis prior to the arrival of the bolus at the upper esophageal sphincter [7]. Since the precise movement of the arytenoid regions is difficult to distinguish in lateral or frontal VF views, VE should be performed for evaluation of this movement.

The purpose of the present study was to confirm that the arytenoid regions dynamically adduct and extend above toward the epiglottis during laryngeal elevation. A cross-sectional area surrounded by the epiglottis and the bilateral arytenoid regions was measured before, during, and after laryngeal closure using VE in individuals without dysphagia.


**Subjects and Methods**

*Subjects*

Healthy non-smoking volunteers were recruited by patting a notice on campus bulletin boards. Persons with neurological or oropharyngolaryngeal abnormalities were excluded by medical interview and clinical assessment performed by a medical doctor. Fourteen healthy volunteers (10 females and 4 males) aged 19 to 32 years old (mean age: 24.1 +/- 4.6 years old) were selected as subjects of examination. After the ethics committee of the institution approved, the purpose of the study and the procedure were explained in detail and written informed consent was obtained from all subjects.

*Videoendoscopic Evaluation of Swallowing*

The participants were ordered to sit on an upright chair with a headrest, and the hips and knees were flexed at a 90° angle. They were instructed to lay back and to keep their neck straight. The tip of a flexible laryngoscope 4mm in diameter (ENF type 4, Olympus Co.) was passed transnasally into the oropharynx (vallecula epiglottica). The laryngoscope was fixed in a position where the epiglottis, aryepiglottic folds, cuneiform cartilage, and corniculate cartilage appeared symmetrical. Air-spray topical anesthesia was not used to avoid attenuation of the swallowing reflex by flow of the anesthetic into the middle and lower pharynx. If the subjects complained of severe pain in the nasal cavity during insertion of the laryngoscope,
a bit of 2 % lidocaine jelly was placed on the wall of the nasal cavity. Care was taken to ensure that the tip of the laryngoscope was not inserted into the laryngeal vestibule.

The participants were ordered to swallow 5ml of white soft yogurt in one gulp without chewing. The movement of the arytenoid regions was observed endoscopically before the bolus was visualized in the pharynx. Each person ate yogurt five times, and each moving image was stored simultaneously on videotape using a digital video recorder (Mini DV, Panasonic Co.) with a speed of 30 frames/sec.

**Selection of Pictures for Analysis**

The recorded data were transferred to a personal computer (Powerbook G3, Apple Co.) and analyzed using image processing software (I-Movie 2, Apple Co.). Each moving image was replayed using the frame step function, and three pictures were selected per swallow; Picture R: a picture taken at the time when the arytenoid regions abducted maximally before the swallowing reflex (resting condition); picture Pr: a picture taken at the time when the arytenoid regions adducted maximally just before laryngeal closure (whiteout); Picture Po: a picture taken at the time when the arytenoid regions adducted maximally just after whiteout. Picture R was meant to capture an image of the entrance to the laryngeal vestibule at its widest. Pictures Pr and Po were meant to capture an image of the entrance to the laryngeal vestibule at its narrowest. The narrower the area at the entrance is, the greater the arytenoid movement should be. A total of 210 pictures (5 swallows
X 3 pictures X 14 subjects) were selected for analysis.

In the course of the analysis of pictures, attention was paid to two other conditions: 1) that all of the arytenoid regions and the epiglottis fit the confines of a picture and 2) that the bilateral arytenoid regions and the epiglottis were symmetrical. A straight line (line A) passing through the anterior borders of the left and right arytenoid regions was drawn, and its length was measured (L). One perpendicular line (line B) was drawn down from the mid-point of line A, and another perpendicular line (line C) was drawn down from the intersection of the bilateral vocal cords to line A. The distance between the two perpendicular lines (D), which was assumed to be imaging error, was measured (Fig. 1). If value \[D/L \times 100\] was more than 3%, the picture was excluded from the analysis.

Data Analysis

The cross-sectional area surrounded by the epiglottis and bilateral arytenoid regions (S) was measured using image analysis software (NIH-Image, Wayne Rasband Co.). The relative area of the entrance in the laryngeal vestibule was calculated as the ratio of the vestibular cross-sectional area to the square of the length between the arytenoid regions \[S/L^2\] before and during swallowing (Fig. 1). The maximum cross-sectional area for picture R and the minimum cross-sectional areas for pictures Pr and Po were extracted as analytical data for each subject.
One-way repeated-measures analysis of variance (ANOVA) was used to compare the mean relative areas between picture R, picture Pr, and picture Po. Data were expressed as means +/- the standard deviation (SD). Statistical significance was defined as a p-value of less than 0.05.
Results

VE Findings in a Representative Subject

Typical VE images before and during swallowing soft yogurt selected by the frame step function are shown in Figure 2. When food was sent to the vallecula epiglottica, the vallecula seemed to expand laterally. Before the epiglottis started to descend, the arytenoid regions were observed to adduct and extend above toward the epiglottis during laryngeal elevation. The height of the arytenoid regions increased as if to prevent laryngeal penetration. It should be understood that laryngeal elevation in itself was not observed by VE. During maximum elevation of the larynx, the laryngeal vestibule could not be seen because of the epiglottal descent. The phenomenon of white light expanding to fill the entire screen (whiteout) indicated laryngeal closure. Although the epiglottis started to ascend just after the whiteout, the arytenoid regions were observed to still be adducting and stuck firmly to the epiglottis. Food had already been sent downward through the pyriform sinus. After the epiglottis returned to its original state, the arytenoid regions were found to have abducted and shrunk gradually.

Comparison of the Relative Area in Three Pictures

The degree of error calculated from picture R was minimal, and successful pictures were obtained from all the participants. However, it was difficult to maintain the symmetry for pictures Pr and Po during
swallowing. Successful pictures for picture Pr could not be obtained from 3 out of 14 subjects, and we were unable to obtain successful pictures for picture Po from other 3 subjects.

The relative areas (S/L^2) were 0.295 +/- 0.108 for picture R, 0.136 +/- 0.051 for picture Pr, and 0.085 +/- 0.069 for picture Po. The areas of pictures Pr and Po were significantly narrower than that of picture R. Although the area of picture Po had a tendency to become narrower than that of picture Pr, there were no significant differences between the two pictures (Fig. 3). The mean areas of pictures Pr and Po were, respectively 45.4 % (14.1 % - 59.0 %) and 31.1 % (7.9 % - 63.3 %) of that of picture R.
Discussion

Generally, epiglottal descent has been believed to be the most important function for the prevention of laryngeal penetration [8]. In the clinic, therefore, efforts have been made to evaluate not only the existence of laryngeal penetration or aspiration, but also movement of the epiglottis using VF. Although glottal closure and epiglottal descent undoubtedly play important roles, we speculate that arytenoid movement is also deeply involved in the prevention of laryngeal penetration [5, 6]. In the present study, using VE, the arytenoid regions were observed to adduct and extend above toward the epiglottis before epiglottal descent. We also recorded and present actual measured values to show evidence of such movement in the arytenoid regions. The area at the entrance to the laryngeal vestibule was shown to become narrower just before epiglottal descent than prior to swallow onset, and became the narrowest just after the epiglottis started to ascend. The mean area narrowed to about 30 %, and the narrowest value was 7.8 %. It must be noted that the variable distance between the tip of the endoscope and the structure to be examined may influence the measurement value.

With regard to airway protection, Kendall et al. reported that closure of the epiglottis against the arytenoid cartilage occurred as early as 0.30 seconds before the arrival of the bolus at the upper esophageal sphincter and as late as 0.06 seconds after the arrival of the bolus at the sphincter in their younger subject group [7]. Since arytenoid movement is difficult to evaluate by VF alone, VE should be
combined with VF to clarify the mechanism of dysphagia. Some research comparing VF and VE has revealed some disagreement in abnormal findings including laryngeal penetration [9,10]. It should be kept in mind that the presence of endoscope in the pharynx undoubtedly interferes with normal swallowing.

Recent advances in digital video recorders and digital versatile discs (DVDs) have made possible the creation of sharp laryngoscopic images. Although the laryngeal vestibule cannot be seen during epiglottal descent, movement of the epiglottis and arytenoid regions around that time can be analyzed using the frame step function or slow-motion replay. With regard to the timing of glottic closure during swallowing, Ohmae et al. reported that true vocal cord closure occurred mainly after the onset of laryngeal elevation [11]. They also noted that the timing of arytenoid adduction and subsequent arytenoid contact varied greatly, although these events occurred as one of the initial events during swallowing. The precise movement of the arytenoid regions might not be observed just before and after laryngeal closure. Similarly, Shaker et al. investigated the time between the onset of vocal cord adduction and their return to full opening during swallowing [12]. They concluded that vocal cord adduction is the initial event during the swallowing sequence, and that abnormal laryngeal kinetics or lack of coordination between the glottic closure mechanism and oropharyngeal bolus transport may play an important role in swallow-induced aspiration. However, they did not mention that the arytenoid regions remained adducted and stuck to the epiglottis even after the epiglottis started to ascend. Ishii first pointed out greater
movement of the arytenoid regions in his animal study [13]. When the swallowing reflex was evoked by stimulation of the superior laryngeal nerve, the arytenoid regions were found to extend above more greatly than imagined during laryngeal elevation.

The normal arytenoid regions are observed as thick, tall, and vigorous walls even in the resting position. Therefore, they seem to serve as the breakwater of the laryngeal vestibule. Even if epiglottal descent is imperfect, not all patients show laryngeal penetration [14]. In addition, it has been reported by some researchers that liquid bolus can also pass into the pyriform sinus before laryngeal elevation in normal persons [15]. It has therefore been presumed that preservation of the structure of the arytenoid regions and recovery of their function are important challenges for medical rehabilitation in patients with aspiration. It should also be kept in mind that these regions may lose their structure and function not only because of motor paresis but also because of long-term nasogastric tube placement [16]. Since many receptors of the swallowing reflex are assumed to exist near the arytenoid regions, future studies are needed to clarify the precise location of those receptors.

In conclusion, the arytenoid regions were observed to adduct and extend above toward the epiglottis during laryngeal elevation. The technique used to measure the cross-sectional area surrounded by the epiglottis and the bilateral arytenoid regions before, during, and after laryngeal closure employing VE was an effective analytical procedure. Further research is needed to determine if this method of measuring
laryngeal closure by endoscopy is also useful for patients with
dysphagia. Moreover, comparative studies of VF and VE with a large
sample size are required to develop dysphagia rehabilitation.
References


Figure Legends

Fig. 1. Methods for measurement of the area at the entrance to the laryngeal vestibule. a: epiglottis; b: arytenoid region; c: corniculate cartilage; d: vocal cords; e: pyriform sinus. A: straight line passing through the anterior borders of the left and right arytenoid regions; B: perpendicular line drawn down from the mid-point of line A; C: perpendicular line drawn down from the intersection of the bilateral vocal cords to line A. L: length between the anterior borders of the bilateral arytenoid regions; D: distance between lines B and C. S: cross-sectional area surrounded by the epiglottis and bilateral arytenoid regions.

Fig. 2. VE images in a representative subject. Each moving image (1-12) was selected using the frame step function. 1: a picture taken before the subject took 5ml of white soft yogurt; 4: a picture taken when the food was sent to the vallecula epiglottica; 6: a picture taken at the laryngeal closure (whiteout); 8: a picture taken at the time when the arytenoid regions adducted maximally just after whiteout.

Fig. 3. Comparison of relative area in three pictures. Picture R: a picture taken at the time when the arytenoid regions abducted maximally before the swallowing reflex (resting condition); picture Pr: a picture taken at the time when the arytenoid regions adducted maximally just before whiteout; Picture Po: a picture taken at the time when the arytenoid regions adducted maximally just after whiteout. S: cross-sectional area surrounded by the epiglottis and bilateral arytenoid regions. L: length of a straight line passing through the
anterior borders of the left and right arytenoid regions. Double asterisks (**) mean p<0.01.
Figure 2
Figure 3

- S/L² values for picture Pr: 0.136 ± 0.051 (n=11)
- S/L² values for picture R: 0.295 ± 0.108 (n=14)
- S/L² values for picture Po: 0.085 ± 0.069 (n=11)

Significant differences marked with **.