

Exploring the Relation between Glottal Closure and Plasma Substance P: A Study Protocol

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Glottal incompetence, i.e., reduced ability to produce effective glottal closure, is a problem often associated with aging. The study protocol is presented to determine whether blood substance P (SP) serves as an indicator of glottal closure function among the elderly. SP is involved in the neuromuscular mechanisms of cough; reduced concentration of SP may be a marker for increased risk of aspiration pneumonia. The antihypertensive angiotensin-converting enzyme inhibitor, which also inhibits degradation of SP, has been found to be effective in preventing aspiration pneumonia by strengthening the cough reflex. In this study, we will employ National Hospital Organization self-controlled vocal exercise (NHOEx), which has been shown to improve glottal closure by strengthening the laryngeal adduction muscles. We hypothesize that improved glottal closure by NHOEx may lead to the increase in the plasma concentration of SP, which reflects effective cough reflex. Maximum phonation time (MPT), an indicator of glottal closure, will be measured to assess improvement of glottis-closing function. The study will include 180 patients over 65 years old with dysphonia, dysphagia, or MPT of less than 15 seconds. Based on the speculation that the improved MPT may be associated with changes in SP expression, we will measure the plasma SP before and after 6-month treatment of glottal incompetence using NHOEx. The goal is to verify the reliability of SP as an indicator for glottal closure and swallowing function. Measurement of plasma SP may be helpful for earlier detection of subjects with higher risk of aspiration pneumonia among the elderly.

Keywords: aspiration pneumonia; glottal closure; maximum phonation time; National Hospital Organization self-controlled vocal exercise; substance P

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Introduction

Glottal incompetence, i.e., reduced ability to produce effective glottal closure, is a problem often associated with aging. Glottal incompetence results in frequent laryngeal ingress and difficulty in expectoration owing to subglottic pressure that is insufficient to remove aspirated substances through coughing (Pitts 2014; Britton et al. 2014). To achieve effective cough for prevention of aspiration, satisfactory glottal closure is needed to increase subglottic pressure. In the elderly with glottal incompetence, cough effectiveness is reduced at the time of greatest risk for pneumonia. A screening test for glottal competence and therefore cough effectiveness is necessary for the elderly. Measurement of maximum phonation time (MPT), which is currently used

as one of indicators of glottal closure, provides a straightforward bedside indication of cough effectiveness (Zhou et al. 2012), but a reliable passive indicator by blood sampling would greatly help to identify cases at risk of aspiration.

We have shown in a previous randomized controlled trial (RCT) that National Hospital Organization self-controlled vocal exercise (NHOEx) improves glottal closure by strengthening the laryngeal adduction muscles (Fujimaki et al. 2017). MPT was significantly increased after a 6-month course of NHOEx, and the incidence of pneumonia was also significantly reduced.

In this study, we will focus on blood substance P (SP). As detailed below, SP has been found to contribute to the cough reflex (Nakagawa et al. 1995; Sekizawa et al. 1996). Clinically, reduction of SP concentration in the elderly is

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associated with reduced cough reflex and may be a marker for increased risk of aspiration pneumonia (Nakagawa et al. 1995). The antihypertensive angiotensin-converting enzyme (ACE) inhibitor has been found to be effective in preventing aspiration pneumonia (Lindgren et al. 1989; Arai et al. 1998; Sekizawa et al. 1998). As a side effect of its antihypertensive function, the ACE inhibitor increases the level of SP by inhibiting SP degradation.

We hypothesize that if the glottal closure is strengthened by training the adduction muscles of glottal closure, SP in the blood will increase, and this may correlate with MPT.

Substance P

Based on a canine larynx study, SP might be involved in the laryngeal sensory innervation and glandular secretion systems (Hisa et al. 1985), although no SP immunoreactive nerve fibers were found in any intrinsic laryngeal muscles. The recurrent laryngeal nerve and the superior laryngeal nerve contain SP immunoreactive nerve fibers and are considered to lie in the pathway of the SP nerve fibers to the larynx (Hisa et al. 1985). SP is also involved in regulation of laryngeal blood flow as well as secretion (Hisa et al. 1999). The presence of SP-related nerve fibers in the larynx causing glottal closure has also been demonstrated (Yoshida et al. 2000). Irritation of laryngeal and pharyngeal mucosa by cough-inducing stimuli may activate capsaicin-sensitive sensory nerves, releasing SP, with the result that protective reflexes are initiated by stimulation of glossopharyngeal and vagal sensory nerves (Sekizawa et al. 1996). The pharyngeal, laryngeal, and tracheal epithelium (the sites most important for initiation of swallowing and cough reflexes) have extensive plexuses of nerves that contain SP (Sekizawa 1998). SP was the first neuropeptide found to be a sensory neurotransmitter in the laryngeal afferent system. To summarize, SP is released by sensory nerves and it contributes to the cough reflex. Clinically, a reduction of SP concentration in sputum in the elderly is associated with reduced cough reflex and may be a marker for increased risk of aspiration pneumonia (Nakagawa et al. 1995).

Cough reflex and glottal closure are needed to increase subglottal pressure. We therefore speculate that if the glottal closure is improved by NHOEx that strengthens the laryngeal adduction muscles, plasma SP concentrations will be increased in parallel with the improvement of MPT.

Purpose of the Study

The purpose of this study is to develop an early marker for predicting the risk of aspiration pneumonia using SP and to verify the validity and reliability of SP as an indicator for glottal closing function and swallowing function. Using NHOEx (Fujimaki et al. 2017), we will examine the transition of SP from before to after treatment of dysphonia/dysphagia and clarify its use as an objective indicator for glottal closure. Glottal closure is one of the most preventable factors for aspiration pneumonia, and NHOEx is effective

to improve the glottal closure by strengthening the laryngeal adduction muscles. In the case of the changes in SP before and after NHOEx, measuring plasma SP may be helpful for early detection of high-risk elderly subjects before the onset of aspiration pneumonia who are expected to increase in the future and also useful for evaluating the effect of exercise.

Endpoints

Primary endpoint

The primary endpoint of this study is to establish the correlation between plasma SP and glottal closing function as measured by MPT and determine reliability and validity of SP as a swallowing function marker.

Eligibility criteria

The inclusion criteria for this study are as follows. (1) Inpatients or outpatients over the age of 65 who require NHO self-controlled vocal exercise; (2) those who provide written consent; (3) those whose MPT is less than 15 seconds; (4) those who complain of breathy hoarseness, cough, and dysphagia; (5) those with glottal incompetence due to vocal folds atrophy; (6) those without inflammation, cancer, tumors, paralysis, polyps, granulations and other elevated lesions in the vocal folds; and (7) those able to visit hospital 6 months after the first session.

The exclusion criteria for this study are patients unable to communicate and perform NHOEx.

Methods

Summary of the study is shown in Fig. 1.

Study design

After the acquisition of written consent, treatment will be performed using of the NHO self-controlled vocal exercise and this will be preceded and followed by blood sampling and measurement of MPT. The research was approved by National Hospital Organization Tokyo Medical Center Research Ethics Committee on November 11, 2018 (approval #H30-1116001). This study was registered in the Clinical Trial Registry (UMIN-CTR) on December 13, 2018 (UMIN000035080).

NHO self-controlled exercise (NHOEx) procedures

Before beginning the exercises, patients are given a brochure explaining of the anatomy and physiology of the glottis and the role of glottal closure and the cause of glottal closure failure owing to aging, how age-related vocal cord atrophy leads to hoarseness and aspiration, recommendations on how to modulate the voice while speaking and singing, and how to prevent aspiration. Then they are shown a DVD of how to perform NHOEx with simultaneous explanation by a physician or a speech therapist. they are instructed to perform NHOEx in the following manner.

1. Sit on a chair and grip the sides of the seat with both hands.
2. While saying each number from one to 10 out loud, pull up firmly on both sides of the seat, then relax and inhale naturally before saying the next number.
3. Repeat this exercise for a total of two sets in both the morning and evening, for a total of four sets per day.

Summary of the study

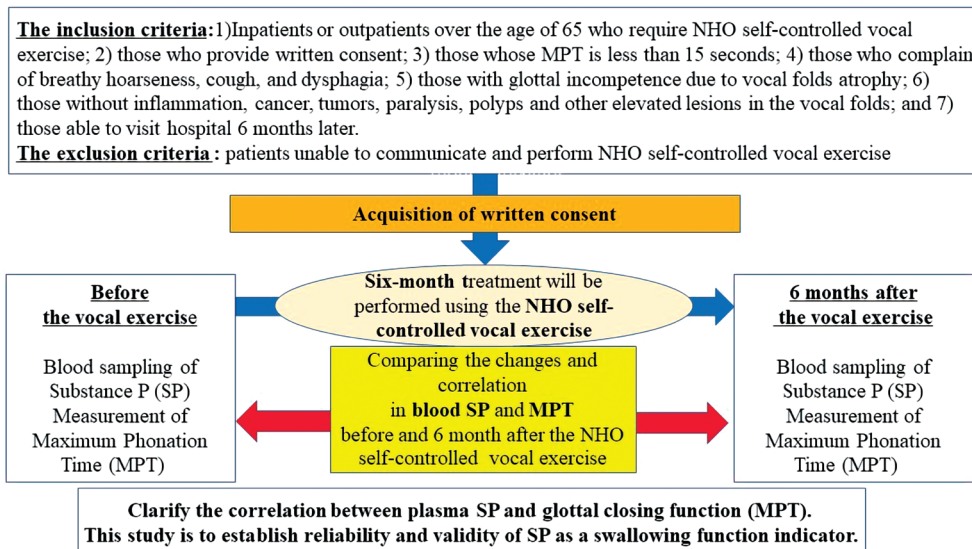


Fig. 1. Summary of the study.

Measurement

Before and after the intervention with NHOEx, venous blood sampling is performed for measurement of plasma SP and MPT is measured using standard procedures. The schedule of the study is outlined in Table 1.

Sample size

The number of registered participants is planned to be 180. The average level of substance P is 90 ± 35 pg/ml (Nakamori et al. 2010) and Standardized Effect Size (E/S) = 0.31 (within 5 errors, standard deviation 31); the required number of cases is 150. The extra 20% is to allow for non-eligibility and dropout, and 180 cases will be considered.

Set as: β error = 0.2, α error = 0.05

Statistical analysis

The number of patients enrolled in the study who performed blood sampling before and after self-controlled vocal exercise is considered an analysis set. The patient age, disease, MPT, and the difference of measured value of SP before and after the exercise will be examined.

Discussion

Both swallowing and cough reflexes are mediated by endogenous SP contained in the vagal and glossopharyngeal nerves (Ohruai 2005). Pharmacologic therapy using angiotensin-converting enzyme inhibitors, which decrease SP catabolism, can improve both reflexes and result in the lowering of the risk of pneumonia (Ohruai 2005).

Based on our RCT showing that NHOEx contributes to strengthened glottal closure (Fujimaki et al. 2017), we propose that the exercises should also contribute to prevention of pneumonia by improving glottal closure for both cough and voice. Also, considering basic and clinical research about SP, we propose that strengthened glottal adduction will cause an increase of plasma SP. Thus, changes in strength of glottal closure and level of SP resulting from NHOEx should be related. We speculate that the increased release of SP may be due to the strengthened contact closure with strengthened intrinsic laryngeal adductor muscles, but there may be other mechanisms. However, we point out that the purpose of this study is not to clarify the

Table 1. The schedule of measurements.

	Before Exercise	After 6-month Exercise
Maximum Phonation Time (MPT)	measure	measure
Plasma substance P	measure	measure

In addition to usual medical care, we will collect about 2 ml of blood for measuring plasma substance P from each patient.

mechanism of this relationship. If the value of SP will be increased after NHOEx, we can speculate that the increase is due to the improvement of forced glottal adduction.

This study is being conducted at multiple facilities, and the equipment that can be used varies depending on the facility, making standardization difficult. Measurement of flow volume in conjunction with MPT would provide a better indication of glottal closure sufficiency. However, such respiratory function test is difficult for each patient in part due to the invasiveness and cost for the patient. To get standardized data from each hospital, we will use only MPT to measure the degree of glottal closure, assuming that lung function does not change.

A simple indicator to evaluate glottal closure to predict aspiration pneumonia is required. If plasma substance P is a swallowing function marker, it could possibly be used for early prediction of aspiration pneumonia. However, there is currently no objective effectiveness index. This study is planned to contribute to the development of better treatments and diagnostics.

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Author Contributions

K.T. and M.T. designed the study and wrote the initial draft of protocol. S.H., H.K., and T.I. brushed up the protocol. All authors agreed with the final protocol and assisted in the preparation of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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