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Research article

Exploring the relationship between plasma substance P and glottal incompetence in the elderly☆

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A B S T R A C T

We speculated that increased blood-plasma levels of Substance P may serve as an indicator of glottal incompetence, which is usually indicated by reduced maximum phonation time. We performed an initial study to test the plausibility of this hypothesis. Patients with dysphonia caused by glottal incompetence were asked to perform vocal exercises for six months to reduce glottal incompetence and we compared the plasma concentration of Substance P before and after the vocal exercise to detect correlation between maximum phonation time and plasma concentration of Substance P. Based on the results, we further hypothesized that patients exhibiting dysphonia with maximum phonation time less than 14 s, in particular less than 10 sec, caused by glottal incompetence may have increased plasma concentration of Substance P with the results of elevated thresholds of cough reflex associated with subclinical aspiration in airways. Further study is needed on patients with decreased Substance P levels, with low scores on Activities of Daily Living and who are hospitalized with aspiration pneumonia.

1. Introduction

Coughing results from a reflex to protect against airway irritation [1]. There is a marked depression of cough reflex in patients with aspiration pneumonia [2]. In the elderly with glottal incompetence, cough effectiveness is reduced at the time in life of greatest risk of

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pneumonia [3]. Glottal incompetence reduces ability to achieve effective glottal closure, resulting in frequent laryngeal ingress and difficulty in expectoration owing to subglottic pressure that is insufficient to remove aspirated substances through coughing [4,5] It also causes breathy hoarseness [3].

In the population at risk of aspiration pneumonia, it is necessary to screen for glottal incompetence, especially because of its effect on cough effectiveness. Measurement of maximum phonation time (MPT), which is how long an individual can maintain phonation of the vowel 'a' without taking a breath, provides a straightforward bedside indication for this purpose [6]. This was exemplified in the work of our clinical research group. A novel self-controlled exercise regime (NHO-exercise) was developed to strengthen the laryngeal adduction muscles and improve glottal competence. In a randomized-control-trial study with 543 patients, 6-months of NHO-exercise was found to significantly increase MPT and reduce the incidence of aspiration pneumonia [3].

Screening for glottal competence using MPT or direct fiberoptic observation of glottal closure is somewhat subjective. It is necessary to establish an objective indicator. We considered that blood sampling of Substance P (SP) may meet this need.

SP was the first neuropeptide found to be a sensory neurotransmitter [7] in the laryngeal afferent system [8]. SP is released by sensory nerves and contributes to the cough reflex. The SP released in response to stimuli may mediate cough and neutral endopeptidase may have a role in modulating SP-induced effects [9]. Coughing during ACE-inhibitor therapy to prevent aspiration is due to an increased inflammatory state in the airways for preventing reduction of SP [10]. Some reports suggested that a reduction of SP concentration in serum [10–14], sputum [15], or saliva [16,17] may be a useful predictive marker for the increased risk of developing aspiration or aspiration pneumonia. Therefore, a relationship between SP, cough reflex, and the risk of aspiration pneumonia has been established. We speculate that increased blood-plasma levels of SP may serve more generally as an indicator of glottal incompetence. This study was designed to test the plausibility of this hypothesis.

In this study, patients with breathy hoarseness (dysphonia) caused by glottal incompetence were asked to perform NHO-exercise for six months. We measured the plasma concentration of SP and the MPT before and after the NHO-exercise to test for correlation between their changes. It was hoped that glottal incompetence, which could lead to early-stage aspiration pneumonia and is expected to occur in more patients in the future, could be reliably detected by a passive indicator using blood sampling of SP in combination with MPT [14]. This would greatly help to identify cases at risk of aspiration.

2. Material and method

This study was focused on elderly patients over the age of 65. To establish the distribution of normal MPT values in this population, MPT measurements were obtained from 131 patients from this age group who had no glottal incompetence according to diagnosis by screening tests. The distribution was found to have a mean of 22.66 and standard deviation of 6.84. Approximating the distribution as Gaussian, its cumulative probability exceeds 2.5% at MPT of 10, 10% at MPT of 14, and 33% at MPT of 20. These values were used to categorize the level of glottal incompetence of study participants according to their MPT: severe below 10, moderate from 10 to 13, and mild from 14 to 20.

Based on data in the protocol paper [14] associated with this study, the recommended sample size of participants was calculated using G*Power (version 3.1) [18] with power = 0.95, $\alpha = 0.05$ and $d = 0.5$, producing an estimate of $n = 105$. The study included 122 patients (outpatients), all over the age of 65, each of whom provided informed written consent. Each of them was examined by laryngeal fiberscopy for diagnosis.

The test group comprised 86 patients who complained of dysphonia (with breathy hoarseness, cough, or dysphagia) and had age-related glottal incompetence due to vocal-fold atrophy with MPT of 4–13 s (sec). Patients unable to perform the NHO exercise and to schedule a follow-up appointment 6 months after the first session were excluded. Any patient with glottal incompetence caused by pathology other than age, including heart failure or pulmonary diseases and rheumatism, was also excluded as was any patient receiving angiotensin-converting-enzyme (ACE) inhibitor therapy, which affects the cough reflex and increases the plasma level of SP. The remaining 36 patients formed the control group. Each had mild MPT in the range of 14–19 s but complained of subjectively experienced dysphonia. Inclusion criteria were similar to those for the test group, except that previous history of otolaryngological conditions including glottal incompetence were exclusionary. The patients with low MPT were divided into two groups based on MPT (severe and moderate). Thus, the respective patient groups were; *severe* [4–9 s] ($n = 41$, mean age 78.55 ± 6.57 years, 68.29% male), *moderate* [10–13 s] ($n = 45$, mean age 79.71 ± 6.91 years, 57.78% male) and *mild* [14–19 s] ($n = 36$, mean age 79.83 ± 5.23 years, 52.78% male). The number in each of the three groups was considered to be large enough to provide statistically reliable results.

During the study, participants performed NHO-exercise every day for 6 months. The exercise regime has been reported in the literature [3]. Before beginning the exercises, patients are given a brochure explaining the anatomy and physiology of the glottis and the role of glottal closure and the cause of failure of glottal closure due to aging, how age-related vocal fold atrophy leads to hoarseness and aspiration, recommendations on how to modulated the voice while speaking and singing, and how to prevent aspiration, They are then shown a video on how to perform NHO-exercise with simultaneous explanation by a physician or speech therapist and they are instructed to perform the exercise 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 1 to 10 aloud, pull firmly on both sides of the seat and then relax and inhale naturally before saying the next number.
3. Repeat this exercise for a total of 2 sets in both the morning and evening, for a total of 4 sets per day.

Before and after the 6-month NHO-exercise, MPT was measured using standard procedures and blood sampling was performed for

measurement of plasma concentration of SP. Blood sampling was performed with EDTA-2Na + aprotinin (NP-EA0305-123D, SRL, Tokyo, Japan). Samples were centrifuged at 0 °C. Plasma SP was stored at −80 °C until assay and analyzed within 2-weeks. Age, disease, MPT, and the difference of measured plasma concentration of SP before and after the NHO-exercise of those who performed all the steps were analyzed at SRL Kitakanto Laboratory Inc., Tokyo, Japan, who were certified to perform these procedures. Relationships of plasma concentration of SP with MPT and the NHO-exercise were analyzed using a two-factor repeated measures mixed ANOVA. The significant differences were specified by post hoc t tests if needed. The statistical analysis was performed in R Studio v.2023.06.1–524 using R v.4.3.2.

The research was approved by National Hospital Organization Research Ethics Committee (approval #H30-1116001). This study was registered in the Clinical Trial Registry (UMIN-CTR) as UMIN000035080. The detail was published as a protocol [14].

3. Result

The effects of the NHO-exercise on SP and MPT are shown in Figure-1. In the two groups (severe and moderate) with glottal incompetence and MPT of less than 14 sec. with dysphonia and choking/cough, plasma concentration of SP decreased after the NHO-exercise and moved below 3000 pg/ml. The plasma concentration of SP never increased and MPTs always increased after the exercise. The results showed significant relation and correlation between decrease of plasma concentration of SP and increase of MPT (Fig. 1a and b). Furthermore, the severe and moderate groups of patients with initial MPTs less than 14 s had higher average levels of plasma concentration of SP than those of the mild group with initial MPTs between 14 and 19 s (3099 vs 2539, $p = 0.011$, $d = 0.415$). Both severe + moderate and mild MPT groups, especially the combination group showed decreased average plasma concentration of SP that moved toward 2500 pg/ml after the NHO-exercise (2706 vs 2378, $p = 0.188$, $\eta p2 = 0.235$).

As shown in Fig. 1a, the plasma concentration of SP was over 3000 pg/ml in both the severe and moderate groups with less-than-14-sec MPTs but significantly fell below 3000 pg/ml after the exercise ($\Delta = -393$ pg/ml; $p = 0.012$; $d = 0.283$). This effect of exercise was more prominent in the moderate group with 10-to-13-sec MPTs ($\Delta = -563$ pg/ml; $p = 0.004$; $d = 0.435$) compared to the severe group with 4-to-9-sec MPTs ($\Delta = -207$ pg/ml; $p = 0.468$; $d = 0.121$). In the mild group with 14-to-19-sec MPTs, the SP value was around 2500 pg/ml before and after the NHO-exercise ($\Delta = -161$ pg/ml; $p = 0.420$; $d = 0.162$). Using the results of our study for the short and moderate cases whose MPTs recovered to more than 14 s after NHO-exercise as representative of those for healthy elderly individuals, the normal level of plasma concentration of SP may be estimated to be 2512 ± 1117 pg/ml (95% CL: 2269–2755 pg/ml).

4. Discussion

Average SP levels are a bit above 3000 pg/ml before exercise regardless of how bad is the average pre-exercise MPT, as long as that MPT is below the normal range (14 s or above), at least without pneumonia. The pattern of changes due to the 6-month NHO-exercise for the three different MPT groups is very different for the SP levels compared to the MPT values. This makes it clear that the information given by SP level is different to that given by MPT so, the utility of SP is not only that it is more objective than measuring MPT.

Increased levels of SP in plasma are associated with persistent cough in humans and might be related to airway sensitivity in asthmatic cough [13]. Clinically, a reduction of SP concentration in the sputum in the elderly is associated with a reduced cough reflex [10].

We could not get the results we expected in the initial study based on our speculation. After this, we further hypothesized that patients exhibiting dysphonia with MPTs less than 14 s, in particular less than 10 sec., caused by glottal incompetence may have

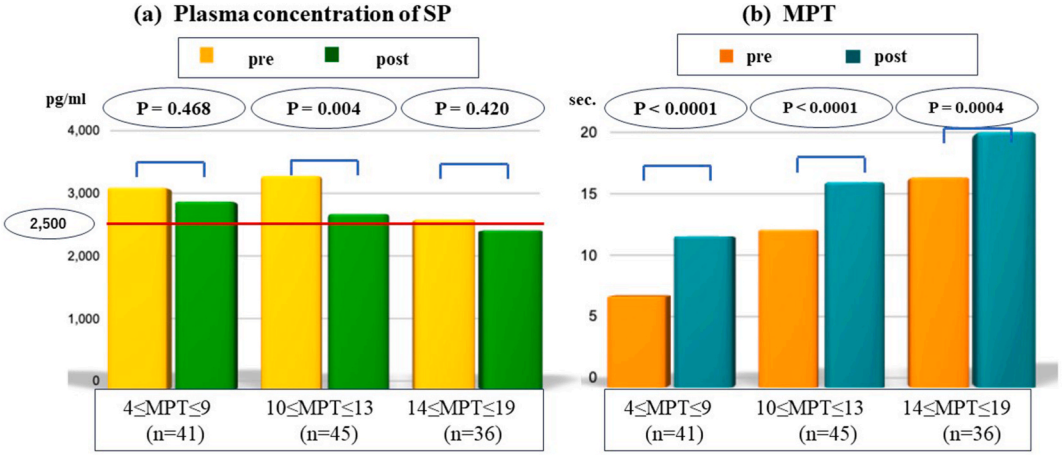

Fig. 1. Mean of plasma concentration of SP and MPT of pre/post NHO-exercise. a) Mean of plasma concentration of SP b) Mean of plasma concentration of MPT.

increased plasma concentration of SP as an inflammatory change with the results of elevated thresholds of cough reflex associated with subclinical aspiration in airways. However, in some cases, patients who have subclinical aspiration never exhibit the aspiration with/without cough. Therefore, if patients whose MPT has decreased to less than 10 sec complain of hoarseness and have elevation of SP to around 3000 pg/ml, or who cough frequently, this might be a sign of the beginning of aspiration (Fig. 2).

It is necessary to maintain satisfactory plasma concentration of SP as neurotransmitter to induce the cough reflex, even with elevated threshold, to prevent aspiration. That is why plasma concentration of SP increases physiologically to compensate for pathological elevated threshold. Also, we hypothesize that improvement of glottal closure with NHO-exercise helps to decrease pathological elevated threshold for cough reflex. Then, physiologically elevated SP based on homeostasis, might return to normal. In cases of severe breathy hoarseness with frequent cough, and with increased plasma concentration of SP, appropriate treatment would be necessary to prevent glottal incompetence. That is the appropriate timing for preventing aspiration pneumonia.

5. Conclusion

Our finding is that raised SP is a sign that the cough is weak and the threshold for the cough reflex needs to be reduced while lowering of the SP level is a sign that the cough has become more effective. Glottal closure is necessary for that cough. In other words, the measurement of substance P in combination with fiberscopic laryngeal examination and MPT sis the first step in a study that may lead to an objective biomarker to detect the risk of aspiration pneumonia and prevent it in advance.

Further study is needed on patients with decreased SP levels, with low scores on Activities of Daily Living indexes, and those who are hospitalized for the treatment of aspiration pneumonia to develop our hypothesis, also, comparing the SP before and after intervention with ACE-inhibitor and Pharyngeal Electrical Stimulation (PES) [16] for such cases, is awaited.

Additional information

No additional information is available for this paper.

Data availability

Data will be made on request.

CRediT authorship contribution statement

Koichi Tsunoda: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Toyota Ishii:** Data curation. **Hiroyuki Kuroda:** Data curation. **Hiroaki Nakatani:** Data curation. **Masaru Tateda:** Data curation. **Sawako Masuda:** Data curation. **Tetsuya Takiguchi:** Data curation. **Fujinobu Tanaka:** Data curation. **Hayato Misawa:** Data curation. **Masamitsu Senarita:** Data curation. **Mihiro**

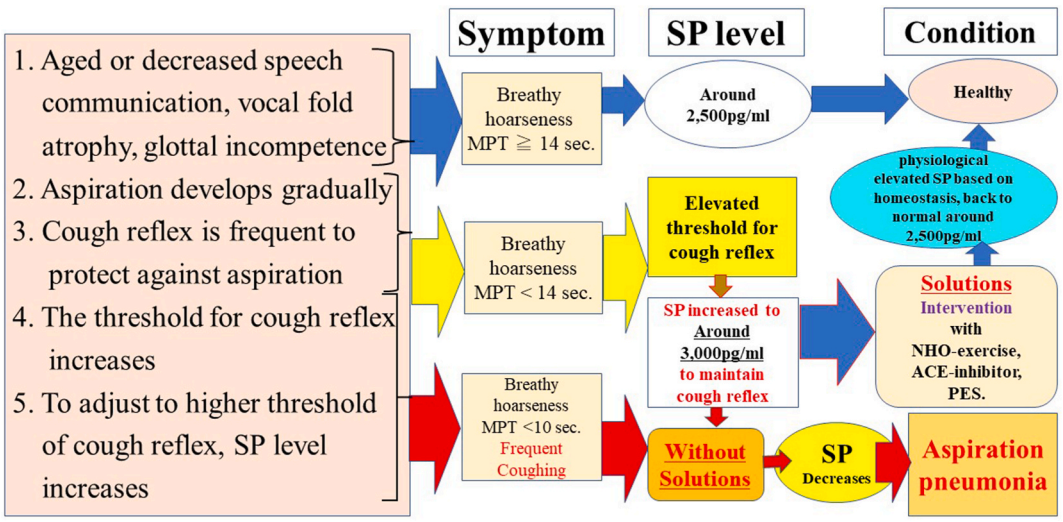

Fig. 2. Relation of aspiration and SP level. Roles of increase or decrease of SP, 1. By aged or decrease speech communication, glottal incompetence due to vocal fold atrophy occurs, 2. Aspiration gradually occurs, 3. To prevent aspiration, the cough reflex occurs frequently, 4. The threshold for cough reflex might be increasing pathologically, 5. To induce cough reflex, the plasma concentration of SP increases physiologically as a neurotransmitter, *With the suitable solution, SP would decrease and moved toward around 2500 pg/ml * Without any solution, SP decreases and aspiration pneumonia would be occurred caused by subclinical aspiration.

Takazawa: Writing – review & editing, Investigation. **Kenji Itoh:** Writing – review & editing, Software, Methodology, Formal analysis. **Thomas Baer:** Writing – review & editing, Software, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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