EFFICACY OF NATAMAME TEA CONTAINING CANAVANINE ON THE NASAL CONDITION AND HALITOSIS IN HEALTHY JAPANESE
— TWO RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDIES —

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Abstract

Objectives: Two clinical studies were done to investigate the efficacy of daily ingestion of "Natamame Tea" containing Canavanine on the nasal condition and halitosis.
Methods: Both studies were performed as randomized, placebo-controlled, double-blind trials for 12 weeks.
Results: As the primary outcome, the study showed significant differences in the subjective assessment on the nasal condition and halitosis. On the other hand, no significant differences were observed in the measurement of causative substances of the halitosis. In addition, as the secondary outcome no abnormal change of safety had been triggered by the ingestion of test drink.
Conclusion: The ingestion of Natamame Tea by healthy people for 12 weeks contributed to the improvement of nasal condition centering on snivel and remediation of halitosis based on self-evaluation. In addition, no safety-related matter occurred during 12-week test period.

Key words: Natamame, Canavanine, nasal condition, halitosis

1. INTRODUCTION

Canavalia gladiata (commonly called the sword bean) is leguminous an annual grass which has a long husk ranging from 25 cm to 30 cm. It is a native of South East Asia and tropical Africa and therefore is tolerant of heat and dryness. From the very beginning of being introduced to Japan, it has been actively cultivated in Satsuma (Kagoshima-Prefecture). In China, the fruit has been used as a Chinese herbal medicine for handling many kinds of symptoms, such as treating a cough, discharging pus, suppressing inflammation, preventing swelling, increasing renal function, improving intestinal environment, controlling vomiting or relieving pain[1]. For eating, on the other hand, its beans are cooked as Nimame (boiled beans) or An (bean paste), and its young husk is cooked as Fukujinzuke (sliced vegetables pickled in soy sauce). The most typical way of ingestion, however, is drinking as tea (i.e. Natamame tea) by grinding and brewing its beans and husks.

In Japan, the prevalence rate of an allergic disease exceeded 30% in 2008, and the rate is increasing each year[2]. Although it is not a life-threatening disease, many patients are forced to suffer from some unpleasant nasal symptoms such as sneezing, snivel and/or sniffles. These symptoms interfere with daily activities, cause impairment in concentration or disruption of sleep, and eventually result in a severe decrease of QOL (Quality of Life). Physiological halitosis, on the other hand, is also a major issue in Japan now: According to the health welfare trend survey conducted by Health and Welfare Ministry (currently, Health, Labor and Welfare Ministry) in 1999, there is a statistical data that about 10% out of all respondent to a survey (approximately 33 thousand people) answered as they mind their bad breath (halitosis)[3]. Halitosis is a generic term of unpleasant odor which comes through the mouth or nose, and about 90% of it is said to be derived from mouth cavity[4]. Its causative substance is volatile sulfur compound (VSC) and especially, hydrogen sulfide and methyl mercaptan account for about 90% of the compound. It is thought that these substances are produced through the activities of bacteria in mouth cavity, and also thought that these activities are accelerated by several factors such as lack of mouth cleaning, tongue coat, disease of the gums, odontonecrosis, dry mouth and/or stress. Everybody has a certain level of halitosis. Nowadays, there is a growing interest in halitosis and many oral-care products such as mouth wash or mouth spray aimed at preventing or alleviating halitosis are sold in the market. The

remediation of halitosis possibly improves the QOL since it is said that due to the problem of their halitosis, a growing number of young people tend to hesitate to talk in front of people or to go outside.

Since Natamame tea is expected to have the positive effects as explained above, many Natamame tea products are sold in the market with the catch line of "good for empyema, rhinitis (nasal inflammation) or halitosis". However, its pharmacological mechanisms are still unclear, and there are few published reports that clearly explain these effects of the tea. In this study, we examined the effect of Natamame tea on nasal symptoms or halitosis, and also the safety of its ingestion. The test targets were healthy person who are worried about their nasal conditions or bad breath (halitosis), and the test method was a randomized, placebo-controlled, double-blind study.

2. METHOD

2.1. Effect of Natamame tea on the nasal condition

2.1.1 Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from Smile-Japan co., ltd. (Fukushima) at two centers, OZ clinic (Tokyo) and JACTA (Tokyo).

The study period was 12 weeks, from August 27th to November 19th, 2015. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study. The clinical trial was registered at UMIN Clinical Trial Registry (Trial ID: UMIN000018782).

2.1.2. Subjects

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo).

2.1.2.1. Inclusion criteria

(1) Healthy people aged between 40 and 59 years;
(2) People suffering from a running nose or nasal congestion;
(3) People suitable for the nasal problem questionnaire (**Table 5**).

2.1.2.2. Exclusion criteria

(1) Individuals undergoing treatment of nasal problems;
(2) Individuals on taking medication, including herbal medicines;
(3) Individuals judged to be unsuitable to participate in the trial by the principle investigator

2.1.3. Randomization

Recruited subjects were 108 persons. Subjects who fulfilled the eligibility criteria were 42 persons. The questionnaire is listed in **Table 5**. The inclusion was judged by the principle investigator. All subjects were sequentially assigned based on a random number table to one of the masked products and randomized to group T (Test sample: 22) and group P (Placebo: 20). The allocation was pre-assigned on the basis of randomized numbers.

Table 1 Nutritional content of the sample per 100 g

Item	Quantity
Moisture	1.4 g
Protein	27.3 g
Lipid	8.1 g
Ash	6.5 g
Carbohydrates	56.7 g
Energy	409 kcal
Sodium	5.5 mg

2.1.4. Description of test drink and blinding

The test drink "Natamame Cha" ("NC") is a tea containing Canavanine. The method of making the tea includes seeping a teabag in a tea pot of cold water and bringing to a boil. Drinking 1,000 ml of this tea per day is recommended. The Placebo tea does not include Canavanine. **Table 1** shows the nutritional content of the test sample (the Natamame bean). Both teas were indistinguishable in color, flavor or taste. Teas were managed by an identification symbol. All involved were blinded.

2.1.5. Experimental procedures

2.1.5.1. Experimental protocol

Subjects consumed almost 1,000 ml of boiled tea every day for 12 weeks. In order to even out the contained amount of Canavanine, the boiled tea is prepared from 500ml of water and 3 g of tea-bag and contained in an aluminum can. The subjects are asked to drink two cans of tea (500 ml x 2 = 1,000 ml) per a day.

Subjects were instructed as follows: to avoid excessive amounts of food, drink or alcohol; to maintain a daily record of lifestyle factors during the test period; and to send the diary to the study coordinator every Friday by mobile email.

2.1.5.2. Outcome

The objective of this study was to verify the nasal condition improvement by ingesting tea containing Canavanine. To evaluate this objective, subjective reporting of nasal conditions were measured as the primary outcomes. The questionnaire covered: nasal mucus buildup; throat mucus buildup; remaining mucus after blowing nose; frequency of sneezing; throat tickling sensation; smelling ability; nasal voice; running nose symptom; and frequency of coughing. Responses to each question were rated on an ordinal scale of 1 to 5, with higher scores indicating a better result. Blood

Table 2　Schedule for the study (nasal condition)

Item \ Term	Screening	Pre Trial Test	Test period		
			4 w	8 w	12 w
Informed consent	●				
Selection and/or allocation	●				
Subjective reporting of nasal condition	●	●	●	●	●
Biochemical analysis of blood		●			●
Urine analysis		●			●
Ingestion of test drinks			↔	↔	↔
Log			↔	↔	↔

● : Implementation
↔ : Daily practice during the test period

biochemical and urine parameters were recorded to evaluate the safety of the test tea as the secondary outcome. These assessments were conducted upon entry into the study (pre-intervention) and after 12 weeks (post-intervention).

To evaluate the safety of the test drink, adverse events were collected by means of a written questionnaire during the study. According to the schedule shown in **Table 2**, we measured parameters on efficacy and safety.

2.1.6. Data analysis

All analyses were performed on the on-treatment population in the study. Data were expressed as mean ± SD. For subjective reporting of nasal condition, changes from baseline in the same group were assessed using Wilcoxon signed-rank test. Mann-Whitney U test was used for intergroup comparisons of changes from the baseline. For biochemical analyses of blood and urine, changes from the baseline in the same group were assessed using the paired t-test. Student's t-test used for intergroup comparisons of changes from the baseline. Student's t-test was used to compare subject backgrounds between groups. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the $< 5\%$ level in the two-sided test.

2.2 Effect of Natamame tea on halitosis

2.2.1. Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from Smile-Japan co., ltd. (Fukushima) at three centers, Bio Clinic Tokyo (Tokyo), OZ clinic (Tokyo), and JACTA (Tokyo). The study period was 12 weeks, from September 15th to December 9th, 2015. This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study. The clinical trial was registered at UMIN clinical Trial Registry (Trial ID: UMIN 000018928).

2.2.2. Subjects

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee., Inc. (Tokyo).

2.2.2.1. Inclusion criteria

(1) Healthy people aged between 40 and 59 years.
(2) People suffering from halitosis.

2.2.2.2. Exclusion criteria

(1) Individuals undergoing treatment of halitosis.
(2) Individuals on taking medication, including herbal medicines.
(3) Individuals judged to be unsuitable to participate in the trial by the principle investigator.

2.3. Randomization

Recruited subjects were 116 persons. Subject who fulfilled eligibility criteria were 41 persons. The inclusion criterion was judged by the principle investigator. All subjects were sequentially assigned based on a random number table to one of the masked products and randomized to group T (Test: 23) and group P (Placebo: 18). The allocation was pre-assigned on the basis of randomized numbers.

2.2.4. Description of test drinks and blinding

The test drink is a tea "Natamame Cha" ("NC"), and the Placebo drink is a tea not including Canavanine as explained in 2.1.4.

2.2.5. Experimental procedures

2.2.5.1. Experimental protocol

Subjects consumed almost 1,000ml of boiled tea every day for 12 weeks as explained in 2.1.5.1.

2.2.5.2. Outcome

The objective of this study is to verify the effect of tea containing Canavanine on halitosis. To evaluate this objective, concentrations of hydrogen sulfide and methyl mercaptan in oral air were measured as the primary outcomes. Questionnaire of halitosis was also observed as

Table 3 Schedule for the study (halitosis)

Item \ Term	Screening	Pre Trial Test	Test period		
			4 w	8 w	12 w
Informed consent	●				
Selection and/or allocation	●				
Medical Examination	●				
Subjective reporting of halitosis	●				
hydrogen sulfide and methyl mercaptan		●			●
Biochemical analysis of blood		●			●
Urine analysis		●			●
Questionnaire of halitosis			↔	↔	↔
Ingestion of test drinks			↔	↔	↔
Log			↔	↔	↔

● : Implementation
↔ : Daily practice during the test period

the primary outcome. The question was "how do you feel your halitosis? ". The response was rated on an ordinal scale of 1 to 5, with lower score indicating a better result. Subjects recorded this self-evaluation every day during 12-week test period. The question asked for self-evaluation of halitosis:
(1) Very low; (2) Low; (3) Moderate; (4) High; (5) Very high.

Blood biochemical and urine parameters were recorded to evaluate the safety of the test drinks as the secondary outcome. These assessments were conducted upon entry into the study (pre-intervention) and after 12 weeks (post-intervention). According to the schedule shown in **Table 3**, we measured parameters on efficacy and safety.

2.2.6. Data analysis

All analyses were performed on the on-treatment population in the study. Data were expressed as mean ± SD. For Questionnaire of halitosis, changes from baseline in the same group were assessed using Wilcoxon signed-rank test. Mann-Whitney U test was used for intergroup comparisons of changes from the baseline. For the concentrations hydrogen sulfide and methyl mercaptan in oral air, and biochemical analyses of blood and urine, changes from the baseline in the same group were assessed using the paired t-test. Student's t-test used for intergroup comparisons of changes from the baseline. Student's t-test was used to compare subject backgrounds between groups. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the $< 5\%$ level in the two-sided test.

3. RESULTS

3.1. Effect of Natamame tea on the nasal condition

3.1.1. Participant demographics

From all of 108 applicants, 66 were eliminated according to the questionnaire (**Table 5**). 42 subjects were randomly assigned to intervention groups and made a start with ingestion. 3 were withdrawn due to personal reasons (physical condition) and the remaining 39 subjects completed the study. Thus, data obtained from 39 subjects (NC group; 19, Placebo group; 20) were used for efficacy analysis (**Fig. 1**). There was no significant difference in the mean age or gender ratio (**Table 4**).

3.1.2. Reporting of the nasal condition

Table 5 shows the results of questionnaire. No significant difference was observed between the two groups in all nine items at the baseline. After 12 weeks ingestion, the NC showed a significant difference in the items of "nasal mucus buildup", "throat mucus buildup", "remaining mucus after blowing nose", "frequency of sneezing", "throat tickling sensation", "nasal voice", "running nose symptom" and "frequency of coughing", whereas the Placebo only showed a significant difference in the items of "frequency of sneezing", "throat tickling sensation", "smelling ability" and "nasal voice". Furthermore the between-group analyses revealed that there was a significant difference of changes in "nasal mucus buildup", "throat mucus buildup", "remaining mucus after blowing nose", "frequency of sneezing", "running nose symptom" and "frequency of coughing" after 12-weeks ingestion.

3.1.3. Blood and urine test

Table 6 and **7** shows blood biochemical and urine parameters. A significant difference was observed in the changes of CK (CPK) of females, Sodium, Chloride, Potassium, Inorganic phosphorus, Urea nitrogen, Creatinine of woman in the blood and urine specific gravity of the NC after 12 weeks ingestion. However, since the difference was within the standard value, the investigator judged it as the range of physiological variation (or clinically safe).

3.1.4. Adverse event

No adverse event was reported during this trial.

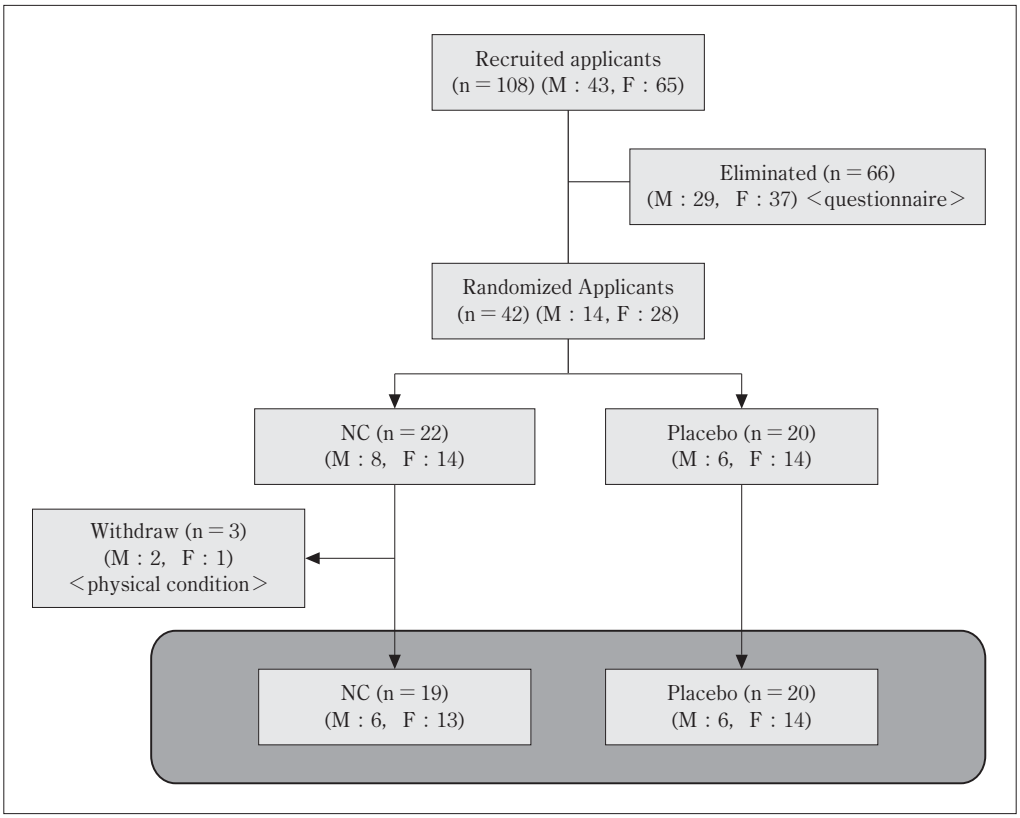

Fig. 1　Flow diagram of subject disposition (nasal condition)

3.2 Effect of Natamame tea on halitosis

3.2.1. Participant demographics

From all of 116 applicants, 75 were eliminated according to the subjective reporting of halitosis. The 41 subjects were randomly assigned to intervention groups and made a start with ingestion. All subjects completed the study. Thus, data obtained from 41 subjects (NC ; 23, Placebo; 18) were used for efficacy analysis (**Fig. 2**). There were no significant differences in the mean age or gender ratios. (**Table 8**)

3.2.2. Concentrations of hydrogen sulfide and methyl mercaptan in oral air

Table 9 shows the results of test analyses. No significant difference was observed intragroup nor intergroup in the two items of concentrations of hydrogen sulfide and methyl mercaptan in oral air.

3.2.3. Questionnaire of halitosis

The result of questionnaire of halitosis is described in **Table 10**. The number shows the mean of cumulative scores of subjective evaluation during 7 days. Significant differences were observed between two groups at week 4 and week 12. In the NC, week 4, week 8, week12 illustrated significant changes compared to week 1.

3.2.4. Blood and urine test

Table 11 and **12** show the blood biochemical and urine parameters. A significant difference was observed in the changes of potassium, creatinine of males in the blood, urine specific gravity of the NC after 12 weeks ingestion.

Table 4　Subject demographics (nasal condition)

Item	Unit	NC	Placebo
Subjects *	—	19	20
Male : Female *	—	6 : 13	6 : 14
Age	years	48.7 ± 5.0	48.2 ± 5.5

* Number of subjects
mean ± SD

However, since the difference was within a range of baseline and a shade of difference, the investigator judged it as the range of physiological variation (or clinically safe).

3.2.5. Adverse event

No adverse event was reported during this trial.

4. DISCUSSION

We conducted two randomized, placebo-controlled, double-blind studies to verify the effect of Natamame tea containing Canavanine on the nasal condition and halitosis. As the primary outcome, the study showed significant differences in the subjective assessment on the nasal condition and halitosis. On the other hand, we could not find any significant differences in the measurement of causative substances of the halitosis. At the same time, as the secondary outcome the observation of clinical findings such as medical interview, blood and urine test

Table 5 Results of questionnaire analyses (nasal condision)

Item	Time points	Scores		P-value
		NC (n = 19)	Placebo (n = 20)	
Nasal mucus buildup	Baseline	2.0 ± 0.7	2.0 ± 0.7	
	Week 4	2.5 ± 1.0 †	2.1 ± 0.9	
	Change	0.5 ± 1.0	0.2 ± 0.9	0.332
	Week 8	3.4 ± 1.0 **	2.2 ± 0.5	
	Change	1.4 ± 0.8	0.2 ± 0.7	< 0.001 ##
	Week 12	3.3 ± 0.9 **	1.9 ± 0.5	
	Change	1.3 ± 1.1	− 0.1 ± 0.6	< 0.001 ##
Throat mucus buildup	Baseline	1.8 ± 0.8	2.3 ± 0.9	
	Week 4	2.7 ± 1.3 **	2.2 ± 0.5	
	Change	0.9 ± 1.2	− 0.1 ± 0.7	0.011 #
	Week 8	2.7 ± 1.2 **	2.4 ± 0.7	
	Change	0.9 ± 1.1	0.2 ± 0.9	0.070 ‡
	Week 12	3.3 ± 1.2 **	1.9 ± 0.6	
	Change	1.4 ± 1.3	− 0.4 ± 0.9	< 0.001 ##
Remaining mucus after blowing nose	Baseline	1.7 ± 0.8	2.4 ± 0.9	
	Week 4	2.4 ± 1.3 *	2.6 ± 1.0	
	Change	0.6 ± 1.0	0.2 ± 0.7	0.267
	Week 8	2.7 ± 1.2 **	2.7 ± 0.9 †	
	Change	1.0 ± 1.2	0.3 ± 0.7	0.049 #
	Week 12	3.3 ± 1.3 **	2.5 ± 1.0	
	Change	1.5 ± 1.2	0.1 ± 0.7	< 0.001 ##
Frequency of sneezing	Baseline	1.7 ± 0.7	2.0 ± 0.8	
	Week 4	2.5 ± 1.2 **	1.9 ± 0.6	
	Change	0.8 ± 1.1	− 0.1 ± 0.6	0.015 #
	Week 8	2.6 ± 1.3 **	2.5 ± 1.1 *	
	Change	0.9 ± 1.2	0.6 ± 1.0	0.319
	Week 12	3.1 ± 1.1 **	2.6 ± 1.0 *	
	Change	1.4 ± 1.1	0.7 ± 1.1	0.038 #
Throat tickling sensation	Baseline	2.1 ± 0.6	2.4 ± 1.1	
	Week 4	2.7 ± 1.2 *	2.9 ± 1.4 *	
	Change	0.7 ± 1.0	0.6 ± 0.9	0.725
	Week 8	2.7 ± 1.1 †	2.8 ± 1.3 †	
	Change	0.6 ± 1.3	0.4 ± 0.9	0.684
	Week 12	3.3 ± 1.1 **	3.0 ± 1.1 *	
	Change	1.3 ± 1.2	0.6 ± 0.9	0.077 ‡
Smelling ability	Baseline	3.9 ± 1.0	3.5 ± 1.2	
	Week 4	4.3 ± 0.7 †	4.3 ± 0.9 *	
	Change	0.4 ± 0.8	0.8 ± 1.1	0.164
	Week 8	4.4 ± 0.7 *	4.1 ± 0.9 †	
	Change	0.4 ± 0.8	0.6 ± 1.2	0.643
	Week 12	4.3 ± 0.6 †	4.5 ± 0.8 **	
	Change	0.4 ± 0.9	1.0 ± 1.2	0.068 ‡
Nasal voice	Baseline	2.4 ± 0.8	2.7 ± 1.0	
	Week 4	2.8 ± 1.1 *	2.9 ± 1.1	
	Change	0.4 ± 0.7	0.2 ± 1.0	0.482
	Week 8	2.6 ± 1.0	3.4 ± 1.3 *	
	Change	0.2 ± 1.0	0.7 ± 1.2	0.299
	Week 12	3.2 ± 1.3 **	3.5 ± 1.3 **	
	Change	0.8 ± 1.1	0.9 ± 0.9	0.899
Running nose symptom	Baseline	1.6 ± 0.7	2.1 ± 0.7	
	Week 4	2.1 ± 1.0 *	2.0 ± 0.8	
	Change	0.5 ± 0.9	− 0.2 ± 0.8	0.045 #
	Week 8	2.2 ± 1.1 *	2.1 ± 0.9	
	Change	0.6 ± 1.0	− 0.1 ± 1.1	0.119
	Week 12	2.6 ± 1.0 **	1.9 ± 0.6	
	Change	1.1 ± 1.0	− 0.3 ± 0.9	< 0.001 ##
Frequency of coughing	Baseline	2.2 ± 0.6	2.6 ± 0.9	
	Week 4	3.1 ± 1.1 **	2.6 ± 0.8	
	Change	0.9 ± 0.8	− 0.1 ± 0.9	0.004 ##
	Week 8	2.7 ± 1.1 †	2.6 ± 0.7	
	Change	0.5 ± 1.1	0.0 ± 0.7	0.140
	Week 12	3.0 ± 1.3 *	2.5 ± 0.8	
	Change	0.8 ± 1.3	− 0.2 ± 0.7	0.012 #

Scores are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group difference in change from baseline.

Table 6　Biochemical blood test (nasal condision)

Item	Unit	Std. Value	Gender	Time points	Values	
					NC (n = 19)	Placebo (n = 20)
Total Bilirubin	mg/dL	0.2-1.2	M/F	Baseline Week 12 Change	0.51 ± 0.18 0.49 ± 0.16 − 0.02 ± 0.16	0.57 ± 0.21 0.51 ± 0.15 − 0.06 ± 0.18
Total Protein	g/dL	6.3-8.3	M/F	Baseline Week 12 Change	7.4 ± 0.3 7.4 ± 0.3 0.0 ± 0.3	7.4 ± 0.4 7.4 ± 0.4 − 0.1 ± 0.3
Albumen	g/dl	3.8-5.3	M/F	Baseline Week 12 Change	4.6 ± 0.3 4.5 ± 0.3 − 0.0 ± 0.3	4.5 ± 0.3 4.4 ± 0.3 † − 0.1 ± 0.2
AST (GOT)	U/L	8-38	M/F	Baseline Week 12 Change	23.4 ± 5.9 22.3 ± 4.9 − 1.1 ± 5.1	21.5 ± 5.0 21.2 ± 5.2 − 0.4 ± 3.8
ALT (GPT)	U/L	4-43	M/F	Baseline Week 12 Change	18.5 ± 7.7 18.4 ± 9.0 − 0.2 ± 8.3	21.5 ± 9.4 21.8 ± 9.0 0.3 ± 7.9
ALP	U/L	110-354	M/F	Baseline Week 12 Change	200.6 ± 59.1 205.7 ± 75.0 5.2 ± 29.1	191.5 ± 54.2 198.9 ± 60.4 7.4 ± 27.8
LD (LDH)	U/L	121-245	M/F	Baseline Week 12 Change	178.5 ± 22.5 175.1 ± 26.8 − 3.4 ± 13.4	169.7 ± 21.6 163.8 ± 29.5 − 5.9 ± 18.6
γ-GT (γ GTP)	U/L	86 and under	M	Baseline Week 12 Change	39.3 ± 22.3 50.3 ± 38.9 11.0 ± 43.8	43.8 ± 23.7 45.5 ± 26.4 1.7 ± 5.7
		48 and under	F	Baseline Week 12 Change	25.8 ± 15.7 24.6 ± 15.0 − 1.2 ± 9.0	29.8 ± 31.3 26.9 ± 25.7 * − 2.9 ± 6.3
CK (CPK)	mg/dL	38-196	M	Baseline Week 12 Change	117.8 ± 38.3 124.3 ± 36.4 6.5 ± 34.3	151.0 ± 77.3 162.5 ± 162.0 11.5 ± 109.9
		30-172	F	Baseline Week 12 Change	104.2 ± 41.6 82.4 ± 21.1 * − 21.8 ± 33.8	86.1 ± 21.9 87.7 ± 30.2 1.6 ± 19.3 #
Total Cholesterol	mg/dL	130-219	M/F	Baseline Week 12 Change	210.4 ± 29.3 215.5 ± 28.9 5.1 ± 18.1	217.8 ± 30.7 216.3 ± 23.1 − 1.5 ± 17.9
Neutral Fat (TG)	mg/dL	30-149	M/F	Baseline Week 12 Change	194.8 ± 138.6 152.6 ± 103.2 † − 42.2 ± 102.5	144.3 ± 135.3 139.4 ± 87.6 − 4.9 ± 79.3 ‡
Sodium	mEq/L	135-150	M/F	Baseline Week 12 Change	143.6 ± 1.6 141.4 ± 2.2 ** − 2.2 ± 2.4	143.4 ± 2.2 141.6 ± 1.5 ** − 1.9 ± 2.1
Chloride	mEq/L	98-110	M/F	Baseline Week 12 Change	104.7 ± 2.3 102.6 ± 1.9 ** − 2.1 ± 2.7	104.4 ± 2.0 103.3 ± 1.4 * − 1.1 ± 2.1
Potassium	mEq/L	3.5-5.3	M/F	Baseline Week 12 Change	4.0 ± 0.3 4.3 ± 0.3 ** 0.3 ± 0.3	3.9 ± 0.3 4.2 ± 0.3 ** 0.3 ± 0.3
Calcium	mg/dL	8.4-10.2	M/F	Baseline Week 12 Change	10.0 ± 0.6 9.8 ± 0.6 † − 0.2 ± 0.4	9.8 ± 0.4 9.5 ± 0.3 ** − 0.3 ± 0.2
Inorganic Phosphorus	mg/dL	2.5-4.5	M/F	Baseline Week 12 Change	3.7 ± 0.5 3.4 ± 0.5 * − 0.3 ± 0.5	3.4 ± 0.4 3.3 ± 0.4 − 0.1 ± 0.5
Urea Nitrogen	mg/dL	8.0-20.0	M/F	Baseline Week 12 Change	13.0 ± 3.3 11.6 ± 2.1 * − 1.4 ± 2.6	12.8 ± 2.3 13.9 ± 2.3 * 1.1 ± 1.9 ##
Creatinine	mg/dL	0.61-1.04	M	Baseline Week 12 Change	0.86 ± 0.25 0.76 ± 0.11 − 0.10 ± 0.24	0.81 ± 0.11 0.80 ± 0.10 − 0.01 ± 0.05
		0.47-0.79	F	Baseline Week 12 Change	0.64 ± 0.06 0.60 ± 0.06 ** − 0.04 ± 0.04	0.64 ± 0.07 0.63 ± 0.07 − 0.01 ± 0.05 #
Blood Sugar (Serum)	mg/dL	60-109	M/F	Baseline Week 12 Change	73.7 ± 12.6 79.1 ± 16.9 5.4 ± 22.0	63.4 ± 10.1 74.9 ± 12.4 * 11.5 ± 7.6

Values are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group difference in change from baseline.

Table 7 Transition of Urinalysis (nasal condision)

Item	Unit	Std. Value	Gender	Time points	Values	
					NC (n＝19)	Placebo (n＝20)
Specific Gravity	mg/dL	1.010-1.025	M/F	Baseline	1.019 ± 0.007	1.018 ± 0.006
				Week 12	1.014 ± 0.007 **	1.018 ± 0.007
				Change	− 0.005 ± 0.006	0.000 ± 0.008 #
pH	g/dL	4.5-8.0	M/F	Baseline	6.0 ± 0.8	6.3 ± 0.8
				Week 12	6.3 ± 0.8	6.5 ± 0.8
				Change	0.3 ± 0.9	0.2 ± 0.7

Values are expressed as the mean ± SD.

** $p < 0.01$ against baseline.

# $p < 0.05$ between-group differences in change from baseline.

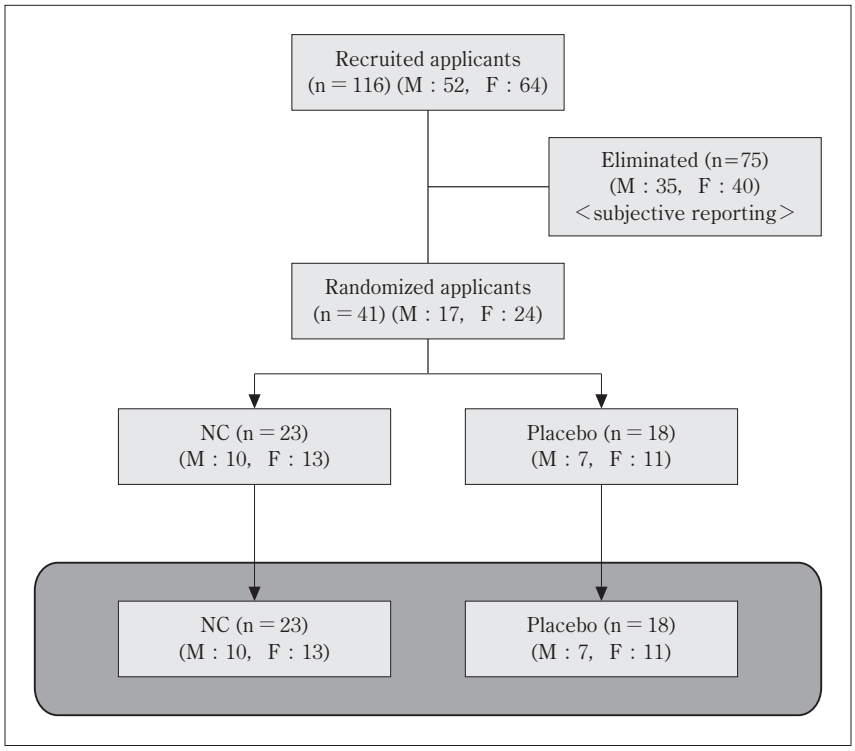

Fig. 2 Flow diagram of subject disposition (halitosis)

revealed no abnormal change had been triggered by the ingestion of test products.

Main Findings

In this study, we observed the significant differences in the subjective assessment on the nasal condition and halitosis, and therefore evaluated that the ingestion of Natamame tea improved the conditions of the subjects. At the same time, the study did not show any significant differences in the causative substances of the halitosis. As for the nasal test, after 12-week test period the NC showed the significant differences in 8 items out of 9 subjective items in total, whereas the Placebo showed in 4 items out of 9. In addition, the intergroup comparisons showed significant differences in 6 items. The items that did not show the significant differences are such as

Table 8 Subject demographics (halitosis)

Item	Unit	NC	Placebo
Subjects *	−	23	18
Male : Female	−	10 : 13	7 : 11
Age	years	48.6 ± 6.0	49.4±5.1

* Number of subjects
mean ± SD

“Throat tickling sensation”, “Smelling ability” or “Nasal voice”, and these items may have a close relationship with symptoms which are rather caused by factors other than allergic symptoms such as nasal inflammation. We also found that the items showing the significant

Table 9 Results of concentrations of hydrogen sulfide and methyl mercaptan in oral air

Item (unit)	Time points	Values		P-value (between-group differences in change from baseline)
		NC (n＝23)	Placebo (n＝18)	
Hydrogen sulfide (ppb)	Baseline	104.8 ± 176.8	64.0 ± 98.5	
	Week 12	133.9 ± 317.0	83.4 ± 165.3	
	Change	29.14 ± 187.5	19.37 ± 210.3	0.876
Methyl mercaptan (ppb)	Baseline	42.4 ± 80.7	5.5 ± 17.7	
	Week 12	38.5 ± 90.6	7.9 ± 24.9	
	Change	−3.91 ± 52.6	2.47 ± 31.8	0.647

Values are expressed as the mean ± SD.

Table 10 Results of questionnaire analyses (halitosis)

Time points	Scores		P-value
	NC (n＝23)	Placebo (n＝18)	
Week 1 (day 1-7)	51.9 ± 18.1	56.1 ± 14.8	
Week 4 (day 22-28)	46.5 ± 115.2 *	58.0 ± 16.5	
Change	−5.3 ± 9.5	1.9 ± 4.9	$0.118^{\#}$
Week 8 (day 50-56)	43.7 ± 15.4 **	54.9 ± 14.5	
Change	−8.1 ± 11.7	−1.1 ± 4.1	0.015
Week 12 (day 78-84)	41.9 ± 13.5 **	57.7 ± 15.6	
Change	−10.0 ± 11.8	1.7 ± 4.8	$< 0.001^{\#\#}$

Scores are expressed as the mean ± SD.

** $p < 0.01$ against week 1.

$^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ between-group differences in change from week 1.

differences in the Placebo are almost identical to those without intergroup significant differences. Although the symptoms such as sneezing or coughing are caused by several factors, we can say that mucus is one of the major causal factors for these symptoms. Based upon the above discussions, it was indicated that the ingestion of Natamame tea especially contributes to the remediation of symptoms pertinent to nasal inflammation such as mucus or nasal congestion.

As for the halitosis test, the result of questionnaire of halitosis in the NC showed the significant differences from 4-week, 8-week and 12-week. In the Placebo group, on the other hand, no significant difference was observed after 12-week ingestion of the test product. The intergroup comparisons after 4-week and 12-week period showed the significant differences. The measurement of the causative substances of the halitosis (hydrogen sulfide and methyl mercaptan), which was conducted to objectively assess the halitosis, did not show any significant differences. The halitosis is caused by a variety of factors or ingredients, and their derivation also varies [5]. It is thought that among these ingredients hydrogen sulfide and methyl mercaptan are the main causative substances [3]: the former smells like a rotten egg and has an odor threshold of 0.0047 ppm, and the latter smells like a rotten onion and has an odor threshold of 0.0021 ppm [6]. Although the level of the obtained figure from the subject in this study was higher than the threshold level, the figures vary widely, and therefore it can be said that the number of the test subject in this study was not enough to adequately understand the tendency. Generally, it is difficult to evaluate the odor itself, and how to sense the halitosis varies widely depending on relative relationship of the people. It is also difficult to judge it, and quite often the evaluation totally different from the general evaluation of the odor is made [7]. In addition, there are many patients with psychogenic halitosis, who have been suffering from the memory in their childhood days that they were said “You have a bad breath!”. In this study we could observe that the result of the self, questionnaire of halitosis showed the ingestion of Natamame tea improved the condition. Since this study adopted a double-blind study and there was no significant difference in the Placebo, it is unlikely that this outcome was a result of placebo effect [8]. Therefore it is reasonable to judge that certain level of remediation of halitosis condition has been achieved from a viewpoint of subjective symptom.

Table 11 Biochemical blood test (halitosis)

Item	Unit	Std. Value	Gender	Time points	Values	
					NC (n = 23)	Placebo (n = 18)
Total Bilirubin	mg/dL	0.2-1.2	M/F	Baseline Week 12 Change	0.59 ± 0.18 0.58 ± 0.19 − 0.01 ± 0.21	0.64 ± 0.29 0.61 ± 0.30 − 0.03 ± 0.15
Total Protein	g/dL	6.3-8.3	M/F	Baseline Week 12 Change	7.5 ± 0.4 7.4 ± 0.3 0.0 ± 0.3	7.5 ± 0.4 7.4 ± 0.4 0.1 ± 0.3
Albumen	g/dl	3.8-5.3	M/F	Baseline Week 12 Change	4.6 ± 0.2 4.6 ± 0.3 0.0 ± 0.2	4.6 ± 0.3 4.6 ± 0.3 0.0 ± 0.3
AST (GOT)	U/L	8-38	M/F	Baseline Week 12 Change	22.9 ± 8.5 24.0 ± 7.5 1.2 ± 8.6	19.6 ± 4.6 22.4 ± 5.1 † 2.8 ± 5.8
ALT (GPT)	U/L	4-43	M/F	Baseline Week 12 Change	22.8 ± 14.3 22.6 ± 12.2 − 0.2 ± 14.3	19.4 ± 10.2 21.3 ± 9.5 1.9 ± 7.8
ALP	U/L	110-354	M/F	Baseline Week 12 Change	205.0 ± 50.2 197.9 ± 54.5 − 7.1 ± 30.3	204.3 ± 60.8 210.2 ± 70.2 5.8 ± 24.3
LD (LDH)	U/L	121-245	M/F	Baseline Week 12 Change	198.3 ± 68.6 188.9 ± 51.4 † − 9.4 ± 26.0	176.6 ± 22.4 180.5 ± 30.5 3.9 ± 15.2 ‡
γ-GT (γ GTP)	U/L	86 and under	M	Baseline Week 12 Change	32.4 ± 17.2 40.3 ± 33.2 7.9 ± 18.0	43.1 ± 18.6 38.7 ± 12.1 − 4.4 ± 10.7
		48 and under	F	Baseline Week 12 Change	21.0 ± 8.5 22.2 ± 9.8 1.2 ± 8.6	20.5 ± 6.6 18.9 ± 5.5 − 1.5 ± 3.9
CK (CPK)	mg/dL	38-196	M	Baseline Week 12 Change	260.3 ± 195.0 223.7 ± 179.1 − 36.6 ± 67.8	155.7 ± 38.1 210.4 ± 158.7 54.7 ± 169.3
		30-172	F	Baseline Week 12 Change	90.8 ± 29.5 101.2 ± 46.3 10.4 ± 45.1	104.9 ± 54.4 95.3 ± 23.0 − 9.6 ± 45.7
Total Cholesterol	mg/dL	130-219	M/F	Baseline Week 12 Change	212.1 ± 25.3 218.6 ± 29.5 † 6.5 ± 17.0	231.0 ± 50.2 242.8 ± 58.2 * 11.8 ± 18.1
Neutral Fat (TG)	mg/dL	30-149	M/F	Baseline Week 12 Change	128.3 ± 73.8 112.0 ± 84.2 − 16.2 ± 84.9	128.1 ± 61.6 139.2 ± 83.4 11.2 ± 60.1
Sodium	mEq/L	135-150	M/F	Baseline Week 12 Change	143.4 ± 2.0 143.3 ± 2.0 − 0.1 ± 2.0	143.2 ± 2.8 143.4 ± 2.0 0.2 ± 2.6
Chloride	mEq/L	98-110	M/F	Baseline Week 12 Change	103.8 ± 2.1 103.9 ± 1.9 0.1 ± 1.7	104.5 ± 2.5 103.8 ± 2.6 − 0.7 ± 2.4
Potassium	mEq/L	3.5-5.3	M/F	Baseline Week 12 Change	4.1 ± 0.4 4.5 ± 0.4** 0.4 ± 0.4	4.0 ± 0.4 4.3 ± 0.4** 0.3 ± 0.3
Calcium	mg/dL	8.4-10.2	M/F	Baseline Week 12 Change	9.6 ± 0.2 9.6 ± 0.3 0.0 ± 0.3	9.6 ± 0.3 9.6 ± 0.2 0.0 ± 0.3
Inorganic Phosphorus	mg/dL	2.5-4.5	M/F	Baseline Week 12 Change	3.5 ± 0.6 3.4 ± 0.5 − 0.1 ± 0.3	3.4 ± 0.6 3.3 ± 0.6 − 0.1 ± 0.4
Urea Nitrogen	mg/dL	8.0-20.0	M/F	Baseline Week 12 Change	13.1 ± 2.9 13.1 ± 2.7 0.0 ± 2.3	13.9 ± 3.7 13.3 ± 3.3 − 0.6 ± 2.7
Creatinine	mg/dL	0.61-1.04	M	Baseline Week 12 Change	0.87 ± 0.11 0.83 ± 0.10 * − 0.04 ± 0.05	0.93 ± 0.14 0.96 ± 0.19 0.03 ± 0.06 #
		0.47-0.79	F	Baseline Week 12 Change	0.64 ± 0.05 0.61 ± 0.04 − 0.02 ± 0.05	0.59 ± 0.10 0.57 ± 0.11 − 0.02 ± 0.04
Blood Sugar (Serum)	mg/dL	60-109	M/F	Baseline Week 12 Change	75.6 ± 14.8 71.7 ± 12.4 − 3.9 ± 16.1	78.1 ± 14.5 72.4 ± 10.5 − 5.7 ± 14.1

Values are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.
‡ $p < 0.1$, # $p < 0.05$, ## $p < 0.01$ between-group difference in change from baseline.

Table 12 Transition of Urinalysis (halitosis)

Item	Unit	Std. Value	Gender	Time points	Values	
					NC (n＝23)	Placebo (n＝18)
Specific Gravity	mg/dL	1.010-1.025	M/F	Baseline	1.019 ± 0.006	1.020 ± 0.007
				Week 12	1.013 ± 0.008 **	1.014 ± 0.007**
				Change	− 0.006 ± 0.007	− 0.006 ± 0.008
pH	g/dL	4.5-8.0	M/F	Baseline	6.0 ± 0.8	5.7 ± 0.5
				Week 12	6.2 ± 0.8	6.6 ± 0.7 **
				Change	0.2 ± 1.1	0.9 ± 0.7 #

Values are expressed as the mean ± SD.

* $p < 0.05$, ** $p < 0.01$ against baseline.

# $p < 0.05$ between-group differences in change from baseline.

Natamame reportedly contains a plenty of concanavalin A (a type of lectin)[9], canavanine (a type of amino acid), phenols, tannin, flavonoid and several types of minerals[10)11)]. Especially, canavanine is an ingredient extracted from Natamame for the first time, and it has functions such as antiviral action, antimycotic action, anti-inflammatory effect, and discharging pus[12)13)]. There is a report that the sword bean extract (Natamame extract) containing canavanine inhibits the growth of Porphyromonas gingivalis or Fusobacterium nucleatum (which are an oral bacteria and periodontopathic bacteria)[14], and also a report that showed inhibiting effect of isolation of histamine by using KU812 cells[15]. In addition, it is reported that the use of the tooth paste containing Natamame contributed to the remediation of mild degree of gingivitis and halitosis[16]. Tannin and flavonoid, on the other hand, also reportedly own functionalities such as depressive effect on allergic nasal inflammation and/or halitosis[17)18)], therefore those contained in Natamame expectedly achieve the same functionalities. In sum, the ingestion of Natamame tea in this study likely led the functions of canavanine and the other ingredients, and contributed to the improvement of nasal condition centering on mucus and the remediation of halitosis.

Secondary Findings

In this study, it was observed that based upon clinical findings such as blood and urine test, no abnormal change was triggered by ingestion of the test product. In the nasal test, significant differences were observed in some blood parameters and urine specific gravity, whereas in the halitosis test significant difference was observed in urine specific gravity; however, since the difference was within a range of baseline, the investigator judged it as the range of physiological variation (or clinically safe). During the test period three (3) subjects discontinued the nasal test, but it was because of their personal reasons such as impossibility of continuing the test due to a cold. Therefore, we observed no harmful influence against biochemical and/or physiological matters of the test subjects which seem to have causal relationship with the test product. These results indicated the safety of the ingestion of the test product (NC) for the 12-week test period.

General Information

In Japan, the number of people who suffer from allergic symptoms (such as hay fever) is growing with each passing year. Among the allergic symptoms, the symptoms related to nasal inflammation such as mucus or nasal congestion force the patient to take breaths through the mouth, and this way of breathing triggers off the discomfort such as a feeling of oppression or difficulty sleeping. In addition, this way of breathing only using mouth may cause the dryness of oral cavity and/or pharyngeal region, and the dryness can lead to susceptibility to viruses such as the flu virus or worsening of halitosis in the oral cavity. The halitosis negatively affects the relationship with others, and at the same time, the actions such as blowing nose or sniffing tend to build a feeling of discomfort among the people around them. The awareness of building such discomfort leads to the excess attention to others and they are eventually forced to bear a huge psychological burden.

Tea is a type of drink Japanese has been drinking regularly since old times, and there are a wide variety of tea products sold in the market now. Switching the type of tea in their daily life from green tea to Natamame tea can contribute to the solution of problems of nasal conditions or halitosis existing among contemporary people. In addition, the ingestion of Natamame tea may alleviate the physical and/or mental burden without any botheration such as “going to the hospital” or “taking medicine”, and is eventually expected to improve the QOL of the user.

Limitations

Although in this study, we found the significant differences in the result of questionnaire of halitosis, the measurement of its causative substances did not show the significant differences. It is generally said that it is difficult to recognize their own halitosis since an olfactory

sensation is a sensation that tends to accept an adaptation easily [19]. This study employed the self- assessment of the test subjects at their home in order to let them monitor the changes sequentially. This may be effective for the remediation of halitosis in the sense of the improvement of QOL, but it does not connect to the objective assessment of the test result. In order to obtain the adequate and sound test result, it should be required to congruously shape the design of further study, which includes an organoleptic examination with intermediation of a third party, or expanding the number of test subjects and measuring the causative substances of halitosis from a perspective of stratified analysis (such as comparison of physiology-oriented halitosis and pathology-oriented halitosis) [19]. In addition, as for the improvement of the nasal condition the further study should scrutinize the relationship between ingredients contained in Natamame and allergic material more closely.

5. CONCLUSION

In conclusion, we found out that the ingestion of Natamame tea by healthy people for 12 weeks contributed to the improvement of nasal condition centering on snivel and remediation of halitosis based on self-evaluation. In addition, no safety-related matter occurred during 12-week test period.

CONFLICT OF INTEREST

Yoshifumi Soeda is the principal of Smile-Japan co., ltd.. All remaining authors have declared no conflicts of interest.

ACKNOWLEDGEMENT

We appreciate the assistance and support of Kazuhiro Kurashima, M.D., Sanno Hospital and International University of Health and Welfare.

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