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Effects of cloth panel containing specific ore powder on patients with Japanese cedar pollen allergy during pollen dispersal season

Suni Lee[1], Shoko Yamamoto[1], Maiko Hamana[2], Hiroshi Okamoto[3], Tamayo Hatayama[1], Fukusou Danbara[2], Yoshio Fujii[3], Youichi Murakami[2], Takemi Otsuki[1,*]

[1] Department of Hygiene, Kawasaki Medical School, 577 Matsushima, Kurashiki City, Okayama 701-0192, Japan; slee@med.kawasaki-m.ac.jp (S.L.). s.yamamoto@med.kawasaki-m.ac.jp (S.Y.), tama@med.kawasaki-m.ac.jp (T.H.), takemi@med.kawasaki-m.ac.jp (T.O.)

[2] Wadakohsan Corporation, 4-2-13, Sakaemachidori, Chuo-ku, Kobe City, Hyogo 650-0023, Japan; maiko-h@wadakohsan.co.jp (M.H.), danbara@wadakohsan.co.jp (F.D.), murakami@wadakohsan.co.jp (Y.M.)

[3] Cosmic Garden Co., Ltd., 1-2-25 Ima, Kita-ku, Okayama City, Okayama 700—0975, Japan; okamoto@cosmic-g.jp 'H.O.), info@cosmic-g.jp (Y.F.)

* Correspondence: takemi@med.kawasaki-m.ac.jp; Tel.:+81-86-462-1111 (T.O.)

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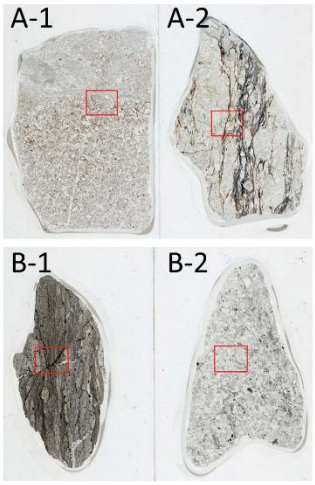
Abstract: Pollen allergy remains a big problem in contemporary societies. We have shown in previous studies that a cloth containing a special natural ore powder (CCSNOP) is effective in relieving symptoms in patients with pollen allergies. However, in that study, subjects were exposed to CCSNOP for only one hour. In the present study, CCSNOP or control (non-woven cloth; NWC) panels were placed in the bedrooms of pollen allergy patients for two weeks during the pollen dispersal season in 2018, and the effects were investigated. Twenty-one subjects were exposed to CCSNOP panels and 10 subjects were exposed to NWC panels. Our investigations showed that use of CCSNOP resulted in relief of symptoms and reduced use of therapeutics. Moreover, the ratio of eosinophil count decrease during exposure was higher in the CCSNOP group. Furthermore, a formula for measuring various cytokines and other parameters was established and clearly showed a distinction between the CCSNOP and NWC groups. In this formula, GM-SCF, IL-12p40, IgG4 and eosinophil count were extracted. These results indicated that CCNSNOP has a beneficial effect on pollen allergy patients. Future studies shall engage in long-term monitoring of pollen allergy patients who will utilize this mineral powder for at least one year.

Keywords: pollen allergy; natural ore powder; cloth; interior material; symptom relief; immunological effect

1. Introduction

Pollen allergies and hay fever typically manifested as allergic rhinitis and allergic conjunctivitis represent seasonal allergic reactions that can limit the ordinary lives of individuals [1-3]. Patients with pollen allergies experience various nasal symptoms such as sneezing, runny nose and stuffy-nose, in addition to eye-related conditions such as redness, watery eyes and itch [1-3]. In Japan, the number of people with allergic responses to Japanese cedar pollen has been increasing during the last two decades [4-6]. Additionally, some patients with pollen allergies can have adverse reactions to as many as 50 types of plants including cypress, rice, ragweed and mugwort. The treatment of pollen allergy is generally limited to suppressing symptoms, with oral antihistamines, nasal drops or eye drops typically being used as therapeutic strategies [7-9]. Although desensitization therapy has been tried [9-11], it has not been fully effective, and patients just wait for the pollen season to pass. Precautions can be taken to avoid or minimize exposure to pollen such as decreasing outdoor activities, closing windows, operating central air conditioning systems with filter attachments, bathing and shampooing the body and hair well to remove attached pollens, and drying clothes well. Notwithstanding these precautions, patients with pollen allergies continue to suffer with symptoms, even in their own homes, during pollen season [4-6].

A



B

Sample	A-1	A-2	B-1	B-2
(wt %)	Sandstone	Mudstone	Mudstone	Sandstone
SiO_2	73.27	71.42	63.94	75.06
TiO_2	0.43	0.49	0.76	0.24
Ai_2O_3	13.64	1.77	18.01	10.47
Fe_2O_3	4.12	6.94	4.20	10.47
MnO	0.04	0.19	0.04	0.07
MgO	1.05	1.72	1.55	0.93
CaO	0.85	1.19	1.58	3.89
Na_2O	3.73	1.76	1.56	3.09
K_2O	2.33	3.10	5.41	1.50
P_2O_5	0.07	0.11	0.13	0.05
H_2O-	0.05	0.04	0.09	0.02
H_2O+	0.42	0.26	2.72	1.47
CO_2	-	-	-	-
Total	100.00	100.00	100.00	100.00

C

1,820 mm × 910 mm × 5 mm

D

Figure 1. The specific natural ore and placement of cloth containing specific natural ore powder (CCSNOP). [A] Appearance of the natural ore obtained near Aso Mountain located in Kumamoto Prefecture, Kyushu Island, Japan. Labels A and B show the mirror-polished flake appearance (normal light) of this natural ore. The red square is where the photomicrograph was taken for analysis (data not shown). The width of the slide glass is 28 mm. A-1 is sandstone, A-2 is mudstone, B-1 is mudstone, and B-1 is sandstone. [B] Results of the chemical analysis using X-ray fluorescence of four parts of the natural ore (A-1, A-2, B-1 and B-2). None of these showed any particularly characteristic findings. [C] Appearance and size of CCSNOP panels used in this experiment. NWC panels had a similar appearance. Each panel was folded and 4 panels were delivered to each subject. Subjects were responsible for placement of the panels within their bedroom. [D] Picture of bedroom containing panels (subject ID 60, posted with permission of the subject).

The Cosmic Garden Co. Ltd. is a custom-home building company located in Okayama City, Japan. Some of the features of their homes include a modified '2x4' construction method for aseismic ability, the avoidance of chemical substances and smells when possible, as well as the construction of well-sealed, super-insulated, durable homes. As we previously reported, this company uses specific natural ore powder within wall materials when constructing homes. This natural ore is obtained near Aso Mountain, Kumamoto Prefecture, Kyushu Island, Japan, and is known to release far-infrared rays. The appearance of this natural ore is shown in Fig. 1A and the chemical analyses of four main parts (shown in Fig. 1A) of this natural ore examined by an X-ray fluorescent (KrF) method is shown in Fig. 1B, as previously reported. The analyses revealed no remarkable characteristics. Additionally, the Cosmic Garden Co. Ltd. has incorporated this natural ore into the wall material of more than 200 homes. Home occupants have expressed improvements in allergic symptoms such as pollen allergy, bronchial asthma and atopic dermatitis. Additionally, many occupants have reported improved sleep quality. Moreover, the office space of this company includes this specific ore powder in the walls, and many customers have claimed immediate improvement of symptoms related to pollen allergy after entering this office space.

In our previous study [12], we investigated the biological effects of cloth containing specific natural ore powder (CCSNOP) on patients with pollen allergy in terms of symptoms, biological markers, mood state via a questionnaire referred to as Profile of Mood Status 2 (POMS2), stress marker using salivary amylase (sAmy), serum immunoglobulin (Ig) E specific to 33 antigens, peripheral blood counts including percentage of white blood cells, and 29 serum cytokines under conditions where subjects were exposed to CCSNOP for 60 min. For the control, subjects were exposed to non-woven cloth (NWC). From this previous study, some symptoms such as nasal obstruction and lacrimation improved, and POMS2 evaluations showed that patients were calmer following their stay with CCSNOP. However, relative eosinophil percentage, non-specific Ig E, epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-α levels were higher in subjects exposed to CCSNOP compared with NWC. However, in that previous study, it was not confirmed whether exposure to

CCSNOP did indeed improve symptoms of pollen allergy, or whether the indoor air circumstances using CCSNOP affected the human immune system [12].

The present study was executed after Wadakohsan Corp. (a real estate business) began selling detached homes containing CCSNOP provided by Cosmic Garden Co. Ltd. In this study, CCSNOP or NWC (Fig. 1C) panels (of indistinguishable appearance) were placed in the sleeping rooms of subjects (Fig. 1D) with pollen allergy (self-reported) for two weeks at least one or more weeks after the beginning of the Japanese cedar pollen dispersal season. Subjects were required to keep a "pollen allergy diary" that included details of symptoms, medication employed, and other comments (Fig. 2). Additionally, biological monitoring was performed one month prior to the anticipated pollen dispersal, immediately prior to and following panel placement, and two months after Japanese cedar pollen dispersal had ceased.

allergy to pollen: Symptom diary ID _____ Age ( ) , Gender ( )

Date (day of week)	M/D (day of week)	M/D (day of week)	M/D (day of week)	M/D (day of week)	M/D (day of week)	M/D (day of week)	M/D (day of week)
Weather (please circle)	☀ ☁ ☔	☀ ☁ ☔	☀ ☁ ☔	☀ ☁ ☔	☀ ☁ ☔	☀ ☁ ☔	☀ ☁ ☔
Symptoms (nose)	■About sneezing and runny nose – how many times did you blow your nose? None→1, 1 ～ 5 times→2, 6 ～ 10 times→3, 11 ～ 20 times→4, >21 times→5. ■About stuffy nose, None → 1 Stuffy nose present but no mouth breathing → 2 Stuffy nose present with some mouth breathing → 3 Stuffy nose present with much mouth breathing. → 4 Nose is completely stuffed → 5						
Sneezing (times)	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Runny nose (times)	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
stuffy nose	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Symptoms (eyes)	Please record, rare→1, slight→2, moderate→3. severe→4						
Redness	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
Watery eyes	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
Itch	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
Everyday life	Please record: No interference → 1; Slight problems → 2; Moderate problems → 3; Severe problems → 4; Impossible → 5						
	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Medicinals	● If you use medicinals, enter the name of the medicine, ○ if you took it as prescribed, X if you forgot to take it, or △ if you did not need to take it. ● If you know the name of the medicine, enter it. ● Please record when using nasal and/or eye drops.						
Others	Please include any additional items such as quality of sleep or sleep status, going out, medical checkups, etc.						

Figure 2. Pollen Allergy Diary. Each subject was asked to keep a diary. In addition to the date, day of the week, and an assessment of the weather, subjects also recorded any symptoms related to the nose or eye on a scale of 1-5. Subjects were also asked to describe the difficulty or impact of their symptoms on their daily life. Additionally, the use of medication (oral drugs, nasal drops and eye drops) was described. Moreover, subjects could list any physical or mental changes they may have noticed. Diary entries were recorded after commencement of pollen dispersal, from one week prior to panel

placement, for two weeks during panel exposure, after panel removal, and the one week where pollen dispersal continued (for a total of four weeks).

2. Experimental Section

2.1. Subjects

All 31 subjects were Japanese. Subjects were recruited following public announcement of the intended study. The subjects comprised 14 males (average age (AA) ± standard deviations (SD): 45.1 ± 14.1 years old) and 17 females (37.1 ± 11.0 years old), with the AA of the 31 subjects being 41.2 ± 12.8 years old. Eight subjects were living near Kobe City, Hyogo Prefecture, Japan. For them, the POMS2 questionnaire, sAmy sampling, and venous blood collection were performed within rooms of Wadakohsan Corp. in Kobe City (Kobe venue). The other 23 subjects were living in Okayama Prefecture. Thus, sampling was performed in rooms at Okayama University in Okayama City, and rooms at Kawasaki Medical School in Kurashiki City, both cities being in Okayama Prefecture (ca. 10 km apart; Okayama venues). Kobe, Okayama and Kurashiki cities are in the western region of Japan and are ca. 120 km apart. However, dispersal of Japanese cedar pollens does not differ markedly between these regions according to weather forecast data and the Japanese Ministry of the Environment. The Kobe venue included eight subjects (M:F = 4:4) while the Okayama venues included 23 subjects (M:F = 10:13). All subjects self-reported pollen allergy to Japanese cedar with various typical symptoms. Subjects with well-controlled lifestyles possessing diseases such as hypertension, diabetes mellitus, respiratory diseases, liver and kidney diseases were included in the study, however, patients with cancers, collagen diseases and other severe diseases were omitted from the study. All subjects continued with their regular daily lives during the study without modification.

The study was designed as a double-blind study. Thus, all researchers measuring POMS2, sAmy and blood sampling were unaware of which panel (CCSNOP or NWC) each subject was being exposed to. Following the final sampling in June, 2018, the distribution of subjects within the CCSNOP and NWC groups was revealed. The Kobe venue had 2 males and 2 females with CCSNOP and 2 males and 1 female with NWC. The Okayama venues included 6 males and 10 females with CCSNOP and 4 males and 3 females with NWC. In total, 8 males and 13 females (21 subjects) comprised the CCSONOP group and 6 males and 4 females (10 subjects) comprised the NWC group. CCSNOP and NWC groups differed since we designed this study not only to compare the two groups but also to analyze changes in biological markers among subjects of the CCSNOP group.

2.2. Ethical matters

This study was approved by the Kawasaki Medical School Ethics Committee (Issue No. 2576, date of approval December, 12th, 2016). All subjects of this study

were approached verbally and in writing, and those from whom written consent was obtained were recruited as subjects in this study. All methods used in this study were performed in accordance with the relevant guidelines and regulations shown by Kawasaki Medical School Ethics Committee as well as the Declaration of Helsinki. Additionally, the monitoring and audit for this study were performed by the Academic Research Organization (ARO), Center for Innovative Clinical Medicine, Okayama University, since favorable results may be used in the advertising of Cosmic Garden Co. Ltd. and Wadakohsan Corp. There were no problems with the monitoring and auditing of this study.

2.3. Study design

As shown in Fig. 3, the study was performed from January to June, 2018. The samplings (POMS2, aAmy and blood collection) were performed 4 times. The first was early January, one month before pollen dispersal. The second was at the end of February, one or more weeks after the commencement of dispersal. The third was two weeks after the second time. During these two weeks each subject was exposed to CCSNOP or NWC panels that had been placed in their bedroom (Fig. 1D). The last sampling was performed in early June, almost two months following the cessation of pollen dispersal. All dates are listed in Fig. 3. One week prior to panel placement and during the 2-week period of panel exposure, all subjects kept a “pollen allergy diary” as shown in Fig. 3.

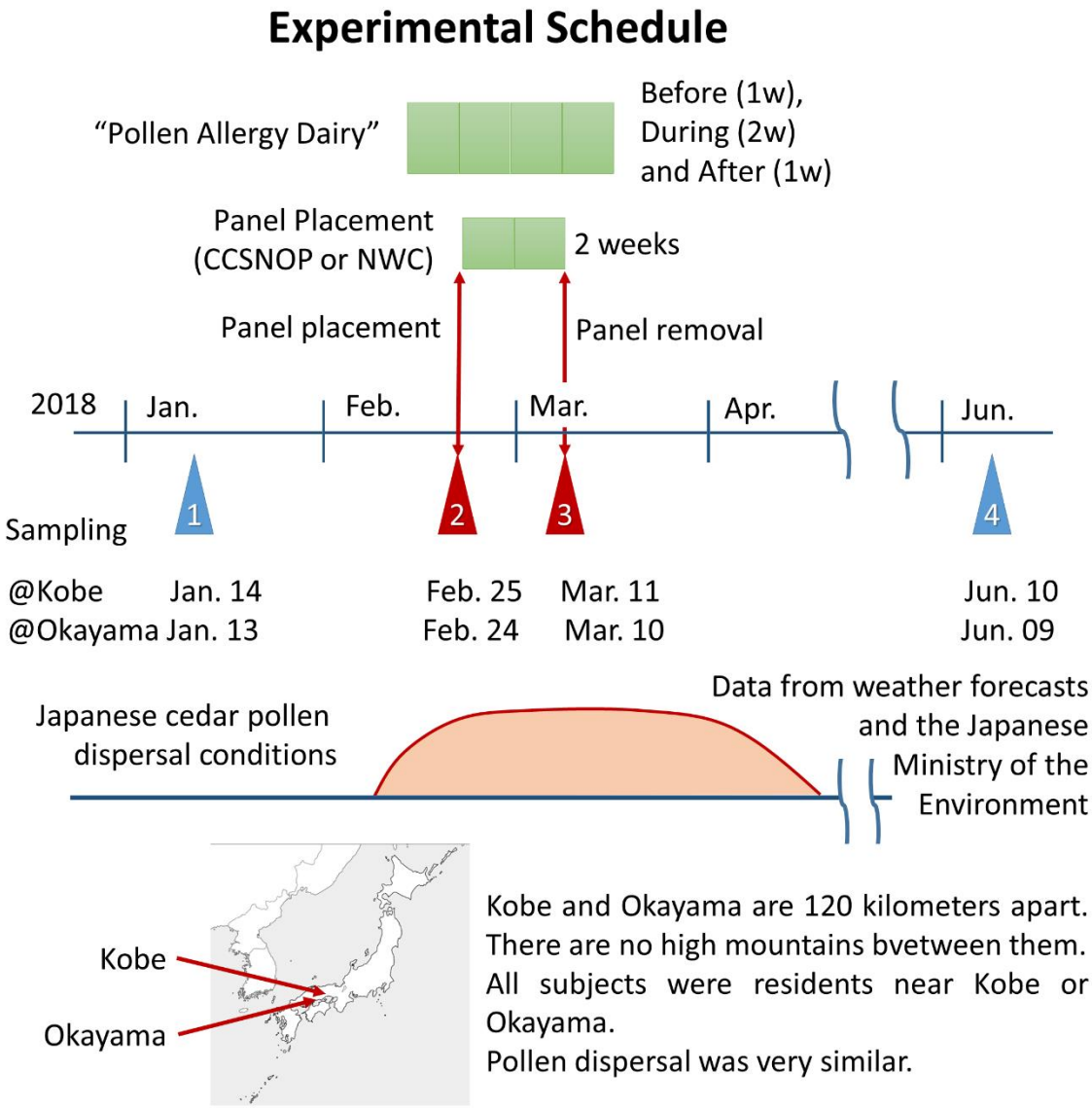
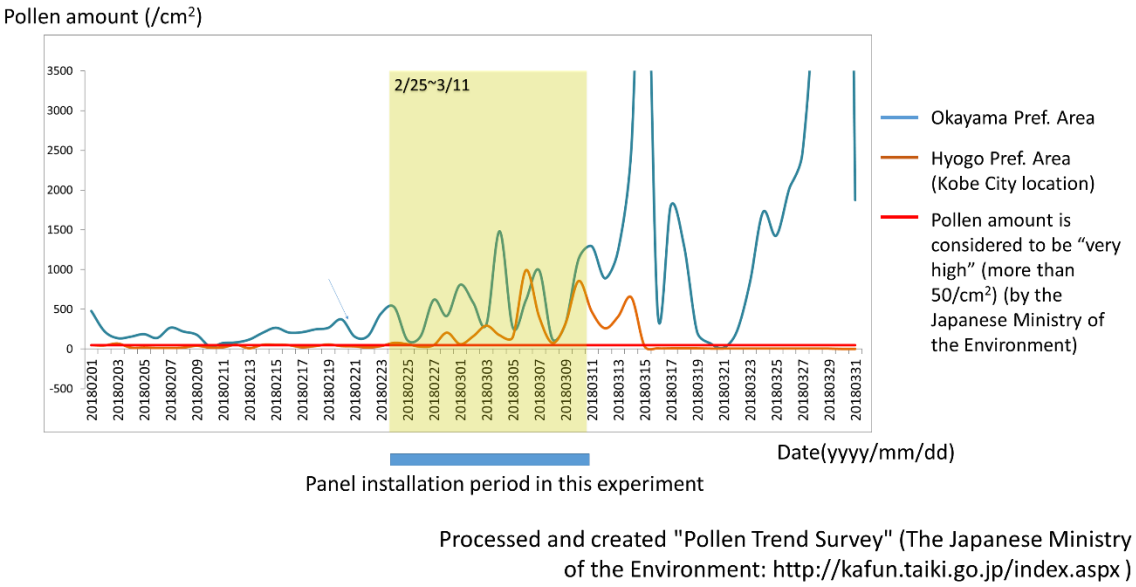


Figure 3. Experimental Schedule. The study was performed from January to June 2018. According to data from Japanese weather forecasts and the Japanese Ministry of the Environment, Japanese cedar pollen dispersal began in early February and ceased at end of April 2018. The degree of pollen dispersal in the Kobe and Okayama areas (where all subjects of this study were living) was similar during this period since both cities are located in the western region of Japan as shown in the map, and are located ca. 120 km apart. There are no high mountains between these areas. The sampling was performed 4 times. The data (mood assessment by POMS2, stress marker by salivary amylase, and blood collection analyses) were collected on Jan. 13 or 14, Feb. 24 or 25, Mar. 10 or 11, and Jun. 9 or 10 at the Okayama and Kobe areas, respectively. The first and last samplings were obtained and represent data reflecting the absence of pollen dispersal. CCSNOP or NWC panels were placed just after the second sampling up until and immediately after the third sampling. Subjects were asked to keep a "pollen allergy diary" as described in the caption of Fig. 2.

The degree of Japanese cedar pollen dispersal was similar in the Kobe and Okayama areas according to weather forecast data and the Japanese Ministry of the Environment. As shown in Fig. 4, the amount of pollen during the period of CCSNOP or control panel installation did not differ significantly in either Okayama City (Okayama Prefecture) or Kobe City (Hyogo Prefecture) where the

198 subjects lived. However, it greatly exceeded the 50/cm² that the Ministry of the
199 Environment of Japan stated as representing a "very high" pollen level.



200

201 **Figure 4.** Changes in the amount of pollen during the panel installation period in Okayama City
202 (Okayama Prefecture, Blue Line) and Kobe City (Hyogo Prefecture, Orange Line) where the subjects
203 of this study were located, as obtained from the pollen trend survey results
204 (http://kafun.taiki.go.jp/index.aspx) of the Japanese Ministry of the Environment. The red line
205 indicates a pollen level of 50/cm², and represents a "very high" level according to the Ministry of the
206 Environment of Japan.

207 *2.4. Pollen allergy diary*

208 As shown in Fig. 3, all subjects kept a "pollen allergy diary" for 4 weeks. An ID
209 number was assigned to subjects by the personal information manager, and was
210 unknown to researchers. Diary entries contained the date, day of the week, and an
211 assessment of the weather. Subjects also recorded any symptoms related to the
212 nose or eye on a scale of 1 (none) to 5 (most severe). Furthermore, problems of
213 daily life caused by pollen allergy was also ranked as 1 (none) to 5 (incomplete).
214 Moreover, the use of medication (oral drugs, nasal drops and eye drops) was
215 described. Other matters related to pollen allergy such as physical and mental
216 condition were recorded in the "others" category. To analyze symptoms "Before",
217 "During" and "After" periods of panel exposure, as well as to compare CCSNOP
218 and NWC groups, recorded scores of 1 to 5 were squared to clarify the differences
219 (symptom weighted score).

220 *2.5. Survey of mood (POMS2)*

221 Investigation of mood was examined using POMS2 [13,14]. Although POMS2
222 is a descriptive questionnaire that conventionally determines the state of mood for

the previous two weeks or so, in our study, subjects were asked to assess their mood state for that present moment.

The questionnaire comprised 30 questions, with answers that ranged in five stages from "not at all" to "very man.". The “feeling condition” was measured according to the following six scales:

- T-A: Tension-Anxiety (Tension-Anxiety), "Feeling tight/under tension" comprised five stages. The higher the score, the more nervous a subject feels.
- D: Depression-Depression (Depression), comprised five stages such as "feeling down or dark." Higher scores indicate increased loss of confidence.
- A-H: 5 stages such as Anger-Hostility and "Bad Mood". A higher score indicates increased anger.
- F: Comprised 5 stages such as "Fatigue" and "I get tired". A higher score indicates an increased feeling of being tired.
- C: Confusion, five items such as "Confusion". A higher score indicates increased confusion and decreased lucidity.
- V: Vigor (Vigor), 5 items such as "Vivid". As this item is a positive item, unlike the other five scales, a lower score indicates that the activity is lost.

Total Mood Disturbance (TMD) was also calculated. This is a comprehensive expression of negative mood state (formula for calculation shown below). The higher the main score, the more negative the mood, and was determined as follows:

TMD = {[anger-hostility] + [confusion-embarrassment] + [depression-depression] + [fatigue-lethargy] + [tension-anxiety]}-[vigor-vitality]

2.6. Saliva amylase

Subjects first rinsed their mouths before sampling, completed the POMS2 questionnaire, and then sAmy measurements were undertaken [15,16]. After placement of the dedicated stick (Nipro saliva amylase monitor chip Product Code 59-010) under the tongue for 30 seconds, the concentration of Amylase was measured using a Salivary Amylase Monitor® according to the manufacturer’s instructions. The results were recorded on a special form.

2.7. Blood sampling

Fifteen milliliters of peripheral venous blood was taken from subjects and the following parameters were measured:

(1) General condition: blood chemistry including liver (AST, ALT and γGT) and kidney (BUN and Creatinine) functions, blood sugar, HbA1c and lipids (including LDL and HDL cholesterols and triglyceride), and peripheral blood counts including white blood cell fraction (granulocytes, eosinophil, basophil, monocytes, lymphocytes, and others) were recorded.

(2) Immunoglobulins (Igs): Ig G, A, M and non-specific Ig E, as well as Ig G4 since Ig G4 is related to food allergy. More recently, Ig G4 is considered to be associated with IG G4-related diseases, which are systemic diseases characterized by elevated serum IgG4, IgG4-positive plasma cell infiltration into the affected tissue, and fibrosis [17-19].

(3) multi-antigen specific Ig E (33 types): This measurement kit typically included antigens for cedar, cypress, house dust, dermatophagoides pteronyssinus, and others.

Measurements (1) to (3) above were performed in a clinical laboratory testing company (BML Inc., Shibuya-ku, Tokyo, Japan).

(4) Cytokines: 29 types of cytokines were measured using a Luminex Cytokine 29-Plex Human Cytokine/Chemokine Panel (HCYTMAG-60K-PX29, Merck Millipore, Billerica, MA). The cytokines included in this kit were EGF, eotaxin, granulocyte-colony stimulating factor (G-CSF), monocyte/macrophage-CSF (M-CSF), interferon (INF)-α2, IFN-γ, interleukin (IL)-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1 receptor antagonist (IL=1ra), IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, Interferon γ-induced protein 10 (IP-10) also known as C-X-C motif chemokine 10 (CXCL10), MCP-1, macrophage inflammatory protein (MIP)-1α also known as chemokine (C-C motif) ligand 3 (CCL3), MIP-1β also known as CCL4, tumor necrosis factor (TNF)-α, TNF-β, and vascular endothelial growth factor (VEGF). Some cytokines showed less than the measurement limitation. In these cases, for convenience, a value of one tenth of the lower limit of the measurement was substituted and utilized for statistical analyses [12,20,21].

2.8. Statistical analyses

Statistical analyses were performed using SPSS version 22 (IBM, Chicago) or Microsoft Office Excel 2013. A significant difference was shown if the degree of risk was less than 5% ($p < 0.05$). To compare the symptom weighted scores of CCSNOP (Ore) and NWC (control; Ctrl), POMS2 score analyses, sAmy concentrations, absolute eosinophil counts, and a comparison of values of the panel-prediction formula in subjects with CCSNOP and NWC, two-way analysis of variance（two-way ANOVA）and Student's t-test (bilateral) were applied. Additionally, the use of allergy-related medicinals by subjects and changes in absolute eosinophil counts were assessed by a χ-squared test. Regarding generation of the formula used to predict subjects who were exposed to CCSNOP or NWC, multiple regression analysis was performed. After obtaining the formula, a receiver operating characteristic (ROC) curve was also generated.

3. Results

3.1. Symptoms related to pollen allergy

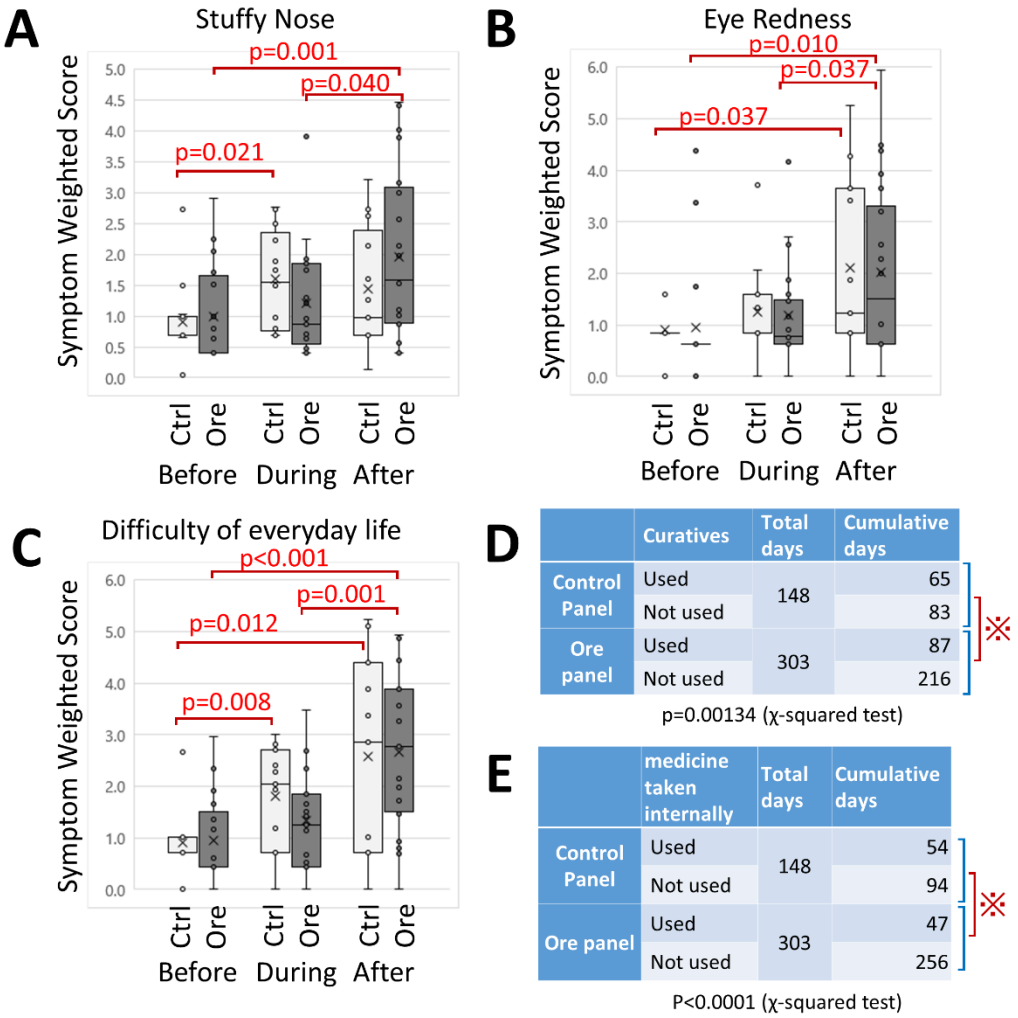


Figure 5. Changes in symptoms. [A] stuffy nose, [B] redness of eyes, and [C] difficulty of everyday life. Data were obtained from the "pollen allergy diary" of individual subjects. Values of severity ranging from 1 to 5 were squared (as "weighted") to clarify the differences. Panels A to C show box-plot graphs. White and gray boxes represent data from NWC (control; Ctrl) and CCSNOP (Ore) subjects, respectively. "Before", "During", and "After" indicate one week before panel placement, two weeks of panel exposure, and one week after panel removal, respectively. The red lines indicate significant difference ($p<0.05$). [D] and [E] show the results of a χ-squared test. [D] use of allergy-related medicinals. [E] use of medicine taken internally.

Changes in symptom severity are shown in Fig. 5. Regarding stuffy nose, subjects with NWC (Ctrl) showed increased severity in the "During" and "After" periods. However, subjects with CCSNOP (Ore) revealed an increase in the severity of this symptom only in the "After" period. Although cedar pollen had been dispersed in the "Before" period, the increase in severity of this symptom in the Ctrl group seemed to suggest a natural course of pollen allergy. However, an increase in the severity of this symptom was not observed for the CCSNOP group in the "During" period (Fig. 5A).

In terms of eye redness, both groups showed increased severity in the "After" period. Thus, there were no differences between the CCSNOP and NWC panel groups (Fig. 5B).

As shown in Fig. 5C, an assessment of the difficulty of everyday life showed similar results. Although subjects with NCW (Ctrl) showed increased difficulties in the "During" and "After" periods, while subjects with CCSNOP revealed increased difficulties only in the "After" period.

Figure 5D shows the results of a χ-squared test regarding days of use of medicinals (general medicine, nasal or eye drops). Figure 5E shows the results of a χ-squared test regarding days of use of medicinals only taken internally. Both results showed that subjects with CCSNOP (ore panel) had lower usage compared with NCW subjects (control panel).

All of these findings indicated that CCSNOP reduced the severity of symptoms associated with cedar pollen allergy. In particular, stuffy nose and difficulty in daily life improved during the installation period. In addition, the CCSNOP environment has led to reductions in medication. These points should be emphasized.

3.2. Changes in mood and stress