

Original Article

Japanese cedar pollen in floating indoor house dust after a pollinating season

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ABSTRACT

Background: Approximately 16.2% of the Japanese population suffers from pollinosis. One of the forms of management is self-care (preventive care), which can be categorized as 'indoor' and 'outdoor'. Outdoor self-care is usually emphasized, but indoor self-care is also important. Considerable pollen is found in indoor dust and this is thought to be one of the factors that worsens pollinosis and enables it to persist for a long time, even after the pollinating period has finished. Taking this into consideration, we investigated the dynamic state of indoor pollen.

Methods: Floating indoor house dust was collected in Petri dishes. The amount of pollen in the house dust samples collected was measured using an LCD laboratory highly sensitive Cry j1 assay kit.

Results: The results showed that, indoors, a lot of Japanese cedar pollen (JCP) was found on the floor (tatami mats, carpets), sofas and curtains. The number of JCP in living rooms peaked in April after the pollinating period and decreased gradually; however, JCP was still found indoors, even as late as the following February. Floating JCP in the house was one-tenth of the JCP levels on the floor. Floating JCP increased on days with low humidity. Air conditioning temporarily increased levels of floating JCP in houses with an air conditioner, but the level of floating JCP decreased rapidly compared with the level of that in houses

without an air conditioner. Nasal signs and symptoms disappeared completely at a level of 30 floating pollen counts/day per Petri dish.

Conclusion: Considerable JCP was found floating indoors with house dust after a pollinating season.

Key words: highly sensitive Cry j1 assay, house dust, Japanese cedar pollen, Japanese cedar pollinosis, self-care.

INTRODUCTION

The frequency of Japanese cedar pollinosis, which develops in early spring, has been increasing and approximately 16.2% of the Japanese population suffers Japanese cedar pollinosis. In the management of pollinosis, one form is medical care (mainly drug therapy) and another is self care (preventive management), such as elimination and avoidance of the allergen. In the latter type of management of the condition, the *Guidelines for the Treatment of Nasal Allergy* describe details for the elimination and avoidance of Japanese cedar pollen (JCP).¹ Outdoor self-care, such as wearing a mask and glasses, is mainly emphasized, but indoor self-care is also important. We have reported previously in many investigations, that considerable amounts of pollen are found in indoor dust, even after a pollinating period.^{2–4} The pollen is believed to collect on clothes or drift into a room through windows and is thought to be one of the factors contributing to the worsening of pollinosis and its persistent for a long time even after the pollinating period is finished. Therefore, in the present study we investigated the dynamic state of indoor pollen.

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Received 14 January 2004. Accepted for publication 19 April 2004.

METHODS

Material investigated and the study period

The material investigated in the present study was house dust collected from 19 houses: six dwellings in which a subject with Japanese cedar pollinosis lived (houses A–F) and 13 homes without a subject suffering from Japanese cedar pollinosis (houses 1–13). To observe the clinical course of allergic rhinitis, a pollinosis diary published by the Japanese Allergy Association was distributed to only the six Japanese cedar pollinosis patients and the subjects were required to write daily entries. In the present study, these subjects suffered from Japanese cedar pollinosis alone, without Japanese cypress allergy and no specific IgE antibody to mites, orchard grass and mugwort.

According to the Nasal Allergy Guidelines,¹ the symptoms at the peak of the pollen dispersion period were classified into five stages as follows: no symptoms and mild, moderate, severe and severest symptoms. Patients A and D had moderate symptom, whereas patients B, C, E and F had severe or severest symptoms. Each nasal symptom was given a numerical score for severity: 0, no symptom; 1, mild; 2, moderate; 3, severe; and 4, severest. Mean scores for nasal symptoms were calculated for each day in every month.

The study period was from April 2002 to February 2003.

Sampling

Samples from a living room, bedroom floor, curtain and sofa

A vacuum cleaner with an LCD Laboratory dust collector was used to collect house dust in a 1 m² area for 2 min and samples were extracted from suspended house dust in 1 mL of 0.01 mol/L phosphate buffered saline (PBS) containing 0.2% bovine serum albumin (BSA). Samples were collected in April, immediately after the end of the pollen dispersion period.

Samples of floating indoor pollen

Figure 1 shows that eight 20 cm diameter Petri dishes were set for 1 day at 2 monthly intervals to collect indoor house dust in the air of a subject's living room at 25 cm intervals from the floor to a height of 175 cm.

The house dust collected on the Petri dishes was swiped with five pieces of 1 m² filter paper immersed in a

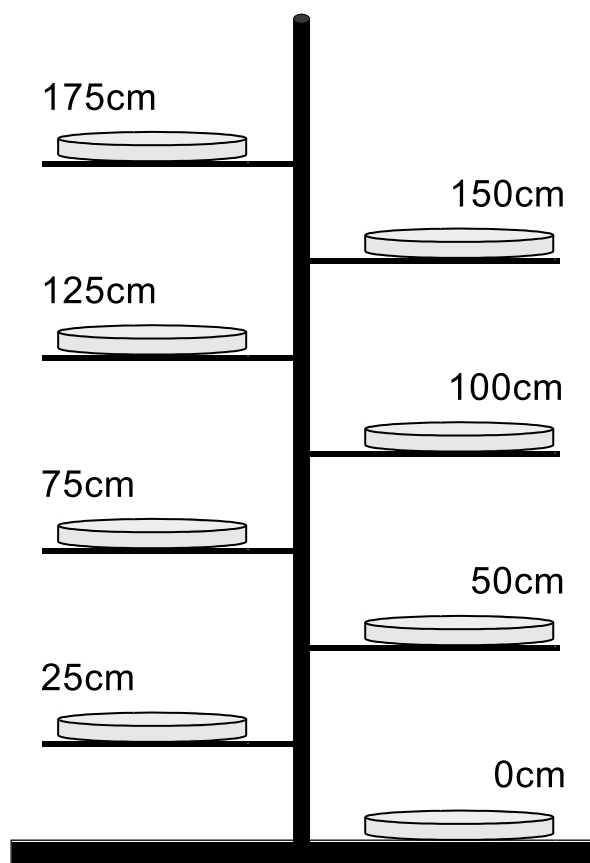


Fig. 1 Method for collecting indoor floating house dust (20 cm diameter Petri dishes are set at 25 cm intervals from the floor to a height of 175 cm).

solution of 0.01 mol/L PBS containing 0.2% BSA. Then, the filter paper was suspended in 1 mL of 0.01 mol/L PBS containing 0.2% BSA solution.

Cry j1 assay

The amount of Cry j1 in extracted samples was determined using an LCD Laboratory highly sensitive Cry j1 assay kit. Briefly, the wells of a Nunc immunoplate were coated with 100 µL anti-Cry j1 antibody (0.2 µg/mL monoclonal antibody; LCD Laboratory) for 2 h at 37°C and washed five times with 300 µL of 0.01 mol/L PBS (pH 7.4) containing 0.5% Tween 20. After being blocked with 300 µL of 0.01 mol/L PBS containing 2% BSA for 2 h at 37°C, the wells were washed again. Then, a 100 µL aliquot of each sample was added to the wells and incubated for 10 h at 25°C. After a third wash, a biotinylated anti-Cry j1 antibody (LCD Laboratory rabbit polyclonal antibody) was added to the wells

and incubated for 3 h at 37°C. After washing, 100 μ L conjugated streptavidin- β -D-galactosidase was added to the wells and samples were incubated for 1 h at 37°C. After washing, 100 μ L of 10 mmol/L o-nitrophenyl- β -D-galactopyranoside was added to the samples to develop

a color reaction for 10 min at 37°C. As the final step, the reaction was terminated by the addition of 1.5 mol/L sodium carbonate and, after stirring, the absorbance of the samples was measured at 415 nm using a micro-plate reader.

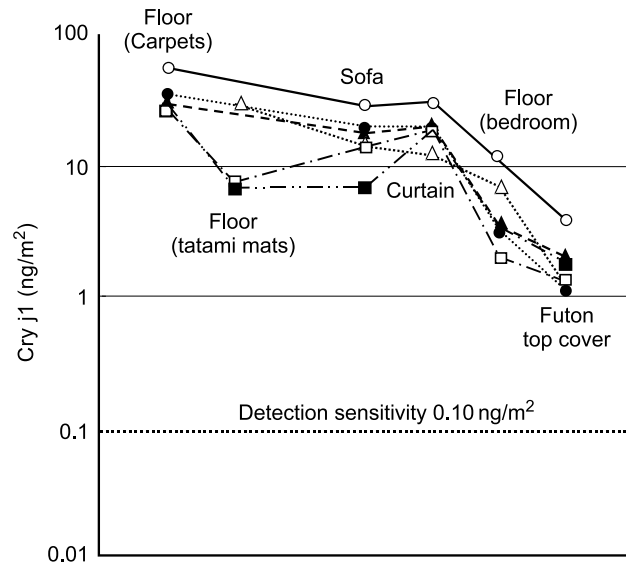


Fig. 2 Pollen remaining in indoor locations in April in houses A–F (○, ●, △, ▲, □ and ■, respectively).

RESULTS

The indoor location of JCP

Figure 2 shows the amount of Cry j1 per m^2 collected in houses A–F. A lot of pollen was found on the floor (carpet, tatami mat), sofa and curtains. The largest amount of pollen was 56.2 ng/m^2 (equivalent to approximately 9367 counts/ m^2) found on the floor (carpets) of a house.

Indoor floating JCP

Indoor floating JCP after the end of the dispersion season

The amount of Cry j1 in Petri dishes placed in houses A–F was measured from April 2002 to March 2003. Figures 3,4 show that houses A and D had a smaller amount of indoor JCP than did the other dwellings.

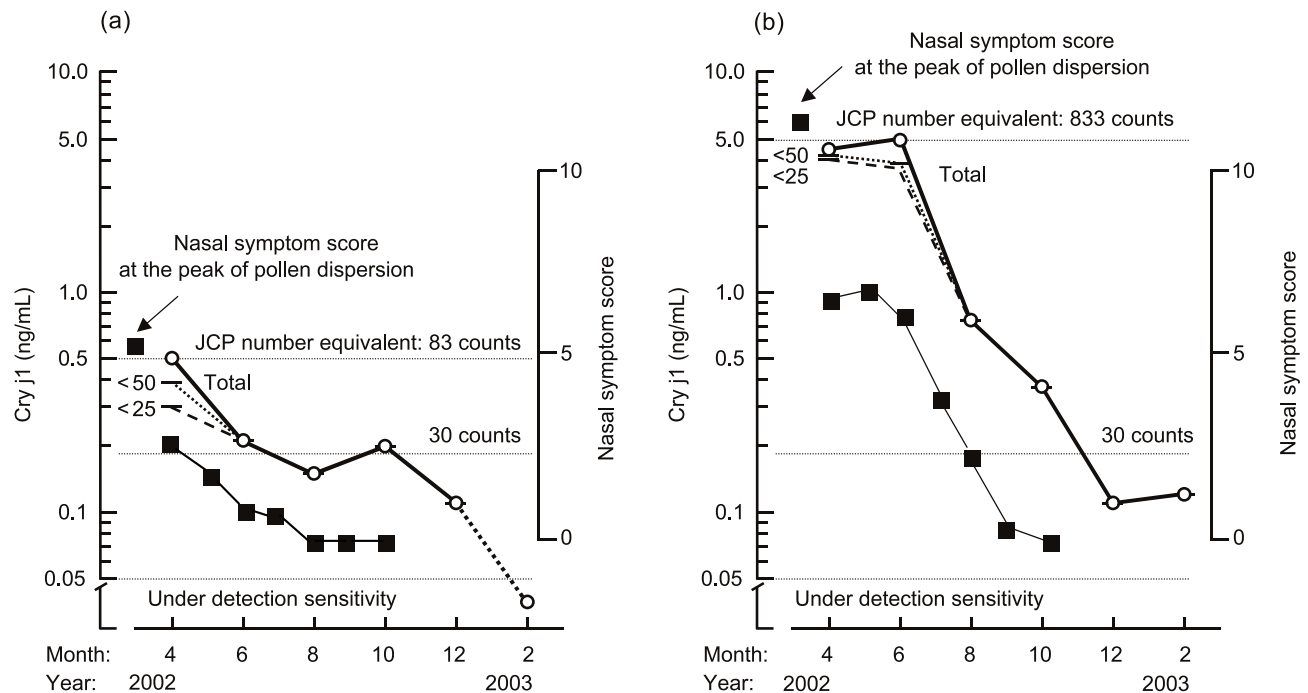


Fig. 3 Floating indoor Japanese cedar pollen (JCP) after a pollinating period in houses A (a) and B (b) at levels below 25 cm (---), below 50 cm (---) and in all Petri dishes (—). (■), nasal symptom score. Nasal symptoms disappeared in patient A from 7 August (a) and in patient B from 10 October (b).

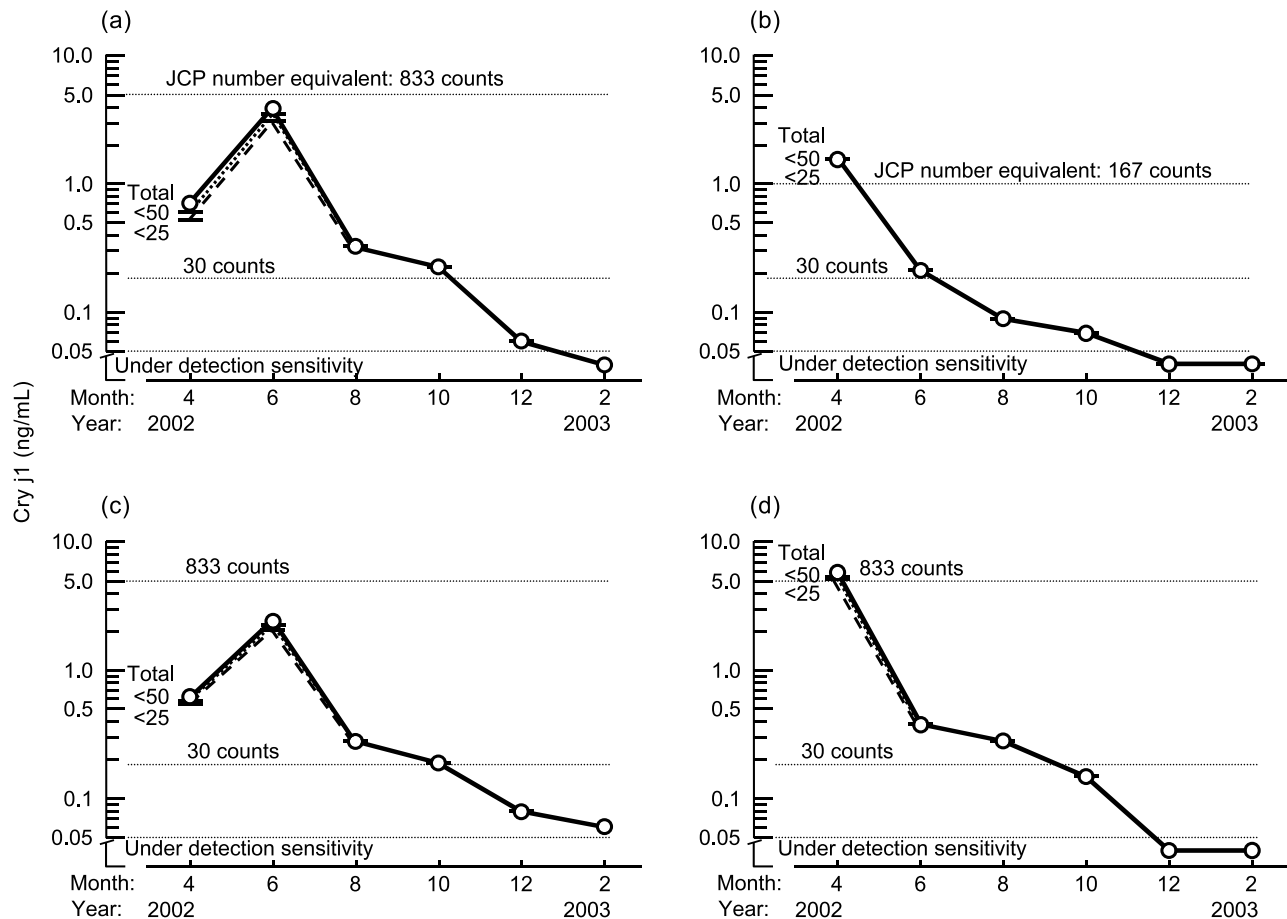


Fig. 4 Floating indoor Japanese cedar pollen (JCP) after a pollinating period in houses C (a), D (b), E (c) and F (d) at levels below 25 cm (---), below 50 cm (---) and in all Petri dishes (—). Nasal symptoms disappeared in patient C from 25 September, in patient D from 7 July, in patient E from 21 October and in patient F from 21 September.

However, every house had almost the same amount of Cry j1 in each Petri dish below a height of 50 cm. These findings suggest that floating JCP is almost non-existent in rooms above a height of 50 cm. Other findings showed that the number of JCP in a room decreased as the pollen dispersion season came to an end and persistent pollinosis after the end of the season disappeared when the pollen count decreased to levels of 30 counts/m² or less.

Figure 3 shows the mean score for nasal symptoms per day in every month for patients A and B. The nasal symptom score of patient A decreased in accordance with the amount of floating Cry j1 and disappeared by 7 August. In contrast, the nasal symptoms of patient B, who lived in a house with a larger amount of floating Cry j1 than did patient A, disappeared later, in October

(Fig. 3). The disappearance of nasal symptoms, as well as a decrease in the amount of floating Cry j1 in the house, of patients C, E and F took a longer time. Meanwhile, the nasal symptoms in patient D improved earlier, as for patient A, because there was a smaller amount of floating Cry j1 in the house (clinical data not shown in Fig. 4).

Correlation between the floor and floating pollen

There was a correlation in pollen counts/m² between the floor and floating pollen. Figure 5 shows that the amount of floating pollen was between one-fifth and one-fortieth (average one-tenth) of the amount of pollen on the floor (although this number fluctuated depending on the month investigated).

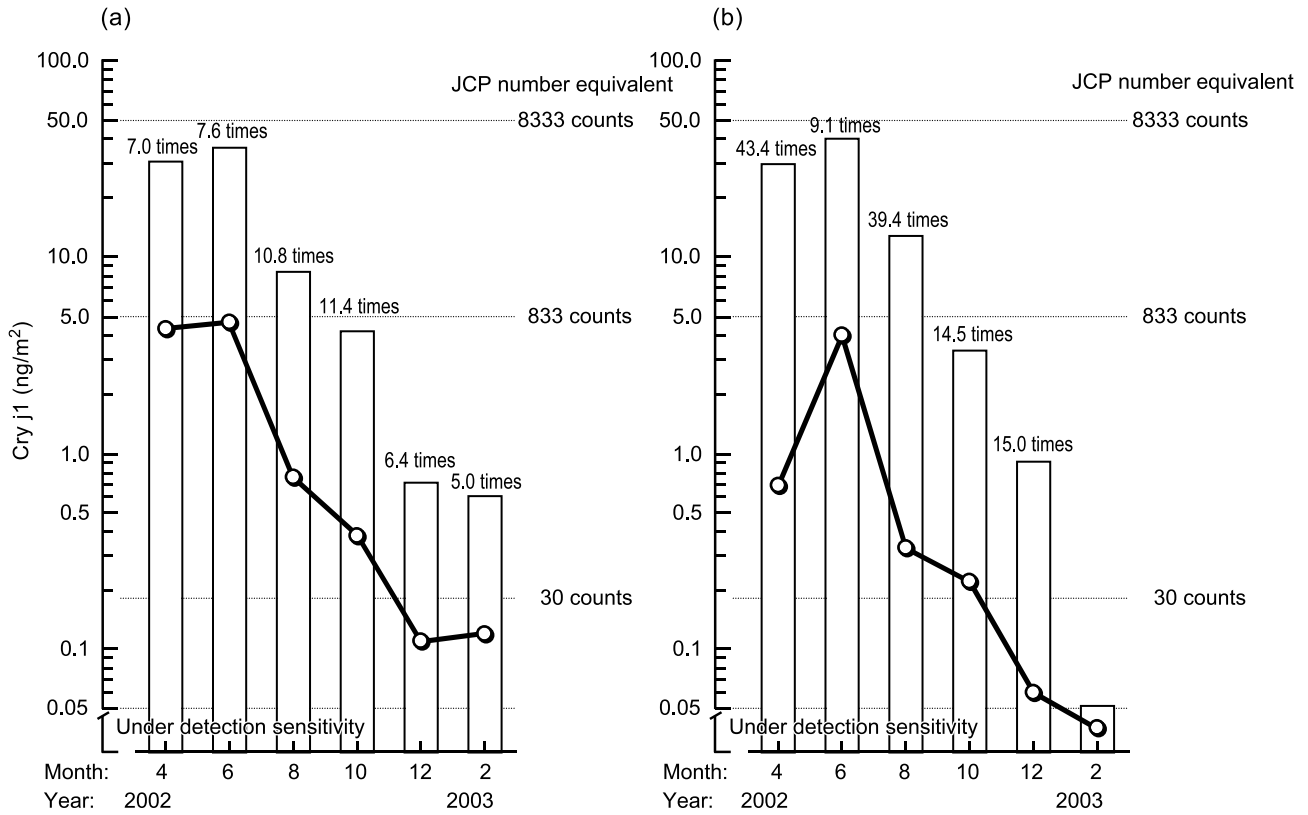


Fig. 5 Correlation between the pollen levels on the floor ($\square$) and floating pollen ($\circ$) in houses B (a) and C (b). JCP, Japanese cedar pollen.

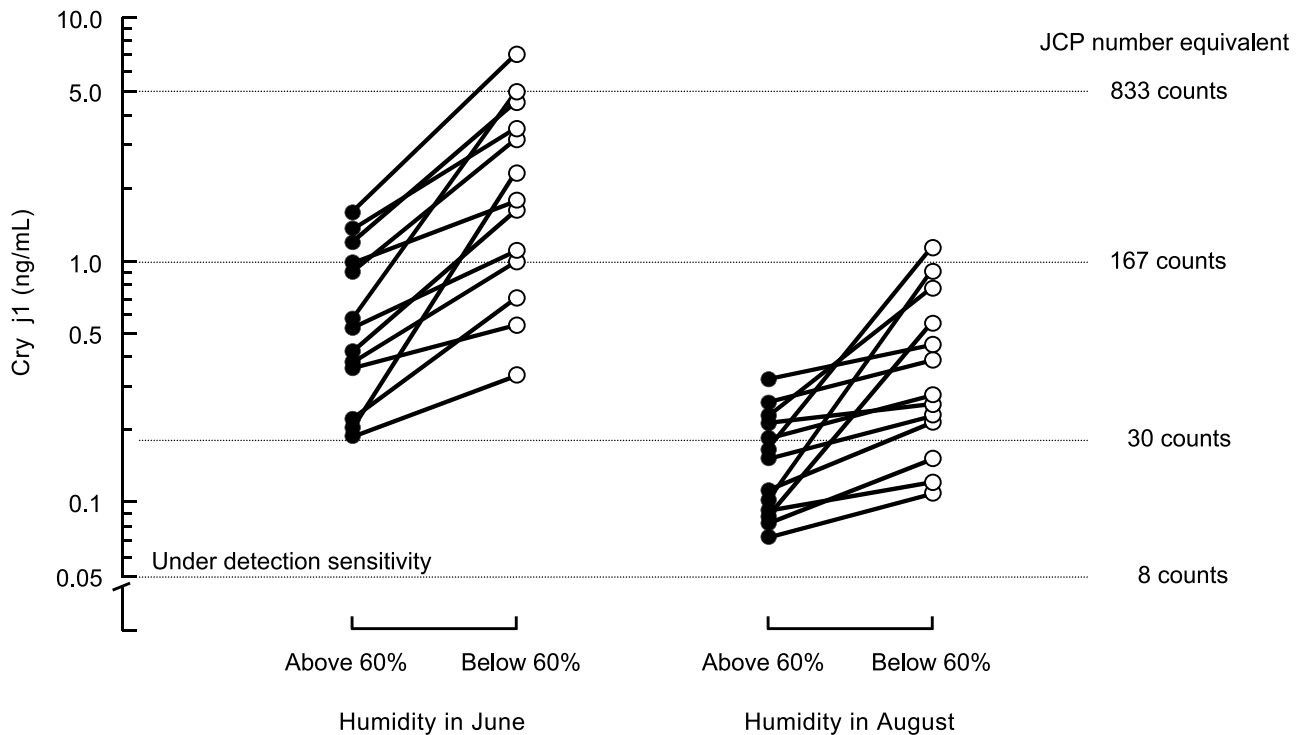


Fig. 6 Relationship between humidity and floating indoor Japanese cedar pollen (JCP). The JCP levels were measured for 1 week in the living room of 13 non-allergic subjects.

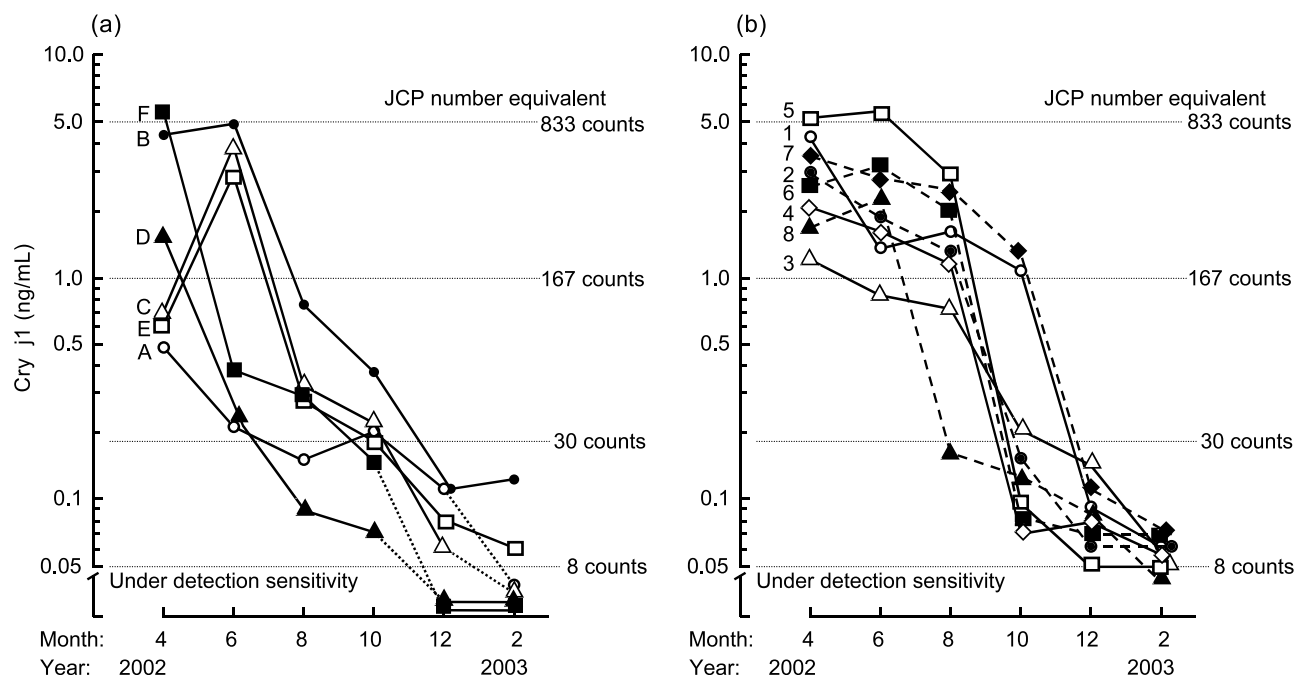


Fig. 7 Floating indoor Japanese cedar pollen (JCP) levels in homes (a) without air-conditioning (houses A–F; allergic subjects) and (b) with air-conditioning (houses 1–8; non-allergic subjects).

Relationship between humidity and floating pollen

The relationship between humidity and floating pollen was investigated in the houses of 13 non-allergic subjects on a day with high humidity (60% or more), as well as on a day with low humidity (less than 60%). The results showed that the amount of floating pollen on days with low humidity was significantly higher than that on days with high humidity (Fig. 6).

Change in the amount of floating-pollen caused by air-conditioning

A comparison of floating pollen was made between houses of six pollinosis patients who did not have air-conditioning and eight non-allergic subjects who did have air-conditioning. In Fig. 7, it can be seen that indoor floating JCP (the amount of Cry j1) persisted longer in houses with the use of an air-conditioner (until August). After that time, the the amount of Cry j1 decreased rapidly in houses with an air conditioner compared with the amount in houses without an air conditioner.

DISCUSSION

The number of Japanese people suffering from Japanese cedar pollinosis has been increasing recently. The

management of Japanese cedar pollinosis is a matter of some concern. One form of management is medical treatment and another is self-care. To reduce health-care costs, self-care in the case of Japanese cedar pollinosis is more important than medical care. The Japanese Guidelines for the Diagnosis and Management of Nasal Allergy¹ stipulate preventive measures, such as elimination and avoidance:

1. Be aware of up-to-date information regarding pollen in the area.
2. Minimize outdoor activity when the pollen count is high.
3. Keep doors closed when the pollen count is high.
4. Wear a mask and glasses while you spend time outside at times of high pollen levels.
5. Do not wear a fluffy fur coat when you go out.
6. Shake off pollen from your hair and clothes, wash your face, gargle and blow your nose when you come home.
7. Clean indoors regularly.

The advice given in point 7 regarding cleaning indoors was presumably written as a preventive tip to protect against pollen drifting into houses, but the above description does not provide specific information. The location, amount and the time period over which indoor pollen persists (i.e. pollen kinetics, as it were) have not been fully investigated yet. One of the main reasons for

this is the difficulty in observing the pollen collected in the gathered indoor dust with the naked eye. Under a microscope, Torii *et al.*⁵ investigated the amount and type of pollen collected in five detached houses in Yokohama City for 1 week in May, August and November 1996 and in February, 1997. These authors reported that the amount of pollen was lowest (2×10^5 counts/g) in February and highest (2×10^6 counts/g) in May (pollen from pine and quercus) and February (JCP). The report also stated that pollen levels were high within a pollinating period, but that pollen was still found even after the pollination period was over. Therefore, pollen remains perennially in indoor dust. However, the problems with the study of Torii *et al.*⁵ are that microscopic observation requires a considerable amount of work and the usefulness of the results is controversial: allergen activity is based on the Ubish body of the anthers attached to JCP and Cry j1 concentrations, which is dependent on cedar species, which vary considerably in Cry j1 concentrations.^{6,7} Reviewing these facts, allergen activity is rather more important in the correct assessment of indoor pollen than the actual amount of pollen. In the collaboration with the LCD Laboratory, we developed highly sensitive Cry j1 assay kit,³ which enables us to measure down to a level of 13 pollen counts. Using this device, it has been reported that a lot of pollen is found in indoor dust during the off-seasons.⁴ Recently, Abe *et al.*⁸ reported that they investigated seasonal changes in concentrations of Dac g, the main allergen of orchard grass belonging to the rice plant family Gramineae, in Yamagata Prefecture.

These days, out-patients with persistent clinical allergic symptoms and signs sometimes present to hospitals. In this regard, the dynamic state of JCP was examined using the highly sensitive Cry j1 assay kit after the Japanese cedar pollinating period was over. The results revealed the following: (i) indoor JCP was found a lot on the floor (tatami mats, carpets), sofa and curtains; (ii) the amount of JCP in the living room peaked in April after the pollinating period and then decreased gradually, but JCP was still found even as late as the following February;

(iii) floating JCP in the house was one-tenth of the JCP levels on the floor; (iv) floating JCP levels increased on days with low humidity; (vii) air-conditioning temporarily increases floating JCP levels in the house, but JCP levels decrease earlier in the end; and (viii) nasal signs and symptoms disappeared completely at a level of 30 floating pollen counts/day per Petri dish. These results will contribute to the improvement the self-care for Japanese cedar pollinosis.

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