

The relationship between Japanese cedar pollinosis and air pollutants deposited on the pollen

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Abstract

In this study, air pollutants such as NO_3^- and SO_4^{2-} which were found deposited on Japanese cedar pollen in a mountainous area (Tsukechi-cho), and in an urban area (Mizuho Ward, Nagoya City) were measured by ion-chromatography in March 1987 and again in March 1991. An investigation of the pollinosis rate in about 400 Junior High School students in both areas was undertaken during the same periods through both a questionnaire study, and a physical examination. Information was also gathered on the number of vehicles passing through the corresponding areas. The results of this research are as follows: (1) The quantity of NO_3^- deposited on the pollen of Japanese cedar was about 2.3 and 2.0 times greater in the urban area than in the mountainous area (1987 and 1991, respectively). The concentration of SO_4^{2-} was about 7.8 and 3.6 times higher in the urban area than in the mountainous area (1987 and 1991, respectively). (2) The results of a questionnaire study taken at the two Junior High Schools from the different districts showed a lower incidence of nasal symptoms in the mountainous area than in the urban area for 1987. The percentage of pollinosis shown by physical examination in the mountainous area rose from 20.1% in 1987 to 30.0% in 1991, and this corresponds to a rise in the number of motor vehicles in the area. Comparatively, in the urban area, the questionnaire results and physical examinations revealed no changes from 1987 to 1991.

Keywords: Cedar pollinosis; Air pollution; NO_x ; SO_x ; Aerobiology

1. Introduction

The incidence of allergy to Japanese cedar (*Cryptomeria japonica*) pollen has increased to a level that allows us to legitimately term it a national disease in Japan (Horiguchi and Saito, 1964; Ishizuka et al., 1987). In a previous study, we showed that about 73.8% of nasal allergy patients have specific IgE to cedar allergen, and suffer from sneezing, nasal discharge, and nasal obstruction in the pollinating season (mainly February, March, and April) (Ito et al., 1995; Suzuki et al., 1995). This increased incidence has been attributed both to air pollution, and increased pollen production

resulting from the extensive planting of Japanese cedar trees after World War II (Muranaka et al., 1986). Cedar trees were planted in over 90% of the mountainous areas in Japan.

However, there is room for argument against this point, considering that cedar trees were abundant prior to the end of World War II, and that cedar pollinosis began to be reported heavily only about 30 years ago (Horiguchi and Saito, 1964).

The increase in pollinosis cannot be explained by the increase in the amount of pollen alone. Thus, a relationship between air pollutants and pollinosis has been suspected (Ishizuka et al., 1987), and there have been some reports suggesting IgE antibody production and air pollution are related. Yet there has been no evidence to show this directly. In Japan, both motor vehicle use

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and cedar pollinosis significantly increased starting about 30 years ago, but the causal relationship of air pollution to pollinosis has not been explained.

This study was conducted to focus on the air pollutants deposited on the pollen. Air pollutant substances: NO_3^- and SO_4^{2-} on the pollens were measured using ion-chromatography, and were monitored and measured in two distinctly different areas. Also, a questionnaire study of nasal symptoms, and physical examination for pollinosis in Junior High School students from these areas were performed. The results were analyzed and correlated with data from passing vehicles in the two areas to clarify the role of air pollutants in the development of allergy.

2. Materials and methods

2.1. Sampling sites

The sampling sites include a National Forest area in Tsukechi-cho, Gifu Prefecture (a mountainous area), and Mizuho Ward, Nagoya City (an urban area of a large city). The population of Nagoya City is two million (2 000 000) people, while that of Tsukechi-cho, is 20 000. Tsukechi-cho is situated 150 km away from Nagoya City. In 1990, a new National Highway was built through the center of Tsukechi-cho.

2.2. Pollen sample collection

The pollen from Japanese cedars was collected from several trees at the two sites. Samples of pollen were collected during the pollinating season, from 10 March to 20 March in both 1987 and 1991. Pollen samples were collected from six branches at about 2 m above ground level from each tree. The measured values are given in terms of pollutant weight, per gram of plant sample.

2.3. Pollen count

Pollen counts were recorded daily with a Durham sampler placed at the sampling sites. After 24 h exposure, the pollen grains were stained, then counted by microscopy. Counts are expressed as the number of pollen grains per square centimeter per day.

2.4. Analytical method

Pollen samples were dried and 1 g of sample was mixed with 10 ml of distilled water. Samples were extracted by ultra-sonification for 15 min, filtration through a 0.45- μm membrane filter (Toyo Co., Japan), then analyzed for NO_3^- and SO_4^{2-} by ion-chromatography (Diamox 20101 type, USA). Mixed samples were

then measured three times and values expressed in $\mu\text{g/g}$.

2.5. Physical examination and health survey

In March 1987 and again in March 1991, about 400 Junior High School students in both Tsukechi-cho and Mizuho Ward, were tested for evidence of pollinosis. This was done through a questionnaire study, and an in-school physical examination of the nose by an otorhinolaryngologist. The students were diagnosed as having pollinosis through the rhinoscopic findings, nasal eosinophilia (Malmberg and Holopainen, 1979), by detecting the cedar-specific IgE in sera using the CAP-RAST system (Kabi Pharmacia Diagnostic AB, Sweden) (Ewan and Coote, 1991), and the incidence of seasonal attacks as evidenced by the questionnaire.

2.5.1. Questionnaire questions

(1) Do you have nasal symptoms (sneezing, nasal discharge and/or nasal obstruction) in the Spring season? (YES/NO);

(2) When did these symptoms start? (a) from this year, (b) 2–3 years ago, (c) several years ago.

2.5.2. Physical examination criteria

There is a positive diagnosis of allergic rhinitis to cedar pollen if: the inferior turbinate in the nasal cavity was pale and swollen with recognizable watery secretion; recognizable eosinophils in the nasal discharge and a positive CAP-RAST result with over 0.35 kU/l.

For these examinations, we obtained informed consent from the students and their parents.

2.6. Cited data

The cited data on air pollution and the number of vehicles travelling on the main roads were measured in 1987 and in 1991 by the Anti-pollution Division of

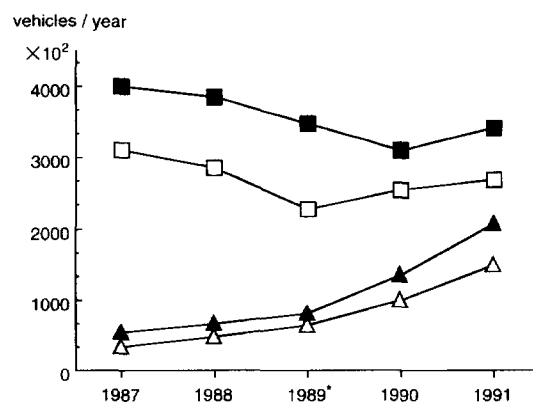


Fig. 1. Annual average number of vehicles: -■- Mizuho-ward (urban area, Route 504); -▲- Tsukechi-cho (mountainous area, Route 257); -□-△- diesel vehicle.

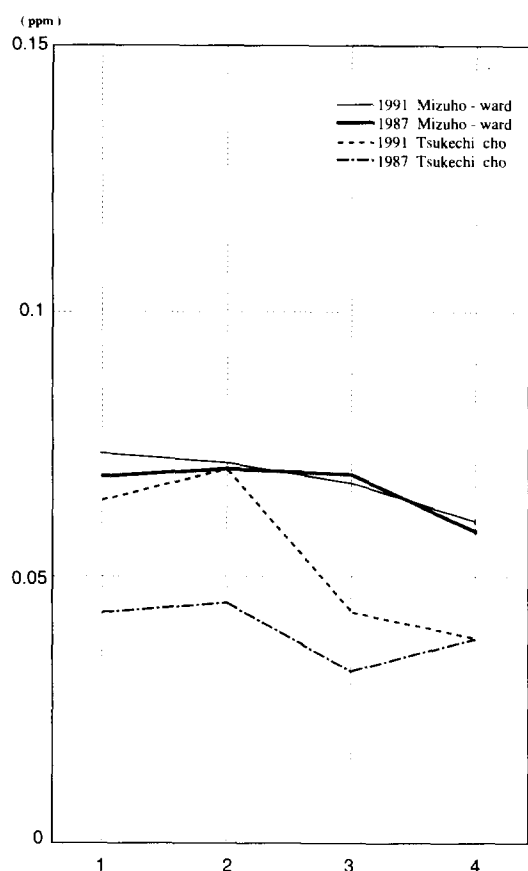


Fig. 2. The averaged NO_x measurement data on air pollution from January to April.

Nagoya City, and Tsukechi-cho, respectively (Figs. 1–3). Each sampling site was the same area used as the air pollution monitoring site.

2.7. Statistical analysis

Statistical analysis of the data obtained was performed by the χ^2 , and the *t*-test.

3. Results

(1) In 1987 and in 1991, the concentration of SO_4^{2-} in pollen samples was respectively 7.8 and 3.6 times higher for the urban area than for the mountainous area ($P < 0.05$). The NO_3^- concentration was 2.3 and 2.0 times higher, respectively for the urban area than for the mountainous area. But significantly, pollutants deposited on to the pollen in the mountainous area increased between 1987 and 1991 (Table 1) (Behredt et al., 1990).

(2) Through the questionnaire study, the incidence of nasal symptoms in students was found to be lower among the students from the mountainous area than in

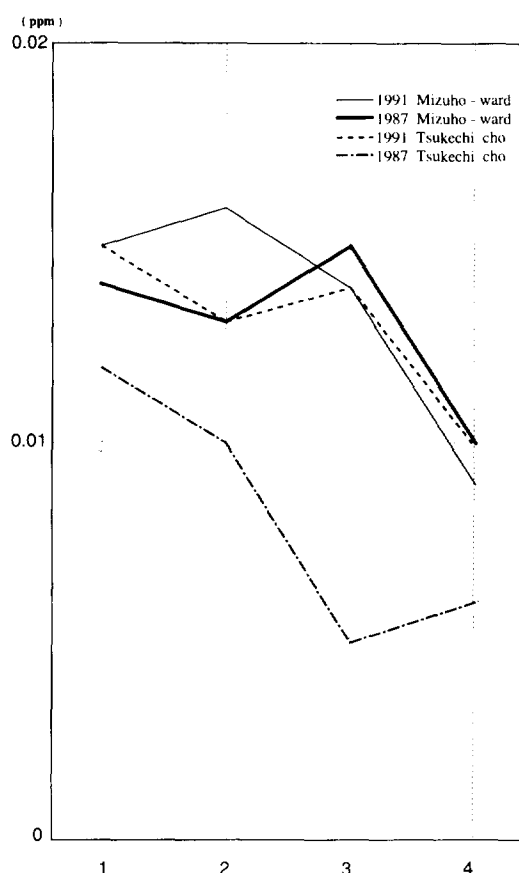


Fig. 3. The averaged SO_x measurement data on air pollution from January to April.

the urban area ($P < 0.01$) for 1987. But in 1991, the percentage of students with nasal symptoms from the mountainous area increased from 16.5% in 1987 to 24.6% ($P < 0.01$). In this period, in the urban area, there was no change. According to the onset times of nasal symptoms, in the urban area, the number experiencing symptoms for over 2–3 years was higher than in the mountainous area in 1991; in 1987, there were no significant differences (Tables 2 and 3). The National Highway passing through Tsukechi-cho was completed and opened to traffic in 1989.

Table 1
Measurement of NO_3^- and SO_4^{2-} deposited on pollens ($\mu\text{g/g}$)

		Mizuho-ku (urban area)	Tsukechi-cho (mountainous area)
NO_3^-	1987	14 ± 4.7	6 ± 3.0
	1991	16 ± 4.6	8 ± 1.6
SO_4^{2-}	1987	1998 ± 692^a	$254 \pm 96^{a,c}$
	1991	1756 ± 422^b	$488 \pm 87^{b,c}$

Values with same superscript letter have a significant correlation at $P < 0.05$ (*t*-test).

Table 2
Questionnaire study results of junior high school students

District	Year	Question 1	Question 2		
		Yes	This year	2–3 years	Several years
Mizuho-ku (urban area)	1987	24.8% (102/410)	12%	36%	52%
	1991	27.5% (110/400)	13%	34%	53%
Tsukechi-cho (mountainous area)	1987	16.5% (68/410)	25%	36%	39%
	1991	24.6% (100/405)	20%	42%	38%

In the physical examination study, the incidence of students with pollinosis was discovered to be lower in the mountainous area than for the urban area in 1987. But in 1991, in the mountainous area, the percentage of students with pollen allergy shows an increase from 20.1% in 1987 to 31.0% ($P < 0.01$). In the urban area, there was no significant change (Table 3).

(3) The average number of vehicles on the road in the two areas studied, shows an increase only for the mountainous area and this corresponds to an increase in pollinosis for that area (Fig. 1). This is reflected by the 1991 data which show an increase in the number of students from the mountainous area suffering nasal symptoms for 2–3 years, and this corresponds to the period when the National Highway was opened to traffic.

4. Discussion

Air pollution is composed of “waste products in the form of gases and small suspended particles in the atmosphere.” (*Webster's New Concise Dictionary and Thesaurus*, Russel, Geddes and Grosset, New York, 1990). The most common of these components are sulfur dioxide, nitrogen oxide, DEP (diesel exhaust particulates), and petrochemical oxidants. In a narrow sense, this pollution is caused by the activities of humans such as the burning of coal and oil, and the subsequent chemical reactions in the atmosphere caused by these activities.

In Japan, air quality standards for polluting substances are defined by the Air Pollution Control Law

(1968), which does not cover diesel-engined vehicles. Of the common air pollutants, sulfur dioxide, nitrogen dioxide, and photochemical oxidants are not monitored by the national and regional public organizations. The DEP component of air pollution is difficult to quantify because DEP is made up of over 2000 organic compounds. DEP has yet to be monitored in Japan. There are many reports that point to a link between air pollution and the pathogenesis of allergy (Fujimaki et al., 1984; Fujimaki et al., 1986; Massiot and Cohen, 1986; Ruffin et al., 1986; Takafuji et al., 1987; Ishizuka et al., 1987; Popp et al., 1989; Braback and Kalvesten, 1991). The various mechanisms for the effect of pollutants are discussed by these reports.

A most likely explanation would be that pollutants increase the permeability of the respiratory mucosa (Holt et al., 1990) thus affecting humans directly. Experiments on guinea pigs have shown that ozone, nitrogen oxide, and sulfur dioxide all increase the risk of allergic sensitization (Matsumara, 1970; Riedel et al., 1988). Popp et al. (1989) suspected that increasing levels of air pollution could undermine local defense barriers and increase mucosal hypersensitivity.

A further adverse mechanism may be the increased antigenicity of pollens exposed to air pollutants (Riedel et al., 1988). A direct effect on the T lymphocytes has also been considered (Warren et al., 1982). Electron microscope studies of airborne pollen indicate that atmospheric pollutants may even act directly on pollen grains by binding (attaching) to their surface thus altering their morphology (Behredt et al., 1990).

Thirty years have passed since the first incidence of Japanese cedar pollen allergy case was diagnosed (Horiguchi and Saito, 1964). Patients suffering from

Table 3
Positive incidence of pollinosis from the physical examination results of junior high school student

District	1987	1991
Mizuho-ward (urban area)	31.3% (125/400) ^a	32.0% (128/400)
Tsukechi-cho (mountainous area)	20.1% (84/400) ^{a,b}	31.0% (124/400) ^b

Values with same superscript letter have a significant correlation at $P < 0.01$ (χ^2 correlation test).

Table 4
Total pollen count for Japanese cedar in 1987 and 1991

District	1987 (Feb. 1–Mar. 30)	1991 (Feb. 1–Mar. 30)
Mizuho-ward (urban area)	1340	1050
Tsukechi-cho (mountainous area)	6534	5463

Values are grains/cm² by Durham sampler.

this severe pollinosis have recently been increasing in number, more than patients of other antigens, especially during February and March (the peak of the pollinating season). In our previous study (Ito et al., 1995; Suzuki et al., 1995), cedar pollen was found to be a major antigen in nasal allergy among young patients in Japan, ostensibly as a result of the increased amounts of Japanese cedar pollen. This increase may have arisen from the extensive reforestation with Japanese cedar after World War II (Muranaka et al., 1986). Yet, as can be seen from the pollen count data taken in 1987 and again in 1991, the pollen count has shown a decrease (Table 4). Air pollution, particularly from particulates emitted by diesel engines, may play a role because they may act as an adjuvant for the antigen of pollen allergy (Takafuji et al., 1987; Takafuji et al., 1989). In Japan, the number of diesel-engined vehicles has increased. Their exhaust causes much more pollution (e.g. NO_x , SO_x , DEP, etc.) than do similar gasoline-engined vehicles.

However, there is no clear indication as to how this induces nasal symptoms. In so far as the pollen is inhaled into the nasal cavity, it is necessary to elucidate the degree of contamination of the pollen by air pollutants. Therefore, the authors have developed a measurement method which directly measures the air pollutants (NO_x and SO_x) deposited on the pollen.

Thus, the concentration of SO_4^{2-} in pollen samples was found to be 7.8 times higher for the urban area than for the mountainous area in 1987 ($P < 0.05$), correlating the increase of incidence of pollinosis in the mountainous area with the increased levels of SO_4^{2-} as evidenced by the pollen samples taken in 1991 matched with the questionnaire study and physical examination results.

With NO_3^- , the concentrations in the urban area were 2.3 and 2.0 times higher respectively in 1987 and 1991, compared to the mountainous area. However, both NO_x and SO_x appear to have a much greater concentration in pollen than in the air. The number of motor vehicles passing through the metropolitan area of Nagoya was about 10 times greater than those passing through Tsukechi-cho in 1987, but after the new highway was opened in 1989, the traffic increased substantially (Fig. 1).

The role that air pollutants attached to pollen play in the mechanism of developing an allergy to Japanese cedar pollen has yet to be discovered. As stated by Ishizaki and Kogure (1984), patients diagnosed as having pollen allergy increased as the number of motor vehicles passing through their area increased. In our investigation of Junior High School students in Tsukechi-cho through questionnaire study and physical examination, this relationship is also suggested. Despite the greater production of pollen in the mountainous area for 1987, the incidence of pollinosis was lower

there than in Mizuho Ward. However, the 1991 data indicate an increase in pollinosis roughly in proportion to the increase in the number of vehicles on the newly built National Highway. In the urban area, the results were similar for both 1987 and 1991.

In this study, the authors have concentrated on the presence of NO_3^- and SO_4^{2-} in Japanese cedar as reflected in our data. However, pollutants other than those discussed in this article may attach to Japanese cedar pollen. This paper is the first to report on the relationship between pollinosis and air pollutants from vehicles attached to pollen, and shows not only an excellent method for examining the contamination found in pollen, but also for measuring air pollution for a particular area. However, it is necessary to clarify the way in which the air pollutant antigen is involved in allergic reactions.

References

- Braback, L. and Kalvesten, L. (1991) Urban living as a risk factor of atopic sensitization in Swedish school children. *Pediatr. Allergy Immunol.* 2, 14–19.
- Behredt, H., Friedrich, K.H. et al. (1990) Allergens and pollutants in the air: a complex interaction (Abstract). *Allergologie* 13, 257.
- Ewan, P.-W. and Coote, D. (1991) Evaluation of a capsulated hydraulic carrier polymer (the Immuno-CAP) for measurement of specific IgE antibodies. *Allergy* 45, 22–29.
- Fujimaki, H., Ozawa, M. et al. (1984) Adjuvant effects of aluminium silicon on IgE and IgG antibody production in mice. *Int. Arch. Allergy Immunol.* 75, 351–356.
- Fujimaki, H., Hirano, S. et al. (1986) Enhanced IgE antibody in mice injected with fly ash. *Int. Arch. Allergy Immunol.* 80, 127–131.
- Holt, P.G., McMenamin, C. et al. (1990) Primary sensitization to inhalant allergens during infancy. *Pediatr. Allergy Immunol.* 1, 3–13.
- Horiguchi, S. and Saito, Y. (1964) Japanese cedar pollinosis in Nikko. *Jpn. J. Allergy* 13, 19–23.
- Ishizaki, M. and Kogure, F. (1984) Epidemiological studies on Japanese cedar pollinosis in Tochigi-Prefecture. *Jpn. J. Allergol.* 33, 678–683.
- Ishizuka, T., Koizumi, K. et al. (1987) Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. *Ann. Allergy* 58, 265–270.
- Ito, H., Nishimura, J. et al. (1995) Specific IgE to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. *Ann. Allergy Asthma Immunol.* 74, 299–303.
- Malmberg, H. and Holopainen, E. (1979) Nasal smear as a screening test for immediate-type nasal allergy. *Clin. Allergy* 8, 31–34.
- Massiot, J.G. and Cohen, S.G. (1986) Epidermalogic and socioeconomic aspect of allergy disease. *J. Allergy Clin. Immunol.* 78, 954–958.
- Matsumara, Y. (1970) The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimentally induced allergic respiratory disorder in guinea pigs. *Am. Rev. Respir. Dis.* 102, 430–447.
- Muranaka, M., Suzuki, S., et al. (1986) Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* 77, 616–623.
- Popp, W., Zwick, K. et al. (1989) Sensitization to aeroallergens depends on environmental factors. *Allergy* 44, 572–575.
- Riedel, F., Kramer, M. et al. (1988) The effects of SO_2 exposure on allergic sensitization in the guinea pig. *J. Allergy Clin. Immunol.* 82, 527–534.

- Ruffin, J., Liu, M.Y.G. et al. (1986) Effects of certain atmospheric pollutants (SO₂, NO₂ and CO) on the soluble amino acids, molecular weight and antigenicity of some airborne pollen grains. *Cytobios.* 46, 119–129.
- Suzuki, M., Itoh, H. et al. (1995) Causative allergens of allergic rhinitis with special reference to silkworm moth allergen. *Allergy* 50, 23–27.
- Takafuji, S., Suzuki, S. et al. (1987) Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. *J. Allergy Clin. Immunol.* 79, 639–645.
- Takafuji, S., Ozawa, M. et al. (1989) Enhancing effect of suspended particulate matter on the IgE antibody production in mice. *Int. Arch. Allergy Immunol.* 90, 1–7.
- Warren, C.P.W., Holford-Stevens, V., Wong, C. et al. (1982) The relationship between smoking and total immunoglobulin E levels. *J. Allergy Clin. Immunol.* 69, 370–375.