

Allergy to pine pollen and pinon nuts: a review of three cases

31269

A. Armentia, A. Quintero, A. Fernández-García, J. Salvador, and J. M. Martín-Santos

INTRODUCTION

Pinus pinea, or the pine nut tree, is indigenous to Europe and western Asia and has been widely cultivated in America. During pollination, which is from March to June, enormous quantities of pollen are released into the air. Contradictory opinions as to its allergenicity have always existed.¹

At the present, few studies on pine pollen allergy are available. Some authors maintain that this pollen is not allergenic because of its large size. Others believe it to be an allergen that must be taken into account when it is found to predominate in the patients' environment, even though the clinical manifestations of sensitization to this allergen are mild.²⁻⁴

The pine nut, fruit of *Pinus pinea*, has gradually crept into our modern diet; nevertheless, only three cases of hypersensitivity due to ingestion of pine nuts have been reported.^{5,6}

This clinical report describes three patients with pine pollen sensitivity demonstrated by clinical assessment, positive skin test, provocation test, and specific IgE to this allergen. The first patient also presented a hypersensitivity reaction to pine nut ingestion. The common factor in these three patients was that they all had lived since birth in regions with pine trees.

MATERIAL AND METHODS

Patients

For this study, a total of 103 possibly atopic patients who lived in re-

gions of pine trees were tested to *Pinus pinea* pollen and pinon nut.

Skin Test

Prick tests were carried out with a battery of the usual inhalants (mites, fungi, grass pollens, weeds, flowers, trees, epithelial cells, and food-stuffs), as well as with specific antigens (pine pollen extract, raw pine nut, and pine wood). Lyophilized pine pollen extract (Abelló Laboratories, Madrid, Spain), was diluted in 0.9% saline solution to an initial concentration of 0.5%, from which three dilutions were made: 1/10, 1/100, and 1/1000. The pine nut extract was produced from raw pine nut that was frozen and later grated. This powder was diluted in 0.9% saline solution to a final concentration of 1/20 wt/vol. The mixture was strained through a filter paper and then cold sterilized by filtering it through a Seitz vacuum filter using a Seitz Sterilizing Pad 3 cm (L3) (Center Laboratories). The pine wood extract was prepared from pine wood shavings, which were dialyzed and filtered, the final concentration being 1/10 wt/vol in 0.9% saline solution.

We used 0.9% saline solution as a negative control, and histamine acid phosphate at 1/100 wt/vol as positive control. Readings were taken after 15 minutes and were considered positive if the largest diameter of the papule obtained was greater than the positive control.

Oral Provocation Test

The oral provocation test was carried out with pine kernels administered in capsules (60 mg of pine nut powder in each capsule). The same capsules filled with an equal weight of lactose were used as placebo. These capsules were administered

every 20 minutes by a person who, like the patient, was unaware of their content, following conventional double-blind technique.⁷

Conjunctival Provocation Test

The technique described by Moller⁸ was adopted. The same lyophilized antigen that was used in the skin test was also used in the conjunctival provocation test. The pollen preparation was prepared with 0.9% saline solution at the time of skin testing and conjunctival provocation testing. Briefly, the technique entails instillation of a drop of the chosen antigen into the conjunctival sac. If there is no reaction after 20 minutes, the next test solution is applied to the other eye. The criteria for a positive result were congestion of the conjunctival mucosa, epiphora, and ocular itchiness.

Inhalation Challenge

This was carried out in accordance with a technique described in previous publications⁹; a pure form of the same antigen was used (0.001 mg of pine pollen per capsule plus 0.99 mg of lactose). The placebo capsules contained 100 mg of lactose. The utilization of whole pollen was based on previous papers in which dry ragweed pollen was used for inhalation tests.¹⁰

All the provocation tests were carried out after obtaining informed consent from the patients and adopting all necessary precautionary measures.

In vitro Tests

Total IgE was determined by Enzignost (Behring, GFR) following manufacturer's instructions.

Specific IgE was determined by Phadebas RAST (Pharmacia Diagnostics, Sweden). Results were de-

From the Rio Hortega Hospital, Department of Allergy, Valladolid, Spain.

Received for publication February 23, 1988.

Accepted for publication July 7, 1988.

scribed in terms of the RAST class (1 to 4). We used discs labelled with the pollen of *Pinus silvestris* because discs labelled with the pollen of *Pinus pinea* are not commercially available. The RAST was also carried out with the pollen of other trees present in the patients' environment (elm, ash), as well as with grass, weed, and flower pollens.

Controls

Skin tests with the same antigens were made in ten control subjects: five patients sensitized to grass pollens and five patients sensitized to mites. The conjunctival provocation test with pine pollen extract was carried out in two patients who were sensitive to grass pollens and in two nonatopic individuals. Likewise, the oral provocation test with pine nut was performed in two patients sensitized to nuts (peanuts, almonds).

All tests were performed during a period when there was a low atmospheric level of pine pollen (October) (Fig 2).

CLINICAL CASES

Patient 1

This is the case of an 8-year-old girl who has lived in the country, surrounded by a pine forest (*Pinus pinea*), since birth. At the age of 4 years, she presented symptoms of rhinoconjunctivitis, cough accompanied by dyspnea, and occasional episodes of wheezing dyspnea. These symptoms appeared during summer and autumn. At 5 years, several minutes after eating raw, peeled pine nuts, she presented angioedema of the lips, followed by generalized urticaria. This has occurred every time she has eaten this fruit or its derivatives (pastries containing pine nuts, chocolate covered pine nuts). She presents no reaction to ingestion of other nuts (hazelnuts, almonds, walnuts, etc). Since she was 6 years old, she has suffered episodes of conjunctivitis-urticaria-angioedema during the summer and autumn months; no precipitating factor has been clearly identified

(she denied prior ingestion of pine nuts). There has also been a gradual disappearance of the episodes of wheezing dyspnea.

Routine investigations to rule out a nonallergic cause of the urticaria were carried out. Skin testing (prick-test) with a battery of the usual inhalants was negative except for the extract of a mixture of tree pollens (6×5 mm as opposed to a diameter of 4 mm to the control histamine). All other laboratory investigations (parasites in stools, serologic testing for cysticercotic cysts, immunoglobulins, complement levels, etc) were negative. Total IgE was 149 IU/mL ($358 \mu\text{g/L}$).

Patient 2

This patient was a 52-year-old male who has been suffering from rhinoconjunctivitis for approximately 30 years. In his youth he also presented occasional episodes of wheezing dyspnea. At the present time, the symptoms are mild and limited to the summer and autumn months. Since birth, he has lived in a rural area in which there are pine trees (*Pinus pinea*). He frequently consumes pine nuts without presenting any appreciable symptoms following their ingestion.

Skin testing performed with a battery of the usual inhalants revealed positive results with an extract of a mixture of tree pollens (6×5 mm) and with grass pollens (6×6 mm), as compared with the control histamine (4×4 mm).

Total IgE was 156 IU/mL ($374 \mu\text{g/L}$).

Patient 3

This case was a 19-year-old male who, during the last few years, has had nasal and ocular symptoms during the summer months and occasional dyspnea following strenuous exercise in the country (farming). Since birth, he has lived in a region with pine trees. The ingestion of pine kernels has not caused symptoms.

Positive skin tests with grass pollens (10×12 mm with pseudopods)

and an extract of a mixture of tree pollens were obtained. Total IgE was 992 IU/mL ($2381 \mu\text{g/L}$).

RESULTS

For this study a total of 103 possibly atopic patients residing in regions with pine trees were tested to *Pinus pinea* pollen and pinon nut. Only three of these patients (2.9%) exhibited skin test sensitivity and provocation test positive to *Pinus pinea* pollen. Another patient presented skin test sensitivity, but the provocation tests were negative.

In patient 1, skin testing to detect specific sensitization to antigens derived from *Pinus pinea*, was positive with the *Pinus pinea* pollen extract (diameter of more than 5×10 mm, versus 4 mm with the control histamine). Testing was also positive with raw pine nut extract at 20% wt/vol (8×9 mm) and negative with pine wood extract.

The oral provocation test was positive three hours after the ingestion of five capsules—three of which were subsequently shown to contain pine nut (60 mg of raw, grated pine nut per capsule)—with the appearance of wheals on the upper thighs and abdomen. One day later, ingestion of three whole pine nuts provoked itchiness of the lips and tongue and angioedema of the lower lip. Both reactions subsided with antihistamines.

The conjunctival provocation test with pine pollen at 1/10 dilution of original pine pollen extract (50% concentration) was positive: 12 minutes after instillation of a drop of this extract onto the conjunctival mucosa, itchiness and epiphora were observed.

An inhalation challenge test was carried out but the results were not evaluated because of the patient's poor cooperation.

The RAST was positive for pine pollen (class 3 of Pharmacia). The RAST with pollens of other trees, grasses, shrubs, flowers, fungi, mites, foodstuffs, and nuts was negative.

In patient 2, skin testing to demonstrate sensitivity to pine antigens was positive for pine pollen extract (7 × 8 mm; versus control histamine: 5 × 5 mm) and negative for the pine nut and pine wood extracts.

In the conjunctival provocation test, the maximum concentration was reached without provoking symptoms other than slight itchiness. Six hours later, however, the patient presented lacrimation, conjunctival reddening and itchiness, and sneezing and watery eyes. The oral provocation test with raw pine nuts was negative: a dose equivalent to ten pine nuts was reached. The inhalation challenge test with pine pollen extract was negative at the maximum concentration and no delayed reactions occurred.

The RAST revealed class 1 positivity for grass pollens and class 2 for pine pollen. The RAST with the other allergens was negative.

In patient 3, skin tests were positive for pine pollen extract (8 × 9 mm with pseudopods, versus histamine 4 mm) and pine nut (6 × 5 mm with pseudopods) but negative for pine wood.

The conjunctival provocation test was positive five minutes after application of a 1/10 dilution of pine pollen extract.

Oral provocation with raw pine nut was negative following the ingestion of a dose equivalent to ten pine nuts.

The inhalation challenge test was

negative at a maximum dose and no delayed reactions were observed.

The RAST was positive for grass pollen (class 3) and pine pollen (class 2) of Pharmacia.

Table 1 shows the results obtained in the *in vivo* and *in vitro* tests in the three patients. The results in control subjects were negative.

DISCUSSION

This clinical case report describes three patients who presented *in vivo* and *in vitro* data suggestive of sensitization, with an underlying IgE-mediated hypersensitivity mechanism to antigens contained in the pollen of *Pinus pinea* (the pine-nut tree).

Even though *Pinus pinea* is one of the main types of forest tree and is widely distributed throughout the world, cases of hay fever caused by its pollen have not been reported.¹ Nevertheless, allergic symptomatology provoked by sensitization to the pollen of other types of pine tree has been described.¹ In 1934, Rowe³ published the case of a 37-year-old woman living in an area of pine trees in California who suffered seasonal dyspnea and had positive skin tests to the pollen of *Pinus radiata* and to other grass and tree pollens. In 1967, Newmark and Itkin⁴ reported the case of a 42-year-old woman with a history of hay fever who presented positive skin tests to pine pollen, as well as positive PK and bronchial challenge test. In

1985, Harris and German² carried out a prospective study involving 200 pollen-sensitive patients. They observed a 2.3% incidence of skin reactivity to pine pollen (*Pinus radiata*), and concluded that the incidence of sensitization to pine pollen in the atopic population is low. Allergic symptomatology caused by sensitization to other types of conifers has been described: *Cupressus sempervirens* (cypress), and *Cryptomeria japonica* (Japanese cedar).¹¹ Other authors, however, believe that pine pollen is not allergenic and have used it as a negative control in inhalation challenge tests.¹²

On the other hand, although the pine nut has entered the modern diet to an ever increasing degree (desserts, chocolate covered pine nuts, pastries, oil, vegetable salads, pesto sauce . . .), only three cases of hypersensitivity reaction due to ingestion of pine nuts have been reported. In 1958, Santos and Unger reported the first case report of a severe allergic reaction following ingestion of one piece of candy containing pine nuts.⁵ Two cases of hypersensitivity reaction due to ingestion of pine nuts have been recently reported by Fine.⁶ The European variety of pine nuts is harvested from the *Pinus pinea*, which grows in southern Europe, especially Italy, southern France and Spain. The European cone is 4 to 6 inches in length and each cone may produce up to 100 seeds (Fig 1).

Table 1. Results Obtained in Our Three Patients

Patient	Suggestive History	Skin Test (Prick)	Total IgE, IU/mL†	RAST to Pine (class of Pharmacia)	Oral Prov* with Pine nut	Conj prov with Extract of Pine Pollen	Inhal. Prov* with Pine Pollen
1	yes	Positive: pine nut and pine pollen Negative: other pollens and pine-wood	149	Class 3 for pine pollen	positive	positive	Negative
2	yes	Positive: pine pollen and gramineal pollens	156	Class 1 for gramineal pollen and class 2 for pine pollen	negative	positive	Negative
3	yes	Positive: pine pollen, gramineal pollens and pine nut	992	Class 3 for gramineal pollens and class 2 for pine pollen	negative	positive	Negative

* Oral prov. = oral provocation test, Conj prov = conjunctival provocation test, and Inhal prov = inhalation provocation test.

† Multiply international units/mL by 2.4 to convert to µg/L.

The Castille area of Spain, where our three patients live, has approximately 662,000 hectares of pine trees.^{13,14} Figure 2 shows the concentration of *Pinus pinea* pollen in the atmosphere of this region from March to October. Only three patients of a total of 103 possibly atopic patients from this area who were tested (2.9%), exhibited skin test sensitivity and provocation test positive to *Pinus pinea* pollen. This suggests that this pollen is a "bad sensitizer" of the atopic population.

Our first patient presented a clinical picture of rhinoconjunctivitis and urticaria-angioedema, probably induced by sensitization to the antigens of pine pollen and pine nut.

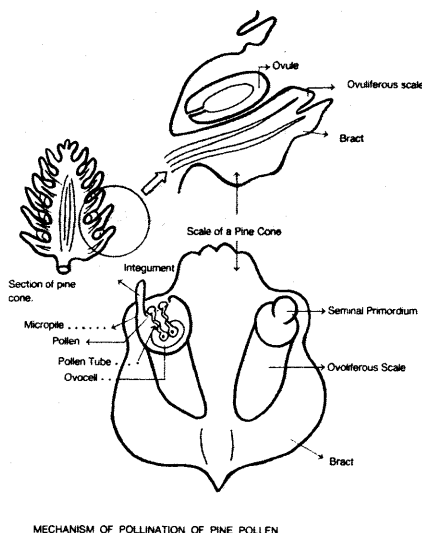


Figure 1. Mechanism of pollination of pine pollen.

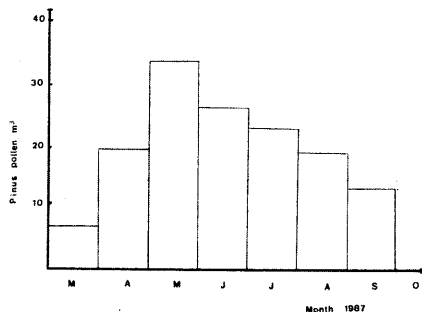


Figure 2. Concentration of pinus pollen (*P. pinea*) in the atmosphere of the Castilla area from March to October 1987.

The seasonal episodes of bronchospasm that she suffered during early infancy could have been caused by this pollen. This suspicion could not be confirmed because of the poor cooperation of this young patient. The seasonal episodes of rhinoconjunctivitis in cases 2 and 3 were presumably caused by sensitization to this pollen and to grass pollen. This suspicion was supported by a positive conjunctival test, positive skin test, and a positive RAST to pine pollen.

Another interesting finding was that the symptoms of rhinoconjunctivitis in these three patients appeared in the summer or autumn, while pine tree pollination peaks in April and May (Fig 2). It is known that when grass pollen comes into contact with the nasal mucosa, it releases its internal proteins spontaneously and rapidly through the exine channels and the pore.¹⁵ The protein material of pine pollen is not so "easily" released. In a large group of gymnosperms, the most extreme example being the genus *Pinus*, there is a considerable delay in gametophyte maturation. Its pollination is anemophilous and the grains of pollen, carried through the air, reach the female *strobili*. The immature ovules are located in the interior of the bracts (Fig 1). Once mature, the ovule releases a drop which stimulates the growth of the pollen tube through which the pollen proteins will be released. This process can take from 2 to 13 months. It seems that the low content of oxido-reductase enzymes in the most primitive gymnosperms, such as the pine tree, could be one of the reasons for its slow germination.¹⁶ Because of its thick and wrinkled exine^{17,18} and large size (44–51 μ m, approximately), pine pollen has been excluded as a cause of pollinosis¹ (Fig 3). But because of its protein release mechanisms, it could be a cause of delayed symptomatology if it were to remain 2 months settled in the nasal mucosa, a sufficient period for antigen re-

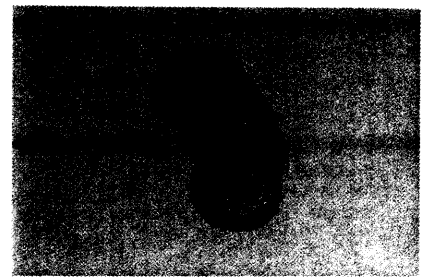


Figure 3. Photomicrograph of *Pinus pinea* pollen. Not stained. Magnification $\times 450$. Diameter = 60 micrometers.

lease. In support of this hypothesis, we recently observed in scrapings of nasal mucosa of pollinic patients, performed in December, fungi, hyphae, and olleaceae pollen grains of a size similar to pine pollen (80 μ m) (unpublished data). These particles were included in the nasal mucosa and accidental contamination of the samples was ruled out. Another finding that supports this explanation is the positivity of the conjunctival test using pine pollen extract (following extraction of its proteins) and the negativity of inhalation challenge test with whole dry pollen.

Against this hypothesis, studies of nasal provocations using radiolabelled pollens showed that they were eliminated by the mucociliary system and after a time they appeared in the pharyngeal mucosa.¹⁹ Nevertheless, other authors affirm that radioisotopic methods using labelled pollen grains are not effective in detecting small numbers of grains in the respiratory mucosa. Further, pollens of pine and other species have been detected in histologic samples of the respiratory mucosa of humans and animals, and even in the peripheral areas of the respiratory parenchyma.²⁰

The mucociliary system under normal conditions cleans out inhaled particles, including those of similar size to pine pollen. It is well known however that mucociliary clearance rate is often reduced in patients with asthma, even when asymptomatic.²¹ Patients with allergic disease are more prone to

chronic respiratory infections, which can result in the loss of ciliated epithelium,²² and long-term deposition of cleared material probably occurs within the lymphoid crypts of pharyngeal lymph tissue.²³

The deposition of small numbers of grains of pine pollen in the respiratory mucosa in amounts sufficient to trigger an allergic delayed response after germination is only a hypothetical mechanism to explain our findings. Further studies are necessary for objective proof of this hypothesis.

Applying the postulates proposed by Thommen², pine pollen satisfies the necessary following criteria to be considered an antigen responsible for hay fever.

1. The pine tree produces pollen.
2. This pollen can be widely distributed in the environment, where it can constitute the most predominant pollen.
3. Large quantities of this pollen can be produced.
4. This pollen can be carried by air.
5. This pollen can be allergenic.

In conclusion, even though the incidence of reactivity to pine pollen in the atopic population is low, it could be valid to perform skin and *in vitro* tests to *Pinus* pollen in patients with delayed seasonal symptomatology who live in pine forest regions or when the possibility of allergic reactions due to the ingestion of pine nuts is suspected.

REFERENCES

1. Wodehouse D: Pollen grains. New York & London, Hafner Publishing Company, 1965, pp 256-261.
2. Harris RM, German DF: The incidence of pine pollen reactivity in an allergic atopic population. *Ann Allergy* 1985;55:678-679.
3. Rowe A: Pine pollen allergy. *J Allergy* 1939;10:377-378.
4. Newmark F, Itkin I: Asthma due to pine pollen. *Ann Allergy* 1967;25:251-252.
5. Santos IM, Unger L: Severe allergic reaction to pignolia nut. *Ann Allergy* 1958;16:459-461.
6. Fine AJ: Hypersensitivity reaction to pine nuts (pinon-nuts-pignolia). *Ann Allergy* 1987;59:183-184.
7. Metcalfe DD: Abordaje práctico de rutina para el diagnóstico de reacciones adversas frente a alimentos. Edición en español. *Proc Allergy* 1987;1:17-21.
8. Moller C, Bjorksten B, Nilsson G, et al: The precision of the conjunctival provocation test. *Allergy* 1984;39:37-41.
9. Losada E, Hinojosa M, Moneo I, et al: Asma bronquial ocupacional por inhalación de papaina: Hallazgos clínicos e inmunológicos y modelos de respuesta bronquial. *Revista Española de Alergología e Inmunología clínica* 1986;1:29-33.
10. Stillwagon PK, Doyle WJ, Fireman P: Effect of an antihistamine decongestant on nasal and Eustachian tube function following intranasal pollen challenge. *Ann Allergy* 1987;58:442-446.
11. Panzani R, Yasueda H, Shimizu T: Cross-reactivity between the pollens of *Cupressus sempervirens* (common Cypress) and of *Cryptomeria japonica* (Japanese cedar). *Ann Allergy* 1986;57:26-30.
12. Frolund L, Madsen F, Gerner S, et al: Reproducibility standardized bronchial allergen provocation test. *Allergy* 1986;41:30-36.
13. Nicolas A, Gandullo J: Ecología de los Pinares Españoles. I. *Pinus Pinaster*. I.F.I.E. (Madrid) 1967, pp 312-316.
14. Cabero V. Espacio Geográfico Castellano Leonés. Valladolid, Ed. Ambito. 1982, pp 143-145.
15. Charpin J, Soler M: Physiopathologie de la pollinose, in *Les Pollinoses*. Ecully, Laboratoires Fisons S.A. 1979, pp 85-91.
16. Stanley RG, Linskens HF: Pollen. Biology Management Biochemistry. New York, Springer Verlag, 1974.
17. Aytug B: Diagnose des pollens de *Pinus silvestris* et *Pinus uncinata* dans les Pyrénées. *Pollen et Spores* 1972;2:283-296.
18. Sivak J, Raz P: Le critère de détermination des *Pinus* Haplostellés et *Doplostellés* d'après les grains de pollen. *Revue de Micropaléontologie* 1973;18:259-263.
19. Wilson AE, et al: Deposition of inhaled pollen and pollen extract in human airways. *N Engl J Med* 1973;288:1056-1059.
20. Michel F, Marty JP, Quet L, et al: Penetration of inhaled pollen into the respiratory tract. *Am Rev Respir Dis* 1977;115:609-616.
21. Mezey RJ, Cohn MA, Fernandez RJ, et al: Mucociliary transport in allergic patients with antigen induced bronchospasm. *Am Rev Respir Dis* 1978;118:677-682.
22. Sakakura Y, Sasaki Y, Hornick RB, et al: Mucociliary function during experimentally induced rhinovirus infection in man. *Ann Otol Rhinol Laryngol* 1973;82:203-208.
23. Proctor DF, Anderson I, Lundavist G: Clearance of inhaled particles from the human nose. *Arch Intern Med* 1973;131:132-138.

Request for reprints should be addressed to:
A Armentia-Medina, MD, PhD
Hospital Del Rio Hortesa
Seccion de Alergia
Calle Cardenal Torquemada SN
Valladolid
Spain