ORIGINAL ARTICLE

Oleaceae-Induced Pollinosis in an Area With Exposure to Olive and Ash Trees

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Abstract

Background: Navarre, in Northern Spain, is an area with moderate exposure to olive and ash tree pollen.
Objective: To assess the relevance of ash as a cause of pollinosis in our region.
Methods: The study sample comprised 85 patients from Navarre with clinical symptoms of pollinosis. Specific immunoglobulin E (sIgE) was determined to Fra e 1, Ole e 1, and a mixture of amino- and carboxy-terminal domains of Ole e 9 (Ole e 9 NC) (ADVIA-Centaur). At the same time, the presence of sIgE to other pollen allergens was studied. Prick tests were performed with ash pollen (n=33) and olive pollen (n=85) and the symptomatic period was recorded (n=85). As a control group, we studied the serum of 98 patients with olive pollen allergy, intense exposure to olive pollen, and no exposure to ash.
Results: Sensitization to Oleaceae was detected in 24/85 patients in the study group (28.2%). In this group, the mean (SD) level of IgE to Fra e 1 was 8.5 (10) kU_A/L and to Ole e 1 6.07 (7.88) kU_A/L ($P<.001$). In the control group, these figures were 103.64 (132.19) kU_A/L and 86.43 (118.5) kU_A/L ($P<.001$), respectively. In all patients with positive sIgE to Fra e 1, IgE to Ole e 1 was also detected (concordance index, $\kappa=1$), both in the study group and in the control group. Patients who were sensitized to Fra e 1 did not present a longer symptomatic period and their symptoms did not have an earlier onset.
Conclusion: We did not find evidence of clinically relevant sensitization to ash in Navarre.

Key words: Allergen. Ash pollen. Olive pollen. Pollinosis.

Resumen

Antecedentes: Navarra, al norte de España, es un área de moderada exposición a polen de olivo y de fresno.
Objectivo: evaluar la importancia del fresno como causa de polinosis en nuestra región.
Métodos: 85 pacientes de Navarra con clínica de polinosis. Se realizó determinación de IgE específica a Fra e 1, Ole e 1, y una mezcla de dominios amino y carboxiterminal de Ole e 9 (Ole e 9 NC) (ADVIA-Centaur). Simultáneamente se estudió la presencia de IgE específica a otros alérgenos polínicos. Se realizaron prick test con polen de fresno (n=33) y de olivo (n=85) y se registró retrospectivamente el periodo de síntomas (n=85). Como grupo control se estudió el suero de 98 pacientes con alergia a polen de olivo, con intensa exposición a polen de olivo y sin exposición a fresno.
Resultados: En 24/85 pacientes del grupo problema (28,2%) se detectó sensibilización a oleáceas. En el grupo problema los niveles medios±DS de IgE a fresno fueron de 8,5±10 kU/L y a olivo de 6,07±7,88 kU/L ($p<0,001$) y en el grupo control de 103,64±132,19 kU/L and 86,43±118,5 kU/L respectivamente ($p<0,001$). En todos los pacientes con IgE positiva a Fra e 1 se detectó IgE a Ole e 1 (índice de concordancia $\kappa=1$), tanto en el grupo problema como en el control. Los pacientes sensibilizados a Fra e 1 no tuvieron mayor número de meses de síntomas ni comenzaron sus síntomas de forma más precoz.
Conclusiones: No hemos encontrado evidencias de que la sensibilización a fresno en Navarra sea clínicamente relevante.

Palabras clave: Alérgeno. Polen de fresno. Polen de olivo. Polinosis.

Introduction

The most significant Oleaceae with regard to allergens are grouped within the genera *Olea* (olive and wild olive) and *Fraxinus* (ash). Olive tree (*Olea europaea*) pollen is one of the main causes of respiratory allergy in the Mediterranean area. It is the second most common cause of pollinosis in Spain, following grass pollen [1]. However, epidemiology and clinical impact vary widely depending on the area, because olive trees are predominantly grown as a crop, and their distribution varies from one part of the country to another [2]. In Navarre, the olive oil sector is based on the production of about 5000 hectares of olive groves. In 2006, olive pollen was present in the atmosphere in Pamplona (the capital of Navarre) from April to June. The annual olive pollen load was 277 grains/m^3, with a peak concentration of 45 grains/m^3, reached on May 16 and 17 [3]. Ash is the principal representative of the Oleaceae family in warm climates in Europe. Ash pollen allergy has been documented in areas without olive, such as Central Europe [4]. Among species of *Fraxinus, Fraxinus excelsior* is predominant in the northern third of the Iberian Peninsula. In 2006, ash pollen was found in the atmosphere in Pamplona from February to April. The annual ash pollen load was 682 grains/m^3, with a peak concentration of 58 grains/m^3, reached on March 15 [3].

Since component-resolved diagnosis is a useful tool for identifying the allergens causing the disease and is particularly necessary in areas with a complex level of exposure and sensitization to pollens [2], we assessed the relevance of ash as a cause of pollinosis in our region.

Material and Methods

The study sample comprised 85 patients with rhinoconjunctival symptoms, seasonal asthma, or both, and no associated food allergies. All the patients had been living in Navarre for at least the previous 5 years. None of the patients had received immunotherapy. The months in which each patient had symptoms were recorded. We used the ADVIA-Centaur platform (ALK-Abelló SA, Madrid, Spain) and purified allergens [5] to determine specific immunoglobulin (sIg) E to recombinant (r) Fra e 1 [6], natural (n) Ole e 1 [7], and a mixture of recombinant amino- and carboxy-terminal domains of Ole e 9 (Ole e 9 NC). At the same time, we studied the presence of IgE to specific allergens in other pollens (nArt v 1, nPar j 1, nCup s 1, nPla l 1, nPhl p 1, nPhl p 5, nBet v 1, and nSal k 1) and to the pollen panallergens profilin (a mixture of 2 isoforms of rMal d 4) and polcalcin (rChe a 3).

Our control group comprised the serum of 98 patients from Cordoba (southern Spain) with olive pollen allergy (clinical symptoms during the olive pollination period, positive skin tests to whole olive pollen extract, sIgE to whole olive pollen extract >0.35 kU_A/L, and sensitization to specific olive pollen allergens: nOle e 1, nOle e 6, nOle e 7, rOle e 9NC, and/or nOle e 11), marked exposure to olive pollen, and no exposure to ash pollen. In these samples, we quantified specific IgE to rFra e 1, and the values were compared with those obtained for nOle e 1.

Levels of sIgE >0.35 kU_A/L were considered positive.

We performed prick tests with whole ash pollen extract (n=33) and whole olive pollen extract (n=85) in the study group patients. A wheal ≥3 mm in diameter was considered positive. We recorded the length of the symptomatic period in all patients.

Statistical Analysis

The association between positivity results for different allergens (nOle e 1 and Fra e 1) and techniques (serum sIgE and prick test) was analyzed using the χ^2 or Fisher exact test. Concordance of positivity was evaluated using the κ statistic. Two-tailed nonparametric tests were applied to compare IgE concentrations to different purified allergens (Wilcoxon) and the length of the symptomatic period (Mann-Whitney).

For all the analyses, *P* values <.05 were considered significant.

Results

In the study group (Navarre), 75 patients (88.2%) were sensitized to grass pollen, and 42 (56%) of these were monosensitized. The most common associated sensitization was to Oleaceae (19/75, 25.3%). Among patients not sensitized to grass pollen, the most frequent sensitization was to Oleaceae (5/10, 50%), followed by Cupressaceae (3/10; 30%). Eleven of the 85 grass-allergic patients (12.9%) were sensitized to profilin and 4 to polcalcin (4.7%).

Positive results for 1 or more *Oleaceae* allergens (nOle e 1 and/or rFra e 1) were observed in 24 of the 85 patients (28.2%). Men accounted for 50% and the mean (SD) age was 29.6 (11.1) years. Only 3 patients were not sensitized to other pollen allergens. Cosensitization was detected to nBet v 1 in 1 patient (4.2%), nPar j 1 in 1 patient (4.2%), nCup s 1 in 7 patients (29.2%), nPla l 1 in 1 patient (4.2%), nSal k 1 in 3 patients (12.5%), and nPhl p 1 and/or nPhl p 5 in 19 patients (79.2%).

In the control group (Cordoba), 89 of the 98 patients (90.8%) had positive levels of sIgE to nOle e 1, 47 (48%) to nOle e 6, 61 (62.2%) to nOle e 7, 50 (51%) to rOle e 9NC, and 61 (62.2%) to nOle e 11. In this group, 32 patients (32.7%) showed sensitization to olive profilin (Ole e 2) and 29 (29.6%) to polcalcin (rChe a 3).

The median (interquartile range) concentrations of sIgE to nOle e 1 and rFra e 1 in patients were 3.15 kU_A/L (1.92-7.30 kU_A/L) and 4.7 kU_A/L (2.34-8.51 kU_A/L) respectively. In the olive-allergic control patients who were not exposed to ash the median nOle e 1 sIgE level was 32.93 kU_A/L (9.6-105.59 kU_A/L) and that of IgE to rFra e 1 was 44.47 kU_A/L (10.7-153.37 kU_A/L).

IgE levels to rFra e 1 were higher than those of IgE to nOle e 1 in 21 of the 24 patients (87.5%) sensitized to either or both of these allergens and in 87 of 98 controls (88.7%). These differences were statistically significant in both groups (Wilcoxon, *P*<.001).

Full concordance [κ=1] in positive test results to rFra e 1 and nOle e 1 was observed for patients and controls, as no patients were positive to one and not the other. No sIgE antibodies were detected to rOle e 9 NC in any of the patients.

A significant association was observed in the study group between a positive test result for sIgE to rFra e 1 and the results of the skin test with whole ash extract (Fisher, $P<.01$), with a κ statistic of 0.69. Likewise, there was a significant association between positive sIgE to nOle e 1 and the skin test result for whole olive pollen extract (Fisher, $P=.017$), with a κ statistic of 0.40. When patients not sensitized to profilin or polcalcin were selected from these patients, the κ statistic increased to 0.56.

Patients who were sensitized to rFra e 1 did not have a longer symptomatic period than patients who were not sensitized to it (Mann-Whitney, $P>.05$) and their symptoms did not commence any earlier (χ^2, $P>.05$).

Discussion

The study of pollinosis in a specific population is based on 3 key points: the exposed population; the presence of the pollen of a particular taxon in the atmosphere, which in turn depends on the flora and local weather conditions; and whether the population is sensitized to these pollens. Our region is on the border between European warm and Mediterranean zones. The presence of olive and ash in the region is irregular, meaning that there are low or moderate levels of these pollens in the atmosphere. In this study, we found that 28% of our patients with pollinosis had sIgE to nOle e 1 and rFra e 1. Although levels of IgE to rFra e 1 were higher than to nOle e 1, the differences were minimal (no more than 5%). The fact that these differences were observed in the study group and control group alike could be explained by the differences in biotin allergen marking; therefore, from an operational point of view, we can consider the responses as equivalent. In this case, sIgE levels to the major allergens Fra e 1 and Ole e 1 did not provide information on the source of sensitization to Oleaceae in the study group. This assertion is based on 2 factors: the same phenomenon was observed in the control group, in which patients were allergic to olive pollen and were not exposed to ash; and patients sensitized to Fra e 1 did not commence their symptoms earlier and did not suffer their symptoms for longer than patients who were not sensitized to Fra e 1, most of whom were allergic to grass pollen, for which the pollination period lasted the same length of time as the olive pollination period. The positive levels of the major ash allergen in the 2 geographical areas under study are explained by their high cross-reactivity with Ole e 1 [4,6,8-10], backed by a sequence identity of 88% [6]. As species simplification is advisable for the diagnosis and therapy of both olive pollen allergy and ash pollen allergy and in light of the scientific evidence we present, it seems that olive can be used as the only allergen for both diagnosis and immunotherapy. In the study group, we did not find any patients who were sensitized to rOle e 9 NC. Since both domains are responsible for 90% of IgE binding to Ole e 9 [11], we can confirm that none of our patients were sensitized to Ole e 9. The frequency of this sensitization increases in areas where there is high allergenic pressure, which can account for over 35% of patients who are allergic to olive pollen [2].

To conclude, both levels and frequency of specific IgE to Fra e 1 are parallel to those of IgE to Ole e 1. This is unrelated to ash pollen exposure and does not modify clinical expression in ash-allergic patients in comparison with olive-allergic patients. Therefore, we did not find evidence of clinically relevant sensitization to ash in Navarre.

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References

1. Alergológica. Factores epidemiológicos, clínicos y socioeconómicos de las enfermedades alérgicas en España. Madrid: Alergia e Inmunología Abelló; 1992.
2. Barber D, Moreno C, Ledesma A, Serrano P, Galan A, Villalba M, Guerra F, Lombardero M, Rodríguez R. Degree of olive pollen exposure and sensitization patterns. Clinical implications. J Investig Allergol Clin Immunol. 2007;17(Suppl)1:11-6.
3. http://www.polenes.com/
4. Hemmer W, Focke M, Wantke F, Gotz M, Jarisch R, Jager S, Götz M. Ash (*Fraxinus excelsior*)-pollen allergy in central Europe: specific role of pollen panallergens and the major allergen of ash pollen, Fra e 1. Allergy. 2000;55:923-30.
5. Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, Quiralte J, Lombardero M, Villalba M, Salcedo G, Rodríguez R. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. Allergy. 2008;63:1550-8.
6. Barderas R, Purohit A, Papanikolaou I, Rodríguez R, Pauli G, Villalba M. Cloning, expression, and clinical significance of the major allergen from ash pollen, Fra e 1. J Allergy Clin Immunol. 2005;115:351-7.
7. Villalba M, Batanero E, López-Otín C, Sánchez LM, Monsalve RI, González de la Peña MA, Lahoz C, Rodríguez R. The amino acid sequence of Ole e I, the major allergen from olive tree (*Olea europaea*) pollen. Eur J Biochem. 1993;216:863-9.
8. Obispo TM, Melero JA, Carpizo JA, Carreira J, Lombardero M. The main allergen of Olea europaea (Ole e I) is also present in other species of the *Oleaceae* family. Clin Exp Allergy. 1993;23:311-6.
9. Niederberger V, Purohit A, Oster JP, Spitzauer S, Valenta R, Pauli G. The allergen profile of ash (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from various plant species. Clin Exp Allergy. 2002;32:933-41.
10. Barderas R, Purohit A, Rodríguez R, Pauli G, Villalba M. Isolation of the main allergen Fra e 1 from ash (*Fraxinus excelsior*) pollen: comparison of the natural and recombinant forms. Ann Allergy Asthma Immunol. 2006;96:557-63.

11. Palomares O, Villalba M, Quiralte J, Rodríguez R. Allergenic contribution of the IgE-reactive domains of the 1,3-ß-glucanase Ole e 9: diagnostic value in olive pollen allergy. Ann Allergy Asthma Immunol. 2006;97:61-5.

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