

Short communication

Assessment of sensitization to holm oak (*Quercus ilex*) pollen in the Mérida area (Spain)

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Sensitization to *Quercus ilex* (holm oak) was studied in 760 patients with clinically suspected sensitization to aeroallergens. Prick tests with commercial extracts of *Q. ilex* proved positive in 27 patients; none were monosensitive. Nasal and conjunctival provocation tests and specific IgE (RAST) performed with *Q. ilex* extracts in these patients were negative in all but one patient, who exhibited specific IgE titer (by RAST) of 7 PRU ml (class 3). We conclude that *Q. ilex* pollen, though obtained in considerable quantities by our collector, does not cause allergies in our area.

Key words: holm oak; Mérida; *Quercus ilex*; sensitization.

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Spain possesses the world's largest area of holm oak plantations, followed by Morocco (17).

The holm oak belongs to the Fagaceae family, genus *Quercus*. Both of the two varieties of *Quercus ilex*, *rotundifolia* and *ilex*, are indigenous to Spain; the latter is predominant in our region.

The blooming period of the holm oak is spring, particularly April and May, and coincides with that of the Gramineae (17).

As our unit is situated in the most densely populated holm oak region in the world, we wondered whether this tree's pollen might be partly responsible for allergies in our patients since *Q. ilex* pollen represents 1.9% of all pollen in our region, and more than 800 grains m⁻³ of air were detected in the first week of May 1991 (unpublished data) by Cour's method (7). Although up to 12% of positive prick tests have been reported with holm oak pollen extracts (17), we found no effects on the clinical repercussions of this pollen in allergic patients.

Material and methods

Patients

From the total number of patients seen in the hospital, 760 were selected (422 females and 338 males;

aged 5–80 years, mean age 28 years) to study sensitization to holm oak (*Q. ilex*), by the following criteria: clinically suspected sensitization to aeroallergens (rhinitis, bronchial asthma, or both), asymptomatic at the moment of the study, not under medication, and not having previously received immunotherapy treatment.

Routine analysis in all patients included complete blood and urine tests, serum immunoglobulins, biochemical profile, and parasites in feces. Chest and paranasal sinus radiographs were also performed.

Assessment of sensitization to holm oak was initiated with skin tests. In patients with positive tests, conjunctival and nasal provocation tests and specific IgE with holm oak extracts were performed.

Skin tests

Skin tests were performed by prick test with a lancet (Prick Lancetter Hollister-Sher) which penetrates only 1 mm, as described by Brown & Thantrey (5).

The initial prick test was performed with a standard aeroallergen battery which included: mites, Gramineae (*Dactylis glomerata*, *Phleum pratense*, *Holcus lanatus*, *Poa annua*, and *Cynodon dactylon*), Oleaceae (*Olea europaea*), Chenopodiaceae (*Cheno-*

podium album and *Salsola kali*), Plantaginaceae (*Plantago lanceolata*), Compositae (*Artemisia vulgaris*), Polygonaceae (*Rumex acetosella*), and fungi (*Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus*).

This was followed by a prick test with holm oak, using in each patient 5% w/v supplied by Abelló Laboratories (Madrid, Spain), 5000 Noon units/ml from Ifidesa-Aristegui Laboratories (Bilbao, Spain), and 2% w/v from Leti Laboratories (Madrid, Spain).

Glycerinated saline serum and histamine at a concentration of 10 mg/ml were used as negative and positive controls, respectively.

The test was considered positive when a wheal equal to or larger than that produced by histamine (10 mg/ml) was obtained, or when the wheal emitted pseudopodia (2). Readings were taken at 15 min and 4 h.

Twenty-five healthy volunteer controls underwent the prick test to assess the specificity of the *Q. ilex* extracts.

Conjunctival provocation test

In the patients with prick tests positive to *Q. ilex*, the conjunctival provocation test was performed with a standardized extract in Noon units (5000 Noon units/ml) supplied by Ifidesa-Aristegui Laboratories (Bilbao, Spain). Three concentrations at 500, 50, and 5 Noon units/ml, respectively, were obtained, using saline serum with 0.03% human serum albumin (diluent supplied by the same pharmaceutical laboratory; this was also used as a negative control).

The test was started with the instillation of a drop of diluent in the lower conjunctival sac; control of ocular symptoms was carried out at 20 min. In the absence of response, a drop of the lowest extract concentration of the above mentioned dilutions was instilled in the opposite conjunctival sac. If this failed to produce a response after a further 20 min, the next higher concentration was instilled into the conjunctival sac opposite to the former, thus alternating until the undiluted extract was reached.

The test was considered positive when chemosis, erythema over 50%, or erythema under 50% but associated with pruritus or tears (8) was observed in bulbar conjunctiva.

Specificity controls were performed in 10 healthy volunteers.

Nasal provocation test

The nasal provocation test was performed in patients with prick tests positive to *Q. ilex* with a standardized extract in Noon units (5000 Noon units/ml), also supplied by Ifidesa-Aristegui Laboratories (Bilbao, Spain). The same dilutions as described for

the conjunctival provocation test were obtained, with the same diluent, which similarly served as a negative control.

Four manual nebulizers (Fisons Ibérica, SA, Zaragoza, Spain) with a delivered dose of 0.132 ± 0.0008 ml per nebulization were used for nasal application of *Q. ilex* extract; differences among doses of each were not statistically significant. Nebulizers were weighed before and after nebulization for dose calculation. The procedure was repeated five times for each, and the arithmetic mean was calculated.

After rhinoscopy to assess nasal permeability status, and with the patient asymptomatic and not having taken any medication which might influence results (as in the two previously described tests), the test was started with nebulization of the diluent into one nostril; nasal symptoms were observed for the following 30 min. If no response was noted, nebulization of the lowest extract concentration (5 Noon units/ml) was initiated in the other nostril, and thus successively with the following concentrations until a positive response was obtained or the undiluted extract was reached.

A result was considered positive if at least two of the following symptoms (1, 11, 13, 16) were clearly observed during the 30-min symptom surveillance period: sneezing, nasal blockage, nasal pruritus, or rhinorrhea.

A specificity control was similarly performed in 10 healthy volunteers.

RAST analysis

RAST was performed using the method described by Wide et al. (17) with Phadezym RAST reagents (Kabi Pharmacia), following the manufacturer's instructions. Solid-phase antigen was obtained by coupling the extract solution (10 mg/ml) to the 6-mm-diameter CNBr-activated paper disks by Ceska & Lundkvist's method (6).

Results

Skin tests

Prick tests with *Q. ilex* extract proved positive in 27 patients (3.5%), none of whom were monosensitive. Accompanying sensitizations and their number are shown in Table 1. Disorders presented by each of the 27 patients are listed in Table 2.

Prick tests with *Q. ilex* extracts were negative in all controls.

Specific IgE to *Q. ilex* pollen extract, estimated by RAST, yielded values lower than 0.6 PRU/ml for all patients, except in two cases, showing 1.2 and 7.0 PRU/ml, respectively.

Table 1. Sensitizations accompanying prick tests positive to *Quercus ilex*

Allergens	n
Gramineae	22
<i>Olea europaea</i>	19
<i>Artemisia vulgaris</i>	12
<i>Chenopodium album</i>	7
<i>Plantago lanceolata</i>	3
<i>Rumex acetosella</i>	1
<i>Salsola kali</i>	1
<i>Dermatophagoides pteronyssinus</i>	5

Table 2. Diagnoses of patients positive to prick tests for *Quercus ilex*

Disease	Patients n
Perennial allergic rhinitis	7
Seasonal allergic rhinoconjunctivitis	8
Seasonal allergic rhinitis and asthma	12

Nasal and conjunctival provocation tests

Both tests, performed with *Q. ilex* extracts, in the 27 patients with positive prick test to the allergen, were negative for all patients and controls.

Discussion

Although holm oak pollen is present in our atmosphere in spring in sufficient quantities to produce sensitization in the population, few positive prick tests with *Q. ilex* pollen extracts were found (3.5%), and none were monosensitive. The most frequent accompanying sensitization was to Gramineae (22 patients), followed by *Olea* (19 patients) and *Artemisia* (12 patients). As no cross-reactivity has been described between Fagaceae and Gramineae, or between Fagaceae and Oleaceae, this does not account for the association; rather, Gramineae and *Olea* pollens are the most frequent cause of respiratory allergies in our region (15). Other potentially allergenic pollens, such as those from *Parietaria judaica* (10) and *Cupressus* spp., are detected in a small amounts. Nevertheless, their possible implication is now being investigated, and preliminary results concerning *P. judaica* have already been reported.

We have found only one reference on holm oak (17), which reported up to 12% positive prick tests with an extract at 5% w/v, and from 3% to 5% with an extract at 1% w/v. The latter data are similar to ours (3.5%), but the former are quite different. We find no explanation for this difference since we also used an extract at 5% w/v. In this reference (17), only the results are given; thus, we are unable to comment further. Extracts standardized in biological units or HEP were not used, since there are none commercially available for *Q. ilex* in Spain.

Similar results for the prick test (4.7%) have been reported by Gergen, but referring to another species of the *Quercus* genus, *Q. robur* (9). It has been noted that *Q. alba* (white oak) contains multiple potentially allergenic proteins (12).

In Italy, Negri & Arobba report 15% prick tests positive to *Quercus*, but do not specify the species tested (14). Bousquet et al., on the other hand, found that *Q. robur* produces a small number of positive prick tests despite the presence of large quantities of the pollen (4). In these studies, the minimal clinical significance of the pollen is noted (3). Our results point to the same conclusion. The flowering of holm oak coincides with that of Gramineae and partly with *Olea*. Although prick tests to *Q. ilex* pollen were positive for the patients showing seasonal disorders, no clinical response was obtained by provocation tests using the same pollen extract. The same results were obtained with the group of patients who showed perennial disorders, thus indicating that *Q. ilex* pollen does not cause seasonal symptoms in the allergic patients (Table 2). We do not consider the class 2 results for RAST relevant, and believe the patient with a positive prick test to *Q. ilex* and class 3 RAST to the same allergen suffered seasonal rhinitis and bronchial asthma due to sensitization to grass and olive pollen, his total IgE being normal. Consequently, we suggest that this result could be a false positive RAST or a kind of subclinical sensitization.

We found no clinical repercussions of *Q. ilex* pollen in patients with prick tests positive to this pollen.

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