Sensitization to *Quercus ilex* pollen is clinically relevant in patients with seasonal pollen allergy

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Holm oak, *Quercus ilex*, is the most abundant tree in the Iberian Peninsula, and its pollen one of the most abundant ones recovered from Madrid pollen collectors during spring season. Former reports dating from 1995 estimated the prevalence of sensitization to this pollen in two Spanish populations among allergic respiratory patients between 3.5% and 14%, with less than 1% of monosensitized patients [1,2]. After these reports, it was considered clinically irrelevant and thus it is not routinely included in the screening allergen panel of allergic respiratory patients. However, Holm oak belongs to the order Fagales that includes species such as birch, alder, hazel and hornbeam, whose pollens are potent triggers of spring pollinosis in central Europe [3]. Likewise, the presence of homolog of Bet v 1, namely Que i 1, has been recently described by our group as the main PR10 sensitizer in Madrid (Spain), a birch free area [4]. In that paper, we also published a frequency of sensitization to *Q. ilex* pollen of 59% in a pediatric population, which was far higher than published before [1,2,5]. With these results in mind, we sought to check the current prevalence in adult and pediatric population in Madrid and to find out the clinic relevance of this sensitization by means of nasal provocation tests.

Patients (148 adults, aged≥16 and 100 children, aged 2-15) suspected of allergic respiratory disease (rhinitis, conjunctivitis and/or asthma) attended at the Allergy Department of La Paz University Hospital were prospectively included (October 2021 and March 2022 adult patients and October 2023 and March 2023 pediatric patients). Skin prick tests (SPT) with *Q. ilex* pollen commercial extract (Roxall, Bilbao, Spain) was

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performed, considering positive a wheal ≥ 3 mm [6]. Nasal provocation test (NPT) was performed in a subgroup of 10 patients; aged 14-46 years, 50% male, and 50% had asthma. All of them were polysensitized to other pollens. A healthy non-sensitized patient and 3 patients sensitized to other pollens were included as negative controls (table 1). NPT was performed with a lyophilized extract of ***Q. ilex*** (Roxall) serially diluted in saline solution at 0.5 mg/ml, 0.250 mg/ml and 0.125 mg/ml. The diluted extract was applied bilaterally using a nebulizer device (0.07 ml/spray). First, two puff of 0.9% saline was administered as negative control. If negative, two puff of successive increasing concentrations were applied at 15-minute intervals until the test was positive or all dilutions had been applied. Nasal examination by anterior rhinoscopy was done before the test started. The test results were assessed using the subjective Lebel symptom scale [7] and the objective acoustic rhinometry (AR). Positivity criteria were established according to the EAACI 2018 Nasal Provocation Position Paper [8]. Patients were observed for one hour after the test was done and instructed on what to do in case of a delayed reaction. It was checked that there were no contraindications or medication that could affect the test result. The local ethics committee approved the study (PI-2243). Written informed consent was obtained from all patients, their parents or their legal representatives whenever it was required.

Frequency of positive SPT to *Q. ilex* pollen was higher in children (56/100, 56.0%) than in adults (34/148, 22.9%). This difference was statistically significant (Chi square test, $p= 1.118 \times 10^{-7}$). All nasal provocation test results were positive (100%, table 1).

Interestingly, the frequency in adults is much lower than in children. Since sensitization profiles often remain stable from childhood to adulthood [9], these differences could be reflecting a tendency to a rise in sensitization rates to this pollen, as these children are the

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adults of the future. However, we cannot ignore the influence of exposure time to pollen, which could be more intense in children, leading to higher rates of sensitization, particularly in Madrid, where *Q. ilex* pollen is one of the largest contributors to the total pollen counts [10].

Nevertheless, the current frequency of sensitization in adults (22.9%) is higher than published in 1995 in a population from the same area (14%) [1]. One possible explanation is the rising amount of this pollen in the environment and the increase in the length of this pollen season due to global warming and higher levels of CO2 in the atmosphere [11]. Leaving aside methodological aspects, the difference between the first study and ours and between children and adults is indicating an increase in sensitization to *Q. ilex* pollen in our population.

An aspect to be highlighted is that *Q. ilex* sensitization is commonly associated with polysensitization, as has previously been described [1], and we cannot offer a proper explanation to the absence of patients monosensitized to *Quercus* pollen. This phenomenon requires a more in-depth investigation, not only to elucidate the mechanism involved in the origin of this sensitization, but to establish a correlation with other non-taxonomically related pollen species, particularly in an area where the pollen counts of birch are extremely low [12]. Moreover, we have not observed any relationship between the severity of the response in the nasal provocation test and the wheal size in SPT. Certainly, the severity of symptoms upon natural allergen exposure does not depend solely on specific IgE reactivity. Some host and environmental factors, including immunological parameters and concomitant exposure to other co-seasonal pollens, can influence the burden of symptoms. Regardless to all these considerations, the whole spectra of pollen sensitizations should be taken into account in order to prescribe the best

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immunotherapy to the patients and to assess its successful outcome [13].

In summary, to the best of our knowledge, this is the first study that objectively reports the clinical relevance of *Q. ilex* pollen through positive NPT. We have compiled a prevalence of sensitization of 22.9% and 56.0% among pollen-allergic adult and pediatric patients, respectively. These results mean that this pollen should be considered as a relevant sensitizer during spring season. Likewise, we have confirmed an increase of the sensitization rate to this pollen over the years in Madrid. Nevertheless, further studies, including populations from other areas are needed to properly establish the allergenic relevance of *Q. illex* pollen.

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Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

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Table 1. NST results of 10 patients and 3 negative individuals tested. The test was assessed using the subjective Lebel symptom scale (0-11) and Acoustic Rhinometry (AR). Positivity criteria: Lebel score increase of ≥3 points; AR a decrease in sum of 2-6 cm^3 ≥27% bilaterally.

patient	**age**	**Total IgE (kU/l)**	***Q. ilex* SPT mm**	**Q. alba IgE (kU/l)**	**Positive dilution 0.5-0.125 mg/ml**	**Acoustic Rhinometry (%)**	**Lebel scale**
1	36	169	7x6	4.64	0.3	- 48	6
2	46	26.9	4x4	0.39	0.3	- 27	4
3	20	1337	5x5	0.87	0.3	- 30	3
4	24	212	10x10	0.54	0.125	- 47	8
5	38	565	8x7	3.30	0.250	- 33	9
6	27	1424	12x10	24.6	0.125	- 35	7
7	40	418	12x10	17.1	0.250	- 43	5
8	25	75.10	5x5	0.23	0.125	- 27	9
9	16	927	5x5	55.50	0.125	- 58	11
10	14	518	13x6	24.50	0.250	-40	7
11	30	13.4	<3	0.01	negative	negative	0
12	28	nd	<3	nd	negative	negative	0
13	39	28.40	<3	nd	negative	negative	0
14	26	nd	<3	nd	negative	negative	0

*nd: not determined

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