
Allergenicity of Grass Pollen From Polluted and Nonpolluted Areas Measured Using the Skin Prick Test and Basophil Activation Test

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Pollution has been associated with several pulmonary diseases, including asthma. The bronchial inflammation caused by contaminants increases the effect of airborne allergens in susceptible patients [1].

Pollen-allergic patients with asthma who reside in cities with high levels of industrial pollution experience worse disease progression and more exacerbations than patients who live in cities with lower levels of pollution [2,3]. Furthermore, industrial pollution has been linked to high enterobacteria counts in grass pollen and increased release of endotoxins, each of which amplifies the immune response and inflammation of the airways when pollen is inhaled [4].

Atmospheric pollution affects plants and their pollen. While several studies have found an increase in the allergenicity of pollen from plants and trees exposed to different contaminants, most were performed under laboratory conditions, with few focusing on grass pollen [5-10].

Previous studies by our group showed that pollution was associated with reduced photosynthetic efficiency and a higher degree of oxidative stress in grass plant and pollen proteins [8].

In the present study, we analyzed the allergenicity of grass pollen from a large city with high levels of environmental pollution (Madrid) and a city with low levels of pollution (Ciudad Real) under real-life conditions of environmental exposure of plants, ie, in their habitat and not in a laboratory setting.

The study was approved by the Ethics and Research Committees of the participating hospitals. All the patients gave their written informed consent to participate.

The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp). The means of quantitative variables were compared using the *t* test when the data followed a normal distribution, and the Mann-Whitney test was used when they did not.

Madrid and Ciudad Real are located in the center of Spain. Both have a dry continental climate with short periods of grass pollination (about 6 weeks) in May and June.

Levels of contaminants and weather data were obtained from the City Council of Madrid and Regional Government of Castilla la Mancha websites. Data were collected from October to May (the period when grass pollen is formed) for the years 2017, 2018, and 2019. Data analysis revealed significantly higher levels of NO₂ and SO₂ in Madrid. There were no significant differences in temperature or precipitation between Madrid and Ciudad Real (Online Supplemental files).

From 1st May to 30th June, daily pollen concentrations were measured in Madrid (Hospital Infanta Leonor) and Ciudad Real (Hospital General de Ciudad Real) using a Burkard spore trap (Burkard Manufacturing Co.) as described elsewhere [2,3]. No significant differences in grass pollen counts were found between the cities (Online Supplemental files).

During the pollination period (May) of the years 2017, 2018, and 2019, *Lolium perenne* plants were collected in Madrid and Ciudad Real from areas near roads with vehicle traffic. The mature pollen was extracted by suction as described elsewhere [8].

Skin prick tests were performed on a sample of 75 patients with allergic rhinitis or asthma and sensitization to grass pollen outside the grass pollen season using each original extract from the pollen collected in Madrid and Ciudad Real. Women accounted for 53.3% of the patients, and the median age was 26 years. The mean wheal diameter was significantly greater with the pollen collected in Madrid (Table).

The basophil activation test (BAT) with both pollen extracts, expressed as the percentage of CD63⁺ basophils, was performed using a BASOTEST kit (GlycoType Biotechnology GmbH). The BAT was performed in 8 patients with pollen-induced respiratory allergy. The mean percentage expression of CD63⁺ (activated basophils) was slightly higher with the pollen from Madrid (Table), although the difference was not statistically significant.

Table. Skin Prick Tests and Basophil Activation Test With Grass Pollen Extracts From Madrid and Ciudad Real

	Pollen A (Ciudad Real)	Pollen B (Madrid)
Skin prick test, mm (n=75; $P<.01$) ^a		
Mean	35.44	43.69
Median	30	35
SD	24.44	31.24
Min	4	9
Max	130	150
Basophil activation test, %CD63 (n=8) ^b		
Mean	56.25	60.58
Median	59.35	70.7
SD	35.17	32.55
Min	9	13.8
Max	95.7	93

^aMean diameter: major × orthogonal diameter.

^bPercentage of CD63⁺ basophils.

In this study of grass plants collected in Madrid and Ciudad Real in their natural environment, we found that wheal diameter was greater with the grass pollen from Madrid in the skin tests performed on allergic patients. Given that pollen levels, temperature, and humidity are similar in both cities, we attribute this effect to the higher levels of the contaminants NO₂ and SO₂ in Madrid.

However, we were unable to demonstrate significant differences in the BAT results using both pollens, probably owing to the small number of patients included in this arm of the study.

Some studies report increased allergenicity in pollen exposed to pollutants such as CO, O₃, NO₂, and SO₂ under laboratory conditions [5-10]. However, Smiljanic et al [9] found that pollen from *Phleum pratense* collected from highly polluted areas expressed lower levels of Phl p 6, 12, and 13 and reported that ELISA revealed less binding to IgE in allergic patients.

Ghiani et al [7] conducted a study with *Ambrosia* pollen exposed naturally to environmental pollution, finding that pollen exposed to high levels of traffic pollution was more allergenic in SDS-PAGE and immunoblotting.

Most of the abovementioned studies are based on in vitro tests with patients' serum. However, García-Gallardo et al [10] found that *Pinus radiata* pollen collected in areas of high O₃ concentrations also yielded more positive results in skin prick tests.

In conclusion, we collected pollen from grass plants exposed naturally to contaminants and analyzed the response in vivo using skin tests and, as a novel feature, in vitro using the BAT. Skin tests revealed that the allergenicity of grass pollen exposed to contaminants was greater than that of pollen not exposed to contaminants.

Environmental pollution constitutes an added risk factor for pollen-allergic patients, not only because of its direct

proinflammatory action on the airways, but also because it increases the allergenicity of pollen itself.

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Conflicts of Interest

The authors declare that they have no conflicts of interests.

Previous Presentations

The data reported here were presented in electronic poster format at 33 Congress of Sociedad Española de Alergología e Inmunología Clínica (SEAIC) in October 2021.

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