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Evaluation of the allergenic capacity of three pollen grains in a tropical region (Medellín, Colombia)

Evaluación de la capacidad alergénica de tres granos de polen en una región tropical (Medellín, Colombia)

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Abstract

Objective: To evaluate the frequency of IgE sensitization and allergenic capacity of pollen grains of *Artocarpus communis, Cecropia peltata* and *Fraxinus uhdei* in allergic rhinitis patients.

Methods: A cross-sectional study with comparative groups: a rhinitis group and a control group. The three pollen extracts were prepared, and IgE sensitization was assessed using the skin prick test and serum-specific IgE. Allergenicity and clinical relevance were subsequently evaluated using the basophil activation test (BAT) and the nasal challenge test (NCT).

Results: A total of 60 people were included in the control group and 60 in the case group. None in the control group and a total of 8 (13.3%) from the rhinitis group were sensitized to one of the 3 pollen extracts: two (3.3%) to *A. communis*, two (3.3%) to *C. peltata* and four (6.6%) to *F. uhdei*. All three extracts were able to induce basophil activation in patients with IgE sensitization, but not in those without sensitization. NCT was performed in the eight patients with IgE sensitization, resulting positive for 1/2 patients with *Artocarpus*, 1/2 with *Cecropia* y 2/4 with *Fraxinus*.

Conclusion: All three pollen sources tested induce IgE sensitization and clinical symptoms, highlighting the importance of evaluating the allergenicity of native plant sources for each region.

Keywords: IgE; Allergenic capacity; Pollen grains; *Artocarpus communis*; *Cecropia peltata*; *Fraxinus uhdei*; Allergic rhinitis.

Resumen

Objetivo: Evaluar la frecuencia de sensibilización por IgE y la capacidad alergénica de los granos de polen de *Artocarpus communis*, *Cecropia peltata* y *Fraxinus uhdei* en pacientes con rinitis alérgica.

Métodos: Estudio transversal con grupos comparativos, uno con rinitis y otro control. Se prepararon extractos de los tres tipos de polen y se evaluó la sensibilización mediante la prueba cutánea por punción (prick test) e IgE específica sérica. Posteriormente, se evaluaron la alergenicidad y la relevancia clínica mediante la prueba de activación de basófilos (BAT) y la de provocación nasal (NCT).

Resultados: Se incluyeron 60 pacientes en el grupo control y 60 en el de rinitis. Solo 8 (13.3%) pacientes del grupo con rinitis tuvieron sensibilización a alguno de los 3 extractos de polen: 2 (3.3%) a *A. communis*, 2 (3.3%) a *C. peltata* y 4 (6.6%) a *F. uhdei*. Los tres extractos fueron capaces de inducir activación de basófilos en pacientes con sensibilización por IgE, pero no en aquellos sin sensibilización. La prueba de provocación nasal se practicó a los ocho pacientes con sensibilización por IgE, resultando positiva en 1 de 2 casos con *Artocarpus*, 1 de 2 con *Cecropia* y 2 de 4 con *Fraxinus*.

Conclusión: Las tres fuentes de polen evaluadas inducen sensibilización por IgE y síntomas clínicos, lo que resalta la importancia de evaluar la alergenicidad de fuentes vegetales nativas en cada región.

Palabras clave: IgE; Capacidad alergénica; Granos de polen; *Artocarpus communis*; *Cecropia peltata*; *Fraxinus uhdei*; Rinitis alérgica.

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INTRODUCTION

Protein particles have the potential to induce respiratory symptoms specially by immunoglobulin E (IgE) sensitization (atopy).[1-3] Sensitization to pollen grains is the main cause of allergies in some subtropical countries,[4-6] however, in most cities located in the tropical region, the frequency of sensitization to pollen grains is comparatively low, and other sources are more relevant in the development of allergies, such as house dust mites and pet dander.[7,8] One hypothesis to explain this observation is that in some areas of the tropics, the distance of dispersion of pollen grains by the wind is lower in terms of quantity and diversity compared to other areas, due to the type of climatic conditions but also due to the type of local flora.[9,10]

Another possible explanation is that in many countries, including most of those located in the Latin American tropical region, aerobiological studies are not available, and allergy test usually use extracts that have been characterized as allergenic in other regions and not necessarily represent what the Latin American population is exposed to.[9,11-13] These is supported by the fact that the plant families commonly reported in European countries causing respiratory allergies had low sensitization frequency in Latin America.[8,14] The plant diversity for the city of Medellín, Colombia has been estimated at more than 2,603 species, grouped into 109 families ("Sistema Árbol Urbano": SAU, (https://www.medellin.gov.co/sau/), being Orchidaceae, Fabaceae, Asteraceae, and Poaceae the most diverse families.

In Medellín, different aerobiological samplings have been carried out in recent which has allowed us to understand the dynamics and trends of these pollen types in the city.[9,10] In these samples it has been found that *Cecropia* pollen is the most abundant in the atmosphere of the city of Medellín, followed by *Urticaceae*, *Fraxinus* (Oleaceae) and *Artocarpus* (Moraceae).[10] In this study, native extracts were prepared to demonstrate whether some pollen types common in the atmosphere of the city of Medellín (*A. communis, C. peltata y F. uhdei*), are sources of IgE sensitization in the region. In addition, the diagnostic performance of the prepared extracts was explored to evaluate their potential usefulness in clinical practice.

METHODS

Study design

The study is divided into two phases: preparation of the protein extracts (Phase 1) and evaluation of their allergenic activity (Phase 2). For phase 1, the extracts were prepared following a previously standardized protocol with the support of the "*Parasitología e Inmunoalergia del departamento de Inmunología, Microbiología y Parasitología de la Universidad del País Vasco*" (Vitoria-Gasteiz, Spain). For phase 2, the presence of IgE antibodies was assessed using cutaneous reactivity (intraepidermal test) and serum specific IgE (sIgE) measurement. Allergenic activity was subsequently evaluated using *in vitro* (basophil activation test, BAT) and *in vivo* (nasal challenge test, NCT) reactivity.

Antigenic extracts preparation (Phase 1).

The species included in this study were: *Artocarpus communis*, *Cecropia peltata*. and *Fraxinus uhdei*. These species were selected considering their abundance in pollen grains/m^3 according to the aerobiological samplings of Alzate et al. (9) and Espinosa et al (10). The samples collected were taken from areas that reduced the risk of contamination of the extract by other plant species, and an aerobiologist also performed microscopic evaluation in different samples from the collected grains to confirm the purity of the extract.

Due to the small size of the flowers of the selected species, pollen grains were obtained by macerating the flowers and then sieving them. The sieved pollen grains were stored at -20°C until processing. Pollen extracts were obtained in three stages: defatting the pollen grains and two successive extractions, always under aseptic conditions.

Defatting was performed using three to four volumes of cold acetone (1 ml per gram of pollen) at 4 °C, followed by stirring and decantation to remove lipids. This process was repeated as many times as necessary until the liquid phase was colorless. The resulting solid was dried in a laminar flow chamber.

In the first extraction, 10 ml of phosphate-buffered saline (PBS) was added per gram of pollen. The samples were kept under gentle, constant agitation overnight at 4°C. They were then centrifuged at 5000 rpm for 30 minutes at a temperature of 6–8°C. The supernatant was stored at 4°C.

The second extraction was performed by resuspending the remaining sediment in PBS under the same conditions as the first extraction, although the agitation time did not exceed four hours. Subsequently, the supernatants from both extractions were combined and filtered twice: first with Whatman No. 1 paper and then through a 0.2 µm pore size filter. Finally, the pollen extracts were lyophilized and stored until use.

The final extract was used for skin prick tests, specific IgE measurement, and nasal challenge tests (NPT). For the extracts used in the skin test, an additional step was added: 50% glycerin was used for suspension stability, and phenol and 0.2% NaCl were used as bacteriostatic agents.

Evaluation of allergenic response (Phase 2)

The evaluation of the allergenic response was done in a cross-sectional study. The case group were patients with a medical diagnosis of chronic rhinitis (rhinitis group) according to the ARIA guideline criteria,[15] over 18 years of age who have resided in the metropolitan area of Medellín for the last 5 years without comorbidities that could alter the interpretation of the results or who cannot suspend medications that interfere with the interpretation of the tests (e.g., chronic obstructive pulmonary disease, rhinosinusitis, immunodeficiencies, dermographism).

The control group were people with the same characteristics as the cases, matched by age and sex but who did not present symptoms suggestive of allergy such as asthma, rhinitis, atopic dermatitis and/or conjunctivitis.

IgE sensitization

IgE sensitization was assessed by skin prick test (SPT) following the international recommendations of the European Academy of Allergy, Asthma and Immunology (EAACI)[16]. For the skin test, the presence of a wheal larger than 3 mm compared to the negative control was considered a positive test. Based in a quantification curve, the protein concentration of each extract used for SPT was 2mg/ml for *Artocarpus communis*, 3.4mg/ml *Cecropia peltata*, and 2.4mg/ml for *Fraxinus uhdei*.

In patients who agreed to donate a blood sample for the study, the presence of serum IgE antibodies was evaluated with a fluoroenzyme immunoassay (ELISA) following a previously described protocol.[17] For standardization of the test, cut-off points for each extract tested were calculated from the results obtained from five healthy non-allergic individuals using two standard deviations as the assessment measure using previously described methodology.[18] IgE levels were expressed in optical density (OD).

Allergenicity assessment

Allergenicity was assessed by BAT and PPN. Basophil activation was evaluated by measuring CD203c expression using a previously described protocol.[18,19] Briefly, basophils from each patient were stimulated with different concentrations according to the protein concentration of each extract (0.01, 0.1, or 1 µg/ml). The basophil activation assay was quantified as the percentage of basophils expressing CD203c above the breakpoint, which was 10.0% of the basophils incubated with buffer alone.[18-20]

The NCT were carried out in accordance with international recommendations.[21,22] Each extract was tested at a concentration of 11 mg/ml, 27.5 mg/ml, and 55 mg/ml. At the start of the test, the presence of nonspecific nasal hyperreactivity was ruled out by exposing the nasal mucosa to the extract solvent (saline solution). Test interpretation followed international recommendations (EAACI), using acoustic rhinometry and/or the Lebel score as evaluation parameters.[21-23]

Ethical considerations

This project was approved by the Ethics Committee of Alma Mater Hospital of Antioquia (Medellín, Colombia) under Code (IN86-2023). Each participant provided written informed consent.

Statistical analysis

For the descriptive analysis of the sociodemographic and clinical aspects of the study population, absolute and relative distributions and summary indicators such as arithmetic mean, median, standard deviation, quartiles, interquartile range, minimum values and maximum values were used, as appropriate to their distribution.

Sample size

Convenience sampling was used. This type of sampling is appropriate for the study because it does not affect its objective. The sample size was calculated[24] according with pollen grains sensitization observed in previous studies of the region[8,14] with an expectative of at least 5% of positive sensitization. Considering the known prevalence of IgE sensitization to pollen grains ranges from 5% to 23% and that of rhinitis in Colombia ranges from 20% to 30% in the study population,[14,25] the following assumptions were made: exposure frequency in subjects with rhinitis was 10%, and in subjects without rhinitis, 2%. The required number per group would be 60 patients and 60 controls. The sample size calculation was performed using an open-access calculator from the "QuiestionPro" package.

Table 1. General characteristics of the patients.

General characteristics		Rhinitis group (n = 60)	Control group (n = 60)
Age median (range)		37 (52)	36 (22)
Sex: female, n (%)		38 (63.3%)	39 (65%)
Atopy to any allergen		48 (80%)	12 (20%)
	HDM	44 (73.3%)	9 (15%)
	Dog	18 (30%)	4 (6.6%)
	Cat	8 (13.3%)	2 (3.3%)
	Pollen grains	3 (5%)	0
Asthma		9 (15%)	0
Conjunctivitis		23 (38.3%)	0
Atopic Dermatitis		0	0
Years with rhinitis median (range)		15 (66)	N/A

Clinical and sociodemographic characteristics of each group. HDM: House dust mites. N/A no apply.

RESULTS

Allergenic extract

The raw material weights were 1.1 g, 0.6 g, and 1.5 g for *Artocarpus, Cecropia,* and *Fraxinus*, respectively. The allergenic extracts obtained weighed 4.8 mg for *Artocarpus*, 8.1 mg for *Cecropia*, and 27.4 mg for *Fraxinus*. The final concentration for skin tests was 120125ug/ml, 101-112ug/ml, 104-130ug/ml respectively.

Prevalence of IgE sensitization

Patient characteristics are presented in **Table 1**. A total of 120 participants were included, 60 in the rhinitis group and 60 in the control group. Eight (13.3%) patients in the rhinitis group were sensitized to one of the study plant extracts (**Figures 1A, 1B**). No patients were co-sensitized to any of the study plant extracts. In the control group, 10 (16.6%) subjects were sensitized to mites and/or pets (**Table 1**), but none were sensitized to any of the study plant extracts (**Figures 1A, 1B**). sIgE and SPT testing were in complete agreement for both positive and negative results (**Figure 1C**).

Allergenic activity according to BAT and NCT

IgE antibodies against proteins from the three study extracts produced basophil activation only in patients with sIgE to the respective extract used as a stimulant in BAT (**Figure 2**). When PPN was performed (concentration 10 ug/500 ul) among patients with IgE sensitization to one of the study extracts, one

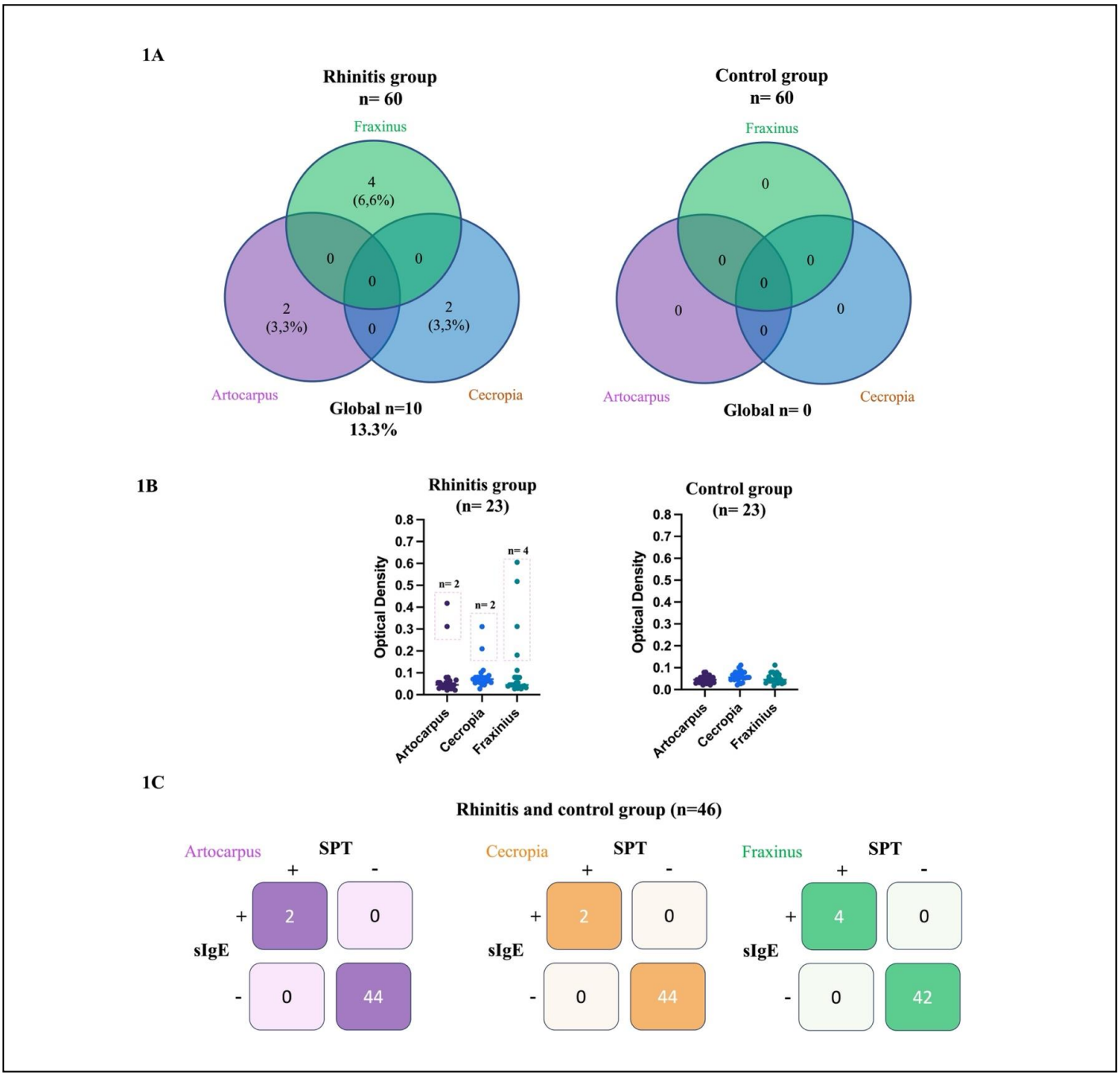

Figure 1. IgE antibodies to the three extracts. IgE sensitization was assessed in all participants by skin prick testing (**Figure 1A**). In those participants who accept donated a blood sample (n = 46), serum specific IgE for each extract was measured (**Figure 1B**). The optical density (O.D.) cut-off was 0.089 for *Artocarpus*, 0.115 for *Cecropia*, and 0.130 for *Fraxinus*. In patients with both skin prick testing and specific IgE measurement, the agreement between the two tests was assessed (**Figure 1C**).

SPT: Skin prick test. sIgE: specific IgE.

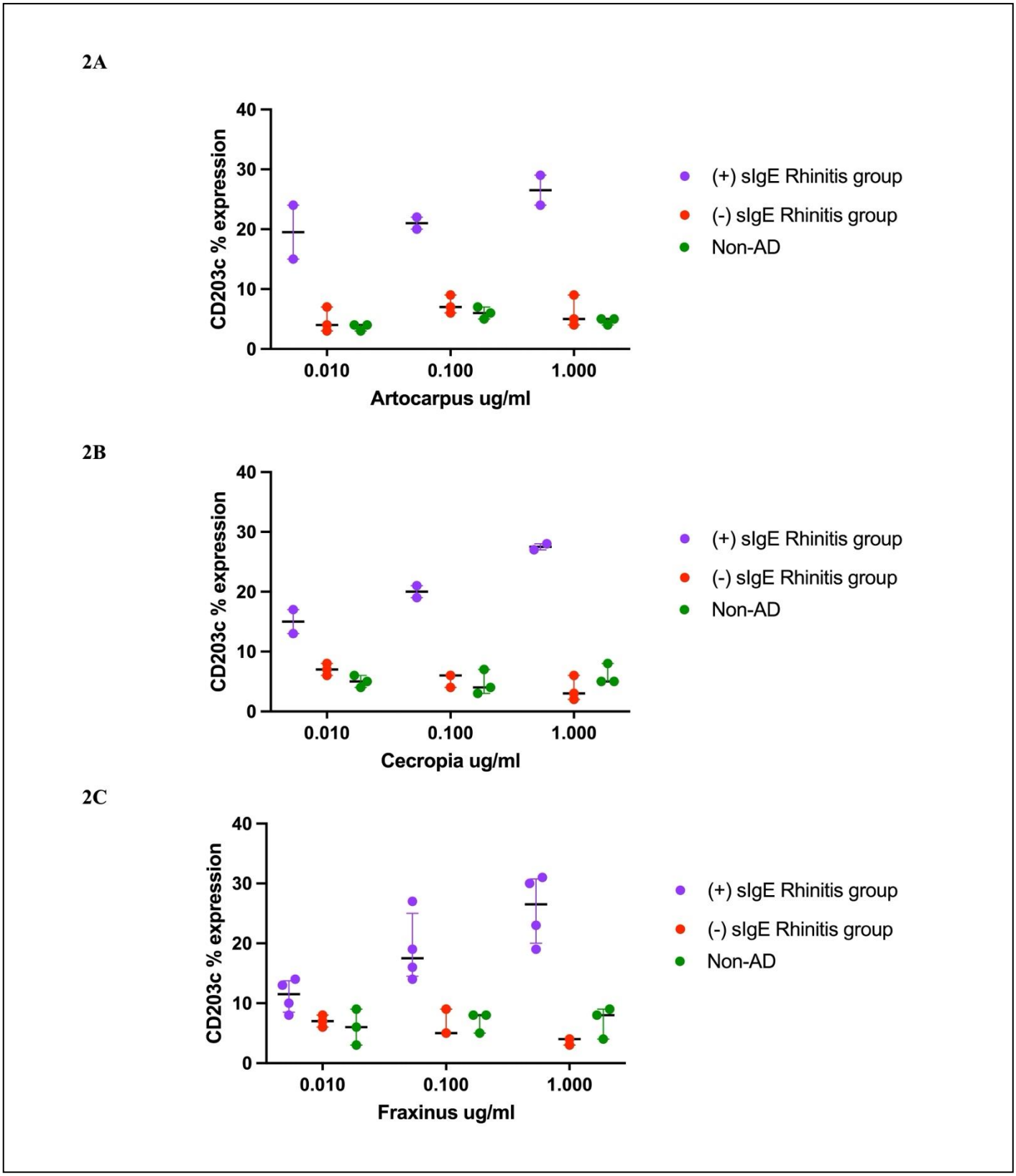

Figure 2. Basophil activation test using proteins from *Artocarpus* (**Figure 2A**), *Cecropia* (**Figure 2B**) and *Fraxinus* (**Figure 2C**).

of two patients with atopy to *A. communis*, one of two to *C. peltata*, and two of four to *F. uhdei* were positive (**Figure 3**).

DISCUSSION

Knowledge of aerobiology allows us to identify the main protein sources to which people are exposed, and which may be related to allergic symptoms. Populations lacking aerobiological information follow the recommendations obtained from other populations, but this can lead to underdiagnosis of allergies, as local allergens may be excluded from diagnostic tests. It has also been reported that an allergenic source may present protein variations in two different regions[11,26-28] both in animal and plant sources, so it is advisable to use the native extracts of each region.

In the most recent aerobiological study carried out in Medellín,[10] carried out between 2019 and 2022, it was found that

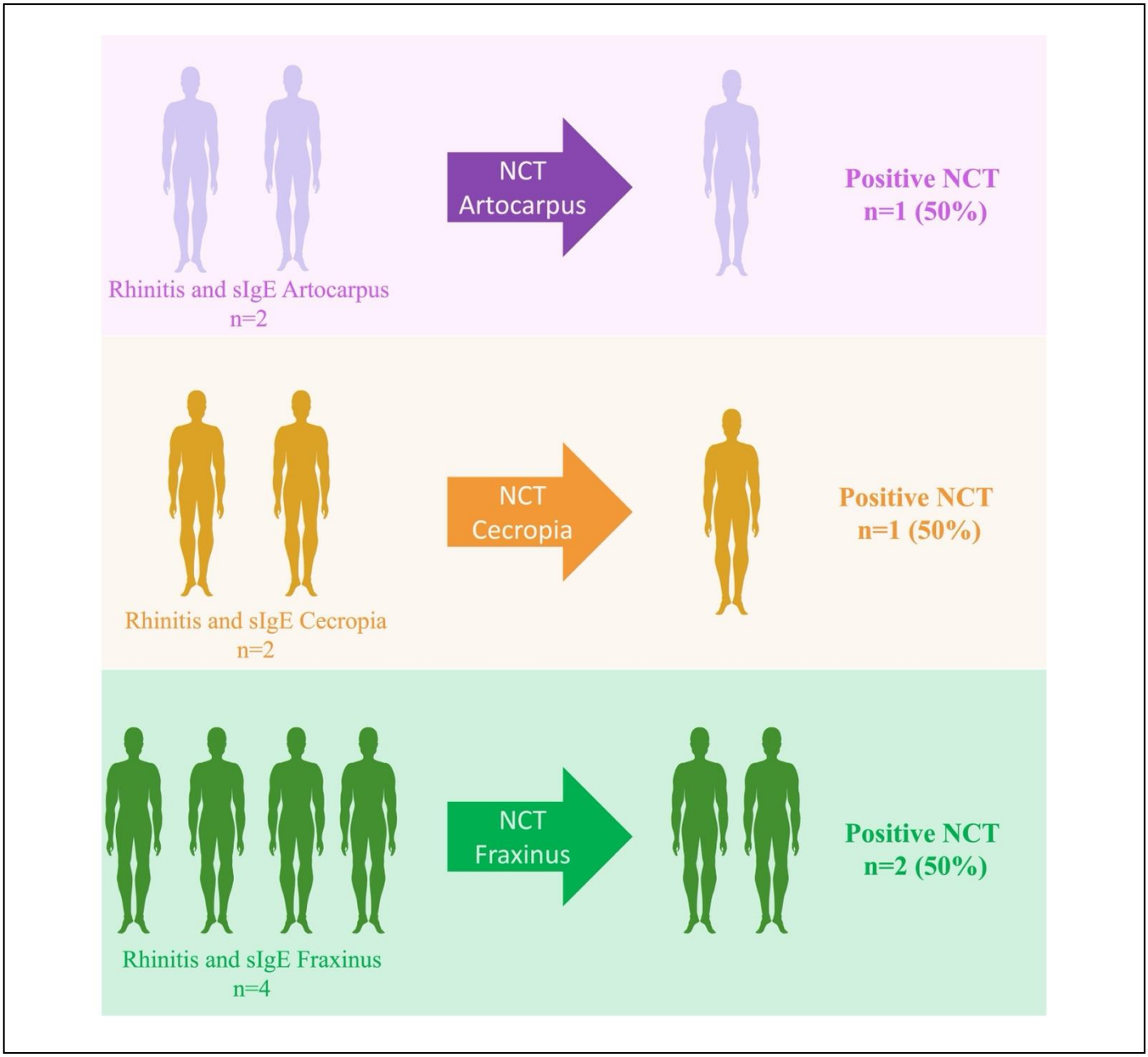

Figure 3. Nasal challenge test among patients with IgE antibodies to *Artocarpus, Cecropia*, or *Fraxinus*.

NCT: Nasal Challenge Test.

the pollen types *Cecropia*, *Fraxinus* and Moraceae (including *Artocarpus*) represent 60% of the city's pollen spectrum, the presence of pollen grains was reported during 24 hours in all days of the sampling period and the pollen concentrations found were comparable to those reported in European cities where pollen allergy is a recognized public health problem. On the other hand, the highest pollen concentrations are recorded after rainy periods, which take place in April-May and October-November.

For the genera *Artocarpus* and *Cecropia*, the available information regarding the frequency of IgE sensitization and its allergenic capacity globally is limited, mostly from case reports. Some studies have reported an oral allergy syndrome after consumption of the fruit of *Artocarpus communis* (breadfruit), attributing to cross-reactivity with birch (Bet v).[29-31] Iddagoda et al., reported a heat-stable allergen of 114 kDa obtained from a patient with a reaction after consumption.[32] Rodríguez et al. reported an evaluation using skin test with *Cecropia* sp. (Yarumo) and *Fraxinus chinensis* Roxb, a species later identified as *F. uhdei* (Mexican ash), in 207 patients with symptoms of asthma or rhinitis; 6 (2.9%) and 20 (9.6%) were positive for *Cecropia* and *Fraxinus*, respectively.[33] *Cecropia* is a genus endemic to the Latin American tropics and of great abundance, growing spontaneously in the city of Medellín. To our knowledge, this study provides the first results evaluating its allergenic activity beyond its IgE sensitization capacity. For *Fraxinus* there are studies especially in the European population,[34,35] but little information on the Latin American population. Our study observed that the pollen species *Artocarpus*, *Cecropia*, and *Fraxinus* could induce the production of IgE antibodies and produce the release of histamine, leading to the development of clinical symptoms demonstrated by NCT. Characterizing the allergen sources in each region is important for establishing public health measures

and for clinical practice aimed at improving the quality of life of patients. These measures include, for example, the construction of pollen calendars to assess the constant change in aeropalynological diversity and dynamics, the development of diagnostic tests, and specific treatments such as immunotherapy.

Although our study allows progress toward the described objectives, it has some limitations that require further evaluation. The frequency of sensitization to the three allergens was less than 10%, and we did not have enough patients to calculate the diagnostic performance of the tests. When performing the provocation tests, we observed that one in two patients had a negative test, suggesting that asymptomatic sensitizations may occur, and that a provocation test is required to confirm clinical relevance. Microscopic examination of the grains collected for extract production by an aerobiologist specializing in pollen studies confirmed that the risk of contamination or the presence of other components in the extracts was virtually zero (section "Antigenic Extract Preparation"). However, complete protein analysis is needed in future studies to characterize the extracts and potential allergens.

Despite the limitations described above, our study also has some strengths. The preparation of extracts using local flora, combined with the assessment of IgE sensitization by skin and serum testing, confirms that even in highly exposed pollen sources such as those studied, sensitization to these pollen types in some tropical regions is comparatively low compared to that observed in other countries. Additionally, these results are hypothesis-generating; This suggests that population characteristics or exposure patterns may reduce the risk of sensitization to pollen grains. Furthermore, in line with the literature, we report for the first time the allergenicity of *Artocarpus* and *Cecropia* using in vitro (BAT) and in vivo (NCT) testing, demonstrating that these sources are allergenic and can induce symptoms in sensitized patients. This leads to a reconsideration of the use of these plants as urban trees, as they could represent a conflict with the population settled in neotropical cities, which would translate into public health problems and higher costs for health systems.

CONCLUSION

A. communis and *C. peltata* demonstrated the ability to induce IgE antibody formation and clinical symptoms, highlighting the importance of evaluating potential allergenic sources in each region. Considering that some Latin American populations have large numbers of these species present in cities, it is important to evaluate their clinical impact. Further studies are needed to adequately establish the prevalence of sensitization and the diagnostic performance of atopy testing with these sources.

Conflicts of interest

The authors declare no potential conflicts of interest with respect to research, authorship or publication of this article.

Financial sources statement

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