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Aeroallergen Sensitization in Adults with Allergic Rhinitis: A Single Center Experience in a Tertiary Hospital

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Abstract

Background and Objectives: Aeroallergen sensitization patterns vary by region, influenced by environmental factors such as climate and vegetation. This study aimed to determine the sensitization profile and identify the most common aeroallergens in adult patients presenting with a preliminary diagnosis of allergic rhinitis (AR) at a tertiary care center in Southeastern Türkiye.

Methods: Adult patients (≥18 years) who presented to our immunology-allergy clinic with suspected AR and underwent skin prick testing (SPT) for inhalant allergens between February 2024-February 2025 were retrospectively analyzed. Demographic and clinical data—including total IgE levels, eosinophil counts, and SPT results—were retrieved from medical records.

Results: A total of 658 patients were included; median age was 31 years, and 65% were female. A family history of atopy was present in 28.3%, and 36.8% had at least one allergic comorbidity, most commonly asthma (26%). Based on symptom severity, 51.3% had mild AR and 48.7% had moderate-to-severe AR. Sensitization to inhalant allergens was identified in 77.4% of patients. The most common aeroallergens were grass pollen mix (40.3%), Dermatophagoides farinae (37.7%), Dermatophagoides pteronyssinus (33.3%), meadow-grass pollen mix (27.8%), and cockroach (24.2%). Sensitization to grass pollen mix, weed pollen mix, olive tree pollens, and cockroach was significantly associated with moderate-to-severe AR. Asthma was more prevalent in this group and was strongly linked to sensitization to house dust mites, and dog epithelium ($p < 0.05$).

Conclusion: Sensitization to pollens and indoor allergens is associated with increased AR severity and asthma. Identifying regional sensitization profiles may support more accurate diagnosis and personalized treatment strategies.

Keywords: Aeroallergen, allergic rhinitis, asthma, skin prick test, sensitization

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Alerjik Rinitli Yetişkinlerde Aeroalerjen Duyarlılığı: Üçüncü Basamak bir Hastanede tek merkez deneyimi

Öz

Amaç: Aeroalerjen duyarlılık paternleri, iklim ve bitki örtüsü gibi çevresel faktörlerden etkilenerek bölgelere göre değişiklik gösterir. Bu çalışma, Güneydoğu Türkiye'deki bir üçüncü basamak sağlık merkezine alerjik rinit (AR) ön tanısıyla başvuran yetişkin hastalarda duyarlılık profilini belirlemeyi ve en sık görülen aeroalerjenleri tespit etmeyi amaçlamıştır.

Yöntemler: Şubat 2024-Şubat 2025 arasında immunoloji-alerji polikliniğine AR şüphesiyle başvuran ve inhalan alerjenlere yönelik deri prik testi (SPT) yapılan 18 yaş ve üzeri yetişkin hastalar retrospektif olarak incelenmiştir. Hastaların demografik ve klinik verileri—toplam IgE seviyeleri, eozinofil sayıları ve SPT sonuçları dahil—tıbbi kayıtlardan alınmıştır.

Bulgular: Çalışmamıza toplam 658 hasta dahil edildi. Hastaların yaş ortancası 31 yıl olup, %65'i kadındı. Hastaların %28,3'ünde ailede atopi öyküsü bulunurken, %36,8'inde en az bir alerjik komorbidite vardı; bunların başında astım (%26) gelmekteydi. Semptom şiddetine göre, hastaların %51,3'ünde hafif AR, %48,7'sinde ise orta-şiddetli AR görüldü. Hastaların %77,4'ünde inhalan alerjenlere karşı duyarlılık tespit edildi. En sık görülen aeroalerjenler sırasıyla: Çayır poleni karışımı (%40,3), Dermatophagoides farinae (%37,7), Dermatophagoides pteronyssinus (%33.3), Çayır otu poleni karışımı (%27,8), Hamam böceği (%24,2) olarak belirlendi. Çayır poleni karışımı, yabani ot poleni karışımı, zeytin ağacı polenleri ve hamam böceğine karşı duyarlılık, orta-şiddetli AR ile anlamlı derecede ilişkiliydi. Astım, bu grupta daha yaygındı ve ev tozu akarları ile köpek epiteline karşı duyarlılıkla güçlü bir bağlantı gösterdi ($p<0.05$).

Sonuç: Güneydoğu Türkiye'deki alerjik rinit hastalarının alerjen duyarlılık profili hakkında önemli bilgiler sunmaktadır. Bölgenin iklimi ve bitki örtüsü göz önüne alındığında, bu veriler klinik uygulamalarda ve halk sağlığı stratejilerinde değerli bir rehber olabilir.

Anahtar kelimeler: Aeroalerjen, alerjik rinit, astım, deri prik testi, duyarlılık

INTRODUCTION

Allergic rhinitis (AR) is a common chronic inflammatory disorder of the upper airways characterized by an IgE-mediated immune response of the nasal mucosa following allergen exposure, leading to symptoms such as rhinorrhea, sneezing, nasal itching, and nasal congestion[1-3]. Additional manifestations, including postnasal drip, frequent throat clearing, cough, palate itching, fatigue, and irritability, are also frequently reported. Globally, AR affects more than 400 million individuals, with its prevalence steadily increasing in recent years due to environmental changes and lifestyle shifts[2,4]. In Türkiye, reported prevalence rates among adults vary between 1.6% and 27.5%[5]. Allergic rhinitis commonly coexists with other atopic conditions, particularly asthma, and is primarily triggered by hypersensitivity to aeroallergens such as pollens, house dust mites, molds, and animal epithelia[2].Aeroallergen sensitization plays a central role in the pathogenesis and clinical expression of AR[6]. Accurate diagnosis relies not only on a thorough clinical history but also on objective testing methods, such as skin prick testing (SPT) and serum-specific IgE measurements. Among these, SPT remains the most commonly used method for identifying allergen sensitization in clinical practice[1].

Regional variability in aeroallergen exposure—shaped by factors such as climate, vegetation, and urbanization—leads to significant differences in sensitization profiles across and within countries[7]. For example, grass pollens are prevalent in most parts of Türkiye, while olive tree pollen dominates in the Aegean and Marmara regions, and pine pollen is more common in Central Anatolia and the Black Sea region[8]. Sensitization to house dust mites is more frequently observed in humid and warm regions such as İstanbul and Antalya[9,10].

Given Türkiye's pronounced ecological variability, region-specific evaluations of aeroallergen sensitization are essential for improving diagnostic precision and facilitating tailored therapeutic approaches. While multiple studies have explored allergen distribution across various geographic regions, ongoing surveillance of local sensitization patterns remains critical in light of evolving climatic conditions and dynamic environmental exposures. Moreover, the interplay between aeroallergen sensitization and clinical characteristics—particularly AR severity and coexisting asthma—requires further elucidation in diverse regional populations. This study aimed to evaluate the aeroallergen sensitization profile and its association with AR severity and asthma comorbidity in adult patients presenting to a tertiary allergy clinic, a Southeastern province of Türkiye with distinct climatic and environmental characteristics.

METHODS

Study Design and Participants

This retrospective study included adult patients (≥18 years) who presented to the Adult Allergy and Immunology Outpatient Clinic of a tertiary care center between February 2024 and February 2025 with symptoms suggestive of AR and underwent SPT for inhalant allergens. Only patients with complete allergy workups and accessible medical records were included.

Diagnostic Criteria and Data Collection

AR and asthma diagnoses and classifications were determined based on the ARIA (Allergic Rhinitis and Its Effects on Asthma) guidelines and GINA (Global Asthma Initiative) guidelines, respectively[11,12]. Patients with allergic rhinitis were classified as having mild allergic rhinitis if they had no impairment in daily activities, no sleep disturbance, leisure, or sports activities, and no decline in school or work performance, as well as no troublesome symptoms. Conversely, those with one or more of these symptoms were categorized as having moderate-to-severe allergic rhinitis. Data retrieved from hospital records included demographic characteristics, symptom profiles and duration, comorbid allergic and non-allergic diseases, rhinitis severity, family history of atopy, total serum IgE levels, peripheral eosinophil counts, and SPT results.

To maintain the confidentiality of the identities of the patients included in the study, their personal data was anonymized. Identifying information such as patient names and surnames was not used in the data collection and analysis processes. The study results are presented only through aggregated statistical data.

Inclusion and Exclusion Criteria

Patients were included if they met the age requirement (≥18 years), exhibited clinical features consistent with allergic rhinitis, and underwent SPT for inhalant allergens. Exclusion criteria were: ongoing use of medications that could not be discontinued and would interfere with SPT accuracy, refusal to undergo testing, or incomplete medical documentation.

Skin Prick Testing

SPTs were conducted after the appropriate discontinuation of medications that might interfere with the test results. Positive (histamine dihydrochloride 0.1%) and negative (0.9% NaCl) controls were used alongside standardized aeroallergen extracts (ALK-Abello® Prick test (Hoersholm, Denmark, Allergopharma® Reinbek, Germany and Lofarma® Milan, Italy), including house dust mites (Dermatophagoides (D.) farinae, D. pteronyssinus), molds (Aspergillus fumigatus, Penicillium notatum, Alternaria alternata, Cladosporium herbarum), pollens (grass mix, meadow, cereal mix, tree mix, Olea europaea, Parietaria judaica, Ambrosia, Plantago lanceolata), and animal epithelium (cat, dog). Grass mix contained Timothy grass, Orchard grass, Kentucky bluegrass, Perennial ryegrass, and Festuca pratensis, while the cereal mix included wheat, rye, barley, and oats. Tree mix included alder, ash tree, birch, oak, maple, poplar, elm, plane tree, and walnut. Meadow polen contained Phleum pretense. SPTs were considered positive if the wheal diameter was ≥3 mm compared to the negative control. All patients

were tested with a 13-allergen panel, and animal epithelium extracts were applied only to patients with domestic animals or frequent exposure to pets. This study was approved by the Local Ethics Committee (Approval No: 414; 28 March 2025), and conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki.

Statistical Analysis

Data were analyzed using SPSS for Windows (version 28.0; SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± standard deviation (for normally distributed data) or median with interquartile range (for non-normally distributed data). Categorical variables were described using frequencies and percentages. Group comparisons for categorical data were performed using either the chi-squared test or Fisher's exact test. For continuous data, the Mann–Whitney U test or Kruskal–Wallis test was used depending on the number of comparison groups. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

Demographics and Clinical Characteristics

A total of 658 adult patients presenting with symptoms of suggestive AR were included in the study. The median age was 31 years (range= 18–83), and 65% (n=428) were female. The median duration of symptoms at presentation was 30 months (range 2–480). A positive family history of atopic disease was reported in 28.3% (n=175/619). Demographic, clinical, and laboratory features are summarized in Table 1.

Table I: Demographic, Clinical, and Laboratory Characteristics of the Study Population (n = 658), Including AR Severity and Comorbidities.

	n, (%)		Median (range)
Sex (Female)	428 (65)	**Age, years**	31 (18–83)
Allergic rhinitis severity (n=606)		**Symptom duration, months**	30 (2–480)
Mild	311 (51.3)	**Family history of atopic disease (n=619)**	175 (28,3)
Moderate-to-severe	295 (48.7)		
Allergic comorbidity	242 (36.8)	**Total IgE**	194 (4-3200)
Asthma	171 (26)	**Eosinophil count,10^9/L**	200 (0-1380)
Urticaria	38 (5.7)		
Atopic dermatitis	17 (2.5)	**Positive skin prick test result**	
Nasal polyps	7 (1.0)	Grass pollen mix	265 (40.3)
Drug allergy	35 (5.3)	D. farinae	248 (37.7)
Other	11 (1.6)	D. pteronyssinus	219 (33.3)
Skin prick test		Meadow grass pollen mix	183 (27.8)
Positive	508 (77.2)	Cockroach	159 (24.2)
Negative	150 (22.8)	Plantain pollen	144 (21.9)
Respiratory tract symptoms		Olive tree pollen	123 (18.7)
Rhinorrhea	631 (95.9)	Weed pollen mix	117 (17.8)
Nasal itching	630 (95.7)	Mugwort pollen	115 (17.5)
Sneezing	552 (83.9)	Tree pollen mix	83 (12.6)
Nasal congestion	141 (21.4)	Cypress pollen	62 (9.4)
Postnasal drip	141 (21.4)	Stickyweed pollen	31 (4.7)
Cough	204 (31)	Cereal pollen mix	15/268 (5.6)
Dyspnea	144 (21.9)	Cat epithelium	33/116 (28.4)
Ocular itching/ discharge	172 (26.1)	Dog epithelium	10/87 (11.5)

Abbreviations: n = number of individuals; Ig = immunoglobulin; L = liter; D. = Dermatophagoides; Data are presented as n (%) or median (range) unless otherwise specified

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Among 606 patients with available data on AR severity, 51.3% (n=311) were classified as having mild AR and 48.7% (n=295) had moderate-to-severe AR according to ARIA guidelines. Allergic comorbidities were observed in 36.8% (n=242) of patients, with asthma being the most common (26%, n=171), followed by chronic urticaria (5.7%), drug allergy (5.3%), atopic dermatitis (2.5%), and nasal polyps (1%).

The most frequently reported nasal symptoms included rhinorrhea (95.9%), nasal itching (95.7%), and sneezing (83.9%). Other complaints included cough (31%), dyspnea (21.9%), postnasal drip (21.4%), nasal congestion (21.4%), and ocular symptoms such as itching or tearing (26.1%).

Laboratory data showed a median total serum IgE level of 194 IU/mL (range= 4–3200), and a median peripheral eosinophil count of 200 cells/µL (range= 0–1380). Overall, SPT positivity for at least one inhalant aeroallergen was found in 77.2% (n=508) of the patients (Table 1).

Aeroallergen Sensitization Patterns

The most common sensitizations were to grass pollen mix (40.3%), D. farinae (37.7%), D. pteronyssinus (33.3%), and cockroach (24.2%). Other relevant sensitizations included Parietaria judaica (21.9%), olive tree pollen (18.7%), ragweed (17.8%), and plantain (17.5%). Sensitization to tree pollen mix and cereal pollen mix was noted in 12.6% and 5.6% of cases, respectively. Among those tested for animal epithelium, 28.4% (n= 33/116) were sensitized to cat epithelium and 11.5% (n= 10/87) to dog epithelium. These findings are summarized in Table 1.

Association Between Allergen Sensitization and AR Severity

When allergen sensitization was evaluated in relation to AR severity, patients with moderate-to-severe AR exhibited significantly higher rates of sensitization to grass pollen mix [36.3% (107/295) vs. 20.9% (65/311), p < 0.001], meadow pollen mix [50.5% (149/295) vs. 33.1% (103/311), p < 0.001], olive tree pollen [24.7% (73/295) vs. 14.1% (44/311), p = 0.001], mugwort pollen [23.1% (68/295) vs. 13.8% (43/311), p = 0.003], cockroach [23.7% (70/295) vs. 18.6% (58/311), p = 0.002], tree pollen mix [17.3% (51/295) vs. 10.0% (31/311), p = 0.008], D. farinae [44.7% (132/295) vs. 33.1% (103/311), p = 0.003], D. pteronyssinus [39.0% (115/295) vs. 28.9% (90/311), p = 0.009], plantain pollen [28.1% (83/295) vs. 17.0% (53/311), p = 0.001], and weed pollen [22.7% (67/295) vs. 14.8% (46/311), p = 0.012] as shown in Figure 1.

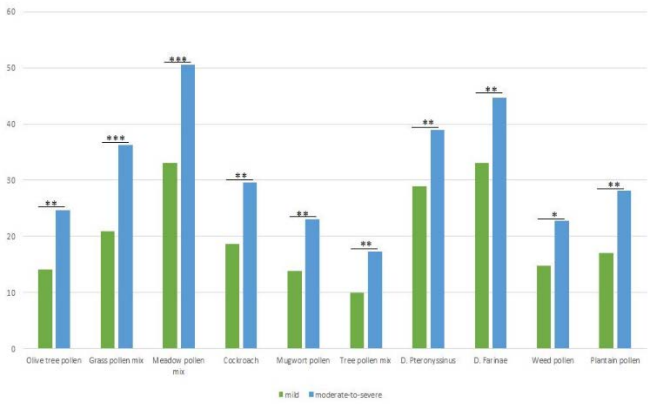

Figure 1. Statistical significance of skin prick test results for aeroallergen sensitization in patients diagnosed with mild vs. moderate-to-severe allergic rhinitis. Bars represent the proportion of patients sensitized to each allergen.

*Significance levels are denoted as follows: *** p < 0.001; ** p < 0.01; * p < 0.05. Abbreviations: D. = Dermatophagoides*

Association Between Aeroallergen Sensitization and Asthma

Among all patients, 26% (n=171) were diagnosed with asthma. Compared to those without asthma, these patients had significantly higher rates of sensitization to D. farinae [44.4%

(76/171) vs. 35.2% (171/486), p = 0.032], D. pteronyssinus [40.4% (69/171) vs. 30.7% (149/486), p = 0.021], and dog epithelium [30% (6/20) vs. 6% (4/67), p = 0.003]. These differences are shown in Figure 2.

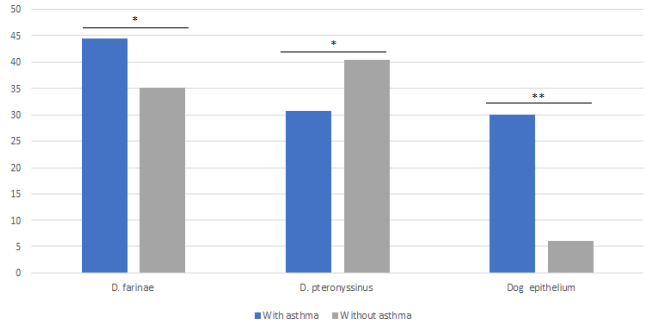

Figure 2. Statistical significance of aeroallergen sensitization in patients with vs. without asthma. Bars represent the proportion of patients sensitized to each allergen.

*Significance levels are denoted as follows: ** p < 0.01; * p < 0.05. Abbreviations: D. = Dermatophagoides*

Relationship Between AR Severity and Asthma Comorbidity

The proportion of patients with asthma was significantly higher among those with moderate-to-severe AR than among those with mild disease [63.4% (102/161) vs. 36.6% (59/161), p < 0.001]. This suggests a strong correlation between increased AR severity and the presence of lower airway involvement.

Comparison of Serum Total IgE and Eosinophil Levels by Allergic Rhinitis Severity and Asthma Status

In the moderate-to-severe allergic rhinitis group, serum total IgE (485.12 ± 645.03 vs. 331.43 ± 460.67 IU/mL; p = 0.047), eosinophil percentage (3.23 ± 2.31% vs. 2.72 ± 1.82%; p = 0.038), and eosinophil count (272.25 ± 216.97 vs. 224.02 ± 144.95 cells/µL; p = 0.015) were significantly higher compared to the mild group. In patients with asthma, serum total IgE (461.01 ± 624.27 vs. 417.00 ± 560.74 IU/mL; p = 0.582), eosinophil percentage (3.02 ± 2.29% vs. 3.02 ± 2.03%; p = 0.985), and eosinophil count (258.76 ± 209.31 vs. 249.28 ± 177.17 cells/µL; p = 0.653) were comparable to those without asthma, and these differences were not statistically significant.

DISCUSSION

In this study, we investigated aeroallergen sensitization patterns in adult patients referred to the adult allergy and clinical immunology outpatient clinic with a preliminary diagnosis of AR in a tertiary hospital in Türkiye, and evaluated their association with disease severity and asthma comorbidity. Our findings showed that sensitization to grass pollens mix, weed pollens mix, olive tree pollen, and cockroach, as well as house dust mites, was significantly associated with moderate-to-severe AR. Additionally, asthmatic patients had higher rates of sensitization to D. farinae, D. pteronyssinus, and dog epithelium.

A previous study conducted at Dicle University in Diyarbakır between 2009 and 2013 evaluated inhalant allergen sensitization patterns in 1,791 patients and reported the most common sensitizations to be grass pollen (70%), wheat pollen (46.5%), and tree pollens (46.1%). Sensitization to house dust mites was observed in approximately 39% of patients and followed these pollens in frequency[13]. In contrast, our study found the most frequent sensitizations to be grass pollen mix (40.3%), D. farinae (37.7%), D. pteronyssinus (33.3%), meadow grass pollen mix (27.8%) and cockroach (24.2%). Sensitization to tree pollens was markedly lower compared to the earlier study. This difference may be explained by various factors, including environmental changes such as the reduction of agricultural land, increased humidity due to dam construction, and forest fires over the past decade, as well as shifts in lifestyle. In addition, differences in the allergen panels and testing methods used across studies may have contributed to this variation. Consistent with our findings, recent studies conducted in other provinces of Southeastern Türkiye have also

reported lower frequencies of tree pollen sensitization[14,15].

When examining studies conducted in other provinces within the same region, similar sensitization patterns emerge. In a study from Şanlıurfa, the most frequently reported sensitizations were to grass pollen (42.6%), cereal mixtures (41.5%), meadow grass (37.9%), and cockroach (37.3%). Sensitization to house dust mites was observed in 27.5% of patients for D.farinae and in 20.8% for D. pteronyssinus. Among patients with both rhinitis and asthma, grass pollens were the most commonly identified aeroallergens[14]. In contrast, our cohort showed higher rates of sensitization to D. farinae, D. pteronyssinus, and dog epithelium in patients with comorbid AR and asthma. Similarly, a study from Bursa by Ediger et al. reported that house dust mite sensitization was more prevalent in AR patients with concurrent asthma, while pollen sensitization was more common in those with AR alone16. In another study conducted in Batman, among patients with positive inhalant allergen sensitization, the most frequent allergens were D. farinae (49.2%), grass mix (45.8%), grass-cereal mix (43.1%), D. pteronyssinus (36.2%), and cockroach (29.6%)[15]. Although study designs and patient characteristics varied, our findings regarding inhalant allergen sensitization patterns appear more consistent with those reported from the Batman region. Additionally, studies conducted in other warmer and more humid regions of Türkiye have consistently identified house dust mites as the most prevalent allergens[9,10,16,17]. Sensitization to grass and weed pollens, along with house dust mites, was significantly associated with moderate-to-severe AR in our study, consistent with the findings of Tuzer's study[15].

Previous studies from Türkiye have reported that the prevalence of sensitization to at least one allergen among patients with AR ranges between 29.3% and 95%[9,10,13,18,19]. This wide variation may be attributed to differences in study design, population characteristics, diagnostic methods, and environmental or lifestyle-related factors such as geographic region and exposure to pets. Increasing urbanization and environmental pollution may also play a significant role in shaping sensitization trends over time. The increasing prevalence of allergic rhinitis in recent years has been partly attributed to rapid urbanization and rising energy consumption, which have led to greater exposure to both higher concentrations and a wider variety of airborne pollutants. Prolonged exposure to traffic-related air pollution, particularly in densely populated and industrialized areas, is considered a significant risk factor for allergic sensitization and the exacerbation of rhinitis symptoms[20-22]. In our study, the rate of sensitization to at least one aeroallergen among patients with AR was 77.2%, which is substantially higher than the 56% previously reported in the same region approximately a decade earlier. Several factors may explain this increase: (1) differences in the allergen panels and techniques used between studies, (2) a true temporal increase in atopy prevalence, (3) increased exposure to environmental allergens due to urban expansion and deteriorating air quality, and (4) improved diagnostic awareness and better access to healthcare services leading to more frequent diagnoses.

In addition to allergen sensitization patterns, demographic characteristics such as sex and family history also play a critical role in understanding AR. AR is more commonly observed in males during childhood. However, sex-related differences in adulthood remain less clear[23]. While several studies have reported a higher prevalence of atopic diseases, including AR, among females, others have found no significant sex-based variation[23-27]. In a study by Lee et al., a significant association between

female sex and AR was identified, which the authors attributed to the possible role of sex hormones[27]. In line with these findings, our study also demonstrated a higher proportion of female patients among those with AR and comorbid asthma.

AR is known to result from a complex interplay between genetic predisposition and environmental exposures. A positive family history of atopy remains a valuable clinical indicator during patient evaluation. In various studies conducted in Türkiye, the reported rate of familial atopy has ranged between 38% and 72.8%; however, this rate was notably lower in our cohort[15,19,28]. We believe this discrepancy may be attributed to the heterogeneity inherent in patient self-reporting, which limits the accuracy of direct comparisons.

In our study, the mean eosinophil count and IgE level in patients with allergic rhinitis were similar to those reported in previous studies[19]. While earlier studies found no difference in IgE levels and eosinophil counts between patients with mild and severe allergic rhinitis, our study revealed a significant difference[15,19]. This variation may be attributed to the limited number of patients with available laboratory data or to interregional differences in patient characteristics.

This study has several limitations that should be considered when interpreting the findings. First, patients' prior residence in other regions or recent travel history may have influenced their sensitization profiles, limiting the ability to attribute allergen exposure strictly to the local environment. Second, due to limited access to specific IgE testing, additional sensitizations may have gone undetected. The retrospective design also posed inherent limitations, including variability in data completeness and documentation quality. Furthermore, we did not assess environmental allergen concentrations, which could have provided more direct correlations between exposure and sensitization. SPT for animal epithelium was performed only in patients reporting regular contact with animals, potentially underestimating the overall prevalence of pet sensitization in the population.

Despite these limitations, the study has notable strengths. To our knowledge, it is one of the few investigations characterizing aeroallergen sensitization patterns in Southeastern Türkiye, a region for which limited data are available. The inclusion of a large sample size and a broad panel of inhalant allergens enhances the generalizability and clinical relevance of our findings.

This study highlights a strong association between sensitization to grass and weed pollens and house dust mites and both increased AR severity and the presence of asthma in adults in Southeastern Türkiye. These findings highlight the clinical value of determining region-specific aeroallergen profiles to determine accurate diagnosis and personalized treatment strategies. Furthermore, given the retrospective design of our study and the lack of direct regional aeroallergen measurement, it highlights the need for future studies that include a prospective design and regional aeroallergen measurement. These studies will not only improve our understanding of sensitization dynamics but will also support the development of more preventive and therapeutic approaches based on local exposure patterns.

Ethics Committee Approval: This study was approved by the Local Ethics Committee (Approval No: 414; 28 March 2025), and conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki.

Conflict of Interest: The authors declared no conflicts of interest.

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