

Abstract

Background: The sensitization profile of patients allergic to house dust mites (HDM) and its molecular diagnosis may determine treatment and evolution of the disease. The present study investigates the prevalence of Der p 23 sensitization and its relation to asthma in a population of HDM-allergic patients.

Methods: We conducted a cross-sectional study of 891 patients with HDM allergy with symptoms of rhinitis and 52.1% of them with asthma. Total and specific IgE (sIgE) was measured against *Dermatophagoides pteronyssinus* and its molecular components (Der p 1, Der p 2 and Der p 23) and the storage mite *Lepidoglyphus destructor* using ImmunoCAP. Prevalence of sensitization and levels of sIgE were analysed according to asthma diagnosis and asthma severity.

Results: Der p 23 was the predominant allergen in this population (83.7%) but IgE levels were lower than those of sIgE to Der p 1 and Der p 2. A good correlation was found between sIgE to Der p 23 and the other allergens. A total of 8.2% patients were monosensitized to Der p 23. Asthma was more frequent in patients with positive sIgE against Der p 23 than in patients without this sensitization (52.8% vs 42.8%, $p=0.027$). A tendency to increase both total IgE and sIgE was observed in relation to the severity of asthma from intermittent mild asthma to persistent moderate asthma but a substantial decrease in total IgE and sIgE was detected in more severe asthmatics.

Conclusion: Der p 23 might be a prevalent allergen in regions with high rates of HDM exposure. Even though sIgE levels against this allergen are usually low, its presence could increase the risk of asthma.

Key words: allergy, Der p 23, house dust mite, asthma, *Dermatophagoides pteronyssinus*.

Abbreviations: House dust mite (HDM), *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), *Lepidoglyphus destructor* (*L. destructor*), Guía Española para el Manejo del Asma (GEMA), Allergic rhinitis and its impact on asthma (ARIA), Receiver operating characteristic (ROC), Immunoglobulin E (IgE), Forced exhaled volume in 1 second (FEV1).

Introduction

House dust mite (HDM) allergy is one of the most prevalent causes of respiratory allergy. Its clinical manifestations are mainly rhinitis, conjunctivitis and asthma. *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) is the most common cause of HDM allergy worldwide (1). Up to 35 different *D. pteronyssinus* allergens have been described (2), being Der p 1 and Der p 2 the most studied allergens. Der p 23 was identified in 2014 by Weghofer et al. as a peritrophin-like protein (3), and from that moment onwards many studies have focussed their attention on this allergen and its role in *Dermatophagoides* species sensitization. In the first description of this allergen, up to 74% of patients with *D. pteronyssinus* sensitization presented specific IgE (sIgE) against it (3).

HDMs are the main cause of respiratory allergic diseases in some regions in Spain, namely in the Northwest (4,5) and the Canary Island (6,7) where weather conditions favours its development. The three major allergens of *D. pteronyssinus*, Der p 1, Der p 2 and Der p 23 (2,3), has been found to be associated with severity of asthma (4,8) and a sensitization to Der p 23 with a higher prevalence of symptoms of allergic rhinitis (9). In addition to *Dermatophagoides*, other HDM also known as storage mites such as *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Acarus siro*, and *Blomia tropicalis*, among others, are common sensitizers in these regions (4-7,10).

The aim of the present study was to investigate in a large sample of patients with perennial respiratory allergic disease and a positive skin prick test to *D. pteronyssinus*, the prevalence of sensitization against Der p 1, Der p 2 and Der p 23, and their relationship with clinical parameters such as the presence of asthma and the severity of the disease in the Northwest region of Spain. We also intended to determine the frequency of sensitization to *L. destructor*, a relevant HDM in our region.

Patients and Methods

We conducted a cross-sectional study of HDM-allergic patients evaluated at the Department of Allergy of Complejo Hospitalario Universitario de Santiago de Compostela, Spain between January 2019 and December 2020. The hospital covers an area of approximately 500,000 inhabitants, most of them living in a rural environment. The study was approved by the Institutional Ethics Committee (code 2018/434) and

complied with the recommendations of the Declaration of Helsinki. The patients had been referred either from primary care physicians or other specialists of our Hospital (mainly, Ear Nose and Throat physicians) for evaluation of respiratory symptoms. Patients, both adults and children, with a positive skin prick test against *D. pteronyssinus* and symptoms related to indoor exposure were included. None of these patients were previously under immunotherapy with HDM allergens.

Study procedures

Skin prick tests with *Phleum pratense*, *Phragmites communis*, *Cynodon dactylon*, *Plantago lanceolata*, *Parietaria judaica*, *Artemisia vulgaris*, *Betula alba*, *Alnus glutinosa*, *Olea europea*, *Fraxinus excelsior*, *Quercus robur*, *Platanus acerifolia*, *Cupressus sempervirens*, *Profilin*, *Polcalcin*, *LTP*, *Latex*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Alternaria alternata*, *Aspergillus fumigatus*, dog dander and cat dander were routinely performed (ALK-Abelló, Madrid, Spain). Histamine was used as a positive control and saline as a negative control.

Asthma was diagnosed based on GEMA 4.4 guidelines and an increase in FEV₁ greater than 12% and 200 mL after 4 successive 100- μ g puffs of a salbutamol inhaler with a spacer was required in patients with typical symptoms. Asthma severity was reclassified according to GEMA 5.0 (11) and GINA (12) before performing the statistical analysis. For the diagnosis of allergic rhinitis, we followed ARIA (13) recommendations. Spirometry was performed in all patients with asthma but also in some patients with rhinitis to rule out asthma.

Total serum IgE was measured using latex-enhanced nephelometry in a BN-II System analyzer (Siemens). Allergen sIgE was measured using the ImmunoCAP-250 system (Thermo Fisher Scientific) and included sIgE against *D. pteronyssinus*, nDer p 1, rDer p 2, and rDer p 23. *L. destructor* sIgE was measured in 807 patients with a positive SPT result. Following the manufacturer's instructions, sIgE levels ≥ 0.1 kU_A/L were deemed positive, although for most analyses the classic 0.35 kU_A/L threshold level was used.

Statistical Analysis

Non-parametric statistics were used due to the non-normal distribution of numerical variables. The Mann-Whitney test to compare numerical variables between pairs of independent groups. The Jonckheere-Terpstra test was used to analyze trend between ordinal independent variables and numerical dependent variables. The Spearman's rank test was used to assess correlations. The chi-square test (with trend analysis, where appropriate) was used to compare proportions. P-values lower than 0.05 were deemed statistically significant.

Results

A total of 891 consecutive patients with HDM allergic rhinitis and/or asthma were included in the study (median age, 29 years [range, 4–69 years]; 43.4% male). Of note, 607 patients presented positive skin prick test results to other sensitizations such as pollens, epithelia, fungi and/or food (68.1%) but symptoms were not directly related to them.

Patients with asthma (456/891, 51.2%) were divided into mild intermittent (172, 37.7%), mild persistent (93, 20.4%), moderate (153, 33.5%), and severe (38, 8.3%) asthma. Only 125 patients (14.0%) were current smokers. Gender, current smoking, or exposure to rural environment were not related to asthma severity. However, older patients were more frequently diagnosed with severe asthma (Table 1).

Prevalence of sensitization and levels of specific sIgE in the studied population

Specific IgE to *D. pteronyssinus* was detected in most patients (850/882 samples available, 96.4%). The percentage of patients with sIgE to Der p 23 was higher (746/891, 83.7%) than that of those reacting to Der p 2 (698/885, 78.9%) and Der p 1 (639/888, 72.0%). However, sIgE levels to Der p 2 and Der p 1 were higher than those to Der p 23 (Table 2). Additionally, 79.3% (640/807) of the patients presented sIgE to *L. destructor*, confirming the high prevalence of this HDM in the region. Of the patients studied with sIgE to *D. pteronyssinus* and its molecular components, 73/882 were monosensitive to Der p 23, which represents 8.2% of the sample.

Regarding age, levels of sIgE to *D. pteronyssinus* and its molecular components (Der p 1, Der p 2, and Der p 23) were significantly higher among younger patients (157 children and/or adolescents <18 years old) than adults (Table 2), following a similar

pattern than total IgE (median 341 UI/mL [143-784 UI/mL] vs median 179 UI/mL [77-400 UI/mL]), $p < 0.001$. In contrast, no differences were found on sIgE to *L. destructor* between older and younger patients (Table 2). Finally, the levels of sIgE to *L. destructor* were higher among patients living in rural areas ($p < 0.001$), but this habitat seemed not to influence sIgE levels to *D. pteronyssinus* or its molecular components (Table 2).

Der p 23 monosensitization seemed not to be related to age, gender, smoking habit, alcohol intake, concomitant asthma or living in a rural environment (data not shown). Patients monosensitized to Der p 23 presented lower levels of both total IgE and sIgE to *D. pteronyssinus* than patients with other *D. pteronyssinus* IgE sensitizations [123 (48-271) UI/mL vs 210 (83-463), $p = 0.001$, for total IgE and 1.84 (0.76-4.54) kUa/L vs 25.8 (8.36-56.9), $p < 0.001$ for *D. pteronyssinus*, respectively].

A significant correlation was found between specific sIgE to Der p 23 and sIgE to *D. pteronyssinus* ($\rho = 0.769$, $p < 0.001$), Der p 1 ($\rho = 0.644$, $p < 0.001$), and total sIgE ($\rho = 0.628$, $p < 0.001$). A lower but significant correlation was found between sIgE to Der p 23 and Der p 2 ($\rho = 0.593$, $p < 0.001$) and *L. destructor* ($\rho = 0.345$, $p < 0.001$).

Total and specific sIgE in patients with asthma

Levels of total IgE and sIgE to *D. pteronyssinus*, Der p 1, Der p 2, Der p 23, and *L. destructor* allergens were significantly higher in patients with asthma than in patients with only rhinitis (Figure 1). A tendency to increase both total IgE and sIgE was observed in relation to the severity of asthma from intermittent mild asthma to persistent moderate asthma. However, a substantial decrease in total IgE and sIgE was detected in more severe asthmatics (Figure 1).

The risk of asthma showed a trend to be higher in patients with more positive sIgE results. Thus, the more positive sIgE determinations, the higher risk for suffering from asthma (Figure 2a). Along this line, asthma was more frequently diagnosed in patients with positive sIgE against Der p 23 than in patients without Der p 23 sensitization (52.8% vs 42.8%, $p = 0.027$). A similar trend was seen in relation to the severity of asthma ($p < 0.001$) (Figure 2b). Finally, a forced spirometry was performed in 495 patients (456 asthmatic patients and 39 with rhinitis) but no differences in FEV1 values or in FEV1/FVC were observed depending on the *D. pteronyssinus* sensitization profile (data not shown).

Discussion

House dust mites (HDM) are a major cause of respiratory allergy and of perennial asthma worldwide. Der p 23, a gut-derived peritrophin present in the outer membrane of mite feces, has been recognized as a major allergen (3,8,15,16,17-20). Even though sIgE-array platforms have included a wide spectrum of HDM allergens (8,14), only Der p 1, Der p 2, Der p 23, and Der p 10 (tropomyosin) are currently available for the component-resolved diagnosis of HDM allergy on ImmunoCAP. Our results, using ImmunoCAP in the largest sample published to date, are similar to those previously reported in terms of prevalence of sensitization (6,8,14,15,17) but we report a high rate of monosensitization to Der p 23 (6,8,15-18,20,21). The clinical relevance of Der p 23 is only partially defined, and the prevalence and relevance of the exclusive sensitization to it has received little attention so far. In this population, patients monosensitized to Der p 23 were similar to the others with respect to age, gender, smoking habit, rural or urban environment and prevalence of rhinitis or asthma. However, when Der p 23 sensitization accompanies other *D. pteronyssinus* allergen sensitizations, the risk of asthma seems to increase. All our patients were primarily diagnosed by means of a positive SPT to *D. pteronyssinus* and most of them were confirmed by a positive serum sIgE to *D. pteronyssinus* whole extract. No positive responses to Der p 23 were found in patients with a negative serum sIgE to *D. pteronyssinus* but these patients have lower levels of total IgE and sIgE to *D. pteronyssinus*.

The fact that sIgE levels decline with age have been previously reported (6,21). In this population, total and sIgE levels to *D. pteronyssinus*, Der p 1, Der p 2 and Der p 23 were also lower in adults compared with children and adolescents. Specific IgE to *L. destructor* seems to follow a different pattern since it did not vary according to age. Mean specific IgE levels to Der p 2 and Der p 1 allergens were higher than those elicited by Der p 23 in terms of quantity in all group ages, but with a close correlation among them and the crude extract of *D. pteronyssinus*.

Considering the clinical expression of the allergic disease (rhinitis with or without asthma), the risk of suffering from asthma has a tendency to increase with the number of allergens recognised by patients which is in accordance with previous results (9,18,22). Letran et al recently found no relationship between Der p 23 sensitization and

asthma. Furthermore, they showed a lower prevalence of asthma when Der p 23 sensitization was present (19). In our sample, asthma was more frequently diagnosed in patients with positive sIgE against Der p 23 than in patients without Der p 23 sensitization, giving Der p 23 a role in asthma development when Der p 23 sensitization accompanies Der p 1 and Der p 2. A similar tendency was shown in relation to the severity of asthma. However, when sIgE levels are analysed, the interpretation changes. While higher levels of total IgE and sIgE to all allergens may be detected in patients with mild intermittent asthma to moderate asthma, a significant drop in all these IgE determinations was detected at the most severe asthma level. It is true that more severe asthmatics were also the eldest but the small size of this group cannot allow to be sure if the effect of this drop in IgE is due to age or the severity of asthma. No similar findings have been reported elsewhere. Severity of asthma could probably be related to bronchial remodelling or pathology where allergy is less relevant, but more studies are needed to define this cause.

Although the large sample of patients described in our study make our results robust, it has some limitations that we would like to highlight. Firstly, we are based on retrospective data, so we cannot draw predictive conclusions because we do not have follow-up information. Second, pulmonary function parameters were performed only in those patients who reported bronchial symptoms, and some patients who only reported rhinitis may have been underdiagnosed. Thirdly, we determined specific IgE to Der p 1, Der p 2 and Der p 23, so the monosensitive concept is exclusively applied to these three allergens. Fourthly, the nasal provocation tests were not performed in those patients who had symptoms with exposure to HDMs and had negative SPT, so that a certain number of patients with local allergic rhinitis could be excluded from our study. And finally, we have taken 0.35 kU/L as the cut-off point for specific IgE positivity. The median sIgE level at Der p 23 in our monosensitive patients was 0.27, so these patients were not detected with the cut-off point used.

In conclusion, our study confirms the high prevalence of sensitization to Der p23 and its clinical implication in asthma. This sensitization could be of clinical relevance as patients who recognize sIgE to Der p 23 present asthma more frequently than those who do not. Specific IgE levels to all allergens increase with asthma severity from mild intermittent to moderate asthma but a drop may be detected in the most severe grade of the disease.

Conflicts of interest.

The authors declare no conflicts of interest

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Competing financial interests.

The authors declare no competing financial interests.

Data Availability

The datasets generated and/or analysed during the current study are available in the RUNA Repositorio de Saúde repository, <http://hdl.handle.net/20.500.11940/16141>.

Author's Contributions.

Laura Romero-Sánchez wrote the main manuscript, collected data and made the database. Andrea Otero and María González-Rivas carried out the data collection and database. Arturo González-Quintela performed the statistical analysis. Santiago Lojo carried out the laboratory studies and analytical determinations. Carmen Vidal designed the project, wrote and supervised the manuscript. All authors reviewed the manuscript.

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Figure legends

Figure 1. Serum concentrations of serum specific IgEs and total IgE in patients with house dust mite allergy, stratified by the presence of asthma and asthma severity. Both bars and numbers represent medians and interquartile ranges.

All specific IgE and total IgE are higher in patients with asthma than in patients without asthma (P values in the figure reflect the comparison between asthmatics and non-asthmatics). Within patients with asthma, specific IgE and total IgE tend to increase with asthma severity to some extent -from severity 1 (mild intermittent) to severity 3 (moderate)-, especially Der p 2 (P=0.012), D1 (P=0.041), *L. destructor* (P<0.001) and total IgE (P=0.032). However, in the most severe cases of asthma, all specific IgE tended to stabilize or decrease.

Figure 2. In patients in whom all three sIgE determinations against *D. pteronyssinus* allergens (Der p 1, Der p 2 and Der p 23) were available: (a) the mean number of positivities (sIgE >0.35 kUA/L) against these allergens tended to increase the risk of asthma, and (b) the mean number of positivities (sIgE >0.35 kUA/L) against these allergens tended to increase with asthma severity: 2.22 in non-asthmatics (n=430), 2.41 in grade 1 asthmatics (n=170), 2.38 in grade 2 asthmatics (n=93), 2.51 in grade 3 asthmatics (n=153), and 2.57 in grade 4 asthmatics (n=38) (P<0.001 in the trend test).

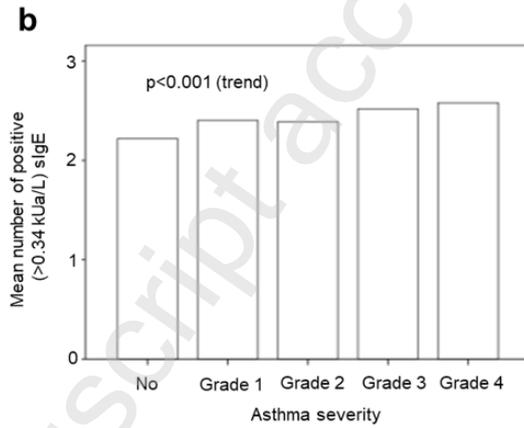
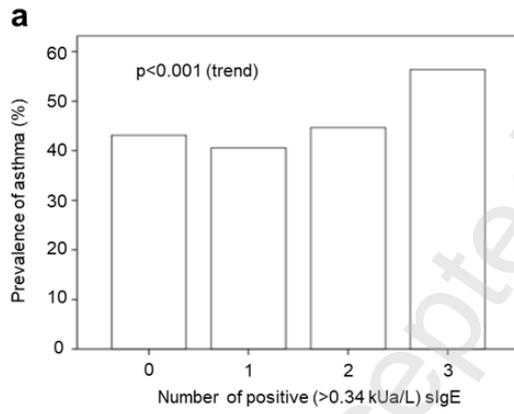
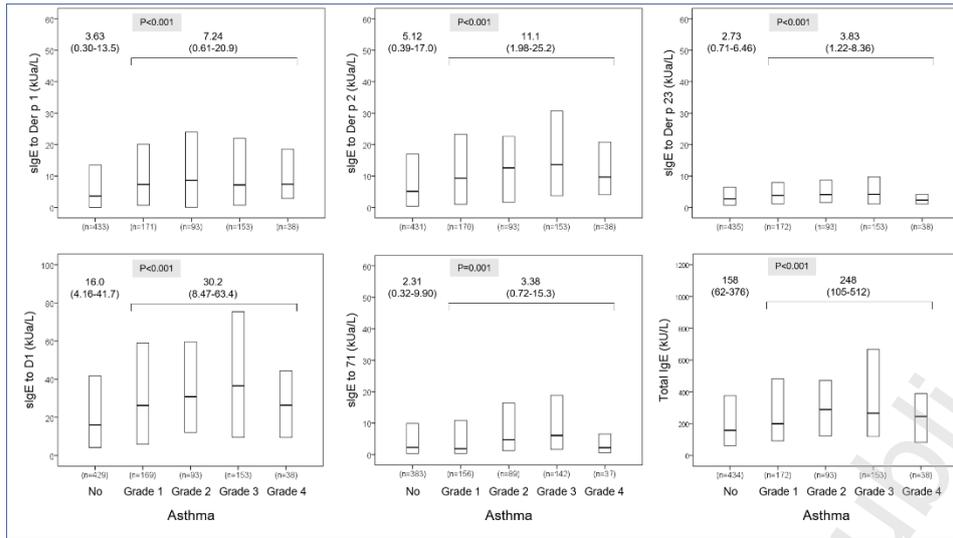


Table I. Factors associated with severity of asthma in house dust mite-allergic patients.

Factor	No asthma (n=435)	Asthma (n=456)				P-value (trend)
		Grade 1 (n=172)	Grade 2 (n=93)	Grade 3 (n=153)	Grade 4 (n=38)	
Age (years)	27 (19-38)	27 (19-37)	31 (20-41)	31 (22-40)	40 (29-44)	<0.001
Sex (male)	202 (46.4)	65 (37.8)	44 (47.3)	62 (40.5)	14 (36.8)	0.174
Smoking (current)	54 (12.5)	24 (14.0)	18 (19.4)	23 (15.0)	6 (15.8)	0.209
Habitat (rural)	254 (58.4)	98 (57.0)	55 (59.1)	82 (56.3)	21 (55.3)	0.381

Age data are medians and interquartile ranges (within parentheses). The remaining data are absolute numbers and percentages (within parentheses). Data about smoking was unavailable for two patients.

Table II. Specific IgE to mites and their allergen components, and total serum IgE in house dust mite-allergic patients.

Factor	Whole sample (n=891)	Age			Sex		
		<18 y (n=157)	≥18 y (n=734)	P-value	Female (n=504)	Male (n=387)	P-value
slgE to D1, kU _A /L	21.7 (5.65-53.6)	38.8 (10.8-78.5)	19.2 (5.21-47.4)	<0.001	19.6 (5.22-52.2)	25.2 (6.12-55.3)	<0.001
slgE to Der p 1, kU _A /L	5.19 (0.10-17.9)	12.1 (0.05-29.8)	4.86 (0.13-15.3)	0.002	4.89 (0.06-17.9)	5.74 (0.15-17.6)	0.002
slgE to Der p 2, kU _A /L	7.82 (0.99-21.4)	15.8 (1.55-33.6)	6.81 (0.90-19.3)	<0.001	6.96 (0.70-20.6)	8.81 (1.33-24.0)	<0.001
slgE to Der p 23, kU _A /L	3.19 (0.94-7.45)	6.10 (2.46-11.6)	2.86 (0.81-6.40)	<0.001	3.10 (0.88-7.35)	3.36 (1.04-7.93)	<0.001
slgE to D71, kU _A /L	2.73 (0.54-12.2)	2.40 (0.63-10.6)	2.74 (0.52-12.4)	0.800	2.73 (0.46-11.8)	2.71 (0.67-12.7)	0.800
Total IgE, kU/L	198 (83-447)	341 (143-784)	179 (77-400)	<0.001	180 (76-383)	240 (100-531)	<0.001

slgE, specific IgE. Figures are shown as median and (IQR). Cases with slgE above the analytical limit (100 kU_A/L) were deemed to have 100 kU_A/L.

slgE to d1, Der p 1, Der p 2, d71, and total IgE were unavailable for 9, 3, 6, 84, and 1 patient, respectively.