

Sensitivity to House Dust Mites Allergens in Patients with Allergic Asthma in Erzincan Province, Turkey

Erzincan'da Alerjik Astımlı Hastaların Ev Tozu Akar Alerjenlerine Karşı Duyarlılığı

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ABSTRACT

Objective: To investigate the sensitivity of allergic asthma (AA) patients to house dust mites (HDM) by conducting skin tests, measuring total and specific IgE antibodies to *Dermatophagoides pteronyssinus* and *D. farinae* mites, and examining HDM fauna in patients' homes.

Methods: The study included 25 patients with AA and 31 healthy controls, who were challenged with Der p and Der f allergens; serum levels of allergen-specific IgE and total IgE were measured. Dust samples were collected from the homes of all participants, and mite species and the number of mites per gram of dust were investigated.

Results: *D. pteronyssinus* was found in the homes of 94.7% patients with positive Der p reactions in the skin test ($p < 0.001$). *D. farinae* was found in the homes of 22.2% patients with positive Der f reactions in the skin test ($p > 0.05$). *D. pteronyssinus*-specific IgE was detected in 75% patients in whose homes *D. pteronyssinus* was also found, while *D. farinae*-specific IgE was detected in 16.6% patients in whose homes *D. farinae* was also found.

Conclusion: A part of AA patients residing in Erzincan are sensitive to HDM allergens, and high numbers of mites leading to allergic sensitization are found in their homes.

Keywords: Allergic asthma, skin prick test, specific IgE, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*

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ÖZ

Amaç: Alerjik astımlı (AA) hastaların ev tozu akarlarına (ETA) karşı olan duyarlılığını deri testi ile değerlendirmek, hastaların *Dermatophagoides pteronyssinus* ve *Dermatophagoides farinae*'ye karşı spesifik IgE ve total IgE antikorlarını ölçmek ve hasta evlerindeki toz akar faunası araştırmak amaçlandı.

Yöntemler: Çalışmaya Der p ve Der f akar alerjenleri ile deri testi yapılan AA'lı 25 hasta ve 31 sağlıklı kontrol alındı ve kan serumunda alerjene özgü IgE ve total IgE seviyeleri ölçüldü. Tüm katılımcıların evlerinden toz örnekleri toplanarak gram tozdaki akar sayısı ve türleri bakımından incelendi.

Bulgular: Deri testinde pozitif Der p reaksiyonu görülen hastaların %94,7'sinin evinde *Dermatophagoides pteronyssinus* ($p < 0,001$), pozitif Der f reaksiyonu görülen hastaların %22,2'sinin evinde *Dermatophagoides farinae* tespit edildi. Spesifik IgE sonuçlarına göre Der p duyarlılığı saptanan hastaların %75'inin evinde *D. pteronyssinus*, Der f duyarlılığı saptanan hastaların ise %16,6'sının evinde *D. farinae* tespit edildi.

Sonuç: Erzincan'daki AA'lı hastaların bir kısmının ev tozu akar alerjenlerine karşı duyarlı oldukları ve hasta evlerinin alerjik duyarlanmaya yol açan akarları yüksek oranda barındırdığı görüldü.

Anahtar Kelimeler: Alerjik astım, deri prick testi, spesifik IgE, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*

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INTRODUCTION

Allergic asthma (AA) is a chronic disease of the lower respiratory tract that is characterized by narrowing of the bronchi and inflammation of the bronchial mucosa in which numerous cells and mediators, mainly mast cells, T lymphocytes, and eosinophils, play a role (1). AA manifests as chronic inflammation, wheezing, particularly at night, shortness of breath, chest tightness, and coughing attacks. While its prevalence varies among countries depending on the respiratory allergen load, it affects about 1%-18% of the population worldwide, particularly children. It is a social and economic burden on communities and causes significant absence from school and work (2, 3).

House dust mites (HDMs), particularly the cosmopolitan species *Dermatophagoides pteronyssinus*, *D. farinae*, and *Euroglyphus maynei* from the family Pyroglyphidae (Astigmata: Acari), may cause atopic diseases known as HDM allergy or HDM atopy in humans. These include allergic rhinitis, atopic dermatitis (eczema), and allergic conjunctivitis. HDMs are the major sources of indoor inhalant allergens facilitating both sensitization of atopic subjects and asthmatic (atopic) attacks in patients. The most common and effective HDM allergens are Der p and Der f, which are the major allergens of *D. pteronyssinus* and *D. farinae*. The allergens are released into the environment through mite feces, which are residues of digestion and contain enzymes, such as peptidase, protease, transferase, and glucosidase (4-7). On an average, an HDM defecates up to 20 times a day. Over time, mite feces and tissue residues arising from fragmentation after death accumulate in carpets, fabric-covered furniture, furry toys, mattresses, and pillows and allergens mix with indoor air. Inhalation of these allergens stimulates the immune system in the respiratory mucosa and causes the initial allergic sensitization mediated by specific IgE. Upon subsequent contact with the allergens, predisposed patients react to the mediators, which are mainly derived from mast cells, causing vasodilation in the bronchial mucosa, edema, mucus secretion, and bronchospasm, leading to acute inflammatory episodes (7-9).

To better understand the etiology of AA and allergic sensitization, it is essential to investigate patients' homes for HDM and to concomitantly conduct skin and serological tests (10-18).

The aim of this study was to investigate AA patients' homes for HDMs that are sources of the allergens Der p and Der f and to compare it with the results of skin and serological tests.

METHODS

Patients and study plan

The study included 25 patients residing in Erzincan Province, who presented to the departments of chest diseases or internal medicine at the training hospital and were diagnosed with AA between March 2014 and June 2014, based on the Global Initiative for Asthma (GINA) criteria (3). The control group comprised 31 healthy individuals without allergic symptoms. The clinical examination of patient and control groups as well as skin and serological testings were carried out in the training hospital, while the Department of Biology collected and examined the dust samples from patients' homes. The Erzincan University Ethics Committee approved the study (Decision No: 2014/2-6), and all the participants provided written informed consent.

Skin Prick Test (SPT)

The interior surface of the forearm was chosen as the test area and cleaned with alcohol. Using sterile lancets (Oryum; İstanbul, Turkey), physiological saline solution was applied to the test area as negative control, and 10 mg/mL of histamine dihydrochloride was applied as positive control. Der p and Der f (Lofarma; Milano, Italy) solutions were used as HDM allergens. The evaluation was conducted by examining the controls after 15-20 min, and indurations of ≥ 3 mm were considered positive (19).

Serological tests

From each patient and each control, 5 mL of venous blood was collected and centrifuged to separate the serum. The levels of Der p and Der f allergen-specific IgE were measured using the immunoblot method with a Rida X Screen device and kit (R-Biopharm AG; Darmstadt, Germany), while the total IgE level was determined using the chemiluminescence immunoassay (CLIA) method with a Siemens Immulite 2000 XPI device and kit (Siemens Healthcare Diagnostic; UK). For evaluation, specific IgE levels of ≥ 0.35 kUA/L and total IgE values of ≥ 87 U/mL were considered positive.

Collection and examination of dust samples

The dust samples were collected during the period of April 2014 to June 2014 from the carpets, fabric-covered furniture, mattresses, and pillows in the homes of 25 patients and 31 controls from various neighborhoods of Erzincan Province by applying vacuum for 2 min/m² using a vacuum cleaner (Bosch; München, Germany). A separate dust bag was used for each house, and the hose and pipe stub of the vacuum cleaner were removed and cleaned between each dust collection. The dust samples were placed in sealed plastic bags and brought to the laboratory within 6 h. The samples were dry sieved using sieves with meshes of sizes 1.5 cm and 1 cm, with the small-sized mesh being placed beneath the large-sized mesh. To determine the number of mites per gram, 1 g of sieved sample dust was weighed in a microbalance and examined for HDMs using the lactic acid precipitation method (5, 20-22). The dust sample was placed in a petri dish, and 10 mL of 90% lactic acid was added. The petri dish was heated for 30 min on a hot table to 70-80°C. The mixture was then distributed as thin layers on other petri dishes. The solution was examined under a stereo microscope (Leica EZ4, Switzerland), and the mites were isolated using a thin needle. The collected mites were transferred to Hoyer's medium to create permanent preparations, labeled, and allowed to dry for 7-10 days in an incubator (Binder, Germany) at 50°C. Mite species were identified under a light microscope equipped with differential interference contrast (DIC; Olympus BX63, Japan) using standard taxonomic keys (5, 23). The permanent mite preparations were stored at the Acarology laboratory of the Department of Biology.

The mean number of mites per gram of dust was calculated by dividing the total number of mites by the number of positive samples.

Statistical analyses were performed using Statistical Package for Social Sciences for Windows, version 18.0 (SPSS Inc.; Chicago, IL, USA). Descriptive statistics were determined for each variable. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. The results for continuous variables

were recorded as the mean ± standard deviation. For continuous variables without normal distribution, the results were demonstrated as median minimum-maximum. For categorical variables, statistically significant differences between the groups were determined using chi-square test. For continuous variables, nonparametric statistics (Mann-Whitney U test) and parametric statistics (t test) were used, as appropriate. A p value <0.05 was considered significant.

RESULTS

Demographic characteristics of patients and controls

The patient group comprised 15 females and 10 males, with ages ranging 7-65 years. The control group comprised 10 females and 21 males, with ages ranging 17-69 years. Upon clinical examination, all the patients were found to have moderate persistent AA according to the GINA classification, and all the controls were found to have no AA symptoms (Table 1).

Skin and Serological Test Results

Positive SPT results with Der p were observed in 19 patients (76%) and 12 controls (38.7%), with a statistically significant difference between the groups (p=0.035). Positive SPT results with Der f were

Table 1. Age and sex of patients and controls

	Patient group (n = 25)	Control group (n = 31)	p
Age (mean±SD), years	36.04±18.11	33.90±15.13	>0.05*
Sex			
Female	15	10	>0.05**
Male	10	21	

SD: standard deviation
*Student t test; **chi-square test

Table 2. Species and total number of mites in patients' homes and results of SPT and total and specific IgE levels

Patient no	SPT		Serum specific IgE (kUA/L)		Serum total IgE (U/mL)	Number of mites in per gram of dust	
	Der p	Der f	Der p	Der f		<i>D. pteronyssinus</i>	<i>D. farinae</i>
1	-	+	+	+	70	0	0
2	-	+	-	-	45.3	0	0
3	-	+	-	+	287	54	0
4	+	-	-	-	6.4	1	0
5	-	+	-	-	54.5	0	0
6	+	+	-	-	21.8	1	1
7	+	-	-	-	115	6	0
8	+	-	-	-	42.5	56	0
9	+	-	-	-	26.7	15	0
10	+	-	-	-	47.3	3	0
11	+	+	-	-	128	2	0
12	+	+	-	-	44.2	82	0
13	-	+	-	-	16.5	1	0
14	+	+	-	-	5	4	0
15	+	+	-	+	89.8	4	0
16	-	+	-	-	21.4	9	0
17	+	+	+	+	457	2	0
18	+	+	+	+	178	64	0
19	+	+	-	-	80.3	2	0
20	+	+	+	+	279	107	54
21	+	+	-	-	10.5	542	24
22	+	+	-	-	39.3	52	10
23	+	-	-	-	87.1	3	0
24	+	+	-	-	63.7	3	0
25	+	-	-	-	203.7	0	0
Total						1,013	89

SPT: Skin Prick Test
Bold values: positive

Table 3. Species and total number of mites in control homes and results of SPT and total and specific IgE levels

Control no	SPT		Serum specific IgE (kUA/L)		Serum total IgE (U/mL)	Number of mites in per gram of dust	
	Der p	Der f	Der p	Der f		<i>D. pteronyssinus</i>	<i>D. farinae</i>
1	-	-	-	-	80.7	0	0
2	+	-	-	-	310	0	0
3	-	+	-	-	19.9	0	0
4	-	+	-	-	1.7	0	0
5	+	-	-	-	7.1	0	0
6	+	-	-	-	56.3	0	0
7	-	+	-	-	45.7	6	0
8	-	+	-	-	62.2	6	0
9	-	-	-	-	10	6	0
10	-	+	-	-	1.5	4	0
11	-	-	-	-	11	2	0
12	-	+	-	-	25.7	4	3
13	-	+	-	-	16.8	3	1
14	+	-	-	-	197.5	0	0
15	+	-	-	-	85.5	0	0
16	-	-	-	-	9.6	2	0
17	-	-	-	-	5.1	1	0
18	-	-	-	-	71.4	0	0
19	+	+	-	-	10	0	0
20	-	-	-	-	211.7	0	0
21	+	-	-	-	39.1	8	0
22	-	-	-	-	19.5	0	0
23	+	-	-	-	86.4	0	0
24	-	+	-	-	8.1	0	0
25	+	-	-	-	17.6	0	0
26	-	-	-	-	11	0	2
27	+	-	-	-	14.9	0	2
28	+	-	-	-	11.5	0	0
29	-	+	-	-	20	0	0
30	+	-	-	-	20	0	0
31	-	-	-	-	5	0	0
Total	42	8					

SPT: Skin Prick Test
Bold values: positive

noted in 18 patients (72%) and 10 controls (32.2%), with a statistically significant difference between the groups ($p=0.007$) (Table 2-4).

Four (16%) patients showed elevated Der p-specific IgE ($p=0.034$) and 6 (24%) showed elevated Der f-specific IgE ($p=0.005$). All the controls were Der p and Der f negative. The total IgE level was high in 9 (36%) patients and 3 (9.6%) controls ($p=0.008$) (Table 2-4).

Microscopic Examination of Dust Samples

D. pteronyssinus was found in the homes of 21 patients (84%) and 10 controls (32.2%; $p<0.001$) (Figure 1, 2). In the homes of patients, 1,013 *D. pteronyssinus* mites were found (min, 1; max, 542; mean, 48.2/g dust), while in the homes of control subjects, 42 mites were found (min, 1; max, 8; mean, 4.2/g dust; $p<0.001$). *D. farinae* (Figures 3, 4) was found in the homes of 4 patients and 4 controls. In the homes of patients, 89 *D. farinae* mites were detected (min, 1; max, 54; mean, 22.2/g dust), and in the homes of

Table 4. Comparing existence of HDMs in homes and results of skin and serologic tests between patients and controls

	Patient group (n=25)	Control group (n=31)	p
Der p SPT positive	19	12	0.035*
Der p SPT positive and existence of <i>D. pteronyssinus</i> in homes	18	1	<0.001*
Der f SPT positive	18	10	0.007*
Der f SPT positive and existence <i>D. farinae</i> in homes	4	1	>0.05*
Der p-specific IgE positive	4	0	0.034*
Der p-specific IgE positive and existence of <i>D. pteronyssinus</i> in homes	3	0	-
Der f-specific IgE positive	6	0	0.005*
Der f-specific IgE positive and existence of <i>D. farinae</i> in homes	1	0	-
Total IgE level (U/mL)	54.50 (5-457)	19.50 (1.5-310)	0.008**
Existence of <i>D. pteronyssinus</i> in homes	21	10	<0.001*
Existence of <i>D. farinae</i> in homes	4	4	>0.05*
Number of <i>D. pteronyssinus</i> in homes	3 (0-542)	0 (0-8)	<0.001**
Number of <i>D. farinae</i> in homes	0 (0-54)	0 (0-3)	>0.05**

HDM: house dust mite; SPT: Skin Prick Test
*chi-square test; **Mann-Whitney U test (median [min-max])

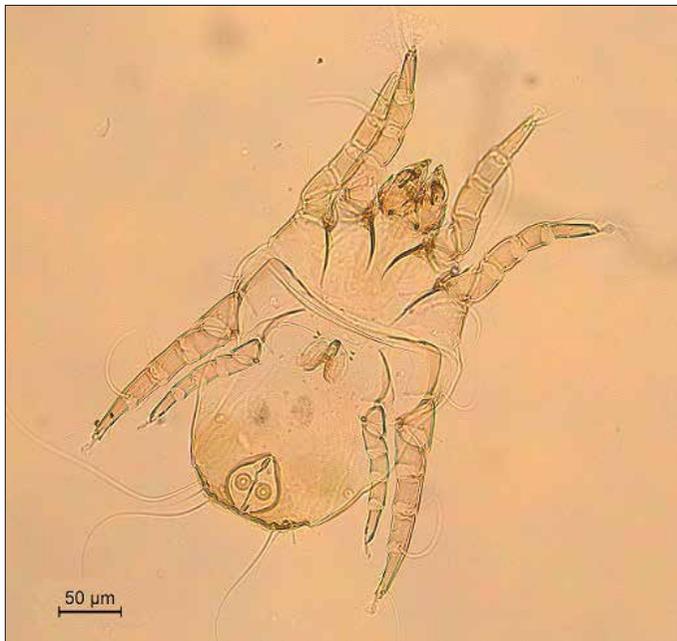


Figure 1. *Dermatophagoides pteronyssinus* (male)

controls, 8 mites were detected (min, 1; max, 3; mean 2/g dust), and no significant difference was found between the 2 groups (Table 2-4).

D. pteronyssinus was found in the homes of 18 of 19 patients (94.7%) positive for the Der p skin test and in 1 of 12 controls (8.3%) positive for the Der p test ($p < 0.001$). *D. farinae* was found in 4 of 18 (22.2%) patients' homes, who were positive for Der f skin test and (10%) controls positive for the Der f test, the differences being statistically nonsignificant (Table 2-4).

Der p-specific IgE positivity was detected in 75% of patients in whose homes *D. pteronyssinus* was found, while Der f-specific

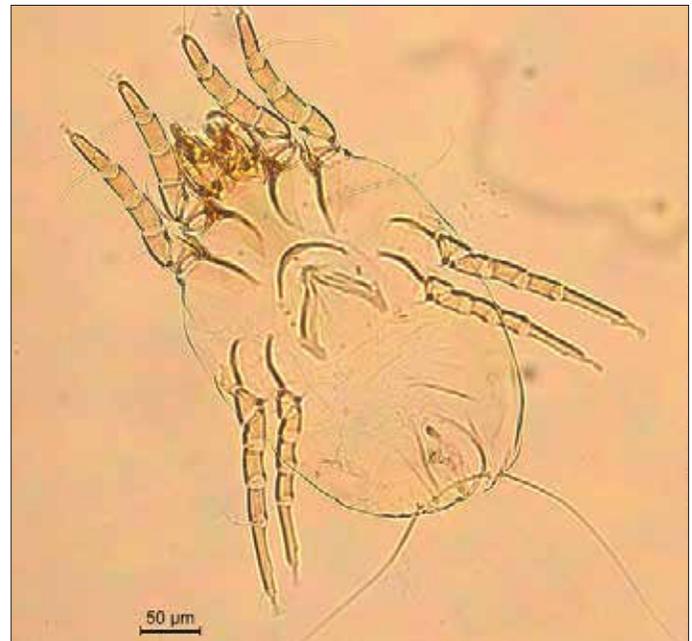


Figure 2. *Dermatophagoides pteronyssinus* (female)

IgE positivity was detected in 16.6% of patients in whose homes *D. farinae* was found. All control subjects were negative for Der p- and Der f-specific IgE (Table 2-4).

D. pteronyssinus was found in 88.8% of patients' homes, while *D. farinae* was found in 11.1% of patients' homes; these patients had high serum levels of total IgE. The serum level of total IgE was found to be high in 3 controls; however, no *D. pteronyssinus* or and *D. farinae* were found in their homes (Table 2, 3).

DISCUSSION

In addition to the medical history and clinical examination, SPT and determination of allergen-specific IgE are the leading diag-



Figure 3. *Dermatophagoides farinae* (male)

nostic criteria recommended for AA diagnosis (3). In the present study, according to the SPT results, 76% of AA patients and 38.7% of control subjects reacted positive to Der p ($p=0.035$), while 72% and 32.2% reacted positive to Der f ($p=0.007$), respectively. Similar results were obtained for other countries with 77.2% and 69.5% in Jakarta, Indonesia; 83% and 88% in Monterrey, Mexico; 70% and 60% in Irapuato, Mexico; and 55% and 25% in Tampico, Mexico; 80.3% and 83.7% in Guangzhou, China; 29.8% and 28% in Gwangju, South Korea; and 53.2% and 49.8% in Yaounde, Cameroon, respectively. Studies conducted in Turkey have reported similar results with 62.2% and 51.3% in Eskişehir; 97.1% and 86.6% in İzmir, respectively, while lower positive tests were obtained in Kocaeli, with 12% and 11.8%, respectively (12, 14, 16-18, 24-26).

In the present study, elevated Der p- and Der f-specific IgE levels were reported in 16% and 24% of patients, respectively. In Guangzhou, China, positive results were obtained in 61.1% and 60.2% of AA patients, and 44% and 42% in Connecticut, USA, respectively (17, 27). Studies conducted in Turkey have reported high levels of Der p- and Der f-specific IgE in 79% and 85% of patients in Eskişehir and 27.5% and 17.5% in Afyon, respectively (25, 28). In a study conducted in Kütahya, Der p-specific IgE was detected in 7.3% of patients, while none of them had Der f-specific IgE (29). The different results obtained could be due to the varying exposure of patients to an HDM in general and to *D. pteronyssinus* and *D. farinae* in particular. In addition, different extraction procedures and concentrations of Der p and Der f as well as the degree of sensitization of the patients examined could have influenced the results.

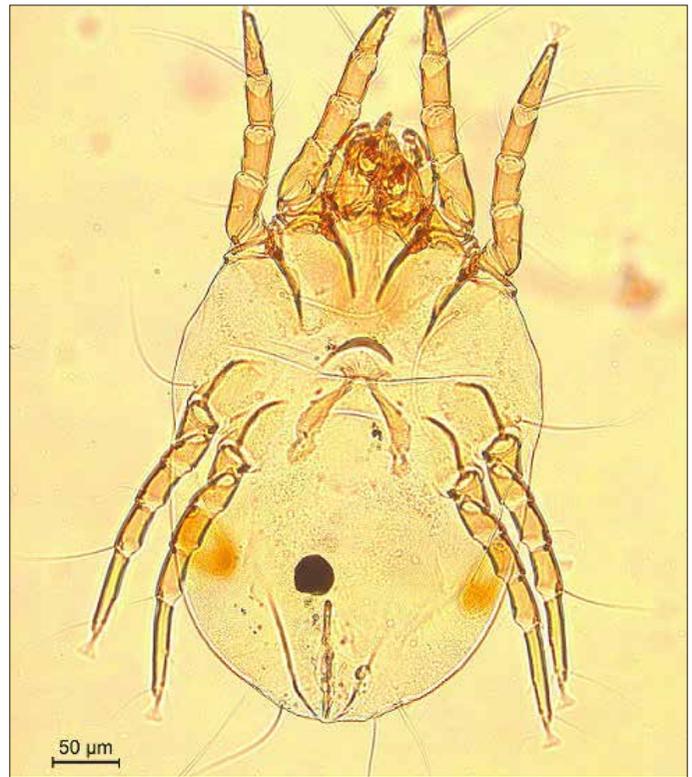


Figure 4. *Dermatophagoides farinae* (female)

Another serological test used to support clinical findings in the diagnosis of AA is the determination of serum total IgE level. Serum levels of IgE have been reported as elevated in allergic and parasitic diseases (30). In the present study, total IgE levels were high in 36% of AA patients. Similar studies in other countries have reported high levels of total IgE in 37.8% of AA patients in Finland and 43.6% in Russia. Studies from Turkey have reported high levels of serum total IgE in 56% of AA patients in Isparta, 19.8% in Afyon, and 31.9% in İzmir (11, 31-33).

In our study, *D. pteronyssinus* was found in the homes of 18/19 patients (94.7%) who were positive for Der p in the skin test ($p<0.001$). *D. farinae* was found in the homes of 4/18 (22.2%) patients who were positive for Der f in the skin test ($p>0.05$). *D. pteronyssinus*-specific IgE was detected in 3/4 (75%) patients in whose homes *D. pteronyssinus* was also found, while *D. farinae*-specific IgE was detected in 1/6 (16.6%) patients in whose homes *D. farinae* was also found. A positive Der p skin test or Der p-specific IgE was detected in 18/21 (85.7%) patients whose homes contained *D. pteronyssinus*, while either a positive Der f skin test or Der f-specific IgE was detected in all 4 patients whose homes contained *D. farinae*. Der p or Der f sensitization in those patients whose homes did not contain *D. pteronyssinus* or *D. farinae* may be the result of cross-reactivity, which is noted in various studies in the literature (5, 8, 34-37). In 84% of the AA patients who were sensitive to Der p and/or Der f allergen, *D. pteronyssinus* and/or *D. farinae* were found in their homes (Table 2). A combination of skin and serological tests as well as acarological examination of the dust samples from the patient's home could provide important indications regarding the sensitization of the patients to HDM.

Acarological studies conducted in the homes of AA patients have reported *D. pteronyssinus* and *D. farinae* in 75% and 25% of patients in Nigeria; 44.4% and 55.5% in Italy, and 77% and 13% in Taiwan, respectively. Studies conducted in Spain have reported 99% and 15% of patients' homes in Tenerife, 98.2% and 5.5% in La Coruna, 98.8% and 4.8% in Lugo, 93.3% and 6.7% in Ourense, 100% and 2.2% in Pontevedra, and 31.8% and 35.6% in Murcia with *D. pteronyssinus* and *D. farinae*, respectively. Studies in Turkey have reported the presence of *D. pteronyssinus* in the homes of 27.5% of patients with allergic suspicion in Afyon, 46.3% in Malatya, and 18.8% in Kütahya, while *D. farinae* was not detected in these homes (10, 13, 29, 32, 38-42). However, faunistic studies of HDM conducted in 7 geographic regions of Turkey showed the presence (0.2%-15%) of *D. farinae* in the homes of healthy individuals (22, 43-47). The present study showed for the first time that *D. farinae* is present in the house of AA patients.

CONCLUSION

In conclusion, the present study revealed that a part of AA patients residing in Erzincan are sensitized to HDMs and that their homes contain high numbers of HDM. Therefore, preventive measures in patients' homes may be beneficial.

Ethics Committee Approval: Ethics committee approval was received for this study from Ethics Commity of Erzincan University (2014-02/6).

Informed Consent: Written informed consent was obtained from participants of this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - E.Z., S.D., F.Ö., E.Ü.; Design - E.Z., S.D.; Supervision - S.D.; Resource - E.Z.; Materials - E.Z., S.D.; Data Collection and/or Processing - E.Z.; Analysis and /or Interpretation - E.Z., S.D., F.Ö., E.Ü.; Literature Search - E.Z.; Writing - E.Z., F.Ö.; Critical Reviews - S.D., F.Ö., E.Ü.

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