Original Article / Özgün Araştırma

The Effect of Indoor Environmental Characteristics on the Detection of House Dust Mite Der p2 and Der f2 in Asthmatics

Astımlı Hastalarda Ev İçi Ortam Özelliklerinin Ev Tozu Akar Allerjeni Der p2 ve Der f2 Saptanmasına Etkisi

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Abstract

Objective: Mites in house dust play a prominent role in the development of allergic sensitization and as a triggering factor that impairs disease control in asthma. The aim of the study was to determine whether the concentration of house dust mite allergens is associated with indoor conditions in stable asthmatics.

Materials and Methods: During the study period, a total of 97 asthmatic patients were queried with a standard survey for their demographical characteristics and living environment. House dust samples from their houses were collected to quantitatively measure *Dermatophagoides farinae* (Der f2) and *Dermatophagoides pteronyssinus* (Der p2) levels by using the Enzyme-linked immunosorbent assay (ELISA) method.

Results: Using the quantitative ELISA method, measurable levels of mite allergens were found in 54.1% of the houses. Higher antigen detection rate was found in houses with visible mould and in those with moisture. The number of household was found to be significantly higher in houses with antigens than in those without antigens. When the indoor characteristics were evaluated by logistic regression analysis, larger number of household (≥4) was found to be a significant risk factor for the presence of mite allergens. The odds ratio for detecting Der p2 and Der f2 antigen was found to be 5.29 (confidence interval 2.18-12.86) ($p<0.001$).

Conclusion: Mite allergen was detected in the house dusts of more than half of the cases by using quantitative ELISA method. Our results did not found any association between concentrations of allergens and indoor characteristics.

Öz

Amaç: Ev tozunda bulunan akarlar, astımda allerjik duyarlılığın gelişmesinde ve hastalık kontrolünü bozan tetikleyici olarak önemli rol oynar. Bu çalışmanın amacı, stabil astımlılarda ev tozu akarı allerjen yoğunluğunun ev içi ortam özellikleriyle ilişkisini incelemektir.

Gereç ve Yöntemler: Çalışma süresi içerisinde polikliniğe başvuran 97 ardışık stabil astım hastası, demografik özellikleri ve ev içi ortam değerlendirmesi için yüz yüze uygulanan standart bir anket yardımıyla sorgulandı. Evlerinden ev tozu örnekleri toplandı ve *Dermatophagoides farinae* (Der f2) ve *Dermatophagoides pteronyssinus* (Der p2) düzeyleri Enzyme-linked immunosorbent assay (ELISA) yöntemiyle kantitatif olarak ölçüldü.

Bulgular: Çalışma grubumuzun %54,1'inin toz örneklerinde ölçülebilir düzeyde antijen saptandı. Evde gözlenebilir küf ve rutubet olduğunda, antijen saptanma oranı daha yüksekti. Antijen saptanan evlerde yaşayan kişi sayısı, antijen saptanmayan evlerde yaşayan kişi sayısından daha yüksekti. Ev içi ortam özellikleri lojistik regresyon analizi ile değerlendirildiğinde evde yaşayan kişi sayısının 4 ve üzerinde olmasının akar allerjeni saptanmasına etkili faktör olarak saptandı. Der p2 ve Der f2 antijen saptanması için Odds oranı 5,29 olarak saptandı (güven aralığı 2,18-12,86) (p<0,0001).
Sonuç: Kantitatif ELISA yöntemi ile olguların yarıdan fazlasında ev tozu örneğinde akar allerjeni tespit edildi. Bulgularımız, ev içi ortam özellikleri ile saptanan allerjen konsantrasyonu arasında ilişki olmadığını düşündürmüştür.

Introduction

Asthma is a disease in which gene-environment interactions are responsible for its development. Exposure to environmental factors and aeroallergens, particularly indoor allergens, in early childhood has a strong effect on the development of allergic airway diseases in individuals with a genetic predisposition (1,2). Evidence that perennial allergens play a prominent role in the development of asthma is increasingly growing (1).

Jaakkola et al. (3,4) showed that atopy is an important risk factor in the development of asthma in adults as well. According to their findings, sensitization to house dusts among indoor allergens is the most frequent cause of asthmatic and allergic diseases occurring in adulthood. Indoor environment is important not only for allergic sensitization but also for exacerbation of allergic diseases (2,4). Living spaces especially houses are the indoor environments which individuals are most exposed to mite allergens (5).

Allergens are significant etiologic factors in the development of atopy, as well as bronchial hyperresponsiveness and asthma. The percentage of asthmatics associated with atopy in adulthood was estimated as high as 30%, which is notably high (3). Increased prevalence of asthma has been reported to be associated with house dust sensitivity (6). *Dermatophagoides pteronyssinus (D. pteronyssinus)* and *Dermatophagoides farinae (D. farinae)* are the best-known types of mites existing in house dusts, their body fluids (Der p2 and Der f2) have been reported to be one of the most important sources of allergens (7). Der p2 is found to be highly correlated with asthma, atopic dermatitis and allergic rhinitis. It has been estimated that 79.2% of patients with asthma, wheezing and/or rhinitis have immunoglobulin E antibodies to Der p2 (8). It has been demonstrated that Der p2 activates proliferation and the expressions of proinflammatory cytokines and toll-like receptors 4 in human B cells (8). Data from the National Cooperative Inner-City Asthma Study (NCICAS) displays a high exposure rate to many allergens in house environment and development of associated sensitization. Additionally, according to NCICAS data, asthma morbidity increases in sensitized cases and cases in which exposure continues (9).

Detection of indoor allergens may be helpful in controlling the environmental factors that increase the severity of asthma. The most important benefit of the measurements of indoor environmental allergen levels is to ensure patients to be aware of the relationship between the allergen and allergic diseases, facilitating the implementation of related measures and the adherence to the treatment.

Studies investigating indoor allergens and their effects on allergic diseases are very limited in Turkey (10-13). The present study aimed at determining whether the concentration of house dust mite group 2 allergens is associated with indoor conditions and whether the clinical findings are associated with the allergens.

Materials and Methods

This study included 97 patients who were admitted to the outpatient clinic of our university hospital between March 1st and May 31st, 2007 and were diagnosed with asthma in accordance with Global Initiative for Asthma (GINA) consensus report (14). Patients older than 16 years of age who were on inhaled steroids due to asthma and were being followed at the pulmonary outpatient clinic were enrolled. Use of systemic steroids for the past six months, acute asthma attack, and pregnancy were the exclusion criteria. Institutional ethics committee approval was received and each patient signed an informed consent form prior to the study. The diagnosis and severity of asthma were established by

a respiratory physician on the basis of GINA consensus report (14). Pulmonary function tests were performed with forced expiratory maneuvers through the use of Jaeger Master Scope PC according to the American Thoracic Society (ATS)/European Respiratory Society guidelines by an experienced technician. Forced expiratory volume in one second was recorded from the best of five blows, which met the ATS criteria (15). Pulmonary function tests with reversibility in the presence of suitable clinical symptoms and family history confirmed the diagnosis.

Questionnaire: A survey including the demographic characteristics, smoking habits and education was applied using the face-to-face interview method. Characteristics of their houses, such as type of the building (apartment/detached house), heating system (central heating/heating stove/air-conditioning), pets, potted plants in the house, floor covering in the living rooms (carpets, wall-to-wall carpet, rug), usage of wool or cotton beds, and pillows, exposure to sunlight, and existence of mould and dampness in the house, were questioned. Sunny houses were defined as those receiving 8 hours of sunlight a day. Respiratory symptoms during the preceding 12 months, allergic symptoms, family history, home and work environment, and use of health care services and medications were questioned. Asthma severity and duration as well as the medication used were recorded. Severity of asthma was determined by clinical symptoms and by pulmonary function tests.

Temperature and humidity: According to the information received from Aydın Meteorology Station for the March-April-May 2007 period during which the study was conducted, the average temperature was 17.25±4.77 ^{o}C, and average humidity was 51.04±15.35% in Aydın. The indoor temperature and the humidity levels in the houses were not measured separately.

Collecting and processing dust samples: The patients were asked to gather dust by standard method of vacuuming the carpets, beds and pillows, from different parts of the house including living rooms and bedrooms using at least 1400-1600 Watt vacuum cleaner for 2 min/m^2 at the end of the 3 days after the house was left uncleaned (16). Vacuum cleaning was carried out using a new, unused dustbag. The samples of fine dust from the dustbag were separated from large particles and fiber by a strainer, and 100 cg was scaled and transferred into 12x75 mm lidded plastic vials containing 2 ml of PBS-T (0.5% tween 20 in phosphate buffered saline, ph, 7.4). The vials were vortexed and then left in a shaker for 2 hours at room temperature; centrifuged at 2500 rpm at +4 °C; supernatants were transferred into eppendorfs and were kept at -20 °C until ELISA processing.

Measurement of allergen levels in dust samples was carried out by ELISA (indoor biotechnologies) in line with the protocols advised by the producer (17). In the context of this study, mite group 2 allergen (*D. farinae* Der f2 and *D. pteronyssinus* Der p2) levels were measured in dust samples. In our country, there are a large number of studies on mite group 1, but few studies on the association of group 2 with allergic diseases.

Statistical Analysis

The data were processed by SPSS software package (SPSS for Windows 14.0). Numerical data were expressed as mean±standard deviation (SD). The Mann-Whitney U test was used for numeric variables that do not meet the normal distribution criteria. Categorical variables were evaluated by the chi-square test. Spearman's correlation and logistic regression analyses were applied for the relationships between the study parameters. A p value of less than 0.05 was considered statistically significant.

Results

A total of 97 adult patients of whom 75.3% were females were included in the study. The mean age of the subjects was 44.1±11.3 years (range: 16.0-63.0 years) and the mean asthma duration was 6.5±5.7 years. While 9.3% of the individuals were active smokers, 73.2% had never smoked. The majority of the patients were mild asthmatics (68%) according to pulmonary function tests on admission. Demographics and disease characteristics of the subjects included in the study are shown in Table 1.

Mite group 2 allergens were detected in 54.1% of all the houses investigated. The higher detection rate was found in houses of participants with low-income than in those with moderate- and high-income and the difference was significant compared to especially with those with high-income (χ^2: 7.344; df: 2, p=0.025).

Mite group 2 antigen was found in 68.3% of houses with 4 and more individuals but was found in 29.7% of houses with less than 4 individuals (Figure 1).

The characteristics of the houses with or without mite group 2 are shown in Table 2. The subjects were living mostly in an apartment building and houses accommodating an average of 3.9 people. The average age of the buildings was 8.2 years and 65.4% of those were sunny.

While dampness was reported to exist in nearly half of the houses, another half of the houses had visible mould growth. The allergens (Der f2 or Der p2) were detected in house dust obtained from 62.8% of the houses with visible mould reported and 61.9% of those with dampness reported. Although the difference was not significant, higher antigen detection rate was observed in houses with visible mould and in those with dampness (p=0.199 and p=0.058, respectively).

When the buildings were categorized according to the construction time, the mite group 2 antigen detection rate was found to be higher in houses built more than eight years ago. The antigen detection rate was 64.6% in older buildings (>8 years old), whereas it was 37.2% in newer buildings (Figure 2). The mean level of the antigen was 7.3±10.4 ng/ml in the dust of old houses and 11.7±9.4 ng/ml in the dust of new houses. The median antigen level was 7.27 (0.50-11.00) ng/ml in older houses and 11.66 (2.88-16.00) ng/ml in newer houses (p=0.027). A significant but weak negative correlation was found between sun-exposure of the house and dust mite detection rate (r=-0.213, p<0.036). The median antigen level was 7.68 (0.60-11.0) ng/ml in the sunless houses and 10.28 (0.65-16.00) in the sunny houses (p=0.472).

Indoor characteristics which were thought to be risk factors for the presence of mite group 2 allergens in house dust were evaluated with logistic regression analysis. When characteristics, such as lack

Table 1. Demographic characteristics of the subjects (n=97)

	n	%
Gender		
- Female	73	74.5
- Male	24	25.5
Income level		
- High-income	43	44.3
- Middle-income	38	39.2
- Low-income	16	16.5
Education status		
- Elementary school	37	38.1
- Secondary school	7	7.2
- High school	31	32.0
- College	22	22.7
Place of residence		
- Village	26	26.8
- Town	19	19.6
- City	52	53.6
Smoking status		
- Smoker	9	9.3
- Never smoked	71	73.2
- Ex-smoker	17	17.5
Severity of asthma		
- Mild intermittent	26	26.8
- Mild persistent	40	41.2
- Moderate	19	19.6
- Severe	12	12.4

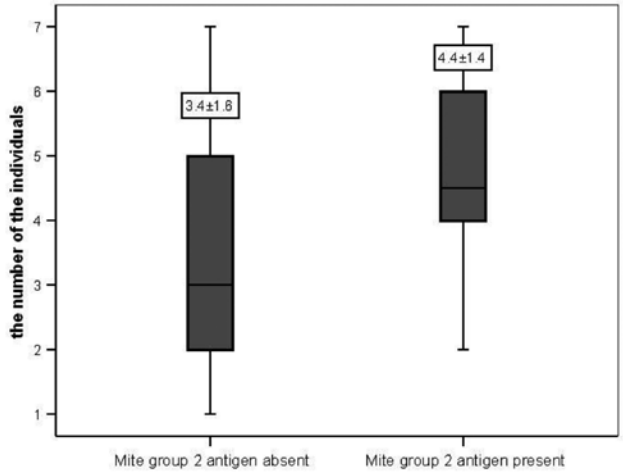

Figure 1. The ratio of mite group 2 antigen detected according to household numbers

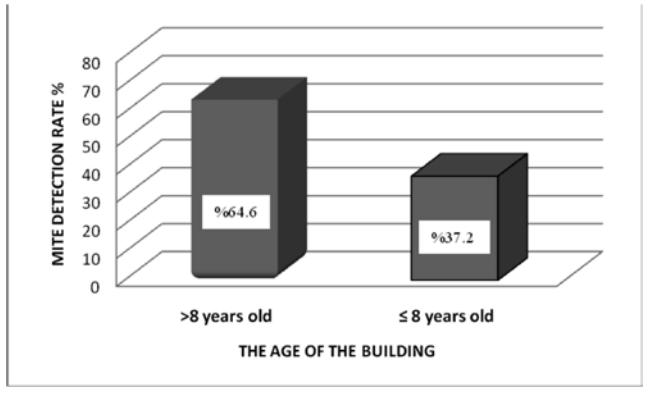

Figure 2. The mite group 2 antigen detection ratio in the houses according to building age

of sunlight, existence of humidity, existence of mould, low-income, aged buildings, 4 and more individuals living in the house, were included in logistic regression model, Household number was determined as a significant risk factor in the detection of mite group 2 antigen. The odds of detecting mite group 2 antigen was found to be 5.29-fold higher in more crowded (≥ 4 individuals) houses [confidence interval of odds ratio (OR): 2.18-12.86] (p<0.001).

Having an asthma attack in the past years and waking up by asthmatic symptoms were more frequent in subjects living in houses in which mite allergens were detected (Figure 3). The rate of night awakenings by asthmatic symptoms, such as dyspnea, cough, and wheeze was 86.5% among patients in whose house dust samples had a detectable level of mite antigen. The rate of night time symptoms in those living at antigen-free house was 62.2% The difference between the groups was found to be significant (χ^2=6,410; SD=1, p=0.011).

Eighty-five percentof patients were on treatment with long-acting beta-2 agonists. The rate of asthma attacks in the past year was 73.1% and 41.3% among patients living in mite antigen (+) and (-) houses, respectively (χ^2=8.86; SD=1, p=0.003). Antigen detection rate was 35% in dust samples from the patients without asthma attack whereas the antigen detection rate was 66.7% in dust samples from the patients with asthma attack in the previous year. The difference between the groups was significant (χ^2=8,247; SD=1, p=0.004).

Table 2. Household characteristics according to mite group 2 antigen detection

Household characteristics	Mite group 2 (+) n=53 (54.1%)		Mite group 2 (-) n=44 (45.9%)		p value
	n	%	n	%	
Building type					
- Apartment building (61.9%)	32	53.3	28	46.7	0.945
- Detached/with garden (37.1%)	20	45.9	17	54.1	
Age of the building*					
- More than 8 years (53.6%)	30	66.7	15	33.3	0.028
- Less than or equal to 8 years (46.4%)	22	41.5	30	58.5	
Income status of the household**					
- High (44,3%)	18	41.9	25	58.1	0.025
- Middle-income (39.2%)	21	55.3	17	44.7	
- Low (16.5%)	13	81.3	3	18.7	
Heating system					
- Central heating (41.2%)	19	47.5	21	52.5	0.582
- Stove heater (47.4%)	27	58.7	19	41.3	
- Air conditioner and/or electric heater (11.4%)	6	54.5	5	45.5	
Wool and cotton beds and pillows					
- Present (49.5%)	30	62.5	18	37.5	0.125
- Not present (50.5%)	22	40.0	27	60.0	
Floor covering of the living room					
- Carpetin piece(s) (44.3%)	20	46.5	23	53.5	0.361
- Wall to wall carpet (51.5%)	29	58.0	21	42.0	
- Rug (4.2%)	3	70.0	1	25	
Presence of pets (20.6%)	12	60	8	40	0.695
Sunlight exposure (64.9%)	29	54.0	34	46.0	0.068
Presence of flower pots in the house (38.1%)	22	59.5	15	40.5	0.485
Presence of visible mould in the house (44.3%)	27	62.8	16	37.2	0.158
Presence of dampness in the house (43.3%)	26	61.9	16	38.1	0.220
Individuals who live in the houses&					
- Four or more individuals (61.9%)	41	68.3	19	31.7	0.0001
- Less than 4 individuals (38.1%)	11	29.7	26	70.3	

*χ^2=4.818, df=1, p=0.028, **χ^2=7.344, df=2, p=0.025, &χ^2=12.206, df=1, p<0.0001

Discussion

Mite allergen level was measured quantitatively in dust samples collected from the houses of asthmatics included in this study and the relationship of allergen levels with asthma symptoms and house conditions were investigated. Mite group 2 allergens were detected in 54.1% of all the houses. The mite detection rate was found to be higher in house dusts of low-income families, crowded household and older buildings. Although not statistically significant, higher detection rate of mite antigen was found in the dust of the houses with visible mould and humidity. Additionally, the severity and frequency of asthma symptoms were higher in patients who live in houses with mite detected in the house dust.

Der p2 and Der f2 are the products of body fluids of *D. pteronyssinus* and *D. farinae* and have been stated to be one of the significant allergen resources (7). Different ratios of measurable levels of mites have been shown in studies, though different methods used in studies are in question. The mite detection rate in houses investigated was found to be 84% in the USA, 78% in Germany, and over 90% in Austria and the United Kingdom (18-21). The scarce number of studies from our country showed 18.6-97.0% mite allergen positivity in house dust samples. The samples of the houses from the coastal area of the country had higher mite detection rates but those from the Southeastern Anatolia Region had the lowest (10).

In a study from İzmir located in Western Turkey, mite allergen was detected in 53.8% of the samples (10). In that study, Der p1 was the most frequently detected antigen with the percentage of 71.4. Due to high levels of mite allergens determined in house dust samples from allergic patients, the authors suggested that environmental factors were important in the development of allergic diseases. They concluded that indoor humidity and residing at seaside could be related to exposure to more intense amounts of mites (10). In parallel with the mentioned study, we found mite group 2 allergens in 54.2% of the samples from the houses of our cases although we evaluated different products of mites.

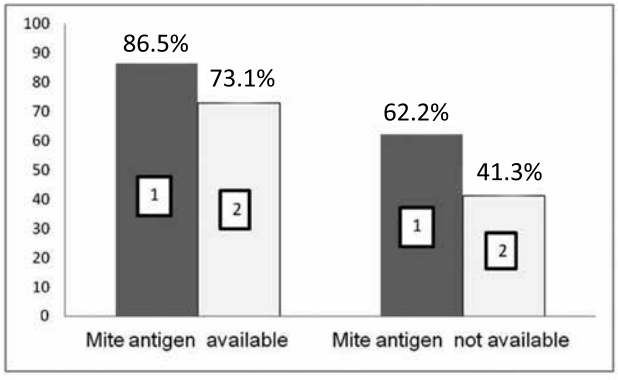

Figure 3. The ratio of awakenings by night symptoms and asthma attacks in the past year according to type of mite detected (the awakenings by night symptoms, 1; asthma attacks in previous year, 2)

Dust mites live in the same environment with humans because they feed on organic debris spilled from humans and other animals. Mites exist mostly in bed sheets, mattresses, pillows, carpets, furniture stuffing, stuffed and live animals. Since mites cannot drink liquid, they need to absorb water from the air. Therefore, they prefer humid climate with a relative humidity of more than 50%. They need an optimal temperature of 18.5-29.0 ^{o}C to grow and multiply. Low altitude that is lower than 1077 meter is convenient for mites to multiply (22,23). The altitude of our city is 60 meters and the distance from the sea is 50 kilometers. In the period when the study was carried out, the average humidity was reported to be 51.04% and average daily temperature was 17.25 ^{o}C which is highly convenient for the growth of mites. Therefore, it would not be surprising to see mites in more than half of the dust samples.

Local environmental factors related to the growth of dust mites have been studied. A study has reported that a higher mite allergen level was found to be associated with factors, such as high humidity level in the house, clothes drying in the house, and lack of a central heating system (24). Bedrooms, because of higher allergen detection rate, are particularly important. Although the level of Der f1 antigen was found to be higher in houses with high levels of humidity in Boston, USA throughout the year, it has been shown that the levels of Der f1 antigen were detected extremely low in houses with central heating system in that relative humidity was kept below 45% year-round (20). Central heating system may reduce relative humidity more than stove-heating. In the present study, there was no effect of the heating system on mite detection.

Age of the house and humidity in bedrooms were found to be the strongest parameters which had a

relationship with house dust mite allergen density in various studies (18,20,21). It has been reported that old carpets, humid areas, and double glazing windows are independent risk factors for mite concentration (21). Older houses were found to be an independent risk factor for higher mite antigen levels as well (18,20,21). The antigen detection rate was found to be higher in houses built more than eight years ago. The explanation might be that water and discharge systems of old houses are older and the humidity from these systems constitutes a basis for mite growth.

It has been shown in a study from Melbourne that Der p1 levels were affected by age and structure of the building, age of the coverlid set and existence of quilt in the bed as well as indoor humidity and existence of mould in the bedroom (20). We detected a higher level of antigen in house dust from the houses with wool and cotton beds and pillows but the difference was not significant (9.53 vs. 8.59 ng/ml; p=0.747). Wool can be a factor that facilitates the growth of mites. Fleece and wool blankets were reported to be a risk factor increasing the level of mite antigen (25). It was also shown in other studies that wool carpets and quilts are associated with higher levels of mite antigens (20,26). Wool materials are charged with static electric as a result of friction and, therefore, can hold a higher amount of antigen particles (27).

In a prospective study, it was shown that the prevalence of asthma increases with house dust mite sensitization in 12 months and asymptomatic bronchial hyperreactivity is a significant risk factor for the development of asthma (6). An increase in asthmatic symptoms in atopic individuals with high indoors allergen contamination was also reported. There are studies showing that high allergen levels in houses were associated with asthma symptoms in allergic individuals (7,19). In the present study, it was found that admission to the emergency department with asthma attack and nocturnal respiratory symptoms were at a higher level in individuals living in houses in which mite allergens were detected in house dusts, suggesting that indoor environment quality might be related with disease control.

In a study from USA in which indoor multi-allergen concentrations were examined with ELISA, minimum six indoor allergens were detected at a measurable level in 51.5% of the houses. Mite, cockroach and rat allergens were detected at a higher level in houses owned by low-income families (28). Higher levels of mite and cockroach allergens were detected in houses with low-income or crowded household in a different study. It was found that asthmatics from a lower socioeconomic status were admitted to hospital more frequently, which was attributed to cockroach antigen (29,30). In the present study, the mite group 2 antigen was detected in 81.3% of subjects with low-income, compared with 41.9% of those with higher income status. However, the mean levels of antigens were found to be higher in houses of individuals with higher income status than those with low- and middle-income status (15.2 ng/ml - high-income, 4.06 ng/ml - middle-income, and 8.92 ng/ml - low-income status).

When dust samples taken from different areas of floor coverings in living rooms of 50 asthmatic patients were examined, it was found that Der p1 levels were higher if the number of individuals who live in the house was three or more (31). When various indoor characteristics were evaluated with logistic regression model in the present study, it was found that only the larger number of household (being four and more) was found to be a significant risk factor for mite group 2 antigen existence. The OR for detecting mite group 2 antigen was found to be 5.29 ($p<0.0001$).

The amount of allergens is particularly important for sensitive individuals. A study by Korsgaard showed that *D. pteronyssinus* and *D. farinae* levels above 2 μg/g in house dust might provoke sensitization in individuals and the a level of above 10 μg/g may cause development of asthmatic symptoms (7). More than half of the samples had mite group 2 allergen at a detectable level in the present study. The level was found to be 0.125 ng/ml, which is the measurable minimum value for mite group 2 allergen by the ELISA method (17).

Conclusion

The existence of atopy, especially against to indoor allergens, is important for asthma management. Determination of indoor allergens may be helpful in controlling the environmental factors. In the present study, mite allergen was detected in the house dust samples of more than half of the cases. Higher detection rate was found in the dust of the houses of low-income families, in crowded houses and aged buildings. The severity and frequency of asthma

symptoms were higher in patients who live in houses in which mites were detected in the house dust.

Due to cost concerns, a limited number of samples and less diverse range of mites have been evaluated. Patient sample data was collected from asthmatic patients who have attended our outpatient clinic within the study period. The cases have also been expected to fulfill the pre-determined criteria. Our centre is a reference clinic that accepts patients from the whole city including the districts. We have considered all the different regions in our sample set. Samples have been collected by using the patients' own vacuum cleaner in their houses that may cause the way of collecting data to be non-standard. However, we strongly believe that we achieved the standards by using new, unused vacuum bag each time before collecting the same amount of dust sample. Measurement with the same vacuum cleaner or a device with similar suction power could have revealed more standard results. This is the other limitation of our study.

In the present study, we emphasized the relationship of indoor environment with atopy and disease symptoms in adult asthmatics. Asthma control may be more successfully ensured by determining indoor allergens and training patients on protection from those allergens for especially those with symptoms continued throughout the year. Factors predisposing to the development of asthma may be assessed better with making detailed determinations for allergens by measuring different subgroups of various allergens.

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Ethics

Ethics Committee Approval&Informed Consent: Institutional ethics committee approval was received and each patient signed an informed consent form prior to the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Fisun Karadağ, Orhan Çildağ, Concept: Emel Ceylan, Sevin Kırdar, Design: Emel Ceylan, Data Collection or Processing: Nimet Demirtaş, Emel Ceylan, Analysis or Interpretation: Nimet Demirtaş, Emel Ceylan, Literature Search: Emel Ceylan, Writing: Emel Ceylan.

Conflict of Interest: No conflict of interest was declared by the authors.

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