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ORIGINAL ARTICLE

Fungi and Indoor Conditions in Asthma Patients

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This study was carried out with 127 asthmatic patients and 127 controls, which aimed to compare and evaluate the environmental conditions in the homes of asthmatic patients and the control group. Air samples were obtained by using an air sampler and the mean mould colony counts were established. *Aspergillus* and *Penicillium* were the most common isolated species. No significant difference was observed with regard to various house conditions and the mean mould colony counts between the houses of patients and controls. The mould colony counts were found to be lower in houses with wooden parquet flooring. The odds ratio for stone floors *vs.* wood floors was 2.3 (95% CI 1.08–4.98) for mould growth.

Keywords asthma, indoor air, living conditions, fungi, colony counts

INTRODUCTION

Allergens in asthma constitute the most important reasons for both airway sensitization and precipitation of asthma attacks. Indoor conditions are important for exacerbation of allergic diseases, such as existing asthma, as well as allergic sensitization (1, 2). Indoor allergens include house dust mites, animal allergens, and fungi (3). While it is well known that house dust mites and animal allergens are the main allergens existing in indoor environments, less research has been carried out on the effect of fungal materials. In a study in which the case referent sample was investigated with a questionnaire, OR was found to be 2.2 for asthma-related mould exposure (4). It is estimated that 10–15% of asthmatic subjects have allergy to moulds, assessed by skin prick testing, most commonly to *Aspergillus fumigatus*, *Alternaria alternata*, *Penicillium*, and *Cladosporium* (5). Mould sensitivity was found to be 9% as a result of allergy tests performed on the patient group, who were referred to our university hospital with respiratory and allergy complaints (Izmir, Turkey) (6).

Although it is not clear by which mechanisms indoor moulds induce asthma, IgE mediated hypersensitivity reactions or effects of mycotoxins are held responsible for fungal allergens. Thorn and Norböck hypothesized that chemicals emitted from the damp materials into the air could be one mechanism through which indoor dampness induces asthma. It is not known what determines the mechanisms in each individual case, but environmental conditions are likely to play an important role (7).

Allergic reactions to house dust mites and moulds are very common among asthmatics, and both are prevalent in damp environments. Indoor environments with dark, damp, and poor ventilation conditions are convenient for fungal growth. Fungal growth is affected by many factors such as temperature, dampness, indoor heating systems, existence of air conditioning, keeping pets in houses, or leakages in the plumbing system (8–10). There are various studies indicating that asthma symptoms are negatively affected by the presence of these factors (1, 11, 12). Besides, good insulation of houses and carpeting on floors create convenient environments for insects, moulds, and bacteria such as house mites and cockroaches increasing in number due to heat and damp conditions. Indoor fungus exposure and allergic symptom development among those living in the non-industrial regions have been the increasing focus of interest recently (9).

An important problem regarding the indoor environment fungus evaluation is whether the data obtained by questionnaires or phone calls reflect the true situation or not. The degree of relation between the indirect evaluations such as the observation of damp spots or mould/fungus growth is another issue. In some studies, the existing mould in the media could be estimated by indirect indicators such as dampness or damp stains on the floor or ceiling, leakages in the plumbing system, ventilation systems, and air conditioning (4, 12, 13). However, fungal load measurement in the media has proved to be more reliable than the questionnaire in epidemiologic studies (2, 9, 14).

Levels of information and awareness of our cases regarding to which extent the living conditions affect their diseases are unknown. Did the existence of respiratory diseases change living conditions of our patient group or force new arrangements in that group compared to the controls? The lack of information regarding indoor mould fungi density in our country and how this ratio is affected by living conditions has caused to emerge a necessity to carry out this study.

The first aim of the present study was to evaluate the relationship of the environmental conditions of asthmatic

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patients in our region and determine the types and densities of fungi and to compare them with the control group. Second, we investigated the environmental features affecting the total mould growth.

Material and Methods

Izmir is located in the western region of Turkey, being in the mild climactic zone. Izmir is situated at the east end of Izmir Bay, which displays a typical Mediterranean climate of hot summers and mild winters. Fungi growth is more evident in summer due to the increase in dampness. However, the lifestyles of our people cause them to stay and spend more time indoors during the winter months. In addition, people rarely open their windows to ventilate their houses in winter months in an effort to decrease heat loss or to avoid air pollution. The ventilation of houses is generally carried out through open windows in our region due to high outdoor temperatures in summer months. Thus, the winter season has been considered as a more appropriate option for our study, which aims at evaluation of indoor environment features.

Study Group

The study population was constituted by 127 volunteer patients who were being monitored for asthma and followed by Dokuz Eylül Medical Faculty, asthma outpatient clinic of the Chest Department.

Control Group

One hundred twenty-seven individuals selected from the neighbors of those patients in the study group with similar socioeconomic levels age and gender were included in the study upon obtaining their consents. Subjects with another respiratory disease or symptom and subjects who refused to enroll in the study were excluded.

Questionnaire

The questionnaire evaluating the home environment was administered to the study and control groups by a pulmonary physician. Questions regarding personal and demographic features (age, gender, educational background, income level, smoking history) were included in the questionnaire. The patients were also asked about how many years ago they were diagnosed with asthma and for how long they have been taking inhaler treatment. In the second part of the questionnaire, features of the houses they live in (the age of the house, ownership, on which floor they live, whether the house gets sunlight, whether it gets dampness, type of heating in winters, whether plumbing system of the house has been repaired recently) were questioned.

Several variables such as the number of individuals living in the house, the number of smokers, whether laundry is dried indoors, existence of pets, use of stores for food, existence of house plants (existence of soil), the flooring, and existence of dampness on the floor or ceiling were also evaluated.

Taking Air Samples and the Mycological Examination

Participants were visited at home in the second part of the winter season (from January 15 to March 30), when air samples were collected from their living rooms and assayed for fungal propagules. Sampling was collected from the living room, because it is where people spend time either watching TV or relaxing and sometimes sleeping.

Air samples of 150 liters were obtained from living rooms by Air Ideal (Biomereux, France) suction system from 9 a.m. to 1 p.m. and before the houses were ventilated. The air IDEAL system is placed downstream a nozzle composed of a specially designed converging pipe and a diverging pipe, into which the measuring probe is inserted. The air flow is adjusted measuring the speed of the air inside the nozzle. The Air IDEAL air sampler has a flow rate of 100 L/min $\pm$6.5 L/min. The samples were collected by absorbing air in the middle of rooms and at 1.5 meters height to ensure standardization. All these processes and house examinations were performed by a physician specialized in pulmonary diseases. Air samples were sprayed into sabouraud dextrose agar and malt extract agar. The media were incubated for 7 to 10 days at 25°C. Colonies of those reproducing were counted and their types were identified.

Measurement of room temperature and dampness level was performed in the living room for at least 10 minutes by a TFA hygrometer hung on a wall at 2 meters height. At each sampling date, relative humidity and temperature were recorded by using a thermo hygrometer device (TFA-Dostmann GmbH, Germany) in main living rooms.

Statistical Analysis

SPSS for Windows, Release 10.0, was used for the statistical analysis. Comparisons between categorical variables were performed by chi-square tests. Continuous variables were compared using the Student's t-test or Wilcoxon sum rank test. P values lower than 0.05 were accepted as significant. To investigate the associations between indoor fungal growth and home characteristics obtained from the questionnaire and observation, logistic regression (backward elimination method) was used.

Results

A total of 254 cases, 65 of whom (26%) were male and 189 of whom (74%) were female, were included in the study. Samples obtained from the houses of 242 cases, 127 of whom were in the study group and 115 of whom were in the control group, were found eligible for evaluation.

Comparisons of asthmatics and controls are presented in Table 1 regarding various demographic characteristics and indoor environment features. Cases and controls were similar in age, gender, income levels, and various indoor environment features. Rate of smoking was lower in asthmatics. Asthma cases were categorized as mild intermittent (37%), mild persistent (34%), moderate persistent (23%), and severe persistent (6%). 53.5% of the asthmatic patients and 35.7% of controls reported symptoms of allergic rhinitis and the frequency of allergic rhinitis was found to be higher in asthmatics ($p = 0.009$). However, no difference was detected regarding the presence of allergic rhinitis according to the severity of asthma.

One hundred seven subjects with allergic rhinitis out of the total participants were evaluated according to indoor environment features. While indoor mean temperature and relative humidity were not found to be different in cases with

TABLE 1.— Sociodemographic characteristics of the asthmatic and control subjects.

	Asthmatic	Control	*p* value
	n = 127	n = 115	
Gender F/M	95/32	86/29	0.55
Mean age	51.8	48.9	0.09
Smokers in household (%)	37.0	49.6	0.05
Income level			0.23
High	17.3%	17.4%	
Middle	68.5%	59.1%	
Low	14.2%	23.5%	
House age (years)	17.8 ± 8.9	18.6 ± 9.1	0.51
Central heating (%)	19.7	20	0.53
Existence of air conditioning (n)	19	23	0.43
Sun light exposure in winter (%)	70.1	70.4	0.53
House plants (%)	55.1	57.0	0.39
Number of residents in the house (n)	3.2	3.2	0.89
Current visible mould %	46.5	40	0.31
Total fungi (CFU/m^3)	36.1	34.7	0.30
Temperature: living room (°C)	21.2 ± 3.6	21.1 ± 3.4	0.94
Relative humidity: living room (%)	44.9 ± 11.5	47.0 ± 10.7	0.14

allergic rhinitis compared with others, the mean amount of total mould was found to be higher. The mean amount of total mould was detected to be 36.8 CFU/m^3 in cases with allergic rhinitis and 34.5 CFU/m^3 in cases without, and the difference was not statistically significant ($p = 0.09$).

There was no relationship determined between visible mould in the living room or existence of mould in any place of the house and the total amount of determined mould upon evaluation considering some living characteristics ($p = 0.321$ and 0.431). Flooring of houses was categorized as parquet and wood flooring for 118 houses (49.2%), stone flooring and marble for 76 houses (31.7%), earth floor and other for 46 houses (19.2%). Flooring types of cases and controls displayed similar distributions ($p = 0.49$).

When house age was grouped regarding 18 years as the mean, in subjects living in houses older than 18 years, the amount of viable fungus was found to be 36.8 CFU/m^3, and this was significantly higher than subjects living in newer houses (34.0 CFU/m^3) ($p = 0.042$). However, mean relative humidity and temperature did not show any difference between the old and new houses. Relative humidity was found significantly different at high-middle and high-low income levels, and room temperatures were found to be significantly different at all income levels upon evaluation of cases altogether (Table 2). Lower dampness and total mould ratios were determined for those with high income level houses.

TABLE 2.—Average monthly income levels and various surrounding conditions*.

Monthly income level	High n = 42	Middle n = 155	Low n = 45	*p* value
Total viable fungi (cfu/m^3)	31.9	35.6	38.2	0.017
Temperature: living room (°C)	22.6 ± 3.1	21.1 ± 3.6	19.8 ± 2.6	0.001
Relative humidity: living room (%)	43.2 ± 11.8	45.7 ± 11.6	49.3 ± 8.03	0.033
Visible mould in living room (n)	5 (11.9%)	33 (21.3%)	18 (40.0%)	0.005
Visible mould in any place of house (n)	11 (26.2%)	66 (42.6%)	28 (62.2%)	0.003

*Cases and controls were evaluated together.

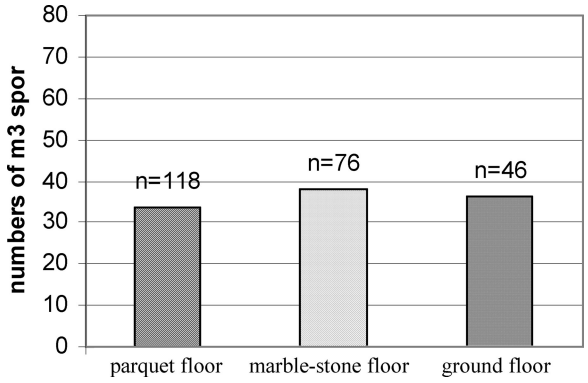

FIGURE 1.—The relationship between flooring and total mould colony counts.

The amount of visible mould spots was higher at low level of income houses.

With regard to sunlight in the house in winter, 170 houses were categorized as getting sunlight (70.2%). There was no difference between groups in terms of temperature; however, relative humidity was found to be statistically different ($p = 0.034$): 44.9% for the houses getting sunlight and 48.3% for the others. Visible mould was observed in 38.2% of houses having access to sunlight and 55.6% of those not having access to sunlight ($p = 0.013$). However, this difference had no effect on total mould growth.

Total amount of determined mould count was found significantly lower in houses with wood or parquet flooring compared to other types of flooring ($p = 0.010$) (Figure 1). Average viable spore counts were determined as 33.6 CFU/m^3 for houses with wood or parquet flooring, 38.0 CFU/m^3 for stone and marble flooring, and 36.4 CFU/m^3 for ground flooring.

Forty-eight houses within all the groups (19.8%) were using central heating. Living room temperature was measured as 25.7°C in houses with central heating and 20.0°C in others. Average amount of living room dampness was found lower in houses with central heating (34.4% *vs*. 48.8%). Although average viable spore count was determined lower in houses with central heating, it was not found to be statistically significant (33.1 CFU/m^3 and 36.0 CFU/m^3; $p = 0.078$).

While almost all cases were using carpets for covering the floor, only 28 of them (11.6%) had wall-to-wall carpets. However, mould growth was not found different between the two types of houses ($p = 0.134$).

Two hundred one individuals (83.1%) were residing in flats and 41 (16.9%) were residing in houses, and some houses had gardens. Visible mould amounts were found significantly higher for those living in houses, but there was no difference determined in terms of total mould growth (Table 3).

Flat residents were categorized as top floor, mezzanine floors and ground floor according to which part of the building they stayed. Visible mould amount in living rooms was detected to be highest in top floor residents ($p = 0.001$). Although there was no difference between groups in terms of temperature and dampness level and growing mould types, a significant difference was found between ground floor and mezzanine floors regarding the amount of growing mould colony. Average total viable spore counts was found 39.3 ±

TABLE 3.—The relationship between the type of home and house characteristics.

	Flat n = 201	Other n = 41	p value
Temperature (°C)	21.3	20.3	0.14
Relative humidity (%)	45.0	50.7	0.006
Age of the building (years)	16.8	25.0	0.000
Living period (years)	10.6	13.6	0.043
Existence of house pet (n)	30 (14.9%)	7 (17.1%)	0.442
Existence of house plant (n)	114 (56.7%)	13 (31.7%)	0.003
Visible mould in living rooms (n)	35 (17.4%)	21 (51.2%)	0.000
Visible mould in other places of house (n)	74 (36.8%)	31 (75.6%)	0.000

9.7 CFU/m^3 on ground floor and 34.7 $\pm$ 11.0CFU/m^3 on mezzanine floors ($p = 0.031$).

To investigate the associations between indoor fungal growth and home characteristics obtained from the questionnaire and observation, logistic regression was used. The total colony counts were grouped as; $<$26.66 (low) and $\geq$ 40 (high) (first and third quartiles). Type and age of house, floor material, heating, and income were the variables included in the model. The odds ratio for stone floors vs. wood floors was 2.3 (95 % CI: 1.08–4.98). After controlling for income, only the type of floor (stone or marble) among the home characteristics remained significant in the model.

Indoor pet exposure was found to be low in the whole group (15.3%), while exposure to indoor house plants (52.5%) was high. Nineteen (51.4%) of 37 persons with domestic pets were keeping lovebirds. Keeping birds at home was not found to affect total mould growth. The percent of keeping house plants was higher in flat residents compared to those residing in houses. It was highest in top floor residents among those living in flats ($p = 0.012$).

A total of 429 fungus colonies with 20 different types were isolated from house environments. While only one type of mould was isolated in 91 cases (35.8%), two or more types were determined altogether in 151 cases (59.4%). There was no difference observed between cases and control groups in terms of determined mould types (Figure 2). Although all houses contained viable moulds, the total CFU per meter cubic counts did not correlate with indoor temperature or relative humidity.

Among 429 colonies determined, 175 (41%) were identified as *Aspergillus spp*., 103 (24%) were *Penicillium spp*., 39 (9.1%) were *Mucor spp*., 32 (7.5%) were *Alternaria spp*., 20 (5%) were *Chrysosporium spp*., 13 (3%) were *Fusarium spp*., 12 (2.8%) were *Acremonium spp*. and 7.6% were identified as other fungi. Other fungi isolated at low frequency and their frequencies were *Cladosporium* (6), *Ulocladium* (5), *Stemphylium* (4), *Aureobasidium* (3), *Paecilomyces* (3), *Scedosporium* (2), *Scopuloriopsis* (2), *Monilia* (1), *Epicoccum* (1), *Nigrospora* (1), *Pseudoallescheria boydii* (1), *Rhizopus* (1), and *Trichotechium* (1).

One hundred seventy-five *Aspergillus* strains were isolated from a total of 162 houses. More than one *Aspergillus* strains were determined in 13 houses. Among 175 *Aspergillus* strains, 22 were identified as *Aspergillus niger*, 5 were identified as *Aspergillus fumigatus* and 1 was identified as *Aspergillus terreus*, and the remaining 147 could not be identified.

DISCUSSION

Damp conditions and moulds in homes have repeatedly been shown to be associated with respiratory symptoms in asthmatics and healthy controls in epidemiological studies (1, 7, 9, 11, 12, 14, 15). In the general population, in persons living in houses with identified damp and mould stains, high respiratory symptom prevalence has been reported whether there is pulmonary disease or not (12, 15). Severity of asthma showed a low positive correlation with total dampness and mould growth in the same study (12). Advanced analysis of a group of community screening investigations demonstrated positive correlations between fungus levels and health effects (16). In the population with identified indoor dampness and mould, prevalence of lower respiratory symptoms was found to be 1.62-fold high in Canada 1.55–1.70 fold high in the Netherlands (15, 17).

The estimates of OR for asthma in relation to visible mould or damp stains from several studies were from 1.25 to 1.56, and there is some evidence of a dose-response relation in which more severe mould problems are related to a higher OR (7, 15, 17). Data obtained from centers with high asthma prevalence showed that the prevalence of indoor mould exposure was high in those regions (11). Asthmatic cases reported a higher indoor dampness before and during the investigation compared with controls (64% in asthmatics and 41% in controls).

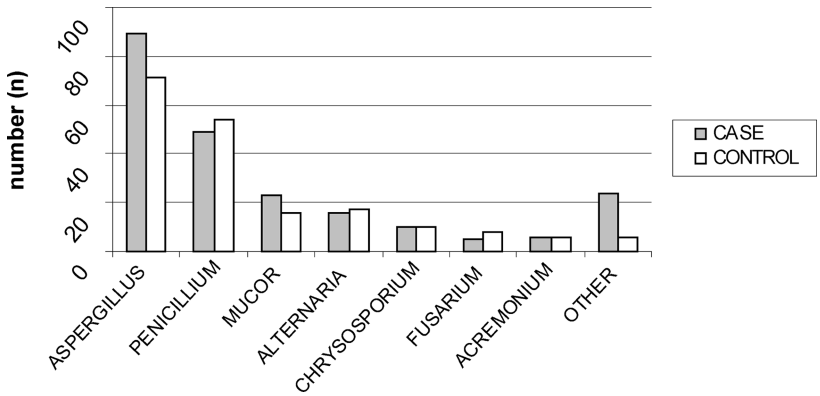

FIGURE 2.—Isolated fungus types in case and control groups.

Burr et al. reported visible mould by 26% of cases and 13% of controls; damp patches by 39% of cases and 38% of controls (5). Data obtained by questionnaires revealed the presence of dampness and/or mould in 25.4% of houses in the Netherlands and in 38% of those in Canada (15, 17). Mould in one or more rooms was reported by 35.1% of respondents in the other population-based study (13). A high rate of indoor mould spots was detected in asthmatics and controls in this study. We carried out the study in winter, and prevalence of mould spots seen in houses without sunlight was 55.6%. Although no association between the observed mould spots and the amount of viable fungus related with the level was reported, the detection of mould in all of the houses can reflect the size and importance of the problem.

In a study carried out in the Netherlands, the total mould concentration was detected to be as high as 669 CFU/m^3(18). The mean indoor mould concentration measured during the whole year in Melbourne, Australia, was reported to be 421 CFU/m^3 (0-6105) (1). Ren et al. detected the mean airborne fungal propagule concentration in the living room using different culture media between 825 to 1038 CFU/m^3 (9). In this study, we determined the mean mould concentration as 35.5 CFU/m^3, and the results were not found to be different in the houses of cases and controls. In a study from our country in which pediatric asthmatic cases from a different geographic region were compared with controls, the mean fungal growth in asthmatics was found to be 13 cfu while it was 2 cfu in controls. In this study carried out during early winter months with a different method (obtained with settlement plate), the amount of total fungal colonies was also found more dense in the houses of asthmatic cases compared with controls (14).

In a study conducted by Yazıcoglu et al. visible fungal patches in the bathrooms (OR = 5.75) and the age of the house (OR = 4.24) were found to be associated with indoor fungal growth. Mould spots in the bathroom was found to be the only factor associated significantly with indoor mould growth. They concluded that bathrooms were the main origin of the fungal propagules (14). Since high correlation was found between the total CFU/m^3 values of living rooms and bedrooms, it is suggested that these areas were the origin of indoor mould (18). In a study by Ren et al., heat, relative humidity, season, and the presence of cat in the house were the factors effective on indoor mould growth (9). In our study, the amount of viable fungus was found to be higher in cases living in older buildings (more than 18 years) than in cases living in new buildings independent from the mean heat and humidity values of the environment. Zock et al. also reported a higher mould exposure in cases living in old buildings with recent water damage (11).

In the present study, a lower concentration of indoor viable fungus growth was detected in cases with a higher income, probably due to better house conditions. Humidity ratio and the amount of visible mould spots were also found to be lower in accordance with fungus growth. Fewer mould spots were reported in subjects with good and excellent home environment in the New Zealand National phone survey. In that study, house design and construction factors that were independently associated with reported mould in the multivariate analysis included: poorer house condition, older house age (>22 years), relative lack of sun exposure, and having no insulation (13). Although various indoor humidity and heat features and lifestyles differed between apartment flats and single houses, no difference was observed in total mould growths in this study. Type and age of house, floor material, heating, and income were evaluated, and only the floor material was found related with mould growth in our study. Stone or marble floor increases the viable fungus growth 2.3-fold compared with parquet or board floor.

Exposure assessment was obtained both with statement of the cases and the observations of a pulmonary specialist in our study. It is known that air samples reflect the fungi at the relevant media, but dust or bulk samples may represent better exposure over a long period of time and thus may be more relevant for chronic health effects (7). The weakness of this study is the lack of evaluation of long time exposure, since a single sample of air taken daily could be inadequate. Perhaps cases can be better evaluated for fungal effect with allergy tests including mould types that grow in this environment. When the method is compared with other studies, although outdoor environment is known to be the origin of indoor fungi, we did not prefer to ventilate the houses of the cases during the whole night before obtaining the samples. Hence we could isolate viable moulds not contaminated with outdoor and most dense in the air. We consider that true airborne viable fungi evaluation was realized by the method we selected.

In the majority of the cases (60%), more than one type of mould was detected in the houses. Katz et al. determined also more than one species of fungal spores in most of the houses in Israel. The most common moulds found in homes were *Aspergillus spp*. followed by *Rhizopus, Penicillium,* and *Alternaria* in that study (19). In 85% of the cases, indoor *Aspergillus* growth was shown in this study; in 78% in another study; while in our study, the frequency was 67% (18, 19). *Aspergillus* and *Penicillium* are less common outdoor moulds and are usually considered the major indoor fungi. *Cladosporium* and *Alternaria* are fungi that grow mainly outdoors (1, 20).

The distribution of fungal spores varies according to regions, seasons, districts, and even according to morning and evening hours (21). When indoor environment of asthmatic patients from the southwest region of Turkey was evaluated, viable moulds were recovered from all houses and 20 different molds were isolated. The most common isolated genera were *Penicillium spp*. (27.9%), followed by *Cladosporium spp*. (26.3%), *Aspergillus spp*. (14.7%), and *Alternaria spp*. (13.1%). No differences in colony numbers were observed between asthmatics and control groups as in our study (22).

Sampling from a single location in the home was suggested to be adequate for the evaluation of home concentration if allergic species of fungi were more important in the investigation. Actual measurements are required for fungal exposure assessment, and to collect samples in one location in a home might be adequate to represent residential levels of fungi (9). We also think that collecting samples from one location is adequate if the sample is air.

There is increasing evidence that indoor moulds increase the severity of existing asthma. There is some evidence that repair of indoor mould problems relieves or eliminates symptoms and signs of asthma (7, 23). No significant difference was observed in living conditions and habits of asthmatics and controls in the first part of our study. Indoor mould types

and concentrations did not differ either. These results suggested that asthmatics did not try to rearrange their living conditions. They might consider that environmental factors do not affect their disease, and they may not be aware of the importance of the situation. However, it is noteworthy that 37% of the asthmatic cases and 50% of the controls were exposed to passive smoking and the difference was statistically significant, which suggests that they made an effort to eliminate passive smoking in their homes.

In the second part of the study, by controlling various house and indoor features with logistic regression analysis, the most effective parameter over indoor mould growth was investigated. Stone or marble floor was found to increase the viable fungus growth 2.3-fold compared with parquet or board floor. No effect of indirect indicators associated with mould on the total mould growth was shown.

In conclusion, the present study is a rare study in our country including evaluation of indoor features and quantitative fungus assessment related to asthma condition. In practice, environmental evaluation of each region should be made since each has its own unique conditions and its effect on airway diseases should be investigated. Routine interrogation of the presence of mould in patients with uncontrolled airway disease and examining fungal allergens among the probable reasons are considered to be useful in patient follow-up and disease control.

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