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The characteristics of indoor and outdoor fungi and their relation with allergic respiratory diseases in the southern region of Turkey

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Abstract Indoor and outdoor fungal exposure has been shown to be associated with the development of allergic respiratory diseases. The aim of the study was to investigate the types and concentrations of airborne fungi inside and outside homes and evaluate the association between fungal levels and allergic diseases in the southern region of Turkey. A total of 61 children admitted with respiratory complaints to the pediatric allergy clinic between September 2007 and November 2008 were included in this study. The air samples were obtained using the Air IDEAL volumetric air sampler longitudinally for 1 year. A comprehensive questionnaire was used for medical history and housing conditions. Skin prick test was performed to determine fungal sensitivity and spirometric indices were employed. The predominant indoor fungal species were *Cladosporium* (69.3 %), *Penicillium* (18.9 %), *Aspergillus* (6.5 %), and *Alternaria* (3.1 %). A strong correlation between indoor and outdoor fungal levels was detected for the *Cladosporium* species ($p < 0.001$, $r = 0.72$) throughout the year. Living in a detached home ($p = 0.036$) and the presence of cockroaches ($p = 0.005$) were associated with total indoor fungal levels. The presence of cockroaches (aOR 3.5; 95 % CI 0.95–13.10, $p = 0.059$) was also associated with fungal sensitization at the edge of significance. The statistical cutoff values of indoor and outdoor *Cladosporium* levels to predict symptomatic asthma were found to be >176 CFU/m^3 ($p = 0.003$, AUC 0.696; sensitivity 65.5 %; specificity 68.7 %) and >327 CFU/m^3 ($p = 0.038$; AUC 0.713; sensitivity 66.6 %; specificity 76.9 %), respectively. Children with respiratory symptoms are exposed to a considerable level of fungi inside and outside their homes. The prevention of fungal exposure may provide valuable intervention for respiratory diseases.

Keywords Indoor fungi · Outdoor fungi · Air sampling · Allergic diseases · Fungal sensitivity · House characteristics

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Introduction

Recent evidence has shown on a global rise in the prevalence of allergic diseases (Masoli et al. 2004). Fungal sensitization is known to be associated with the development of allergic diseases such as asthma and rhinitis (Weinmayr et al. 2013; O'connor et al. 2004; Dharmage et al. 2002). The detrimental effects of dampness and fungal growth in homes have been reported in World Health Organization guidelines, which underline that residents of moldy buildings are

at increased risk of respiratory problems (World Health Organization 1990). Other studies have revealed that exposure to moldy environments is associated with cough, upper respiratory symptoms, wheezing, and asthma (Verhoeff et al. 1994; Bornehag et al. 2001, 2004, 2005; Cabral 2010). The prevalence of lower respiratory symptoms was found to be 1.62-fold higher in the population with indoor dampness and mold in Canada and 1.55–1.70-fold higher in the Netherlands (Dales et al. 1991; Brunekreef 1992). The estimates of odds ratio for asthma in relation to visible dampness and mold vary from 1.25 to 1.56 in prior studies (Jaakkola and Jaakkola 2004; Dales et al. 1991; Brunekreef 1992). In another study, exposure to high levels of indoor *Penicillium* was found to be significantly associated with wheezing episodes among infants (Rosenbaum et al. 2010).

Understanding the factors that potentially give rise to environmental fungal exposure allows for better evaluation of risk factors for respiratory diseases. Some previous studies used questionnaires for assessing exposure to dampness and mold (Dharmage et al. 1999; Ceylan et al. 2006; Yazicioglu et al. 2004). Housing characteristics, such as age of the house, type of construction, presence of visible mold and musty smells, reported water leaks, air conditioning use, presence of pets and cockroaches, ventilation, carpeting, indoor temperature, and relative humidity, have potential to contribute to fungal growth and exposure (Crawford et al. 2015; Ren et al. 2001; Li and Hsu 1997; Dharmage et al. 1999). Nevertheless, self-reported fungal exposure and housing conditions may not be sufficient to demonstrate actual mold exposure. Quantitative measurements of fungi have been shown to be more reliable than questionnaires are. However, direct measurements of fungi have several limitations because of the ubiquitous presence of fungi in all environments, a lack of standardized methods (such as spore sampling methods or culture-based methods), and seasonal differences (Crawford et al. 2015).

Mersin is one of the biggest cities located in the southern region of Turkey characterized by a temperate climate with mild winters and humid summer months. There is limited data relevant to fungus exposure in this region (Inal et al. 2007, 2008). Thus, the first aim of the present study was to evaluate the types and concentrations of airborne fungi inside and outside the homes in Mersin. The second aim was to investigate whether there was any association between indoor and outdoor spore concentrations and allergic respiratory diseases.

Materials and methods

Study design

The present study comprised two phases. Phase 1 involved completion of a questionnaire about medical history and housing conditions and evaluation of fungal sensitization, allergy markers, and lung functions. In phase 2, researchers conducted indoor and outdoor air sampling in the homes of the study subjects.

Subjects

Children admitted with respiratory complaints to the pediatric allergy department of Mersin University between September 2007 and November 2008 were included in this study. A total of 61 patients with a diagnosis of allergic rhinitis, asthma, chronic cough, laryngitis, recurrent lower respiratory tract infections, adenoiditis, and/or sinusitis were enrolled in the study. The study was approved by the clinical research ethics committees of Mersin University. All parents of patients provided written informed consent prior to taking part in the study.

Allergy tests

A skin prick test was performed to evaluate the fungal sensitivity after the baseline questionnaire. The test was performed according to the International Study of Asthma and Allergies in Childhood (ISAAC) protocol (Weiland et al. 2004). The following antigens were applied to the volar surface of the forearm in addition to histamine and saline controls: *Dermatophagoides pteronyssinus*, *D. farinae*, cockroach, cat and dog dander, mixed grass, tree pollen, *Alternaria alternata*, *Cladosporium*, *Aspergillus*, and *Penicillium*. A test was considered positive if the maximal diameter of the wheal was 3 mm larger than the maximal diameter of the negative control. Fungal sensitization was defined as having a positive skin prick test to at least one of the fungi in the panel. Also, serum total IgE levels and absolute eosinophil counts were measured as markers of an allergy.

Questionnaire

A questionnaire regarding medical history and housing conditions was administered to the study group.

Questions about demographics (age, gender, history of family atopy) and residential characteristics, such as the number of residents; age and type of home; presence of visible mold and musty smells; occurrence of water leaks; use of air conditioning; presence of pets, cockroaches, and house plants; type of ventilation; heating; type of carpeting and cleaning routines, were included. The patients were also asked whether they had wheezing, dyspnea, or recurrent cough or had been diagnosed with asthma, rhinoconjunctivitis, atopic dermatitis, sinusitis, or adenoiditis ever or within the last 12 months.

Evaluating lung functions

Lung function evaluations were conducted in accordance with the American Thoracic Society and the European Respiratory Society (ATS/ERS) guidelines (Miller et al. 2005) using a spirometry device (Jaeger, Germany). The values of forced expiratory volume in 1 s (FEV1) and FEV1/FVC were recorded for data analysis. Forced vital capacity (FVC) is the amount of air that a person can expire after a maximum inspiration. FEV1/FVC represents the proportion of a person's vital capacity that he or she is able to expire within the first second of forced expiration. These indices are used to detect respiratory disease in patients presenting with respiratory symptoms. They are used to diagnose and assess the severity of asthma.

Collecting indoor and outdoor air samples and fungal analysis

Indoor and outdoor air sampling were performed at nine different times during the study period. Indoor air samples were obtained during each home visit from 61 homes. Air samples of 200 L were obtained using the Air IDEAL (bioMerieux, France) suction system from 8 a.m. to 5 p.m. before the homes were ventilated. The samples were collected by absorbing air in the middle of bedrooms at a standardized height of 1.5 m. Outdoor samples were obtained from either a balcony or garden of the 28 selected homes. Air samples were seeded into sabouraud dextrose agar. The culture media were incubated for 5 to 8 days at 25 °C. The breeding colonies were counted, and conventional methods were used to identify types. Results were reported in colony-forming units (CFU) per cubic meter of air. The temperature and humidity of the bedrooms were found using a hygrometer, recorded at each sampling date.

Statistical analysis

Statistical analyses were performed using the SPSS 11.5.1 statistical software for Windows. Descriptive statistics were computed including frequencies, means, standard deviations, and medians. Comparisons for continuous variables were made by the Mann-Whitney *U* test and categorical variables were compared by the chi-square test. The Friedman test was used to test whether there was a significant change in indoor and outdoor levels of fungi throughout the year. Parametric tests were performed with the logarithm of absolute eosinophilic count and total IgE values. Correlations were assessed using Spearman's correlation. A logistic regression analysis was used to investigate the associations between fungal sensitization and home characteristics. The adjusted odds ratio (aOR) and its 95 % confidence interval (CI) were calculated. Receiver operating characteristic (ROC) curve analysis was performed to find the cutoff values for indoor or outdoor fungal levels to predict symptomatic asthma. The area under the curve (AUC) was calculated using MedCalc® (MedCalc Software, Mariakerke, Belgium). A p value of <0.05 was considered statistically significant.

Results

A total of 61 patients, 25 (40.9 %) of whom were female, were included in the study. The median age was 10 years (min 5 years, max 18 years). Among these children, 8 (13.1 %) had allergic rhinitis, 29 (47.5 %) had asthma, 7 (11.4 %) had urticaria and chronic cough, 3 (4.9 %) had chronic cough, 2 (3.3 %) had recurrent lower respiratory tract infections, 1 (1.6 %) had laryngitis, 6 (9.8 %) had allergic rhinitis and sinusitis, 4 (6.5 %) had adenoiditis and sinusitis, and 1 (1.6 %) had urticaria and asthma. According to the skin prick test results, 31 patients were sensitized to at least 1 of the fungal allergens and 30 patients were not sensitive to fungal allergens. Among the children with fungal sensitization, 19 (61 %) were male. *Alternaria* species sensitization was most prevalent (36 %). Sensitization to *Cladosporium*, *Aspergillus,* and *Penicillium* species was found in 15, 8, and 3 % of children, respectively. Comparison of groups with and without fungal sensitization are presented in Table 1 regarding demographic characteristics and housing conditions. The groups were similar in age, gender, and various housing characteristics.

Table 1 Demographic and housing characteristics of the patients with and without fungal sensitization

Characteristic	Group 1 with fungal sensitization ($n=31$) (%)	Group 2 without fungal sensitization ($n=30$) (%)	p value
Median age (years)	10 (5–18)	9 (5–17)	0.268
Male gender	19(61.3)	17(56.7)	0.714
Presence of family atopy	16(51.6)	8(26.6)	0.046
Log total IgE	2.46 ± 0.49	1.99 ± 0.56	0.002
Log absolute eosinophilic count	2.50 ± 0.40	2.17 ± 0.38	0.003
FEV1%	91.55 ± 18.80	93.59 ± 17.24	0.733
FEV1/FVC	83.83 ± 8.53	83.27 ± 10.26	0.857
Number of residents in home	4.00 ± 0.73	4.2 ± 0.76	0.299
Age of house (years)	12.48 ± 8.22	15.86 ± 10.86	0.175
Living in a detached house	12(38.7)	9(30.0)	0.474
Presence of dampness or visible mold	7(22.5)	4(13.3)	0.348
Ventilating houses frequently in winter	29(93.5)	27(90)	0.616
Sun exposure of home	30(96.7)	28(93.3)	0.612
Using air conditioning	13(41.9)	10(33.3)	0.488
Old carpeting	21(67.7)	24(80)	0.277
House plants	19(61.2)	18(60)	0.918
Existence of pets	6(19.3)	4(13.3)	0.731
Reported cockroach	12(38.7)	5(16.6)	0.055

Categorical variables are summarized as frequency (percentage). Normal distributed continuous variables are summarized as mean ± SD. Non-normal distributed variables are summarized as median (min-max)

The serum total IgE and absolute eosinophil count were significantly higher in the group with fungal sensitization compared the group without ($p=0.002$ for total IgE level, $p=0.003$ for absolute eosinophil count). There was no significant difference between the groups in terms of spirometric indices such as FEV1 and FEV1/FVC (Table 1).

A total of 203.143 CFU/m^3 fungal colonies relating to 31 different genera were isolated from indoor samples of 61 homes. A total of 95.077 CFU/m^3 fungal colonies were identified from outdoor samples of 28 selected addresses. The predominant indoor fungal species were found to be *Cladosporium* (69.3 %), *Penicillium* (18.9 %), *Aspergillus* (6.5 %), *Alternaria* (3.1 %), *Fusarium* (0.7 %), and others (1.2 %). *Cladosporium* was the most frequently isolated outdoor fungus (76.8 %), followed by *Penicillium* (10.7 %), *Aspergillus* (5.8 %), *Alternaria* (3.9 %), and others (2.45 %) (Table 2).

While the total levels of indoor fungi were significantly higher in May (median indoor fungi levels 556 CFU/m^3) and June (median indoor fungal levels 394 CFU/m^3) ($p<0.001$), total levels of outdoor fungi were significantly higher in May (median outdoor fungal levels 927 CFU/m^3) and November (median outdoor fungal levels 423 CFU/m^3) ($p<0.001$).

During the sampling period, the mean temperature varied between 9.4 and 30.1 °C throughout the year. The average humidity level ranged from 51 to 74 %, reaching peak levels during the summer. Also, monthly rainfall was in the range of 4.6 133.9 kg/m^2.

The mean indoor temperature and relative humidity in the homes of children with fungal sensitization was not significantly different from the homes of children without fungal sensitization ($p=0.874$ for temperature, $p=0.639$ for relative humidity). The total indoor fungal counts did not

Table 2 The distribution of isolated indoor and outdoor fungi

	Indoor fungi		Outdoor fungi	
Genus	CFU	%	CFU	%
Alternaria	6414	3.16	3786	3.98
Aspergillus	13235	6.51	5591	5.88
Cladosporium	140857	69.34	73100	76.89
Penicillium	38488	18.95	10263	10.79
Fusarium	1588	0.78	891	0.94
Others	2561	1.27	1446	1.52
Total	203.143	100	95.077	100

significantly correlate with indoor temperature ($p = 0.266$) or relative humidity ($p = 0.307$).

The total levels of outdoor fungi were not significantly different between groups with and without fungal sensitization (median outdoor total fungal levels in both groups 376 CFU/m^3), except in October (median outdoor total fungal levels in fungal sensitive group 341 CFU/m^3; and 264 CFU/m^3 in group without fungal sensitivity, $p = 0.002$). In particular, in October, the outdoor levels of *Cladosporium* and *Penicillium* were significantly higher in the group with fungal sensitivity compared the group without ($p = 0.015$ for *Cladosporium*, $p = 0.021$ for *Penicillium*). There was no significant difference between the groups in terms of total indoor fungal colony numbers.

Total indoor fungal concentrations positively correlated with total outdoor fungal levels ($p < 0.001$, rho = 0.450). Indoor and outdoor total fungal concentrations throughout the year are shown in Fig. 1. Table 3 presents baseline correlations between various fungi. While a strong significant correlation between indoor and outdoor fungal concentrations was detected for *Cladosporium* species ($p < 0.001$, rho = 0.72), significant but weaker correlations were seen between levels of *Cladosporium* and *Penicillium* both indoors and outdoors. Also, weaker correlations were seen between indoor and outdoor concentrations of *Penicillium* and *Cladosporium* and outdoor concentrations of *Aspergillus* and *Penicillium* species (Table 3).

We did not find a significant correlation between respiratory symptomatology and total indoor and outdoor fungal concentrations. Also, no significant associations between indoor and outdoor fungal levels and spirometric indices were detected.

Living in a detached home ($p = 0.036$) and the presence of cockroaches ($p = 0.005$) were associated with total indoor fungal levels, as shown in Table 4. To investigate the associations between housing characteristics and fungal sensitization, multivariate logistic regression model was used. The presence of cockroaches (aOR 3.5; %95 CI 0.95–13.10, $p = 0.059$) was associated with fungal sensitization at the edge of significance (Table 5).

We assessed a threshold for *Cladosporium* to predict symptomatic asthma because it was found to be the most frequently isolated indoor and outdoor fungus in our region. The statistical cutoff values of indoor and outdoor *Cladosporium* levels to predict symptomatic asthma were found to be >176 CFU/m^3 ($p = 0.003$, AUC 0.696; sensitivity 65.5 %, specificity 68.7 %) (Fig. 2a) and >327 CFU/m^3 ($p = 0.038$, AUC 0.713; sensitivity 66.6 %, specificity 76.9 %) (Fig. 2b), respectively. No statistical cutoff values for other fungi were found.

Discussion

In the present study, we investigated the types and concentrations of airborne fungi inside and outside of homes in Mersin and whether there was any association between indoor and outdoor spore concentrations and allergic diseases. The predominant indoor fungal species were found to be *Cladosporium* (69.3 %), *Penicillium* (18.9 %), *Aspergillus* (6.5 %), and *Alternaria* (3.1 %). Total levels of indoor and outdoor fungi were found to be significantly higher in May. A strong correlation between indoor and outdoor fungal levels was detected for *Cladosporium* throughout the year. We did not find any significant correlation between indoor and outdoor fungal concentrations in the houses of patients with different respiratory symptomatology and fungal sensitization. Living in a detached home and the presence of cockroaches were associated with total indoor fungal levels. The presence of cockroaches was also found to be associated with fungal sensitization. The statistical cutoff values of indoor and outdoor *Cladosporium* levels to predict symptomatic asthma were found to be >176 and >327 CFU/m^3, respectively.

A striking rise in the prevalence of asthma and allergic diseases has focused attention on possible risk factors such as exposure to dampness and mold in homes (Hu et al. 1997; Verhoeff et al. 1995). Epidemiological studies have shown that fungal exposure in homes is related to respiratory symptoms in atopic and healthy populations. ISAAC phase II study revealed that dampness-related molds being present at home during the first year of life was a significant risk factor for current rhinitis (Kuyucu et al. 2006; Hu et al. 1997; Bornehag et al. 2004; Verhoeff et al. 1995).

The Commission of the European Communities has established that fungal concentrations below 500 CFU/m^3 are low, those between 500 and 999 CFU/m^3 are intermediate, and those above 1000 CFU/m^3 are high (Comission of the European Communities CEC 1994). In the present study, the mean indoor and outdoor mold concentrations were 370 and 379 CFU/m^3, respectively. Nevertheless, in May, mean indoor fungal (681 CFU/m^3) and outdoor fungal levels (839 CFU/m^3) rose to

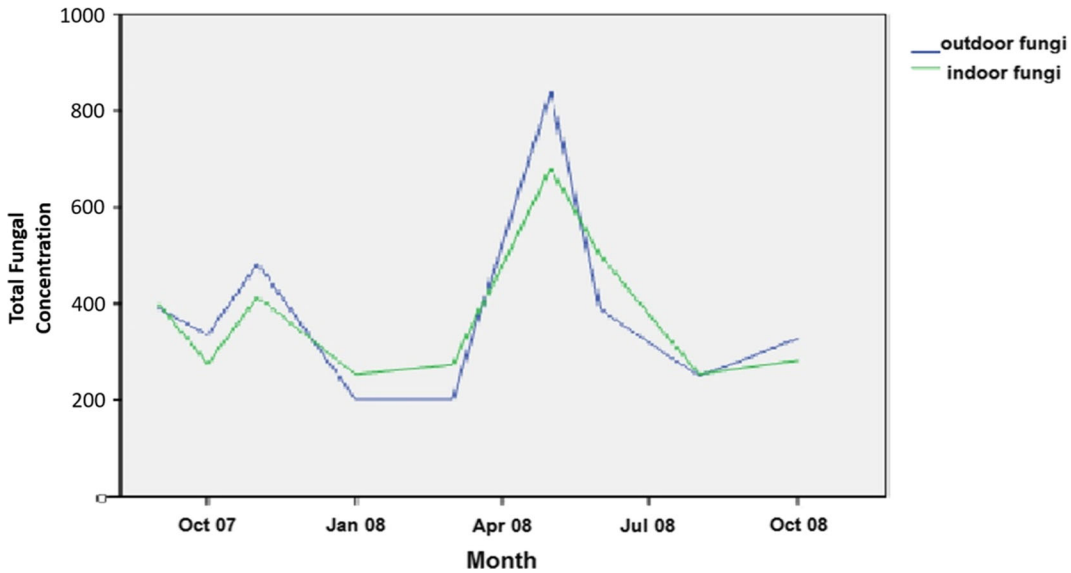

Fig. 1 Indoor and outdoor total fungal concentrations throughout a year

levels classified as intermediate to high. The total mold concentration was detected to be as high as 825–1038 CFU/m^3 in a previous study (Ren et al. 2001). In other studies, the mean indoor mold concentrations were determined to be 669 CFU/m^3 in Netherlands (Verhoeff et al. 1992) and 421 CFU/m^3 in Australia (Dharmage et al. 2002). Ceylan et al. detected the mean fungal level as 35.5 CFU/m^3 (Ceylan et al. 2006), while Boyacioglu

Table 3 Baseline correlations between fungi taxa

		Alternaria	*Cladosporium*	*Aspergillus*	*Penicillium*
		Indoor fungi			
		r (*p* value)	*r* (*p* value)	*r* (*p* value)	*r* (*p* value)
Indoor fungi	*Alternaria*	1.000	0.229(0.076)	0.017(0.898)	0.083(0.524)
	Cladosporium		1.000	0.277(0.031)[a]	0.484(<0.001)[a]
	Aspergillus			1.000	0.208(0.107)
	Penicillium				1.000
		Outdoor fungi			
Outdoor fungi	*Alternaria*	1.000	0.332(0.085)	−0.072(0.716)	−0.016(0.937)
	Cladosporium		1.000	0.001(0.997)	0.449(0.013)[a]
	Aspergillus			1.000	0.369(0.049)[a]
	Penicillium				1.000
		Outdoor fungi			
Indoor fungi	*Alternaria*	0.317(0.101)	−0.108(0.572)	−0.360(0.055)	−0.195(0.293)
	Cladosporium	0.279(0.151)	0.719(<0.001)[a]	0.113(0.561)	0.063(0.737)
	Aspergillus	0.208(0.289)	0.284(0.129)	0.376(0.044)[a]	0.175(0.347)
	Penicillium	0.062(0.752)	0.434(0.016) [a]	−0.045(0.817)	0.207(0.264)

Correlations are shown with spearman correlation coefficient *r* (*p* value)

[a] The correlations that are statistically significant

Table 4 The housing characteristics related with the levels of total indoor fungi

		Total indoor fungal levels Median [min.-max.]	*p* value
The nature of house	Detached house	385.22 [184.11–957.33]	0.036
	Flat	274.78 [134.78–827.22]	
Visible mold	Present	340.44 [159.56–957.33]	0.690
	Absent	368.78 [134.78–827.22]	
Old carpeting	Present	340.78 [134.78–735.78]	0.077
	Absent	401.61 [142.33–957.33]	
Reported cockroach	Present	396.11 [237.11–957.33]	0.005
	Absent	305.50 [134.78–735.78]	
Presence of pets	Present	417.83 [184.11–569.78]	0.302
	Absent	357.11 [134.78–957.33]	
Using air conditioning	Present	354.33 [134.78–827.22]	0.666
	Absent	372.78 [159.56–957.33]	

et al. found 501 CFU/m^3 in İzmir (Boyacioglu et al. 2007). The variations in the results of total fungal levels could be related to air sampling method, the season, different geographic locations, and housing conditions (Crawford et al. 2015).

In the present study, the most commonly recovered fungi from indoor and outdoor air were *Cladosporium* and *Penicillium*. Another study by Inal et al., from a similar geographic region, revealed that the most isolated fungi from outdoor air were *Cladosporium* and *Alternaria*, and from indoor air, *Cladosporium* and *Penicillium* (Inal et al. 2007, 2008). The level of total outdoor fungi was found to be less than the indoor fungi in the present study. However, this is likely only due to the fact that more indoor samples were taken than outdoor samples. Indoor samples were taken from 61 homes while outdoor samples were taken only from 28 addresses.

It is important to include seasonal variations when testing for levels of fungi, since fungal levels determined from an air sample taken at one time during the year cannot reflect fungal levels throughout the year. Therefore, air sampling was performed at nine different times during the study period. This perennial sampling revealed that total levels of indoor fungi were significantly higher in May and June, while May and November showed the highest counts for outdoor fungi.

We did not find any significant correlation between indoor and outdoor fungal concentrations in the homes of patients with different respiratory symptomatology and fungal sensitization. The lack of significant association in indoor and outdoor levels of fungi within the different diagnostic groups could be due to the fact the symptoms were questioned based on the questionnaire. Therefore, the parents may not have remembered the

Table 5 Multivariate logistic regression analysis of house characteristics related with fungal sensitization

Independent variable	Odds ratio (95 % CI)	*p* value
Age	1.148(0.959–1.374)	0.133
Male Gender	0.460(0.129–1.641)	0.232
Parental atopi	2.826(0.899–8.881)	0.075
Living in a flat house	0.593(0.139–2.532)	0.480
Age of house	0.945(0.887–1.006)	0.076
Old carpeting	0.348(0.076–1.593)	0.174
Reported cockroach	3.533(0.952–13.108)	0.059
Visible mold or musty odor	2.936(0.540–15.957)	0.213
Using air conditioning	1.658(0.404–6.801)	0.483
Ventilating houses frequently in winter	1.463(0.146–14.711)	0.746
Existence of house plants	0.968(0.230–4.074)	0.964
Existence of pets	1.348(0.221–8.226)	0.747

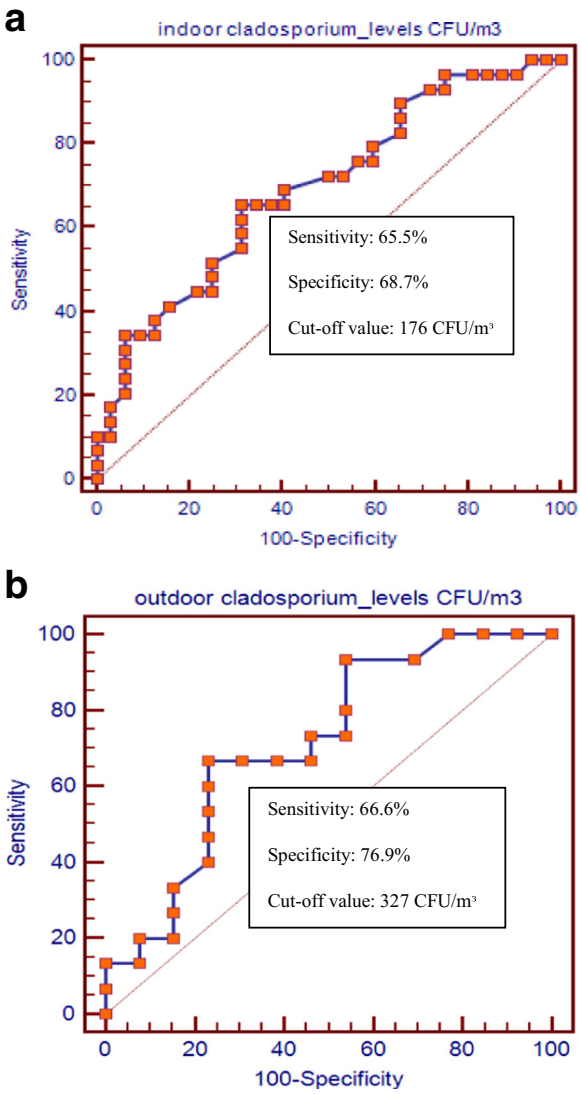

Fig. 2 **a** The cutoff value of indoor *Cladosporium* levels to predict symptomatic asthma in ROC analysis. **b** The cutoff value of outdoor *Cladosporium* levels to predict symptomatic asthma in ROC analysis

severity of symptoms. On the other hand, the potential health risks associated with indoor fungi depend on the timing and extent of exposure to different fungal components such as fungal spores, hyphae, cell wall structural component β-glucans, and mycotoxins (Cabral 2010). Thus, the contribution of fungal fragments to indoor exposure and subsequent development of fungal sensitization should be taken into account. In the present study, we were not able to measure the allergy triggering fungal fragments that have been shown to aerosolize simultaneously with spores in lungs and lower airways.

We observed that total indoor fungal concentrations were positively correlated with total outdoor fungal levels. Although the levels may vary widely because of humidity, temperature, season, and geographic location, outdoor fungal levels were shown as the most consistent predictors of indoor levels (Cabral 2010; Crawford et al. 2015). Similar to the present study, many previous studies demonstrated significant correlation between indoor and outdoor fungal levels (Chew et al. 2003; Dassonville et al. 2008; O'connor et al. 2004).

We found the highest concentration of *Alternaria* to be 23 CFU/m^3 inside the homes and 32 CFU/m^3 outside the homes in May in the present study. Both of these levels were below the critical level of 100 CFU/m^3, which has been reported to be related to hyperresponsiveness of airways in children sensitive to *Alternaria* (Downs et al. 2001). *Alternaria* was shown to contribute to severe asthma in localizations where the exposure to the fungus is high (Salo et al. 2005). We did not find any relation between the symptoms of asthma and *Alternaria* levels. However, our study found the cutoff values of indoor and outdoor *Cladosporium* levels for predicting symptomatic asthma to be >176 and >327 CFU/m^3, respectively. There is currently a lack of data available in the literature related to predictive levels of other fungi.

The present study found a relation between living in a detached home and the presence of cockroaches to total indoor fungal levels. An inner-city asthma study reported a positive association between cockroaches and *Aspergillus* concentrations (O'connor et al. 2004). In our study, the presence of cockroaches significantly predicted total indoor fungal levels, as well as fungal sensitization at the edge of significance. Cockroaches require water for survival and thrive in warm and humid environments. Their presence may indicate chronic moisture at home, which supports fungal growth (Crawford et al. 2015). Therefore, the presence of cockroaches should be questioned in surveys designed to predict indoor fungi levels. Ren et al. demonstrated that heat, humidity, season, and the presence of a cat at home were predictors of indoor fungal growth (Ren et al. 2001). Zock et al. reported that living in old buildings with recent water damage correlated with higher fungal exposure (Zock et al. 2002). In other studies, presence of dampness, musty odors, moldy patches, limited ventilation, household pets, and carpeted floors were found to be predictors of indoor fungal growth (Verhoeff et al. 1992; Wickman et al. 1992; Dharmage et al. 1999; Garrett et al. 1998). On the other hand, Zubairi et al.

reported that there was no association between the concentrations of fungi and housing characteristics (Zubairi et al. 2014).

Self-reported fungal exposure and housing characteristics may not be sufficient to accurately demonstrate the real levels of mold exposure (Ren et al. 2001; Ceylan et al. 2006). In our study, fungal exposure data and most of the housing characteristics collected through the questionnaire were not predictive of indoor fungal levels. Therefore, quantitative measurements of fungi might be more reliable than are questionnaires. Air sampling can contribute to a greater understanding of indoor exposures and their relation to housing characteristics.

The strength of the present study is that it provides data on a geographic region in Turkey where there was previously limited information about mold spectrums and concentrations. The study also provided new information on the comparison of indoor and outdoor fungal levels in homes as it relates to fungal sensitization and respiratory symptomatology in children. Another strength of this study was the use of quantitative assessment which allowed for more reliable evaluation of fungal exposure. In addition, this study used air sampling from various times throughout the year instead of only one, since one example cannot represent the levels throughout the year. The final strength of our study is the determination of a threshold level for indoor and outdoor fungi to predict symptomatic asthma.

One of the limitations of our study was the small sample size. Another limitation was the lack of assessment of fungal exposure in other environments where children spend a lot of time, such as school. The final limitation may be the lack of measurements of fungal fragments other than the spores and hyphae, for more accurate assessment of fungal exposure.

Conclusions

In conclusion, children with respiratory symptoms are exposed to a considerable level of fungi inside and outside their homes within our region. The prevention of fungal exposure may provide valuable intervention for respiratory diseases. Future investigations are needed to identify factors that affect indoor and outdoor fungal levels and the impact of these factors on the children's respiratory health.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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