

Determination of fungal frequency and comparison of allergic symptoms related with buildings and fungi in Afyon, Turkey

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ABSTRACT

Objectives: To investigate the respiratory symptoms and atopic sensitization to fungi and its relation with the building type where school children were attending. We also compared the prevalence of sensitization to different fungi and respiratory symptoms in 2 different school buildings in a particular area of Turkey.

Methods: Using simple random sampling, 301 school children aged 16-19 years attending a high school in 2 different school buildings (old and new buildings) were recruited and agreed to participate in the study. The study was conducted in Afyonkarahisar, Turkey from August 2003 to August 2004. Information on allergic diseases, building related symptoms and respiratory symptoms of the students was collected by questionnaire. Skin prick test (SPT) was performed to all students. Fungal concentrations and genera were determined by using Petri Plate Gravitational Settling Method.

Results: In both buildings, throughout all seasons, the most common fungi species seen were *Penicillium* and *Cladosporium*. When SPT was performed, the most allergen fungi seen in new building was *Epidermophyton* and *Penicillium* in the old building. The SPT results were also related with building related symptoms, except *Alternaria*.

Conclusion: When the 2 groups were compared, it was observed that there were no statistically significant differences between them in regards with asthma, allergic diseases, building related symptoms, and respiratory symptoms. No significant difference was determined between these 2 buildings in accordance to fungus concentration, although the humidity rate was higher in new buildings.

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It is well known that environmental factors might affect asthma and allergy prevalence. Apart from these environmental factors, types of buildings, where people are living, are also considered as one of the risk factors of asthma and allergy possibility causing other related risks such as fungi growth. Fungi are ubiquitous on this planet and exposure to them is a fact of life nearly all over the world.¹ Mould spores are prevalent in houses and flourish

in the presence of optimal growing conditions, including high humidity.² Some studies reported that there is a relation between parental reports of respiratory symptoms and the presence of fungi in the buildings.³⁻⁸ Typical respiratory findings related with buildings are recurrent respiratory infections, asthma-like conditions such as wheezing or prolonged cough, and even asthma in addition to other symptoms such as conjunctivitis.^{9,10} Asthma and allergy are

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considered as multifactorial diseases, consisting of genetic and environmental susceptibility. Since genetic susceptibility is not changeable in individuals, elucidation and elimination of environmental causes in the country-specific basis to prevent asthma and allergy comes to an important position. Most recently, emphasis has been placed on the indoor air quality of school and day-care center buildings.^{5,11} Our aim was to investigate respiratory symptoms and atopic sensitization to fungi and its relationship with the building type where school children were attending. We also compared the prevalence of sensitization to different fungi and respiratory symptoms in 2 different school buildings in a particular area of Turkey.

Methods. The study was carried out between August 2003 and August 2004 in Afyon, Turkey. Afyon High-school had 1600 pupils in total, and 310 of them were selected by random sampling. They were in school for at least 3 years, in 2 separate buildings. The age range was from 16 to 19 years. One hundred and fifty-three students occupied the old building (110 years old), while 157 of the students were in the new building (19 years old). Both old and new buildings have no air-conditioning apparatus and heating is provided with central heating unit. The students in these 2 buildings were compared in respect to building types and age related symptoms like asthenia, headache, vertigo, concentration disorders, and respiratory symptoms and allergic diseases (such as asthma, allergic rhinitis, eczema), as well as sensitization to different fungi genera based on the skin prick tests (SPT). At the beginning of the study, the students were informed regarding the questionnaire and the SPT. Subjects were asked to complete a questionnaire which was specifically designed for the study. Children's health informations were collected by questionnaires with no identity informations. The questionnaire was composed of questions of: doctor-diagnosed asthma, cough and repeated wheezing episodes (≥ 2 during 12 months). We assessed vertigo, headache, asthenia, phlegm, dyspnea, allergic symptoms such as rhinitis, urticaria, and eczema of the adolescents from the questionnaire. The data collected from children have no details such as years of symptoms, symptoms prior to attending these high schools, family history, and pets. The SPT was performed on the ventral side of the forearm. After cleaning the forearm with alcohol, 0.01-0.02 ml of antigens (1:50 dilution) were placed in a parallel array to the axis of each sterile forearm at a distance of 2 centimeters apart. The Stallerpointe device was used to perform the prick test. Sites were then pricked with No. 26 hypodermic needle. *Alternaria*,

Penicillium, *Cladosporium*, *Trichophyton*, *Aspergillus*, *Epidermophyton* fungal allergens in addition to *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), *Dermatophagoides farinae* (*D. farinae*), eastern trees, mixture of 4 cereals, *Salicaceae*, *Compositae*, and feather mixture were used according to a blind protocol. An allergenic extract is considered to have a value of 100 IC/ml when the manufacturing parameters result in the same mean dilution ratio as that of standardized extracts from the same group with a value of 100 IR/ml and which are used as reference standards. For groups without a standardized reference extract, a value of 100 IC/ml corresponds to an extract for which the dilution ratio has been established by clinical experience (Stallergenes SA, Antony, France). Prick tests were also carried out with a positive control to confirm the state of reactivity of skin. Histamine diphosphate (1 mg/ml) and buffered saline diluent were used as positive and negative control. The results were recorded after 20 minutes and allergic reactions were graded as '+', '++', '+++', '++++', and '-' based on the intensity of reactions, erythema or wheal formation. For this, no reaction occurred was marked as "-", when the occurred edema diameter is equal or higher than 3 mm was accepted as positive. In positiveness, the diameter of the edema which smaller than positive control and bigger than negative one was considered as "+". When the diameter of the edema is half size of positive control, this was graded as "++". If the edema diameter is in the same size of positive control it was grade as "+++". When the edema diameter is 2-fold size of positive control, it was grade as "++++". Moisture from the building was measured by Barigo-% normal hygrometer A. This was carried out in monthly intervals. The Petri Plate Gravitational Settling method was applied for the isolation of airborne fungi due to its practical usage and low cost. Rose bengal chloramphenicol agar (Oxoid GM549, Basingstoke, Hampshire, England) and Malt Extract Agar (Merck, 1.10130, KGaA 64271, Darmstadt, Germany) in petri dishes were exposed to the air at the height of 1.5 m above the ground. Samples were collected at one month intervals from August 2003 to August 2004. Twelve localities were chosen and 3 petri samples were taken from each locality. During the sampling, windows were kept closed. Indoor air normally reflects the outdoor air. Collecting the specimens during school days or holiday months, all over the year, were carried out in order to identify the fungi flora of the study area. Cultures were incubated at room temperature ($27 \pm 2^\circ\text{C}$). Plates were initially inspected on the 4th day of incubation up to the 14th day.¹² Subsequently, mould colonies were transported

Table 1 - Prevalence of allergic diseases, respiratory and building related symptoms in newer and older buildings.

Symptoms	Newer building N (%)	Older building N (%)	P-value N (%)
Lung			
Cough	34 (21.7)	34 (22.2)	0.50
Phlegm	21 (13.4)	15 (9.8)	0.21
Dyspnea	11 (7)	14 (9.2)	0.31
Wheezing	8 (5.1)	7 (4.6)	0.52
Hemoptysis	1 (0.6)	0 (0)	0.50
Asthma	4 (2.6)	3 (2)	0.50
Skin			
Eczema	5 (3.2)	3 (2)	0.37
Urticaria	5 (3.2)	1 (0.7)	0.11
Nose			
Allergic rhinitis	21 (13.4)	15 (9.8)	0.21
Building related symptoms			
Asthenia	58 (36.9)	65 (42.5)	0.18
Headache	48 (30.6)	46 (34.6)	0.27
Vertigo	22 (14.1)	21 (13.7)	0.52
Concentration disorder	27 (17.2)	34 (22.2)	0.16

Table 2 - Prevalence of sensitization to common airborne and fungal allergens in both buildings.

Symptoms	Newer building N (%)	Older building N (%)	P-value N (%)
Allergen			
<i>Penicillium</i>	11 (7)	13 (8.5)	0.39
<i>Cladosporium</i>	9 (5.7)	7 (4.6)	0.42
<i>Trichophyton</i>	11 (7)	12 (7.8)	0.47
<i>Alternaria</i>	17 (10.8)	5 (3.3)	0.00
<i>Aspergillus</i>	8 (5.1)	6 (3.9)	0.41
<i>Epidermophyton</i>	20 (12.7)	12 (7.8)	0.10
Other allergens			
<i>D. farinae</i>	11 (7)	12 (7.8)	0.47
<i>D. pteronyssinus</i>	15 (9.6)	11 (7.2)	0.29
Eastern trees	6 (3.8)	9 (5.9)	0.28
Mixture of 4 cereals	9 (5.7)	12 (7.8)	0.30
<i>Salicaceae</i>	6 (3.8)	6 (3.9)	0.59
<i>Compositae</i>	6 (3.8)	10 (6.5)	0.20
Feather mixture	4 (2.5)	5 (3.3)	0.48

to the tubes containing Potato Dextrose Agar (Merck, 1.05398, KGaA 64271, Darmstadt, Germany). Light microscope and stereomicroscopes were used for determination of colonial characteristics and morphological structures of fungi. We identified the colonies to the genus category.¹³

All data obtained from research were evaluated statistically, using the Statistical Package for Social Science Version 10.0. Chi-square, Fisher's exact and t-tests were applied for statistical analysis. Approval for the study was obtained from the Human Research Ethics Committee of Afyon Kocatepe University.

Results. There were 98 males and 55 females from the old building and 100 male and 57 female students from the new building. The mean age of the school children from the old building was 16.01 ± 0.52 years, while from the new building 16.44 ± 1.27

years. The climate was dry and hot in summer and cold in winter, with a mean temperature of 11°C over the year. The city is placed far from the sea and the altitude is more than 1000 meters. The air humidity varied between 53.8% and 72.2%. In our study, the mean humidity was measured as 46% in the old building and 54% in the new building. The overall prevalence of allergic diseases (asthma, urticaria, allergic rhinitis, and atopic eczema) of children was 14.5% (n=22) from old building and 22.4% (n=35) from the new building. Prevalences of respiratory and building related symptoms and doctor diagnosed allergic diseases are shown in **Table 1**.

According to the SPT, *Penicillium* was the most common allergen found in the old building, while *Epidermophyton* was the most common in the newer building ($p > 0.05$) (**Table 2**). During a 12 month period, 8 fungal genera (*Penicillium*, *Cladosporium*,

Table 3 - Distribution of the total colony species according to the seasons (n).

Season	Newer building	Older building
Winter	139	80
Spring	140	108
Summer	275	198
Autumn	145	115
Total	699	501
$p>0.05$		

Table 4 - Distribution of the colony species of newer building (NB) and older building (OB) in different seasons (%).

Allergens	Spring		Summer		Autumn		Winter	
	NB	OB	OB	NB	OB	NB	OB	NB
<i>Penicillium</i>	33	39	27.2	20.7	27.6	26.5	33	45.3
<i>Cladosporium</i>	24.8	18.6	20.6	22.4	21.2	24.3	30.9	10.4
<i>Alternaria</i>	15	15.25	21.7	22.8	20	25	15.5	24.5
<i>Aspergillus</i>	6	8.5	10.87	10.4	11.8	8.8	6.2	4.7
<i>Mucor</i>	6.76	6.8	7.6	9.9	5.9	5.9	5.15	3.8
<i>Fusarium</i>	5.26	3.4	5.4	5.8	5.3	4.4	2.1	5.7
<i>Geathricum</i>	0.75	3.4	3.3	2	2.9	2.2	3.1	-
<i>Rhinosporidium</i>	3	5.1	3.3	5.8	5.3	2.9	4.12	5.7

Alternaria, *Aspergillus*, *Mucor*, *Fusarium*, *Geathricum*, *Rhinosporidium*) were isolated from Afyon high school's 2 buildings. **Table 3** shows the total number of fungal colonies according to season ($p>0.05$). **Table 4** indicates the distribution of different fungi colonies in different season. As observed in **Table 4**, the coincidence of colonies in the air was higher in summer time and the dominant genus in both buildings was *Penicillium*, followed by *Cladosporium* and *Alternaria*. In winter, *Alternaria* was the dominant genus, followed by *Penicillium*.

Discussion. Presence of moist damage in school buildings is an important risk factor for respiratory symptoms in school children. The association between moisture problems or fungi growth in buildings and respiratory symptoms of inhabitants has been documented. As there is yet no available data on building related respiratory diseases in Turkey, we evaluated respiratory and building related symptoms in respect with building type and fungi species of indoor air. At the beginning, our hypothesis was expecting to find higher fungi and moisture concentration in the old building, and more students affected with health problems. However, our results showed that the fungi concentration ($p>0.05$) and the humidity rate was higher in the new building. This might be due to the old building's structure which

was in agglomeration feature and the walls being made of stone. On the other hand, the new building was built from concrete and bricks. The frames of the windows may be another factor in this difference. While new building had polyvinyl chloride windows, the old one had wooden window frames. The levels of indoor fungi in D'harmage's et al¹⁴ study was high, and the most commonly identified fungi genera from houses were *Cladosporium* and *Penicillium*; which is relatively common fungi found in our study. Meklin et al¹⁵ found that in brick-concrete schools, the effect of moisture damage was clearly seen in higher concentrations when compared with the reference schools, and the moisture damage of the building did not alter the fungal concentrations in wooden school buildings. In our study, there was no humid damage in both buildings and the moisture amount in the old building was lower than the new building. The wooden material in window's frames can be a factor that keeps the humidity lower in the air due to the allowance of more air circulation. Although there were humidity differences in the 2 buildings, compared with the symptoms of the students, we could not find any statistically significant difference, in accordance to building-related symptoms, respiratory symptoms, asthma, and other allergic diseases ($p>0.05$). Similar to our data, Meyer et al¹⁶ reported that moulds in floor dust and building-related symptoms in adolescent school children did not show any positive correlation

between building related symptoms and the extent of moisture and mould growth in the school buildings. Our results also did not support the association among the respiratory symptoms, allergic sensitization and fungi exposure. Epidemiological data have suggested that moisture and fungi exposure may increase the risk of asthma, up to 5-folds, in school children aged 16-18 years. Although well known environmental factors such as climate may affect asthma prevalence, this was not a specific factor as other environmental factors causing related problems, such as life style in a certain area, may be a cause. Previous studies have suggested that atopic diseases and atopic symptoms, unlike respiratory infections, have no association with moisture or fungi exposure at home or in schools.¹⁷⁻¹⁹ Robertson et al,²⁰ carried out a study on 13-15 years old children (12 280 children) in order to investigate the prevalence of asthma, and he reported the prevalence of wheeze in 13-14 year olds, in the past 12 months, as 18.6%. In our study, wheezing frequency was 4.6% in the old building and 5.1% in the new building.

The difference between our findings and Robertson et al²⁰ results could be due to the variation in the ages of the studied children. Taskinen et al¹⁷ performed a clinical study in 99 children, attending school with moisture problems and compared the findings with those of 34 children from a reference school. He found that asthma was diagnosed in 9 (6.7%) children. In our study, the asthma prevalence was 2.6% in children from the old building and 2% from the new building. We found no statistically significant difference between the 2 groups in regard with allergic diseases; however, the allergic disease ratio was lower in the same childhood age. We suggest that the decreased humidity rate of our area may be responsible for this finding. Immonen et al²¹ found positive SPT to fungi in 6 out of 144 children (4%), 5 of these 6 children were from the school which had moisture problems. A general population based on epidemiologic survey in the United States²² indicated that 3.6% of the population was sensitized to the fungus *Alternaria*. Our results indicate *Alternaria* positivity of 10.8% from the new building and 3.3% from the old building ($p < 0.05$). Peat et al²³ reported that among 6388 children (age 8-11 years), the prevalence of positive SPT to *Alternaria* was 4-23.1% in 7 different climatic regions. D'Amato et al²⁴ studied the fungal skin test reactivity in various European countries among patients with suspected respiratory allergy. Approximately 3% of the patients in Portugal and 20% of the patients in Spain had a positive skin test reaction to either *Alternaria* or *Cladosporium species*. Katz et al²⁵ reported that despite of the lack of a statistically significant correlation between high fungi count, positive SPT and atopic symptoms,

there seems to be a tendency towards higher rates of symptomatology when positive SPT and fungi counts were high.

In our study, positiveness of SPT was determined. We found 7% *D. farinea* and 9.6% *D. pterenooussinus* from the new building and 7.8% *D. farinea* and 7.2% *D. pterenooussinus* from the old building. Taskinen et al¹⁶ found that house dust mites were present in 11% of the 99 children from the dampness-problem schools. Also, we found out no correlation between positivity to SPTs and fungi concentration in indoor air, in both buildings. As a result, we found a higher average humidity rate in the new building, although we started this study supposing that there were more fungi in the older building due to its age. Because it is more than 100 years old. There was no statistically significant difference between the fungal concentration in both buildings, thus there was no statistically significant difference between the school children attending the old and new buildings in view of their respiratory system symptoms, asthma, allergic diseases, building related symptoms and SPT (except *Alternaria*).

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