Investigation of indoor molds and allergic diseases in public primary schools in Edirne city of Turkey

Coskun Celtik¹, Suzan Okten², Ozlem Okutan¹, Halide Aydogdu³, Musa Bostancioglu¹, Galip Ekuklu⁴,

Ahmet Asan² and Mehtap Yazicioglu¹

Summary

Background: Studies evaluating the role of indoor molds in the development of allergic or respiratory symptoms in schools are few in childhood.

Objective: This study aimed to investigate relation between indoor molds and allergic diseases or respiratory symptoms in primary school's children in Edirne, Turkey.

Methods: Ten public primary schools were included into the study. A thorough assessment. using a questionnaire and inspection surveys was carried out. The concentration of culturable mold was assessed in the dust samples in the schools. Indoor temperature and humidity were measured. A total of 1374 students who completed valid questionnaires were included in the study, and dust-samples were collected from the schools.

Results: Cumulative and current prevalence rates of wheezing, asthma, allergic rhinitis, and atopic dermatitis were found as 31.4%, 9.3%, 16.2%, 6.0% and 13.4%, 11.9%, 15.1%, 2.1%, respectively. The most frequent mold-species detected in indoor dusts were *Cladosporium*, *Penicillium*, *Alternaria and Aspergillus*. Although the prevalence rates of allergic diseases and respiratory symptoms were high, indoor mold amounts were low in the schools in our region and no significant correlation was determined between indoor mold amount and the prevalence of these diseases in schools or classrooms.

Conclusion: Even though allergic molds are present in schools, the mold-exposure may not be an important predisposing factor for development of allergic and respiratory diseases the schools in our region. (*Asian Pac J Allergy Immunol 2011;29:42-9*)

Key words: prevalence, allergic diseases, respiratory diseases, asthma, mold, school, student, children

Introduction

Children spend most of their time indoors, whether at home or at school. Therefore, it is important to recognize and control the indoor environment for child health. Indoor molds are important biological contaminants which affect child health.^{1,2} Molds and other fungi may cause adverse effects on human health through three processes: allergy, toxicity, and infection. Many allergic diseases such as asthma, hypersensitivity pneumonia, rhinitis, eczema and urticaria may occur in asociation with exposure to indoor molds.^{2,3} The epitopes of some mold types such as Cladosporium, Aspergillus, Alternaria, and Penicillium may be the cause of allergic symptoms of varying severity in genetically predisposed persons.4-6

Most of the previous studies have reported adverse effects from dampness and visible fungal growth in homes on respiratory health.^{1,2,7} However, the studies investigating the relation between mold in the school environment and respiratory and allergic symptoms are few.

In a previous study performed in the children's homes in Edirne, it was suggested that indoor mold exposure may contribute to childhood asthma.⁷ In this study, we aimed to investigate the relation between allergic diseases or respiratory symptoms and indoor molds in the primary schools in our region.

From the ¹Medical Faculty, Department of Pediatrics, Edirne, Turkey

 $^{^{2}\}mbox{Faculty}$ of Arts and Sciences, Department of Biology, Turkey

³Arda Vocational College, Turkey

^{*}Medical Faculty, Department of Public Health, Trakya University, Edirne, Turkey Corresponding author: Coskun Celtik Email: <u>cceltik2001@yahoo.com</u>

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Methods

This epidemiological study included ten public primary schools (one rural and nine urban schools) located in Edirne city-center. The study was supported by Trakya University Academic Investigation Unit (TUBAP-533). The Ethical Committee at the Trakya University Medical Faculty approved this study. After receiving permission from the Department of National Education in Edirne, the study packages were distributed to the schools. Study packages included an introductory letter for the families, a parental consent form, and the questionnaires. A total of 2000 students from ten schools were selected from student lists of 2nd - 5th grade students (age range: 8-11 years). The numbers of classrooms and students for each school were tested by uniform distribution (p = 0.915). The questionnaires were distributed to these students. The students were selected by stratified sampling method according to sex and weighted numbers of students.

Questionnaires:

The questionnaires were the improved versions of those previously used^{8,9} (Appendix 1). Before distributing the questionnaire, it was tested with 30 parents of pupils in a classroom-excluded study. Frequently misinterpreted questions were identified and rewritten for use in the study.

A total of 1374/1500 forms (91.6%) satisfactorily completed by parents of the students were evaluated. According to the answers of the questionnaire, the students who ticked 'yes' to the presence of allergic disease diagnosis and allergic symptoms were classified as allergic subjects. The prevalence of cumulative (lifetime) and current (last 12 months) of the allergic diseases in students were determined.

In Turkey, terms such as allergic bronchitis, recurrent bronchitis, and chronic bronchitis are used instead of "asthma" by some doctors. The students who were diagnosed as having asthma or allergic bronchitis formed the "asthma group," while other students with recurrent bronchitis or chronic bronchitis were evaluated as "persistent wheezing group".

The evaluation of the questions in questionnaire part 1:

The total number of cases who had wheezing at least once in their lives was the "Cumulative wheezing prevalence". The total number of other cases who had wheezing during the last 12 months was the "current wheezing prevalence". The prevalence of "the doctor-diagnosed asthma" and "the doctor-diagnosed persistent wheezing" were identified from the questions in the first part. Also, the rate of the doctor-diagnosed asthma was accepted as the cumulative prevalence of asthma. The rate of "in the past 12 months, the symptoms associated with asthma" was evaluated as current asthma prevalence.

The evaluation of the questions in questionnaire part II:

The existence of allergic rhinitis symptoms throughout life was "the cumulative allergic rhinitis prevalence", while existence of the symptoms in the last 12 months was "the current allergic rhinitis prevalence". Moreover, at any time, the number of children who were diagnosed as having allergic rhinitis by a doctor was "the doctor-diagnosed allergic rhinitis prevalence".

The evaluation of questions in questionnaire part III:

The existence of atopic dermatitis/eczema symptoms throughout life was "the prevalence of cumulative atopic dermatitis-symptoms"; while the existence of atopic dermatitis-symptoms in the last 12 months was "the prevalence of current atopic dermatitis-symptoms". Moreover, the number of students who were diagnosed with atopic dermatitis by the doctor at any time was "the doctor-diagnosed atopic dermatitis prevalence".

Sample collection and assessment of dampness in the schools:

The dust samples were collected by using a vacuum cleaner (BEKO BKK-1182) from canteens, corridors and classrooms in the selected schools. In each dust-collection area, the equipment cleaned by was the diluted hypochlorite solution, and a new dust-filter packet was used to prevent the mold contamination. The dust samples were collected between 04.00 P.M and 05.00 P.M before the scheduled cleaning at each school. The collected dust samples were put into disposable and sealed packages and sent to the laboratory, and were processed on the same day. These procedures were carried out between December 2003 and January 2004. The reason for choosing the winter season was the fact that these months are very cold in Edirne, and windows/doors are closed, so air exchange



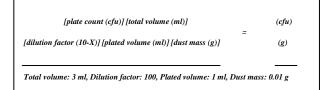
between the inside and outside is kept to a minimum.

During the collection process, the temperature and relative humidity were recorded by using thermo-hydrometer device (TFA-Dostman GmbH, Germany) inside the schools. Also, an experienced researcher noted evidence of moisture-damage; such as dampness, discolouration, musty odors and visible dust in areas of the schools. Moreover, the age of the buildings and the cleaning processes used in the schools were determined.

The production and identification of dust molds:

The method was devised according to the literature.¹⁰⁻¹² The general dust processing protocol was as follows: weighed-dust samples were suspended in measured volumes of sterile peptone water [1.5 g peptone and 0.5 g NaCl per liter of distilled water] with 0.1 g Tween 80. The mass of dust and volume of suspending liquid varied so as to provide sufficient material for individual tests. The dust samples were incubated for 10 min at room temperature to allow the dust to absorb the liquid, after which the samples were vigorously agitated. Serial dilutions were made, as required, to achieve appropriate plate counts. Dust was processed in test tubes of sufficient size to allow vigorous mixing (i.e., 10 and 25 ml of liquid in test tubes of 25 and 75 ml capacity, respectively). Suspended material was allowed to settle for 15 min (unless otherwise indicated) before aliquots of the dust suspensions were removed with a pipette at the midpoint between any floating material and the settled dust. Plating was done in duplicate or triplicate.

From the culture media only microfungi were studied only at genus level. A Rose-Bengal Streptomycin Agar was used as culture medium. The numbers of colonies were counted for microfungi between 7 and 14 days after inoculation. The concentration of culturable microfungi in a dust sample was expressed as cfu/g and calculated as shown in the following equation:



For the first-step isolation of microfungi, Rose-Bengal and Streptomycin added Pepton Dextrose Agar (RSA) was used. Streptomycin was used to prevent bacterial reproduction; Rose-Bengal was used for limitation of overgrowth of rapidly reproducing fungi such as *Rhizopus* and *Trichoderma*.

Each fungal colony was inoculated on malt extract agar (MEA) (Merck, Germany), czapekdox agar (CZ) (Merck, Germany) and potato dextrose agar (PDA) (Difco, USA) media for identification to the genus and incubated at room temperature (27 °C \pm 1) for seven days. Petri plates were first examined under a dissecting microscope (stereomicroscope). Then, we used a light microscope in order to determine the colonial features and morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on material mounted in a modified mounting medium, Lacto-Cotton Blue, proposed by Sime *et al.*¹²

Statistical analysis:

The statistical analysis was performed via statistical software package Minitab Release version -13. Normality distribution of the variables was tested by One sample Kolmogorov Smirnov test. Results are expressed as "median; mean ± SD (min-max)" or as "n (%)". Categorical variables were compared by the chi-square test; numeric variables were compared by the Mann-Whitney U-test due to the non-normal distribution. The Spearman test was used to determine the correlation between the variables. A p-value < 0.05 was considered as statistical significant.

Results

The number of valid questionnaires was 1374 (91.6%). The mean age of the students completing valid questionnaires was 9.5 ± 1.5 (8-11) years. The sex distribution was determined as 672 males (48.9%), 701 females (51.1%). Their life- time in Edirne was 5.8 ± 3.1 (1-11) years.

The prevalence rates associated with allergic disease and respiratory symptoms are shown on Table 1. In our study, as a result of valid questionnaires, cumulative and current prevalence rates of wheezing, asthma, allergic rhinitis, and atopic dermatitis were found as 31.4%, 9.3%, 16.2%, 6.0% and 13.4%, 11.9%, 15.1%, 2.1%, respectively. The prevalence rates are shown in Table 1. In our study, life time wheezing was the

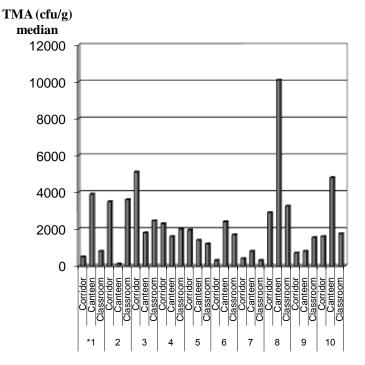
Table	1.	The	prevalence	rates	for	wheezing,
asthma	, all	ergic	rhinitis and	atopic	dern	natitis

Diseases / symptoms	Prevalence
	n (%)
Wheezing/Asthma	
Life time wheeze symptoms	432 (31.4)
In the past 12 months, wheeze symptoms	184 (13.4)
Persistent wheezing	97 (7.1)
Doctor diagnosed-asthma	128 (9.3)
In the past 12 months, the symptoms	163 (11.9)
associated with asthma	
Allergic rhinitis	
Life time symptoms associated with allergic	222 (16.2)
rhinitis	
In the past 12 months, the symptoms	208 (15.1)
associated with allergic rhinitis	
Doctor diagnosed-allergic rhinitis	101 (7.4)
Atopic Dermatitis	
Life time symptoms associated with atopic	82 (6.0)
dermatitis	
In the past 12 months, the symptoms	29 (2.1)
associated with atopic dermatitis	
Doctor diagnosed-atopic dermatitis	28 (2.0)

most frequently reported symptom; however none of the prevalence rates related with asthma, allergic diseases and respiratory symptoms among the selected schools was significantly different (p > 0.05). The distribution of total mold amounts in

the schools is shown at Figure 1. The total mold amount in school-number two and eight were significantly higher than the other schools (p < 0.05). In these two schools, we observed discolouration and dampness in the corners of the walls; in corridors and in one class of school number-2, and two classes of the school number-8. In these schools, floor moping was carried out twice weekly using only water & detergent, while in the other schools, this process was repeated four times a week by using water & detergent with added hypochlorite. Moreover the buildings of these two schools was older than those of the other schools (>50 years).

The median value of total indoor molds from the schools was 1900 cfu/g. The mold amounts when indoor areas are compared were found to be 1900; 3281 ± 2320 (680-7875) cfu/g in the classrooms, 1700; 2771 ± 2949 (110-10100) cfu/g in the canteens, 1775; 1925 ± 1573 (300-5100) cfu/g in the corridors (Figure 1). The mold amounts in the classrooms were higher than the other areas. However, there was no significant difference between the mold amounts in the indoor areas (p > 0.05).



TMA: total mold amounts;*School number.

Figure 1. Total mold amounts in indoor areas of the schools



The mean indoor temperature (°C) and relative humidity (%) were 20.3 ± 2.5 (15-25) °C and 55.4 ± 10.1 (37-86) %. The correlation between indoor mold amount and indoor temperature and indoor moisture ratio was tested with the Spearman test. A positive correlation was determined between indoor mold amount and indoor moisture ratio (r: 0.40, p = 0.001). On the other hand, a negative correlation (r: -0.54, p= 0.001) was found between indoor mold amount and indoor temperature.

Among the reproducing molds in the dust samples, *Cladosporium (30.8%)*, *Penicillium (25.8%)*, *Alternaria (%8.8) and Aspergillus (6.6%)* species were the most common. Each of the other mold species formed under 5% of total mold amount. *Stachybotrys* species were found in only two schools, both of which had moisturedamage. The reproducing mold-species in the schools is shown in Table 2.

The relation between the mold amounts and asthma, and allergic symptoms:

With the Spearman test, we tested the correlation between the amount of the mold in the schools and the cumulative prevalence rates of wheezing, doctor diagnosed-asthma, persistent wheezing, doctor-diagnosed allergic rhinitis, allergic rhinitis symptoms, doctor-diagnosed

atopic dermatitis, atopic dermatitis symptoms, total doctor-diagnosed allergic diseases, and the current prevalence rates of wheezing, allergic rhinitis symptoms, atopic dermatitis symptoms and total atopic symptoms. There was no significant correlation between the mold amount and the prevalence rates associated with respiratory symptoms or allergic diseases. The prevalence rates and total mold amounts for each school are shown in Table 3 and Table 4.

Moreover, we statistically compared the prevalence score of the two schools with the highest level mold, with that of the schools which had the lowest mold amount, by a chi-square test. We did not find a significant difference between them (p > 0.05).

Discussion

Indoor molds in schools may be hazardous to the health of children. The exposure to indoor molds can be decreased through renovation of buildings. Meklin *et al.*¹³ have suggested that the prevalence of respiratory symptom is higher among students in school with moisture-damage and this problem could be solved by repair of their living areas.

In most studies, it was determined that the species of *Cladosporium, Penicillium, Alternaria*

Table 2. The distribution of fungus species in indoor dust in schools

Fungus species	Mold amount (cfu/g)							
	Total	%	Median	Mean ± SD	(Range)			
Cladosporium spp.	85600	30.77	5800	8560 ± 7429	1500-25400			
Penicillium spp.	71700	25.77	4450	7170 ± 7603	1500-27300			
Alternaria spp.	24600	8.84	1950	2460 ± 2209	200-8200			
Aspergillus spp.	18250	6.56	1325	1825 ± 1699	100-5700			
Mycelia sterilia.	13150	4.73	1200	1345 ± 795	300-2700			
Ulocladium spp.	5900	2.12	250	590 ± 931	0-2700			
Rhizophus spp.	5050	1.82	500	525 ± 244	100-900			
Scopulariopsis spp.	4350	1.56	350	435 ± 497	0-1300			
Nonidentified	3900	1.40	0	390 ± 1130	0-3600			
Drechslera spp.	2700	0.97	200	270 ± 327	0-1100			
Verticillium spp.	2600	0.93	150	260 ± 306	0-800			
Paecilomyces spp.	2300	0.83	0	230 ± 727	0-2300			
Acremonium spp.	2210	0.79	100	221 ± 315	0-800			
Phoma spp.	2100	0.75	100	210 ± 277	0-900			
Trichoderma spp.	1700	0.61	100	170 ± 205	0-500			
Fusarium spp.	800	0.29	100	80 ± 79	0-200			
Mucor spp.	300	0.11	0	30 ± 95	0-300			
Stachybotrys spp.	300	0.11	0	30 ± 67	0-200			
Trichothecium spp.	200	0.07	0	20 ± 63	0-200			
Mycophyta spp.	100	0.04	0	10 ± 32	0-100			
Chaetomium spp.	100	0.04	0	10 ± 32	0-100			
Curvularia spp.	100	0.04	0	10 ± 32	0-100			
Humicola spp.	100	0.04	0	10 ± 32	0-100			



School No	\mathbf{n}^{+}	I*	Ш	ш	IV	v	Total mold amount [cfu/g (%)]
1	172	50 (29.1)	8 (4.7)	18 (10.5)	15 (8.7)	6 (3.5)	16600 (6.7)
2	178	42 (23.6)	27 (15,2)	5 (2.8)	9 (5.1)	26 (14.6)	49190 (19.3)
3	135	47 (34.8)	18 (13.3)	6 (4.4)	16 (11.9)	15 (11.1)	36300 (14.0)
4	142	45 (31.7)	19 (13.4)	6 (4.2)	13 (9.2)	18 (12.7)	18700 (7.8)
5	109	41 (37.6)	11 (10.1)	9 (8.3)	11 (10.1)	10 (9.2)	16350 (6.8)
6	120	33 (27.5)	13 (10.8)	8 (6.7)	10 (8.3)	9 (7.5)	18850 (7.5)
7	192	76 (39.6)	26 (13.5)	21 (10.9)	21 (10.9)	23 (12.0)	4900 (1.9)
8	121	41 (33.9)	28 (23.1)	14 (11.6)	14 (11.6)	27 (22.3)	55300 (22.8)
9	118	30 (25.4)	22 (18.6)	9 (7.6)	11 (9.3)	18 (15.3)	17400 (7.1)
10	87	27 (31.0)	12 (13.8)	1 (1.1)	8 (9.2)	11 (12.6)	13700 (6.1)
							278190 (100)

Table 3. Prevalence rates for wheezing and asthma, and Total Mold Amounts for Each School

⁺n: The valid questionnaire count; *I: Life time wheeze symptoms; II: In the past 12 months, wheeze symptoms; III: Persistent wheezing; IV: Doctor diagnosed-asthma; V: In the past 12 months, the symptoms associated with asthma.

and Aspergillus were the most common among the indoor molds in the environment, and that they showed seasonal changes.^{3,10,12,14,15} Gomez de Ana et al.¹⁴ evaluated the seasonal distribution of the species of Alternaria, Aspergillus, Cladosporium and Penicillium both at indoors and outdoors. It was determined that in the autumn and winter months, these molds were found at a higher level indoors than outdoors. In our region, in a previous study carried out between 2001-2002 and in January, the concentration percentages of indoor molds such as Penicillium, Cladosporium and Alternaria species in the schools were found to be 42.8%, 19.3% and respectively¹⁶. 10.0%, In our study, Cladosporium, Penicillium, Alternaria and Aspergillus species were determined to be 30.8%, 25.8%, 8.8%, and 6.6%, respectively. According to our data, the indoor concentration of Cladosporium spp. was higher than that of the previous study carried out in our region. Probably, the dominant indoor mold type changed in the period between 2002 and 2004.

Indoor mold concentrations can be affected by those outdoors. In a study, *Cladosporium* (81.5%), *Penicillium* (5.2%), *Chrysosporium* (4.9%), *Alternaria* (2.8%) and *Aspergillus* (1.1%) species were found to be the most frequent in the outdoors, among all molds.¹⁷ Also, in this study, it was found that the mold species indoors and outdoors were similar, but the concentrations of indoor *Penicillium* and *Aspergillus* species were higher than outdoors. Thus, it was suggested that indoor *Penicillium* and *Aspergillus* species played a primary role in allergy development.¹⁷ On the

other hand, there are studies which suggest that *Cladosporium, Aspergillus* and *Alternaria* species are also important in the development of allergic diseases.^{1,2,18} In our study, the dust samples were collected between December and January in order to decrease the contamination from the outside. Also, the same indoor molds have been determined to be similar to those identified in previous studies.

In a study by Santilli and Rockwell¹⁹, it was emphasized that for a healthy environment, the amount of mold must be less than 1000 spores/ m^3 . Also, in the European Collaborative Action (ECA) report prepared by Wanner *et al.*²⁰, according to the airborne dust-collecting method, mold contamination of less than 200-500 cfu/m³ or 20000 cfu/g has been classified as low contamination. A study by Levetin et al.²¹ indicated that even on a single day, the indoor mold amounts were variably associated with geographic and climatic changes in environment and a concentration of viable fungi up to 6000 cfu/m^3 might be a problem for atopic students. The mold levels in our study couldn't be compared with the values given as cfu/m^3 , for in our study it was measured as cfu/g. According to the data in our study, total indoor mold amount was found to be 1900 cfu/g. This value was a low level according to the classification in the ECA-report.²⁰

The other aim of our study was to investigate the relationship between the indoor mold amount and allergic diseases. In our study, as a result of valid questionnaires, cumulative and current prevalence rates of wheezing, asthma, allergic

School No	\mathbf{n}^+	I*	п	ш	IV	v	VI	Total Mold Amount [cfu/g (%)]
1	172	24 (14.0)	18 (10.5)	13 (7.6)	7 (4.1)	6 (3.5)	5 (2.9)	16600 (6.7)
2	178	10 (11.5)	26 (14.6)	15 (8.4)	5 (2.8)	3 (1.7)	4 (2.2)	49190 (19.3)
3	135	27 (15.2)	28 (20.7)	13 (9.6)	13 (9.6)	4 (3.0)	2 (1.5)	36300 (14.0)
4	142	26 (18.3)	24 (16.9)	12 (8.5)	8 (5.6)	2 (1.4)	1 (0.7)	18700 (7.8)
5	109	21 (19.3)	14 (12.8)	8 (7.3)	8 (7.3)	1 (0.9)	3 (2.8)	16350 (6.8)
6	120	14 (11.7)	10 (8.3)	9 (7.5)	10 (8.3)	5 (4.2)	5 (4.2)	18850 (7.5)
7	192	35 (18.2)	29 (15.1)	12 (6.3)	7 (3.6)	4 (2.1)	1 (0.5)	4900 (1.9)
8	121	31 (25.6)	25 (20.7)	5 (4.1)	13 (10.7)	2 (1.7)	4 (3.3)	55300 (22.8)
9	118	24 (20.3)	21 (17.8)	10 (8.5)	9 (7.6)	1 (0.8)	3 (2.5)	17400 (7.1)
10	87	10 (11.5)	13 (14.9)	4 (4.6)	2 (2.3)	1 (1.1)	0 (0.0)	13700 (6.1)
								278190 (100)

Table 4. Prevalence rates for allergic rhinitis and atopic dermatitis, and Total Mold Amounts for each school

*n: The valid questionnaire count; *I: Life time symptoms associated with allergic rhinitis; II: In the past 12 months, the symptoms associated with allergic rhinitis; III: Doctor diagnosed-allergic rhinitis; IV: Life time symptoms associated with atopic dermatitis; V: In the past 12 months, the symptoms associated with atopic dermatitis; VI: Doctor diagnosed-atopic dermatitis.

rhinitis, and atopic dermatitis were found as 31.4%, 9.3%, 16.2%, 6.0% and 13.4%, 11.9%, 15.1%, 2.1%, respectively. In Ankara, the capital city of Turkey, in a study which was conducted on primary school students, the cumulative and current prevalence rates of wheezing, asthma, allergic rhinitis and atopic dermatitis were determined to be 22.5%, 16.8%, 18.7%, 6.5%, and 13.3%, 9.8%, 14.1%, 4.3%, respectively.⁸ If the results of these two studies are compared, it can be seen that the cumulative wheezing prevalence is higher; however cumulative prevalence rates of asthma and the other allergic disturbances are the lower in our city. This condition may with be related low industrialization in Edirne region.

The overall prevalence of fungal hypersensitivity ranges from 3% to 91% in the general population, and from 7% to 50% in children with asthma.² The rate of monosensitization to mold-species is not known in children, because there are few studies investigating mold allergy in children; however, this frequency was measured as approximately 5% in a study by Kang *et al.*²²

Most of the studies in children have showed a relationship between allergic symptoms, especially respiratory symptoms, and indoor molds.^{2,23,24} However, some studies have not reported such a relationship.^{25,26} In a study from Turkey, it was found that the mold amounts were low in the homes of children who had mold hypersensitivity, and therefore the relationship between mold amount and allergic symptoms could not be determined. They suggested that this result could be due to the number of only mold-sensitized children was less in their case

series, or that the mold amount was low in the environment.²⁷ In our study, we did not find a significant correlation between the mold amount and the prevalence of asthma, allergic diseases and allergic symptoms in the schools. This could be due to the low mold exposure in the schools in our region. On the other hand, the sensitization against other allergens such as pollens and dust mites may play the most important role in the progression of allergic diseases in children in our region.

Allergic diseases are known to have multifactorial origin. In a previous study on allergic children in our region it was found that mite and pollen sensitivities were higher than that for molds as dertermined by skin prick tests. In this study, no mono-sensitization against molds was determined, however the sensitization rate of mixed-allergen included mold was 32.3%.²⁸

The indoor mold concentration was low at the primary schools in our region, thus any relation between indoor school molds and allergic diseases or respiratory symptoms could not found. Moreover, non-mold allergens can be more important factors in allergic and respiratory diseases in our region. However, this study is preliminary. Therefore, additional studies in which the diagnosis is confirmed by skin-prick tests should be planned.

Appendix

The questions in the questionnaire

A. PART 1: Questions related with asthma and respiratory symptoms

- 1) Has your child <u>ever</u> had wheezing or whistling in the chest?
- 2) Has your child had wheezing or whistling <u>in the last 12 months</u>?



- 3) Has your child <u>ever</u> had asthma or allergic bronchitis diagnosed by a doctor?
- 4) Has your child <u>ever</u> had recurrent bronchitis or chronic bronchitis diagnosed by a doctor? (Except asthma).
- 5) Has your child <u>ever</u> had respiratory symptoms such as dispnea, shortness of breath, persistan dry cough provoked by smoke, perfumes, pollen exposure, and exercise? (without having cold or chest infection)

B. PART 2: Questions related with allergic rhinitis

- 1) Has your child <u>ever</u> had recurrent sneezing, or runny, or a blocked nose, or a problem associated with itchy-watery eyes without cold or flu?
- 2) <u>In the past 12 months</u>, has your child had one or a few of above mentioned complaints? (without cold or flu)
- 3) Has your child <u>ever</u> had hay fever or allergic rhinitis/rhino-conjunctivitis diagnosed by a doctor?

C. PART 3: Questions related with atopic dermatitis/atopic eczema

- 1) Has your child <u>ever</u> had itchy rash continued for his/her infancy period?
- 2) Has this itchy rash <u>at any time</u> affected the body areas such as folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?
- 3) Has your child ever had eczema?
- 4) <u>In the past 12 months</u>, has your child had this itchy rash/eczema attack?
- 5) Has your child <u>ever</u> had atopic dermatitis/eczema diagnosed by a doctor?

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