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CHILDREN'S ALLERGIC DISEASES AND MICROBIAL CONTAMINATION OF INDOOR AIR – A CASE REPORT

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Abstract: The assessment of exposure to bioaerosols in damp houses of two children who suffered from perennial rhinitis and asthma was performed. The paper presents an approach to the complex (i.e., medical and environmental) treatment of allergic diseases.

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Key words: children, allergic sensitization, indoor air, damp houses, bacteria, fungi.

INTRODUCTION

Sensitization of children to indoor allergens has been demonstrated to be one of the major risk factors for development of allergic diseases, especially for asthma and allergic rhinitis [1, 2, 5]. In the indoor environment, where children spend more than 80% of their time, such effects of exposure to microbial allergens (if occurred) can be significantly pronounced. Hence, the determination of the degree of exposure to agents of microbial origin seems to be of a great value [4]. The aim of the study was to evaluate an exposure to bacterial and fungal aerosols of two children living in two independent dwellings and suffering from perennial allergic rhinitis and difficult to treat asthma, respectively.

METHODS

Medical examination. The anamnesis, routine physical and laryngological examinations, X-ray examinations of chest and sinus, spirometric examination, and routine laboratory tests were performed.

Immunological tests. Immunoglobulin IgA, IgM, IgG serum concentrations were determined by the turbidimetric method (Behring, Germany). Total IgE and specific IgE serum concentrations were determined using chemoluminescence method. Lymphocyte populations and subpopulations were determined by flow cytometry.

Allergy skin prick tests (Allergopharma, Germany) were performed according to the recommendations of the European Academy of Allergology and Clinical Immunology [9]. Tests were considered positive if skin wheals had a mean diameter at least of 3 mm.

Bioaerosol measurements. Bacterial and fungal aerosol samples were taken using a 6-stage Andersen impactor. Microorganisms were collected on agar plates: bacteria on trypticase soy agar and fungi on 2% malt extract agar. After sampling, all plates were incubated for 7 days at room temperature. The microbial concentrations were calculated as colony forming units per cubic meter of the air (cfu/m^3) using the positive hole correction table. Identification of fungal strains was conducted microscopically according to taxonomic monographs.

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CASE DESCRIPTION

Case 1. Clinical characteristics of patient A.K.

Patient's history. The 13-year-old patient with perennial rhinitis and a negative history of atopy was admitted to the Pediatric Department because of 2 years' sneezing, rhinorrhoea, blockage of nose, and periodical nasal bleeding. An exacerbation of symptoms was observed during rainy days, autumn and winter, and remission during holidays.

Medical examination showed rhinitis, swelling of nasal mucosa and deviation of the nasal septum. No abnormalities within other organs were found. Sinusitis was excluded based on sinus X-ray examination. The results of spirometry ranged within the normal values.

***In vitro* tests.** Immunoglobulin concentrations in serum were as follows: IgA - 81 mg/dl, IgM - 167 mg/dl, IgG - 833 mg/dl, IgE total - 6.33 kU/l (normal value). Populations and subpopulations of lymphocytes T and B: lymphocytes B - 18% (normal value, N: 12-22%), lymphocytes T - 54% (N: 66-76%), lymphocytes Th - 38% (N: 33-41%), lymphocytes Ts - 25% (N: 27-35%), Th/Ts ratio - 1.52 (N: 1.1-1.4), lymphocytes NK - 15% (N: 9-16%), active lymphocytes T - 8% (N: 9.5-17%).

Allergy skin prick tests: positive control (histamine) - 4 mm wheal size; negative control - negative; positive skin prick tests for: moulds mixture - 3 mm wheal, flush - 8 mm, *Candida albicans* - 3 mm wheal, *Rhizopus nigricans* - 4 mm wheal, flush - 10 mm, *Fusarium moniliforme* - 4 mm wheal, *Alternaria tenuis* - 3 mm wheal, flush - 5 mm (delayed reaction), *Cladosporium herbarum* - 3 mm wheal, flush - 5 mm (delayed reaction).

Final diagnosis. Perennial allergic rhinitis resulting from sensitization to moulds.

Household characteristics. Flat located in 20-year-old building on the second floor with a leaking roof. Area of the flat: 60 m^2, 3 rooms occupied by 4 persons. In the kitchen and child's room there were visible mould growths on the walls and fitted carpets. Inhabitants were non-smokers. Pets were not present. Microclimate parameters (September): temperature: indoor 22ºC, outdoor 10ºC; relative humidity: indoor 62%, outdoor 68%.

Case 2. Clinical characteristics of patient Sz.K.

Patient's history. The 9-year-old patient with bronchial asthma was admitted to the Paediatric Department to investigate the cause of frequent asthma exacerbations. A family history of atopy (mother and brother) was positive. The patient suffered from atopic dermatitis and recurrent wheezing in pre-school age. Bronchial asthma was diagnosed at the age of 7. One year after, seasonal (May and June) rhinitis and conjunctivitis started to be noticed. Despite asthma treatment, a persistent cough and wheezing were present on the majority of the days and mainly at night were observed. The remission of symptoms was reported after longer periods when leaving home.

Table 1. Bacterial and fungal aerosol concentrations (cfu/m^3) in the child A.K.'s flat.

Particle size (μm)	Bacteria		Fungi	
	indoors	outdoors	indoors	outdoors
>7.0	106	106	21	85
7.0-4.7	92	85	42	318
4.7-3.3	71	71	106	516
3.3-2.1	85	21	205	919
2.1-1.1	339	21	184	389
1.1-0.65	57	0	0	0
Total	750	304	558	2,227

Medical examination. Laryngological examination revealed deviation of the nasal septum, blockage of nose, and hypertrophy of tonsils. Sinusitis was excluded based on sinus X-ray examination. Physical examination of the chest showed bilateral expiratory wheezing without crackles. Pneumonia, atypical infection and other pathology were excluded by the chest radiography and laboratory tests. Spirometric tests revealed obturation.

***In vitro* tests.** Immunoglobulin serum concentrations were as follows: IgA - 160 mg/dl, IgM - 185 mg/dl, IgG - 1420 mg/dl, IgE total - 19 kU/l, Specific IgE for Grass Mix Early Blooming - 51.43 IU/ml - V class, IgE specific for Tree Mix 1-1.12 IU/ml - II Class, IgE specific for Fruit Mix 1 IU/ml, mites *Dermatophagoides pteronyssinus* and *D. farinae* < 0.20 IU/ml - negative. Populations and subpopulations of lymphocytes T and B: lymphocytes B - 19% (N: 12-22 %), lymphocytes T - 60% (N: 66-76%), lymphocytes Th - 38% (N: 33-41%), lymphocytes Ts - 25% (N: 27-35%), Th/Ts ratio - 1.52 (N: 1.1- 1.4), lymphocytes NK - 12% (N: 9-16%), active lymphocytes T - 9% (N: 9.5-17%).

Allergy skin prick tests. Positive control (histamine) - 4 mm wheal; negative control - negative; positive skin prick tests: mould mixture 1-3 mm wheal (delayed reaction), mould mixture 2-3 mm wheal (delayed reaction), *Aspergillus fumigatus* - 3 mm wheal, reaction persistent to 72 hours, *A. tenuis* - 3 mm wheal, *Penicillium notatum* - 3 mm wheal (delayed reaction), hazel - 3 mm wheal, birch - 4 mm wheal, grass pollen - 5 mm wheal, flush - 30 mm, rye pollen - 4 mm wheal; positive skin prick tests for food allergens: rye flour - 4 mm wheal, egg yolk - 3 mm wheal.

Final diagnosis. Atopic bronchial asthma with sensitization to mould, seasonal rhinoconjunctivitis, and food allergy.

Table 2. Bacterial and fungal aerosol concentrations (cfu/m^3) in the child Sz. K.'s flat.

Particle size (μm)	Bacteria		Fungi	
	indoors	outdoors	indoors	outdoors
>7.0	629	290	28	14
7.0-4.7	728	212	78	71
4.7-3.3	1590	233	212	42
3.3-2.1	700	120	883	85
2.1-1.1	834	42	1823	49
1.1-0.65	297	57	14	7
Total	4778	954	3038	268

Household characteristics. Flat located in a more than 50-year-old building on the ground floor. Area of the flat: 45 m^2, 2 rooms occupied by 6 persons. Fungal growth and moisture condensation were visible on the external wall of the building as well as in the living room and bedroom walls and fitted carpets. Animals at home: dog and rabbit. All 4 small children living there were exposed to cigarette smoke. Microclimate parameters (March): temperature: indoor 23ºC, outdoor 10ºC; relative humidity: indoor 62%, outdoor 68%.

RESULTS OF BIOAEROSOL MEASUREMENTS

The results of determinations of bacterial and fungal aerosol concentrations and fungal identification are presented in Tables 1-3. Since the limit values for bioaerosols in non-industrial indoor environments are not widely accepted, the obtained results need to be compared with the available proposals for such values. Based on the Polish proposals [4], the obtained bioaerosol concentrations did not exceed residential limit values, i.e., 5,000 cfu/m^3 for total mesophilic bacteria and fungi, respectively. However, in the child Sz. K.'s flat, both bacterial and fungal indoor concentrations were relatively high and significantly higher than outdoors. Regarding the fungal contamination (presence of species that are frequently found in homes with moisture problems: *A. versicolor* was the most prevalent species, followed by *Cladosporium sphaerospermum*, and *Penicillium brevicompactum*), such an improper relation between indoor and outdoor concentrations confirms the presence of indoor emission source of these microorganisms, their abundant dissemination in the air, and may be responsible for the diagnosed serious health outcomes of this child.

Table 3. Species composition of fungal aerosol in the child Sz. K.'s flat.

Species	Number of identified strains	Percentage (%)
Aspergillus versicolor	160	42.9
Mycelia sterilia	119	31.9
Penicillium brevicompactum	24	6.4
Penicillium verrucosum	22	5.9
Cladosporium sphaerospermum	34	9.1
White yeasts	1	0.3
Unknown	10	2.7
Doratomyces purpureofuscus	2	0.5
Aspergillus ornatus	1	0.3
Total number of strains	373	100

DISCUSSION

In the analyzed cases the therapeutic difficulties were connected with home environment. The concentration of bacterial and fungal aerosols in the studied flats were higher than that measured simultaneously in the outdoor environment, and were also higher than those admitted by the scientific literature as "normal" or "healthy" [3, 4]. In patient A. K. the symptoms of perennial allergic rhinitis appeared during intensive rainfalls occurring in southern regions of Poland. Since runny and stuffy nose still remained, despite of the treatment, the patient was diagnosed towards the allergic etiology. The performed skin tests revealed sensitization to moulds occurring usually indoors (*R. nigricans*, *F. moniliforme*) as well as to these associated with outdoor environment, which are a frequent cause of allergic reactions (*A. tenuis*, *C. herbarum*). The exposure to mould allergens may be responsible for immunological reactions mediated by IgE or IgG, as well as for cellular (lymphocyte) type reactions [6]. The etiology of symptoms observed in the diagnosed patient was established based on skin prick tests. Delayed hypersensitivity reactions to several fungal allergens was observed. As this type of immunological response may occur in sensitization to moulds, the skin prick test should be assessed not only after 15 minutes but also after 20-30 minutes and later (6-72 hours). Clinical diagnosis excluded in the patient A. K. such diseases as: atopy, immunity disorders and the presence of inflammatory foci in sinuses. The remission of symptoms of allergic rhinitis was noticed after the therapy lasting for 2 years and simultaneous remediation action performed in this flat.

In the case of patient Sz. K, a typical "allergic march" was observed. Administered proper pharmacotherapy for bronchial asthma did not give expected results. A detailed clinical diagnostics excluded immunity disorders, and the presence of inflammatory foci stimulating exacerbation of bronchial asthma. The symptoms in the form of cough and dyspnoea retreated outside the household environment, which encouraged us to conduct environmental investigations. The cramped conditions of the flat, the presence of animals, moisture and visible mould growth on indoor surfaces in the children's bedrooms were major factors responsible for the elevated level of bioaerosols. Besides, several identified fungal species belonging to the *Aspergillus* and *Penicillium* genera may be responsible for mycotoxins production and volatile organic compounds emission [1, 2]. These mould products can elicit adversely influence both the respiratory and immunological systems.

Exposure to *Aspergillus* spores is connected with an increased risk of atopy [2, 5]. The spores of *Aspergillus* and isolated representatives of genus *Penicillium* belong to the most important allergenic fungi indoors [2]. In the examined child, there was observed an intensified reaction to *A. fumigatus* in skin prick test, however, 2 different species were isolated in the household environment. Nevertheless, it is important to remember that many fungi share common antigens, even if they have distinct taxonomical classification [7, 8]. After 2 years of the change of living conditions and stopping of cigarette smoking in the presence of the child (which was strongly recommended after environmental evaluation) a stable course of disease without exacerbations was observed.

CONCLUSION

Correlation between the medical treatment and environmental exposure assessment is of a great value for the complex treatment of allergic diseases in children.

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