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The dynamics of the clinico-functional indices in patients with bronchial asthma on the background of antifungal sublingual allergen-specific therapy

Key words: *bronchial asthma, fungal allergy to domestic mold, sublingual allergen-specific immunotherapy, parameters of external respiration function, cellular sputum composition, side effects.*

Despite the fact that research on fungal allergy has been ongoing since the 19th century, remain many unresolved questions due to influence of micromycetes on the pathogenesis of bronchial asthma (BA) and other allergic diseases. Today, studies on the importance of fungi from the genera Alternaria, Cladosporium, Penicillium, Aspergillus, and Malassezia are well documented in the development or deterioration of the course of allergic diseases [4, 7]. In particular, A. alternata spores are powerful source of environmental aeroallergens that can increase the severity of asthma [5]. Among the allergenic proteins of this micromycete, the main allergen Alt A 1 has been described, which can be a marker of primary sensitization to A. alternata, as well as a starting factor in the development of polysensitization to many related and unrelated allergens.

In practice, when IgE antibodies against one fungal allergen bind to structures of other allergens the precise diagnostics of fungal allergy is difficult due to the existence of cross reactivity. Particularly, from 23 allergenic proteins of A. fumigatus in 13, a high similarity of structure with allergens of other micromycetes was demonstrated. It can be explained by the evolutionary similarity of organisms. which causes a high degree of similarity of protein structures with the presence of cross reactivity on IgE [2, 11, 13]. At the same time, half of the cross-reacting fungal allergens show cross reactivity with non-fungi (in particular, with human proteins), which can cause the formation of severe chronic allergic diseases in humans based on reactions with autoantigens that can form and become defenseless and accessible if the cells damaged of inflammatory process [9, 11].

There are many questions about allergen-specific therapy (ASIT) with extracts of fungi when fungal allergy is detected. Thus, most researchers believe that immunotherapy with fungal extracts is possible, but in most countries is not widely recommended because of problems with standardization of extracts and frequent occurrence of side effects [6, 8, 9, 14]. In addition, the use of fungal extracts for immunotherapy prevents huge number of fungal species that may be a source of allergies in the absence of knowledge on the influence of many forms.

Generally, in recent years, on the base of using molecular biology techniques was isolated and characterized a lot of number of fungal allergens (recombinant forms were obtained), some of which are tested in clinical studies and demonstrated a high specificity in the diagnosis fungal allergy, such as allergic bronchopulmonary aspergillosis [1, 3, 10, 12].

The aim of this work was to study the efficiency of sublingual allergen-spesific immunotherapy in patients with persistent-mild and persistent-moderate asthma with positive skin test to fungal mix allergens (household mold mix).

Materials and methods

This study was performed as a prospective, randomized, open-labeled, clinico-functional, laboratory and allergy

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examination of 106 allergy patients with asthma [mean age (52,7 ± 1,2) years, including 80 women (75.5%)]. 45 patients (42.5%) of them had asthma in remission with positive skin test to fungal allergens mix (aged 20 to 70 years), the average age (46,6 ± 1,3) years, including 39 women (86 7%). Patients with asthma II stage (mild persistent) were 18 people (40.0%), moderate persistent – 27 people (60.0%). Disease duration was (7,7 ± 1,1) years, the incidence of asthma exacerbations – (2,2 ± 0,4) times / year. Forced expiratory volume in 1 second was on average (88,7 ± 1,8)%, forced vital capacity – (88,9 ± 1,7)%, peak expiratory flow rate – (83,8 ± 1,6)% (measurements were carried out using an «Пульмовинд», Ukraine). To evaluate cellular characteristics of sputum composition (spontaneous or induced) conducted its microscopic examination of Gram staining of smears. Patients were examined before the test, after 6 months and after 12 months of treatment.

Allergic study conducted by skin tests (prick test) with fungal mix allergens (household mold mix), mainly interior (Aspergillus fumigatus, Aspergillus niger, Penicillium spp., Mucor spp., Rhizopus spp.) (Production Sevafarma, Czech Respubliyka) with the test fluid and positive controls (histamine) controls. About of specific reaction to the allergen evaluated by the lack of response to test control and in the presence of a positive reaction to histamine. Evaluation of skin tests performed in 15–20 minutes (immediate type reactions). The response was assessed by measuring of 3 mm induration or more, on average – (8,4 ± 0,5) mm.

Treatment was conducted by sublingual method using fungal mixed allergens mainly of interior (Aspergillus fumigatus, Aspergillus niger, Penicillium spp., Mucor spp., Rhizopus spp.) (Sevapharma, CZ) during the year under the scheme, which is specified in instructions, with a gradually increase the dose of allergens. During the year of treatment, patients received 6 bottles of the drug from different concentrations of allergens. No patient withdrew from the study.

Storage of research results and their mathematical processing was carried out using licensed software products that are included in the package Microsoft Office Professional 2000 (license Russian Academic OPEN No Level № 43437596).

The arithmetic mean value (M), mean deviation (σ) and error of the arithmetic mean (m) were determined. Comparison of the average values of the group and assessment of the significance of differences was performed parametric and non-parametric methods of variation and rank statistics using t-test probability Students or two selective Wilcoxon test [8]. Calculation of criterion values and confidence intervals (CI) was held at a given confidence level $p \le 0,05$.

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Results and Discussion

During the ASIT, positive dynamics of the main ERF indices (FVC, FEV1, PEFR, FEF 25–75%) was established in 6 months ASIT, and also after 12 months ASIT (Table 1).

Simultaneously, study of the dynamics of the cellular composition of sputum showed a significant decrease of leukocytes, mainly due to eosinophils (Table. 2).

After 12 months of treatment reexamination showed the skin sensitivity to antifungal mixed-allergens decreased from (8.4 ± 0.5) mm to (1.8 ± 0.4) mm, $p < 0.05$.

Thus, against the background of sublingual ASIT with antifungal allergens of asthma patients during the

Table 1

Dynamics of indices of external respiratory function in asthma patients under the influence of antifungal antigen-specific therapy, (M ± m)%, (n = 45)

Indices	Groups of patients		
	Before treatment	6 months after ASIT	12 months after ASIT
FVC (Forced vital capacity)	88,7 ± 1,8	96,4 ± 1,3	98,8 ± 1,2
	p1,2, p1,3		
FEV1 (Forced expiratory volume in 1 second)	88,9 ± 1,7	96,9 ± 1,5	101,8 ± 1,3
	p1,2, p1,3	p2,3	
PEFR (Peak expiratory flow rate)	83,8 ± 1,6	93,5 ± 0,9	99,2 ± 0,8
	p1,2, p1,3	p2,3	
FEF 25–75% (Forced expiratory flow 25–75% FVC)	77,9 ± 2,0	92,3 ± 1,5	99,4 ± 1,5
	p1,2, p1,3	p2,3	

Notes:
1) p1,2 – the difference between this indicator and the indicator of the group examined 6 months after ASIT was statistically significant (< 0,05);
2) p1,3 – the difference between this indicator and the indicator of the group examined 12 months after ASIT was statistically significant (< 0,05);
3) p2,3 – the difference between this indicator and the indicator of the group examined 6 months after ASIT was statistically significant (< 0,05).

Table 2

Dynamics of cellular contents in sputum of patients with asthma under the influence antifungal allergen-spesific therapy, (M ± m) cells in the field of view, (n = 45)

Indices	Groups of patients		
	Before treatment	6 months after ASIT	12 months after ASIT
Leukocytes	24,3 ± 2,0	16,4 ± 1,4	12,8 ± 1,2
	p1,2, p1,3	p2,3	
Granulocytes	2,4 ± 0,7	1,8 ± 0,6	1,3 ± 0,5
Eosinophils	4,0 ± 0,7	2,3 ± 0,6	1,8 ± 0,6
	p1,2, p1,3		

Notes:
1) p1,2 – the difference between this indicator and the indicator of the group examined 6 months after ASIT was statistically significant (< 0,05);
2) p1,3 – the difference between this indicator and the indicator of the group examined 12 months after ASIT was statistically significant (< 0,05);
3) p2,3 – the difference between this indicator and the indicator of the group examined 6 months after ASIT was statistically significant (< 0,05).

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Table 3

Frequency of side effects in 45 asthma patients in carryng antifungal allergen-specific therapy, % of patients, n – their absolute number (persons)

Side effect	Frequency of occurrence		Side effect	Frequency of occurrence	
	%	n		%	n
Cough	53,3	24	Weakness	4,4	2
Runny nose	46,7	21	Fatigue	4,4	2
Sneezing	42,2	19	Swelling of the tongue	2,2	1
Itching	35,6	16	Difficulty breathing (shortness of breath)	2,2	1
Swelling of the throat	31,1	14	Hives	2,2	1
Lacrimation	13,3	6	Fever	0,0	0
Sleepiness	6,7	3	Anaphylaxis	0,0	0
Pyrosis	4,4	2	Broncho-spasm	0,0	0

year showed an increase in external respiration rates, improvement of sputum cell composition (a decrease in the leukocyte content mainly due to eosinophils) and decrease in skin sensitivity to fungal mixed -allergens – which indicated the effectiveness of ASIT in complex treatment of patients.

None of the patients had serious side effects, but some kind of reactions were noted in 44 patients (97.8%). The most often simptoms as coughing, runny nose, sneezing, swelling of the throat, itching (in 53% –31% of patients) were determined (Table 3). All the side effects were not severe, mainly in 30–60 minutes after the first taking of allergens (the patients used an antihistamine drug pill), disappeared after 2–3 hours. In the vast majority of patients with repeated taking of the medicines (without increasing the dosage) the side effects were less pronounced and did not occur on the 3–5th day of treatment.

Conclusions

1) Positive reactions of immediate type to fungal skin allergens in adults with mild persistent and moderate bronchial asthma (BA) are indications for antifungal sublingual allergen-specific immunotherapy (ASIT).

2) Antifungal sublingual ASIT on the background of basic therapy in patients with BA during the year, has a clear positive effect, which is manifested in the increase of indices of external respiration (FVC, FEV1, PEFR, FEF 25–75%), improvement in sputum cell composition (decrease in leukocyte count mainly due to eosinophils) reduction of skin sensitivity to fungal mixed-allergens.

3) Mild side effects are detected in 98% of patients with asthma during antifungal sublingual ASIT, most often in the form of cough, runny nose, sneezing, tickle in throat, itchy skin (in 53% –31% of patients).

4) The positive effect of antifungal sublingual ASIT in patients with mild persistent and moderate bronchial asthma with positive immediate reactions to fungal skin allergens suggested the important role fungal allergy to the household mold in the course of the disease.

Список літератури

1. Crameri R, et al. Fungi: the neglected allergenic sources. Allergy. 2014;69(2):176-85.
2. Denning DW, et al. Fungal allergy in asthma–state of the art and research needs. Clinical and Translational Allergy. 2014;4:14. Available from: https://ctajournal.biomedcentral.com/articles/10.1186/2045-7022-4-14.
3. Fukutomi Y, et al. Serological diagnosis of allergic bronchopulmonary mycosis: Progress and challenges. Allergol Int. 2016;65(1):30-6.
4. Fukutomi Y, Taniguchi M. Sensitization to fungal allergens: Resolved and unresolved issues. Allergol Int. 2015;64(4):321-31.
5. Gabriel MF, et al. Alternaria alternata allergens: Markers of exposure, phylogeny and risk of fungi-induced respiratory allergy. Environ Int. 2016;89-90:71-80.
6. Helbling A, Reimers A. Immunotherapy in fungal allergy. Current Allergy and Asthma Reports. 2003;3(5):447-453.
7. Knutsen AP, et al. Fungi and allergic lower respiratory tract diseases. J Allergy Clin Immunol. 2012;129(2):280-91.
8. Ozdemir O. Mold Allergy, Its Prevention and Therapy – Part 2. MOJ Immunol. 2015;2(5):00057.
9. Simon-Nobbe B, et al. The Spectrum of Fungal Allergy. Int Arch Allergy Immunol 2008;145:58-86.

References

1. Crameri R, et al. Fungi: the neglected allergenic sources. Allergy. 2014;69(2):176-85.
2. Denning DW, et al. Fungal allergy in asthma–state of the art and research needs. Clinical and Translational Allergy. 2014;4:14. Available from: https://ctajournal.biomedcentral.com/articles/10.1186/2045-7022-4-14.
3. Fukutomi Y, et al. Serological diagnosis of allergic bronchopulmonary mycosis: Progress and challenges. Allergol Int. 2016;65(1):30-6.
4. Fukutomi Y, Taniguchi M. Sensitization to fungal allergens: Resolved and unresolved issues. Allergol Int. 2015;64(4):321-31.
5. Gabriel MF, et al. Alternaria alternata allergens: Markers of exposure, phylogeny and risk of fungi-induced respiratory allergy. Environ Int. 2016;89-90:71-80.
6. Helbling A, Reimers A. Immunotherapy in fungal allergy. Current Allergy and Asthma Reports. 2003;3(5):447-453.
7. Knutsen AP, et al. Fungi and allergic lower respiratory tract diseases. J Allergy Clin Immunol. 2012;129(2):280-91.
8. Ozdemir O. Mold Allergy, Its Prevention and Therapy – Part 2. MOJ Immunol. 2015;2(5):00057.
9. Simon-Nobbe B, et al. The Spectrum of Fungal Allergy. Int Arch Allergy Immunol 2008;145:58-86.

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10. Singh B, et al. Allergic aspergillosis and the antigens of Aspergillus fumigatus. Curr Protein Pept Sci. 2014;15(5):403-23.
11. Soeria-Atmadja D, et al. IgE sensitization to fungi mirrors fungal phylogenetic systematics J. Allergy Clin. Immunol. 2010;125(6):1379-86.
12. Tanimoto H, et al. Molecular-based allergy diagnosis of allergic bronchopulmonary aspergillosis in Aspergillus fumigatus-sensitized Japanese patients. Clin Exp Allergy. 2015;45(12):1790-800.
13. Thakur R, Shankar J. In silico Identification of Potential Peptides or Allergen Shot Candidates Against Aspergillus fumigatus. Biores Open Access. 2016;5(1):330-341.
14. Twaroch TE, et al. Mold Allergens in Respiratory Allergy: From Structure to Therapy. Allergy Asthma Immunol Res. 2015;7(3):205-220.

10. Singh B, et al. Allergic aspergillosis and the antigens of Aspergillus fumigatus. Curr Protein Pept Sci. 2014;15(5):403-23.
11. Soeria-Atmadja D, et al. IgE sensitization to fungi mirrors fungal phylogenetic systematics J. Allergy Clin. Immunol. 2010;125(6):1379-86.
12. Tanimoto H, et al. Molecular-based allergy diagnosis of allergic bronchopulmonary aspergillosis in Aspergillus fumigatus-sensitized Japanese patients. Clin Exp Allergy. 2015;45(12):1790-800.
13. Thakur R, Shankar J. In silico Identification of Potential Peptides or Allergen Shot Candidates Against Aspergillus fumigatus. Biores Open Access. 2016;5(1):330-341.
14. Twaroch TE, et al. Mold Allergens in Respiratory Allergy: From Structure to Therapy. Allergy Asthma Immunol Res. 2015;7(3):205-220.

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