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DETERMINATION OF SENSITIZATION TO POLLEN AND FUNGAL ALLERGEN EXTRACTS IN CHILDREN WITH ALLERGIC PATHOLOGY BY IMMUNOCAP METHOD

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The purpose of the study was to investigate the sensitization of children with allergic pathology to weed pollen and tree pollen, as well as fungal allergens. 220 children with various allergic diseases in the age range from 1 to 17 years were examined. Allergological examination included determination of specific IgE-antibodies in blood serum by ImmunoCAP method (pollen, fungal). Relatively high level of sensitization was noted for cypress evergreen pollen allergen (t23), sIgE level was 2.232 ± 1.515 kU/l (2nd class). Relatively high level of fungal allergens was observed for *Alternaria alternata* allergen (m6), sIgE level was 2.358 ± 1.215 kU/l ($pF=0.912$; $pU=0.067$) (2nd class). Among pollen allergens, sensitization to lanceolate plantain, evergreen cypress and European olive was the most frequent. Hypersensitization to antigens of *Alternaria alternata* fungus was proved by the conducted studies.

Key words: sensitization, pollen allergens, fungal allergens, children, allergic pathology, ImmunoCAP method.

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ВИЗНАЧЕННЯ ЧУТЛИВОСТІ ДО ЕКСТРАКТІВ ПИЛКУ ТА ГРИБКОВИХ АЛЕРГЕНІВ У ДІТЕЙ З АЛЕРГІЧНОЮ ПАТОЛОГІЄЮ МЕТОДОМ IMMUNOCAP

Метою дослідження було вивчення сенсibiliзації дітей з алергічною патологією до пилку бур'янів і деревних рослин, а також до грибкових алергенів. Обстежено 220 дітей з різними алергічними захворюваннями у віці від 1 до 17 років. Алергологічне обстеження включало визначення специфічних IgE-антитіл у сироватці крові методом ImmunoCAP (пилкові, грибкові). Відносно високий рівень сенсibiliзації відзначений до алергену пилку кипариса вічнозеленого (t23), рівень sIgE склав $2,232 \pm 1,515$ кОд/л (2-й клас). Відносно високий рівень грибкових алергенів спостерігався для алергену *Alternaria alternata* (m6), рівень sIgE склав $2,358 \pm 1,215$ кОд/л ($pF=0,912$; $pU=0,067$) (2-й клас). Серед пилкових алергенів найчастіше зустрічалася сенсibiliзація до подорожника ланцетового, кипарису вічнозеленого та оливи європейської. Проведені дослідження підтвердили наявність гіперсенсibiliзації до антигенів гриба *Alternaria alternata*.

Ключові слова: сенсibiliзація, пилкові алергени, грибкові алергени, діти, алергопатологія, метод ImmunoCAP.

Pollen is a prevalent environmental factor contributing to the onset of allergic rhinitis and represents a significant public health concern. In individuals with a predisposition to atopy, exposure to pollen initiates the release of allergic mediators – such as histamine – from the upper respiratory tract, ultimately provoking hypersensitivity reactions. Each year, pollen-induced allergic rhinitis affects

approximately 60 million people in the United States, with associated healthcare costs surpassing 3 billion USA dollars [5].

Across Europe, around 33 million individuals are sensitized to ragweed pollen, and projections estimate that this number may more than double between 2041 and 2060. The diversity and concentration of airborne pollens are influenced by factors such as local vegetation, geographical conditions, temperature variations, and climatic patterns [2, 3].

The study of the spectrum of pollen sensitization in children, prediction of the realization of the atopic phenotype of bronchial asthma and possible factors influencing its development is an urgent issue of modern allergology [6, 10, 11]. In the practice of pediatricians, allergy problems are becoming more common, and the picture of the disease has changed significantly [8].

An important place among the known sources of aeroallergens is occupied by microscopic fungi (micromycetes) [4]. According to various studies, the prevalence of hypersensitivity to mold fungi ranges from 3 to 10 % in the general population. Relationships between sensitization to micromycetes and the development of severe bronchial asthma have been described [7].

Diagnosing clinical allergies in pediatric patients involves integrating a suggestive symptom history with objective evidence of sensitization to suspected allergens, typically confirmed through skin prick testing or measurement of specific IgE antibodies. While a well-structured and detailed symptom history is essential in the evaluation of both food and airborne allergen sensitivities, relying solely on reported symptoms can lead to considerable rates of false-positive or false-negative conclusions [2]. Likewise, the mere presence of allergen sensitization does not equate to a clinically significant allergy. Therefore, an accurate diagnosis necessitates a comprehensive and multifaceted assessment strategy.

The purpose of the study was to investigate the sensitization of children with allergic pathology to weed pollen and tree pollen, as well as fungal allergens.

Materials and methods. The study was conducted at the base of Department of Children's diseases II of Azerbaijan Medical University in the period of 2018–2022. We examined 220 children with various allergic diseases in the age range from 1 to 17 years, mean age (7.5 ± 0.1 years). The distribution of children by sex revealed that boys prevailed among the examined patients, 130 boys (59.1 %) and 90 girls (40.9 %). We divided the children into two representative groups. Group I included children who did not suffer from bronchial asthma (BA) but had various allergic pathologies: allergic rhinitis, atopic dermatitis, atopic conjunctivitis in 151 (68.6 %) cases (group BA (-)). II group consisted of patients suffering from bronchial asthma in 69 (31.4 %) cases (group BA (+)). Allergological examination included determination of specific IgE-antibodies in blood serum by ImmunoCAP method (pollen, fungal). The criterion of sensitization was considered to be an increase in the level of specific IgE above the threshold level according to the manufacturer's instructions: more than 0.35 kU/l. The probability of differences was considered statistically significant at $p < 0.05$.

For the purpose of correct application of statistical procedures, the mean values of the obtained samples (M), their standard deviations (σ), standard errors (m), 95 % confidence intervals (95 % CI), minimum (min) and maximum (max) values of the series were calculated, in the groups were determined. The analysis of variance (F-Fisher) and nonparametric Wilcoxon U-criterion (Mann-Witney) and Kruskal-Wallis criterion (Kruskal-Wallis) were used for comparison and probabilistic evaluation of differences between the values of the compared groups. Pearson's χ^2 method (Pearson Chi-Square) was used to determine the degree of contiguity between qualitative features. The statistical significance was set at $p \leq 0.05$. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and National Research Committee and with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards.

Results of the study and their discussion. The distribution of traits was different from normal, so median and interquartile range were used to estimate parameters. A total of 59 cases of sensitization to some extract of weed and tree pollen allergen were identified, of which 24 (40.6 %) occurred in the group of patients with BA (+) and 35 (59.3 %) in the group of patients with BA (-). The most frequent sensitization was to European olive pollen extract (t9) in 16 (27.1 %), evergreen cypress (t23) in 14 (23.7 %) and lanceolate plantain (w9) in 13 (22.0 %) cases. A relatively high level of sensitization among all studied allergens in the general group of children was noted for cypress evergreen pollen allergen (t23), sIgE level was 2.232 ± 1.515 kU/L (class 2). Table 1 presents the values of sIgE-antibody levels to allergen extracts of pollen allergens of weeds (plantain lanceolate) and trees (hazel, sycamore, European olive, eucalyptus, acacia and cypress evergreen).

Sensitization level (sIgE -antibodies) to weed and tree pollen allergen extracts

Allergens	Patients with AD (+)	Patients with AD (-)	Reliability	
	N Me±m (min-max)	N Me±m (min-max)	P _F	P _U
w9 plantain lanceolata (<i>Plantago lanceolata</i>)	6 0.060±0.009 (0.0-0.1)	7 3.613±1.611 (0.0-9.3)	0.067	0.033
t9 European olive (<i>Olea europaea</i>)	5 0.018±0.005 (0.0-0.0)	11 1.726±0.873 (0.0-9.9)	0.216	0.031
t4 common hazel (<i>Corylus avellana</i>)	1 0.010±0.000 (0.0-0.0)	1 1.370±0.000 (1.4-1.4)	0.317	0.317
t11 sycamore (<i>Platanus</i>)	2 0.025±0.015 (0.0-0.0)	4 0.253±0.106 (0.0-0.4)	0.226	0.264
t18 eucalyptus (<i>Eucalyptus</i>)	1 0.020±0.000 (0.0-0.0)	2 0.015±0.005 (0.0-0.0)	0.667	1.000
t19 acacia (<i>Acacia</i>)	3 0.020±0.000 (0.0-0.0)	2 0.855±0.845 (0.0-1.7)	0.277	0.221
t23 cypress evergreen (<i>Cupressus sempervirens</i>)	6 0.088±0.057 (0.0-0.4)	8 3,840±2.568 (0.0-21.1)	0.234	0.215

When assessing the prevalence of sensitization to weed and tree pollen extracts studied by ImmunoCAP, sensitization to lanceolate plantain was found in 6 (46.1 %) children with AD (+) and 7 (53.8 %) with AD (-). Sensitization to European olive was detected in 5 (31.2 %) children with BA (+) and 11 (68.7 %) with BA (-). Sensitization to common hazel was detected in 2 children, of whom 1 (50.0 %) was in the BA (+) group and 1 (50.0 %) in the BA (-) group. Sensitization to sycamore was noted in 2 (33.3 %) children with BA (+) and 4 (66.6 %) with BA (-). Sensitization to eucalyptus – in 1 (33,3 %) child with BA (+) and in 2 (66.6 %) with BA (-). Sensitization to acacia – in 3 (60.0 %) children with BA (+) and in 2 (40.0 %) with BA (-). Sensitization to cypress evergreen – in 6 (42.8 %) children with BA (+) and in 8 (57.2 %) with BA (-).

In the group of patients with BA (+) the levels of sensitization (sIgE) were: to pollen extracts of plantain lanceolate (w9) – 0.060±0.009kU/L (grade 0), European olive (t9) – 0.018±0.005kU/L (grade 0), common hazel (t4) – 0.010±0.000kU/L (grade 0), sycamore (t11) – 0.025±0.015kU/L (grade 0), eucalyptus (t18) – 0.020±0.000kU/L (grade 0), acacia (t19) – 0.020±0.000kU/L (grade 0), cypress evergreen (t23) – 0.088±0.057kU/L (grade 0).

In the group of patients with BA (-) the levels of sensitization (sIgE) were: to pollen extracts of plantain lanceolate (w9) – 3.613±1.611kU/L (3rd class), European olive (t9) – 1.726±0.873kU/L (2nd class), common hazel (t4) – 1.370±0.000kU/L (2nd class), sycamore (t11) – 0.253±0.106kU/L (class 0), eucalyptus (t18) – 0.015±0.005kU/L (class 0), acacia (t19) – 0.855±0.845kU/L (class 2), cypress evergreen (t23) – 3.840±2.568kU/L (class 3).

When analyzing the contribution of pollen allergens to the development of various allergopathologies in the group of patients with BA (-), the share of allergen “plantain lanceolate” was 0.45 units, the share of allergen “European olive” – 0.17 units, the share of allergen “common hazel” – 0.13 units, the share of allergen “acacia” – 0.08 units, the share of allergen “cypress evergreen” – 0.50 units.

A total of 59 cases of sensitization to some weed and tree pollen allergen extract were identified, of which 24 (40.6 %) occurred in the group of patients with BA (+) and 35 (59.3 %) in those with BA (-). The most frequent sensitization was to European olive pollen extract (t9) in 16 (27.1 %), evergreen cypress (t23) in 14 (23.7 %) and lanceolate plantain (w9) in 13 (22.0 %) cases. A relatively high level of sensitization among all studied allergens in the general group of children was noted for cypress evergreen pollen allergen (t23), sIgE level was 2.232±1.515 kU/L (class 2).

The relative values of IgE antibody levels to the pollen allergen panels were further calculated:

- tx2 (late flowering trees)
- gx1 (pollen of cereal grasses)

The late flowering tree pollen panel consisted of: maple (t1), birch (t3), beech (t5), oak (t7) and walnut (t10). The pollen panel of cereal grasses consisted of: hedgehog (g3), meadow fescue (g4), tares (g5), meadow timothy (g6) and bluegrass (g8).

A total of 59 cases of sensitization to some weed and tree pollen allergen extract were identified, of which 24 (40.6 %) occurred in the group of patients with BA (+) and 35 (59.3 %) in those with BA (-). The most frequent sensitization was to the pollen extract of European olive (t9) in 16 (27.1 %), evergreen cypress (t23) in 14 (23.7 %) and plantain

When assessing the incidence of sensitization to pollen allergen extracts, it was found that sensitization to the allergen panel tx2 (late flowering trees) was detected in 1 (50.0 %) patient with BA (+) and in 1 (50.0 %) patient with BA (-). Sensitization to allergen panel gx1 (grass pollen) was detected in 18 (37.5 %) children with AD (+) and 30 (62.5 %) patients with BA (-). The results obtained are presented in Table 2.

Table 2

Level of sensitization (sIgE-antibodies) to panels of pollen allergens and to fungal and bacterial allergen extracts

Allergens	Patients with AD (+)	Patients with AD (-)	Reliability	
	N Me±m (min-max)	N Me±m (min-max)	P _F	P _U
tx2	1 0.000±0.000 (0.0-0.0)	1 0.000±0.000 (0.0-0.0)		
late blooming trees	18 0.222±0.101 (0.0-1.0)	30 0.273±0.084 (0.0-1.2)	0.705	0.733
m3	14 0.251±0.225 (0.0-3.2)	13 0.263±0.154 (0.0-1.7)	0.967	0.504
Aspergillus fumigatus	2 0.040±0.000 (0.0-0.0)	2 0.030±0.010 (0.0-0.0)	0.423	1.000
m4	6 0.018±0.003 (0.0-0.0)	6 0.305±0.263 (0.0-1.6)	0.302	0.317
Mucor racemosus	15 2.215±2.094 (0.0-31.5)	16 2.492±1.371 (0.0-20.7)	0.912	0.067
m5	1 0.020±0.000 (0.0-0.0)	2 0.030±0.020 (0.0-0.1)	0.821	1.000
Candida albicans	6 0.018±0.003 (0.0-0.0)	6 0.305±0.263 (0.0-0.1)	0.821	1.000
m6	26 0.154±0.072 (0.0-1.0)	47 0.191±0.058 (0.0-0.0)	0.692	0.689

In the group of patients with BA (+), sensitization (sIgE) to allergen extracts from panel tx2 (late flowering trees) was not determined. The values of sensitization levels to the allergen extract of panel gx1 (cereal grass pollen) were 0.222±0.101kE/l (class 0). In the group of patients with BA (-), sensitization (sIgE) to the allergen extract of panel tx2 (late flowering trees) was also not determined. The values of sensitization levels to the allergen extract of panel gx1 (cereal grass pollen) were 0.273±0.084kE/L (grade 0).

A total of 50 cases of sensitization to any extract of the pollen allergen panel were identified, of which 19 (38.0 %) occurred in the group of children with BA (+) and 31 (62.0 %) in the group of children with BA (-). The most frequent sensitization was to the pollen allergen panel gx1 (cereal grass pollen) – 48 (96.0 %) cases. The highest level of sensitization among all studied allergens in the general group of children was observed for pollen panel gx1. The sIgE level was 0.254±0.064 kU/L (grade 0).

The distribution of the signs was different from normal, so the median and interquartile range were used to estimate the parameters.

When assessing the prevalence of sensitization to fungal allergen extracts, sensitization to *Aspergillus fumigatus* allergens was found in 14 (51.8%) children with BA (+) and 13 (48.2 %) with BA (-). Sensitization to *Mucor racemosus* was detected in 2 (50.0 %) children with BA (+) and in 2 (50.0 %) with BA (-). Sensitization to *Candida albicans* was detected in 6 (50.0 %) children with BA (+) and in 6 (50.0 %) with BA (-). Sensitization to *Alternaria alternata* was detected in 15 (48.3 %) children with BA (+) and in 16 (51.6 %) with BA (-). Sensitization to *Aspergillus niger* was detected in 1 (33.3 %) of children with BA (+) and in 2 (66.6 %) with BA (-). Sensitization to a mixture of mold allergens was detected in 26 (35.6 %) children with BA (+) and in 47 (64.4 %) with BA (-).

In the group of patients with AD (+), the value of sensitization level (sIgE) to *Aspergillus fumigatus* (m3) allergen extract was 0.251 ± 0.225 kU/L (grade 0), *Mucor racemosus* (m4) – 0.040 ± 0.000 kU/L (grade 0), *Candida albicans* (m5) – 0.018 ± 0.003 kU/L (grade 0), *Alternaria alternata* (m6) – 2.215 ± 2.094 kU/L (grade 2), *Aspergillus niger* (m207) – 0.020 ± 0.000 kU/L (grade 0), to a mixture of mold allergens (mx2) – 0.154 ± 0.072 kU/L (grade 0). In patients of the BA group (–), sensitization levels to *Aspergillus fumigatus* allergen extract (m3) corresponded to 0.263 ± 0.154 kU/L (grade 0), *Mucor racemosus* (m4) – 0.030 ± 0.010 kU/L (grade 0), *Candida albicans* (m5) – 0.305 ± 0.263 kU/L (grade 0), *Alternaria alternata* (m6) – 2.492 ± 1.371 kU/L (grade 2), *Aspergillus niger* (m207) – 0.030 ± 0.020 kU/L.

When analyzing the contribution of fungal allergens in the development of atopic bronchial asthma in children, it was found that in the group of patients with BA (+) the share of *Alternaria alternata* allergen amounted to 4.32 units. When analyzing the contribution of fungal allergens to the development of various allergopathologies in the group of patients with BA (–), the share of *Alternaria alternata* allergen was 2.98ed. In general, 160 cases of sensitization to any fungal allergen extract were established, of which 71 (44.3 %) occurred in the group of children with BA (+) and 89 (55.6 %) in the group of children with BA (–). The most frequent sensitization was to the extract of a mixture of mold allergens, in 73 (45.6 %) cases. A relatively high level of sensitization among all studied allergens in the general group of children was noted for *Alternaria alternata* allergen (m6), sIgE level was 2.358 ± 1.215 kU/L (pF=0.912, pU=0.067) (grade 2).

Analysis of mold allergen panel – mx2 (*Penicillium chrysogenu* m1, *Cladosporium herbarum* m2, *Aspergillus fumigatus* m3, *Candida albicans* m5, *Alternaria alternata* m6, *Setomelanomma gostrata* m8) showed a very low class of IgE antibody reactivity – 0.178 ± 0.045 kU/L (class 0).

Similar results were noted by other researchers. So, a retrospective analysis was performed by Li X, et al (2024). They observed 1,028 pediatric patients diagnosed with allergic rhinitis and asthma, in which allergen-specific IgE antibody testing was utilized. Based on clinical presentation, the participants were categorized into three groups: allergic rhinitis, cough variant asthma, and bronchial asthma. The analysis revealed that the most frequently encountered allergens were dust mites, household dust, and mold. Notably, children in the bronchial asthma group exhibited sensitization to a broader range of inhalant allergens, with a higher prevalence of strong and multiple sensitizations. The researchers concluded that the presence of multiple allergen sensitizations significantly increases the risk of progression from cough variant asthma to classic bronchial asthma [4].

In the other study – multicenter investigation, which was carried out between October 2019 and March 2021 across 11 clinical sites located in 9 provinces of mainland China, Luo W, et al, evaluated 736 pediatric patients under the age of 18 who were clinically diagnosed with allergic rhinitis by structured questionnaire designed to collect data on demographic variables, mode of delivery, family history of allergies, exposure to tobacco smoke, environmental influences, and AR severity, enabling a comprehensive clinical assessment. The overall positive responses to the 14 pollen allergens in mainland China from highest to lowest was 31.39 % for *Artemisia vulgaris*, 21.60 % for *Cynodon dactyl*, 21.06 % for *Ambrosia artemisiifolia*, 20.92 % for *Chenopodium album*, 20.79 % for *Ulmus campestris*, 20.11 % for *Populus alba*, 16.30 % for *Platanus acerifolia*, 15.63 % for *Betula verrucosa*, 15.22 % for *Salix fragillis*, 14.95 % for *Brassica napus*, 11.68 % for *Phleum pratense*, 9.38 % for *Phragmites communis*, 8.83 % for *Triticum aestivum*, 7.20 % for *Cupressus sempervirens*. These results differed from ours, it can be explained by geographical features of China compared with our region [5].

In some studies, the new methods of diagnosis were discussed. Thus, Kleine-Tebbe J, et al (2021) noted that molecular allergy diagnosis is able to distinguish broad cross-reactivity due to allergen-specific IgE to pollen panallergens (i.e. profilins Bet v 2 or Phl p 12; polcalcins Bet v 4 or Phl p 7; and, in the future, cyclophilins Bet v 7 or Ole e 15) from primary IgE sensitizations to so-called marker allergens represented by important pollen major allergens: Bet v 1 for the birch and beech family (Fagales), Ole e 1 for olive and ash (Oleaceae), Phl p 1 for temperate climate grasses (Poaceae), Art v 1 for mugwort (*Artemisia*), Amb a 1 for *Ambrosia* species (*Ambrosia*). Five typical cases (A – E) with positive skin prick test results to tree, grass, and weed pollen extracts demonstrate typical patterns of IgE sensitization with a variable impact of pollen panallergens: A – profilins, B – polcalcins, C – profilins and polcalcins, D – presumably cyclophilins, E – primary polysensitization to tree, grass, and weed pollen without interference from profilins or polcalcins. Differences between pollen extract-based skin prick test diagnosis and molecular allergen-specific IgE testing are explained using the presented concept. This approach allows to reduce the number of allergen extracts – presuming they are also clinically relevant – for allergen immunotherapy (i.e., only tree and/or grass pollen extracts), particularly in pollen-polysensitized patients [3]. In our study we used ImmunoCAP method, which demonstrated high efficiency.

Preda M, et al (2024) revealed, that the wind-pollinated weeds from the Asteraceae family are highly allergenic and constitute an important source of airborne allergens during late summer and autumn. In Europe, ragweed and mugwort pollen allergies have emerged as a considerable public health concern. They noted, that individuals with respiratory allergies to weed pollen may also experience hypersensitivity reactions to certain herbal remedies derived from cross-reactive members of the Compositae family, such as chamomile, marigold, and purple coneflower. The authors emphasized, that general practitioners, otorhinolaryngologists, and pulmonologists should be familiar with the diagnostic tools used by allergists to ensure accurate identification of such cases. In addition, allergists must consider the recommendations of the European Medicines Agency's Herbal Medicinal Products Committee and the wide range of herbal treatments in order to properly advise patients about possible risks [7].

Conclusions

Thus, the conducted studies allow us to analyze the spectrum of IgE deterministic sensitization to specific pollen allergens and fungal allergens in various allergopathologies in children.

1. When analyzing the frequency of sensitization to pollen allergens in children with BA (+), it was found that sensitization to lanceolate plantain (n=6; 25.0 %) and evergreen cypress (n=6; 25.0 %) was the most frequent among allergens. The highest values of sensitization level (sIgE) were noted to cypress evergreen allergen extract (t23) – 0.088±0.057kU/L.

2. When analyzing the frequency of sensitization to pollen allergens in children with BA (–), it was found that sensitization to European olive was the most frequent among allergens (n=11; 31.4 %). The highest values of sensitization level (sIgE) were noted to cypress evergreen allergen extract (t23) – 3.840±2.568kU/l and plantain lanceolate (w9) – 3.613±1.611kU/l. The conducted studies proved hypersensitization to *Alternaria alternata* fungus antigens both in bronchial asthma BA (+) and in other allergic diseases BA (–).

3. The established prevalence of fungal molecules of *Alternaria alternata* allergen among patients not only with bronchial asthma but also with other allergic pathologies is an alarming prognostic marker for this category of children.

The widespread prevalence of the allergen in the environment necessitates the diagnosis of hypersensitization to develop effective measures to prevent the development and exacerbations of allergic diseases.

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