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Ye.M. Dytyatkovska [1],
S.V. Biletska [1, 2]

DYNAMICS OF IL-4 AND IFN-γ CYTOKINE PROFILE UNDER THE INFLUENCE OF COMBINED ALLERGEN-SPECIFIC IMMUNOTHERAPY (ASIT) IN PATIENTS WITH POLLENOSIS AND COMBINED SENSITIZATION TO POLLEN AND HOUSEHOLD ALLERGENS

SE «Dnipropetrovsk medical academy of Health Ministry of Ukraine» [1]
Department of Occupational Diseases and Clinical Immunology
Department of Family Medicine FPE
V. Vernadsky str., 9, Dnipro, 49044, Ukraine
Medical Center "Unimed" [2]
Mlodej Polski str., 7, Krakow, 30-131, Poland
ДЗ «Дніпропетровська медична академія МОЗ України» [1]
кафедра професійних хвороб та клінічної імунології
(зав. – д. мед. н., проф. К.Ю. Гашинова)
кафедра сімейної медицини ФПО
(зав. – доц., д. мед. н., І.Л. Височина)
вул. В. Вернадського, 9, Дніпро, 49044, Україна
Медичний центр «Unimed» [2]
вул. Молодої Польщі, 7, Краків, 30-131, Польща
e-mail: biletskasv@gmail.com

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Abstract. Dynamics of IL-4 and IFN-γ cytokine profile under the influence of combined allergen-specific immunotherapy (ASIT) in patients with pollenosis and combined sensitization to pollen and household allergens. Dytyatkovska Ye.M., Biletska S.V. *The article presents the results of the clinical evaluation of the cytokine profile (IL-4, IFN-γ) in combined allergen-specific ASIT immunotherapy in patients with pollenosis and combined sensitization to pollen and household allergens. Studies were conducted in 49 patients aged 19-57 years (24 women and 25 men) suffering from pollenosis from 1 to 38 years. Patients underwent an allergological examination, including history taking, diagnostic skin tests - prick test, immunological studies of blood by enzyme-linked immunosorbent assay and allergic component diagnosis ALEX, on the basis of which polysensitization to pollen and household allergens was revealed in all patients. All patients underwent pre-season ASIT. The main group of 31 patients received a combined ASIT with solutions of pollen and household allegens. 18 patients of the comparison group received ASIT only with pollen allergens. The observation groups were comparable by sex, age, and disease duration. Studies were conducted during the period of remission of the disease before the onset of ASIT and 1 year after. It was found that under the influence of combined ASIT, an immunospecific effect was observed in patients with a significant decrease in the level of IL-4 in simultaneous increase in the production of interferon-gamma by 59.8%, which indicates the activation of the process of switching the immune response from Th-2 to Th-1 cells. The positive results of clinical and immunological studies convincingly prove the advantages and effectiveness of the use of ASIT in patients with pollenosis and combined sensitization to pollen and household allergens.*

Реферат. Динамика показателей цитокинового профиля IL-4и IFN-γ под влиянием комбинированной аллерген-специфической иммунотерапии (АСИТ) у больных с полинозом и сочетанной сенсибилизацией к пыльцевым и бытовым аллергенам. Дитятковская Е.М., Белецкая С.В. *В статье представлены результаты клинической оценки цитокинового профиля (IL-4, IFN-γ) при комбинированной аллерген-специфической иммунотерапии АСИТ у больных с полинозом и сочетанной сенсибилизацией к пыльцевым и бытовым аллегенам. Исследования проведены у 49 пациентов 19-57 лет (24 женщины и 25 мужчин), страдающих полинозом от 1 до 38 лет. Больные прошли аллергологическое обследование, включающее сбор*

анамнеза, диагностические кожные пробы - prick test, иммунологические исследования крови иммуноферментным методом и аллергокомпонентную диагностику ALEX, на основании чего у всех пациентов выявлена полисенсибилизация к пыльцевым и бытовым аллегенам. Всем пациентам была проведена предсезонная АСИТ. Основная группа из 31 пациента получала комбинированную АСИТ с растворами пыльцевых и бытовых аллегенов. 18 больных группы сравнения получали АСИТ только пыльцевыми аллергенами. Группы наблюдений были сопоставимы по полу, возрасту и длительности заболевания. Исследования были проведены в период ремиссии болезни до начала АСИТ и через 1 год. Установлено, что под воздействием комбинированной АСИТ у больных наблюдался иммуноспецифический эффект достоверного снижения уровня IL-4 при одновременном повышении на 59,8% продукции интерферона-гамма, что свидетельствует об активации процесса переключения иммунного ответа с Th-2 на Th-1 клетки. Позитивность результатов клинических и иммунологических исследований убедительно доказывает преимущества и эффективность применения АСИТ у больных с поллинозом и сочетанной сенсибилизацией к пыльцевым и бытовым аллергенам.

Nowadays allergic diseases present an acute problem for clinical medicine that has a global spread and social character [3, 4, 6]. Thus, 4-10% of the population suffers from allergic rhinitis in the world and 3.2-19.6% of those in European countries [3, 4, 8]. In Ukraine, especially in industrially polluted cities, this situation is even more complicated. The state of technogenic pollution of life-supporting environments (atmospheric air) affects the aggressiveness of pollen allergens, which contributes to a significant increase in incidence of pollenosis among the population of cities. According to the analysis conducted by Dytyatkovska Ye.M. (10 thousand questionnaires of the city dwellers), the number of patients with pollenosis is 6.6 times higher than according to official statistics based on patient referrals [4, 9]. Technogenic air pollution potentiates the aggressiveness of pollen allergens, which contributes to a significant increase in the prevalence of pollenosis in the population.

Among the methods of treatment of pollenosis the leading place belongs to allergen-specific immunotherapy of causative allergens (ASIT), the advantage of which is the direct effect on the pathogenesis of the disease, in which a very important role is given to various cytokines [11]. According to current concepts, the production of IgE or IgG in the immune response is determined by T-lymphocyte helper cells, which, as a result of antigen recognition, can differentiate from Th0 into Th-type 1 cells, one of the markers of activity of which are cytokines IL-12, IFN-γ or Th -cells of type 2, one of the markers of activity of which are cytokines IL-5 and IL-4. The action of ASIT is naturally directed to the immunological phase and leads to a change in the immune response from Th-2 type to Th-1 type with a change in the profile of the corresponding cytokines: the production of IFN-γ is increased and the secretion of IL-5 and IL-4, IL-13 is suppressed [2 , 5, 10].

Despite all the advances in medical science, many questions regarding the diagnosis and treatment of pollenosis in Ukraine, and especially in industrial cities remain, this urges the importance of the problem and requires finding new ways to improve the effectiveness of ASIT [6, 8].

In view of the above, the aim of this study was to evaluate the dynamics of cytokine profile (IL-4, IFN-γ) under the influence of combined allergen-specific immunotherapy (ASIT) in patients with pollenosis with combined sensitization to pollen and household allergens.

MATERIALS AND METHODS OF RESEARCH

The study included 49 patients, aged 19 to 57 years (mean age – 35.5±1.5 years), including 24 (49.0%) women and 25 (51.0%) men. All patients suffered from pollenosis, which was clinically manifested by seasonal rhinoconjunctive syndrome and year-round allergic rhinitis, which had less manifested clinical symptoms. Patients were treated at the Consultative-Diagnostic Center and Allergic Department of the Communal Non-Profit Institution "Clinical Emergency Care Hospital" of City Council, Dnipro. The duration of the disease ranged from 1 to 38 years (average – 9.2±1.1 years). All patients underwent an allergic examination, which included history taking, diagnostic skin tests - prick test with pollen (wormwood, ragweed, quinoa, sump weed, maize, sunflower, spring and summer herbs) and household (house dust, mites) allergens, produced by Vinnytsya ME "Immunolog" (Ukraine) and/or methods of allergic component diagnosis ALEX. According to the results of the tests, polysensitization to pollen and household allergens was found in all patients.

All patients under study underwent pre-season ASIT with etiologically significant allergens according to the express scheme using water-salt allergen solutions (1 ml of solution – 10000 PNU of allergen), produced by Vinnytsya ME "Immunolog" (Ukraine) for 1 course. The main study group consisted of 31 patients receiving combined ASIT with solutions of pollen and household allergens. The comparison group consisted of 18 patients who received ASIT only with pollen allergens, although they had sensitization to household allergens.

Both groups were compared by gender, age and disease duration (p>0.05) (Table 1).

Diagnosis of IL-4 and IFN-γ was performed in serum by enzyme-linked immunosorbent assay (quantitative analysis) at VIK-MEDIK laboratory (license of the Ministry of Health of Ukraine AG No. 602777 dated August 4, 2011). The study was conducted in the period of remission of pollenosis before the onset of ASIT and one year after.

Table 1

General characteristic of patients study groups

Group	Gender (abs. / %)		Age, years (M±m)	Disease duration,, years (M±m)
	women	men		
Main (n=31)	14/45.2	17/54.8	35.3±2.0	10.3±1.5
Comparison (n=18)	10/5.6	8/44.4	36.0±2.3	7.2±1.1
Difference between groups (p)	0.483*		0.813	0.107

Note. * – significance level (p) calculated by χ^2 criterion, in other cases – by Student's t-criterion

STATISTICA v.6.1 (Statsoft Inc., USA), serial number AGAR909E415822FA was used for statistical data analysis. Taking into account the deviations of the distribution of quantitative indicators from the normal law by the Shapiro-Wilk criterion, both parametric and non-parametric characteristics and methods of comparison were used only in separate groups or at separate stages of the study: arithmetic mean (M), standard error (m), median (Me), 95% confidence interval for the mean (95% CI), Student's t test for bound (T) and unrelated samples (t), Mann-Whitney (U) and Wilcoxon (W) [1, 4]. Comparisons were made using Pearson's Chi-square test (χ^2) and Fisher's exact test (TFT) [1, 4].

The critical level of statistical significance (p) was assumed to be ≤0.05 when testing all hypotheses, and a trend was determined at p<0.10.

RESULTS AND DISCUSSION

Analysis of baseline levels of IL-4 in the blood of patients with pollenosis (before ASIT) showed their increase in comparison with the norm (0-4 pg/ml) in the majority of patients (69.4%), including 19 (61.3%) patients in the main group and 15 (83.3%) patients in the comparison group (p=0.107 by χ^2 criterion). Concentration of IFN-γ in the blood of 15 (30.6%) patients, in contrast, was reduced relative to normal (2-15 pg/ml), including 8 (25.8%) and 7 (38.9%) patients from the study groups, respectively (p=0.338 by χ^2). However, both groups were statistically comparable (p>0.05) by the average levels before treatment: the concentration of IL-4 in the blood was 4.50±0.36 pg/ml in the main group and 5.08±0.38 pg/ml in the comparison group (p=0.296 by Student's t-test); the average IFN-γ content was 4.13±0.51 pg/ml and 4.42±0.92 pg/ml, by groups (p=0.759 by t-test) respectively.

The results of the study of immune status in patients with pollenosis under the influence of ASIT showed characteristic changes in the cytokine profile in both groups, namely: a decrease in the production of IL-4 and an increase in IFN-γ synthesis (Tables 2, 3). The most pronounced changes were observed in the patients of the main group, where a significant (p<0.05) decrease in the level of IL-4 from 4.50±0.36 pg/ml to 3.41±0.24 pg/ml occurred against the background of combined ASIT, i.e. by 24.2%. As the analysis of individual data showed, such dynamics was characteristic of the majority of patients under study (58.1%). The most pronounced positive effect of ASIT was observed in the dynamics of IFN-γ content - its concentration in the blood increased by 77.4% (n=24) patients, it corresponded to the limits of the reference interval in 100% of patients, and the average level in the group after treatment increased by 59.8% – from 4.13±0.51 pg/ml to 6.60±0.60 pg/ml (p<0.001 by t and U criteria).

So, the obtained data suggest the development of a process of switching the immune response from Th-2 to Th-1 cells against the background of combined ASIT with pollen and household allergens.

Table 2

Dynamics of cytokines in patients of main group under impact of combined ASIT (n=31)

Value	Before treatment		After treatment		p_1 / p_2
	deviation from the norm, abs./%	M±m (Me)	deviation from the norm, abs./%	M±m (Me)	
IL-4, pg/ml, N (0-4)	19/61.3	4.50±0.36 (4.40)	13/41.9	3.41±0.24 (3.20)	0.012/ 0.034
INF- γ, pg/ml N (2-15)	8/25.8	4.13±0.51 (3.54)	–	6.60±0.60 (6.71)	<0.001 / <0.001

Notes: N – reference interval; p_1/ p_2 – significance level of difference of mean values in dynamics by Student's (T)/Wilcoxon (W).

Positive changes in the studied findings of the cytokine profile after ASIT were also observed in the patients of the comparison group, but were less pronounced (Table 3). Thus, despite the decrease in the production of IL-4 in the blood of 11 (61.1%) patients, they remained above normal and changes in the mean value in the group by 16.5% (from 5.08±0.38 pg/ml to 4.24±0.32 pg/ml) did not reach the level of statistical significance and were of a trend ($p<0.10$). Therefore, at statistically comparable mean values of serum IL-4 levels in both groups at the beginning of the study ($p>0.05$), after combined ASIT, IL-4 levels in the main group were by 19.6% lower than in comparison group – 3.41±0.24 pg/ml versus 4.24±0.32 pg/ml ($p=0.043$ by the t-test; $p=0.050$ by the U-test) (Fig.).

Table 3

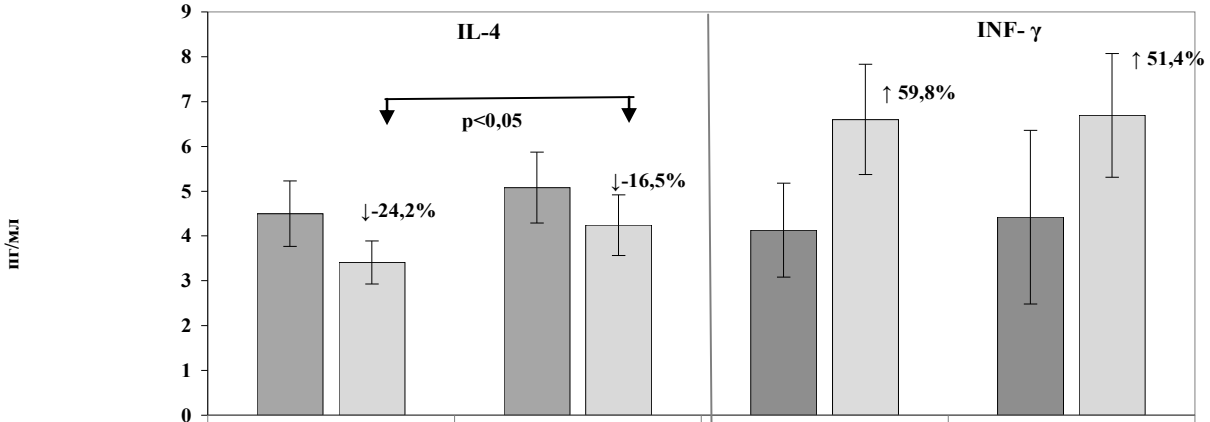
Dynamics of cytokines in patients of comparison group under impact of ASIT with pollen allergens (n=18)

Value	Before treatment		After treatment		p_1/ p_2
	deviation from the norm, abs./%	M±m (Me)	deviation from the norm, abs./%	M±m (Me)	
IL-4, pg/ml, N (0-4)	15/83.3	5.08±0.38 (4.73)	11/ 61.1	4.24±0.32 (4.25)	0.061/0.071
INF- γ, pg/ml N (2-15)	7/38.9	4.42±0.92 (3.84)	–	6.69±0.66 (6.20)	0.003/0.006

Note. p_1/ p_2 – significance level of difference of mean values in dynamics by Student's (T)/Wilcoxon (W).

The dynamics of INF-γ production in patients with pollenosis with combined sensitization to pollen and household allergens against the background of ASIT with only pollen allergens was similar to the main group (Table 3). The level of INF-γ increased in 83.3% of patients and did not go beyond the reference interval in 100% of patients. The average level increased by 51.4% – from 4.42±0.92 pg/ml to 6.69±0.66 pg/ml ($p<0.01$), and was statistically comparable with the same one in patients of the main group ($p=0.920$ and $p=0.901$ by t- and U-criteria) (Fig.).

Thus, the results of the study showed that conducting a course of pre-season combined ASIT in patients with pollenosis with combined sensitization to pollen and household allergens increases the activity of type 1 Th-cells, significantly reducing the production of Th-2 cells.



	Main group	Comparison group	Main group	Comparison group
before treatment	4,50	5,08	4,13	4,42
after treatment	3,41	4,24	6,60	6,69

Dynamics of mean levels (M, 95% CI) of cytokines under impact of ASIT in study groups: changes of value as compared to baseline before treatment

CONCLUSIONS

1. Under the impact of allergen-specific combined immunotherapy, an immunospecific effect of significant decrease in the level of IL-4 from 4.50±0.36 pg/ml to 3.41±0.24 pg/ml ($p<0.05$) with increase in interferon-gamma production by 59.8% ($p<0.001$) is observed, indicating the activation of the process of switching the immune response from Th-2 to Th-1 cells.
2. The use of allergen-specific combined immunotherapy in patients with pollenosis with combined sensitization to pollen and household allergens causes a more significant effect on the activity of Th-2 cells, which was manifested by a decrease in production of IL-4 by 19.6% compared with patients who received pollen allergens only ($p<0.05$).
3. Positivity of the results of immunological studies convincingly proves the advantages and effectiveness of the use of allergen-specific combined immunotherapy in the treatment of patients with pollenosis with combined sensitization to pollen and household allergens.

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