https://doi.org/10.31288/oftalmolzh202352226

Assessing serum cytokine and immunoglobulin levels in patients with allergic rhinitis and allergic rhinoconjunctivitis before and after treatment supplemented with macromycetes

V. K. Bohdanov [1], S.M. Pukhlik [1], M.B. Makarova [2], S.I. Poliakova [2], K.G. Bohdanov[1]

[1] Odesa National Medical University

[2] SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the NAMS of Ukraine",
Odesa (Ukraine)

Background: *In recent decades, the prevalence of allergic rhinitis (AR) has been increasing all over the world including Ukraine. Allergic rhinoconjunctivitis (ARC) is one of the most common clinical forms of AR. An imbalance between pro-inflammatory and anti-inflammatory cytokines is known to have a key role in allergic inflammation.*
Purpose: *To compare cytokine and immunoglobulin levels among patients with AR and ARC treated with different therapeutic options.*
Material and Methods: *Forty patients with AR and ARC (age range, 20 to 46 years) were included in the study. Disease duration ranged from 6 months to 2 years. Patients were divided into two subgroups of 20 patients each. Patients of subgroup 1 received the basic therapy (a 10-mg loratadin tablet daily and mometasone furoate nasal spray at a dosage of 200 μg once daily), whereas patients of subgroup 2, the basic therapy plus polypore macromycetes (Astmagan), one capsule twice daily. Treatment course duration was 90 days. The control group was composed of 25 healthy individuals. A comprehensive clinical immunological examination was conducted at baseline and on the completion of the treatment course. Enzyme-linked immunosorbent assay kits were used to determine serum levels of immunoglobulins A (IgA), IgM, IgG, and IgE, and cytokines (gamma interferon (IFN-γ) and IL4).*
Results: *At baseline, serum levels of IgA, IgM and IgE were almost twice as high (p = 0.0008; 0.0005; and 0.0001, respectively); IgG, 1.2 times higher (p = 0.001); pro-inflammatory cytokine IL4, 3.5 times higher (p = 0.0001); and anti-inflammatory cytokine IFN-γ, 2.4 times lower (p = 0.0001) in patients with AR and ARC compared to controls, and these differences were significant. Astmagan, when used as an adjunct to the basic treatment of AR and ARC, contributed to 8%, 17%, 16.2%, 7.3% and 6.0% greater decreases in the serum levels of IgA, IgM, IgE, IgG and IL4, respectively, and a 16.6% greater increase in the serum level of IFN-γ compared to the basic treatment only, and these differences were significant, with an improvement in immune response to therapy.*

Keywords:
allergic rhinitis, allergic rhinoconjunctivitis, immunoglobulins, IL4, IFN-γ, polypore macromycetes

Introduction

In recent decades, the prevalence of allergic rhinitis (AR) has been increasing all over the world including Ukraine [1]. Approximately 15% to 30% of people in the United States [2] and about 25% of the general population in Europe [3] have the disease. AR is a disease that significantly impacts the quality of life of affected individuals. Environmental factors, stress and climate-related factors are among the factors determining the development of AR. Today, AR is considered an inflammatory disorder of the nasal mucosa caused by IgE-mediated T helper 2 (Th2) hypersensitivity responses [4].

Allergic rhinoconjunctivitis (ARC) is one of the most common clinical forms of AR. Perennial allergic conjunctivitis is a persistent mild or moderate inflammatory disorder of the conjunctiva that runs with periods of exacerbation which are not associated with seasonality, and is manifested by hyperemia and mild conjunctival edema. In addition, it is manifested by tearing, photophobia, itching and burning eyes in periods of exacerbation.

Mast cells, lymphocytes and eosinophils are the major cells involved in allergic inflammation of the nasal mucosa and conjunctiva. It should be noted that ARC can affect sleep and inflict damage in multiple spheres of patient's life.

World Health Organization (WHO) guidelines require that the treatment of allergic diseases should include pharmacotherapy, elimination therapy and allergen-specific therapy. Pharmacotherapy can relieve allergic symptoms without causing side-effects and allows maintaining physical and social activity. The basic therapy includes intranasal corticosteroids and antihistamines, and their administration is a mainstay treatment strategy for

allergic diseases. Some authors believe that leukotriene receptor antagonists should be also included in the basic therapy. Symptomatic medications are prescribed mostly to alleviate allergic symptoms. Medicamentous therapy, however, exerts effects only on certain components of allergic disease pathogenesis, but does not prevent disease progression. Ganoderma lucidum and Ganoderma applanatum are polypore macromycetes species containing biologically active substances and their use as a supplement to the basic therapy facilitates the removal of toxic substances from the body and, consequently, an improved patient's well-being [5].

An imbalance between pro-inflammatory and anti-inflammatory cytokines leading to hyperactivated immune response and increased immunoglobulin (Ig) E and IgG production is known to have a key role in allergic inflammation. Cytokines are produced in very low concentrations (5-10 ng per cell), mediate cell-to-cell interactions, and generate initiation, amplification and suppression signals to form physiological and pathological body responses. Various cytokines can exert different and complementary effects on the same target cells. Therefore, studies on cytokine and immunoglobulin levels in, and a search of new approaches to treatment of patients with AR and ARC are of special interest.

The purpose of the study was to compare cytokine and immunoglobulin levels among patients with AR and ARC treated with different therapeutic options.

Material and Methods

Forty patients with AR and ARC were included in the study. Of these, 15 (37.5%) were men and 25 (62.5%) were women. Mean patient age plus or minus standard deviation (SD) was 37.58 ± 14.3 years. Disease duration ranged from 6 months to 2 years. Patients were divided into two subgroups of 20 patients each. Patients of subgroup 1 received the basic therapy (a 10-mg loratadin tablet daily and mometasone furoate nasal spray at a dosage of 200 μg once daily), whereas patients of subgroup 2, the basic therapy plus polypore macromycetes (Astmagan), one capsule twice daily. Treatment course duration was 90 days. The control group was composed of 25 healthy individuals.

A comprehensive clinical immunological examination was conducted at baseline and on the completion of the treatment course. After an overnight fast, 8-ml blood samples were collected from the cubital vein into blood collection tubes. Blood sera were separated by centrifugation at 1200 rpm for 10 mins. Hemolyzed sera were discarded. Enzyme-linked immunosorbent assay (ELISA) kits (from Granum Laboratory, Ukraine; Khema, Ukraine; and IBL, Hamburg, Germany) were used to determine serum IgA, IgM, IgG, IgE, gamma interferon (IFN-γ) and IL4 levels. Photometric measurements were performed on an ELISA plate reader (Stat Fax 2100, Awareness Technologies Inc, Palm City, FL). Analyzes were conducted using conventional ELISA techniques [6]. A calibration curve was used to assess the results. The major components of ELISA kits are monoclonal antibodies adsorbed on the surfaces of ELISA polystyrene plate wells. The results of ELISA analysis were calculated according to ELISA kit manufacturer's instructions. The incubation duration is 4 hours. 100-μl serum samples are required for the analysis, and the control samples are supplied by the manufacturer. The sample is added to the well, and the antigen within the sample binds to antibodies on the well surface. Unbound material is removed by washing. Enzyme-labeled conjugate is added to the well. After a further washing step, the activity of the enzyme bound on the well surface is detected by adding substrate. Absorbance values are read at 450 nm. Absorbance at 450 nm is read using an ELISA microwell plate reader. The color intensity is directly proportional to the concentration of the studied immunoglobulin or cytokine present in the test sample.

We investigated serum levels of the anti-inflammatory cytokine interleukin (IL)-4, a natural inflammation inhibitor and a key cytokine produced by Th2 cells. Its most prominent biological activities are to increase eosinophilia and stimulate growth and accumulation of mast cells. In addition, IL4 increases the expression of IgE Fc receptors, is essential for B cell secretion of IgE and is an antagonist of IFN-γ. Increased serum IL4 levels have been found in allergic patients, especially in those with exacerbated allergic diseases.

IFN-γ, a pro-inflammatory cytokine secreted by activated Th1 lymphocytes and natural killer (NK) cells and has an antiviral activity.

The study followed the ethical standards stated in the Declaration of Helsinki, the European Convention on Human Rights and Biomedicine, and the relevant WHO regulations and laws of Ukraine. This study is a part of the research project "Developing Differential Diagnosis Criteria and Etiopathogenetic Treatment Methods for Allergic, Inflammatory and Tumor Diseases of the Upper Respiratory Tract and Ear" (state registration No. 0121U100260) by ENT Department at Odesa National Medical University. The study was approved by approved by the Ethics Committee of Odesa National Medical University (Committee Meeting Minutes No.31 of May 31, 2021).

Parametric and non-parametric statistical methods were used as appropriate. Statistical analyses were conducted using Statistica 8.0 (StatSoft, Tulsa, OK, USA) software. The data obtained were entered into a spreadsheet database. Data are presented as mean (M) and error of the mean (m). Statistical studies were performed using the Student's t test for paired and unpaired samples as appropriate. The Shapiro-Wilk test was applied to determine whether the quantitative variables were normally distributed.

Results

All patients were diagnosed both with perennial AR and perennial ARC. Rhinoconjunctival syndrome was the most common symptom (100% or 40 patients), followed by reduced work performance (95% or 38 patients), impaired

nasal respiration (82.5% or 33 patients), reduced olfactory performance (55% or 22 patients), and sleep impairment (25% or 10 patients). Household sensibilization was supposed by history collected and confirmed by allergic tests in 14 patients (35%).

The results of serum immunoglobulin analysis of patients with AR and ARC are presented in Table 1. At baseline, serum IgA, IgM and IgE levels were almost twice as high, and serum IgG levels, 1.2 times higher in patients compared to controls, and these differences were significant (p=0.0008; 0.0005; 0.0001 and 0.001, respectively). On completion of the comprehensive treatment, both subgroups of patients with AR and ARC exhibited reductions in serum IgA, IgM, IgE and IgG levels. In subgroup 1, IgA levels decreased by 15.2% (p = 0.0008); IgG levels, by 5.5% (p = 0.07); IgM levels, by 14.0% (p = 0.0005); and IgE levels, by 8.2% (p = 0.0001), compared to baseline, after treatment with the basic therapy only. In subgroup 2, IgA levels decreased by 23.2% (p = 0.0003); IgG levels, by 12.8% (p = 0.0001); IgM levels, by 31.1% (p = 0. 000001); and IgE levels, by 24.4% (p = 0. 0004), compared to baseline, after treatment with the basic therapy plus Astmagan. Therefore, Astmagan, when used as an adjunct to the basic treatment of AR and ARC, contributed to 8%, 17%, 16.2%, and 7.3% greater decreases in the levels of IgA, IgM, IgE and IgG, respectively, compared to basic treatment only, and these differences were significant.

Table 2 compares the serum cytokine levels in patients with AR and ARC at baseline and on completion of the treatment course, and in controls. At baseline, serum IL4 levels were almost 3.5 times higher in patients than in controls (p1-2 = 0.0001). In addition, serum IFN-γ levels were 2.5 times lower in patients than in controls (p1-2 = 0.0001). The results obtained indicate substantially abnormal cytokine profiles in patients with AR and ARC.

Serum IL4 levels in subgroup 1 and subgroup 2 decreased to 37.6 ± 1.7 pg/ml and 32.6 ± 1.94 pg/ml, respectively, after completion of their treatment course, and these decreases were statistically significant (p1-2 = 0.0001). In addition, serum IFN-γ levels in subgroup 1 and subgroup 2 increased to 31.3 ± 3.4 pg/ml and 34.1 ± 3.2 pg/ml, respectively, after completion of their treatment course, and these decreases were statistically significant (p1-2 = 0.0001). Of note that these processes were more marked in patients treated with the basic therapy plus Astmagan (p2-3 = 0.001). Given that, after completion of their treatment course, both treatment groups showed subnormal serum immunoglobulin and cytokine levels, but patients treated with the basic therapy plus Astmagan exhibited greater and more statistically significant improvement, it would be reasonable for them to receive another course, because Astmagan is a natural extraction of Ganoderma lucidum and Ganoderma applanatum and has demonstrated antitoxic effects.

Discussion

Therefore, the current study demonstrated that patients with AR and ARC exhibited characteristic changes in the immune signature, with activation of the humoral immune response and increased serum levels of the cytokine IL4, a Th2 response regulator. In addition, these

Table 1. Serum levels of immunoglobulins A, M, G and E in patients with allergic rhinitis and allergic rhinoconjunctivitis before and after completion of treatment and healthy controls

Characteristics	Healthy controls, n=25 (1)	Patients with AR and ARC (basic therapy), n = 20 (2)		Patients with AR and ARC (basic therapy plus Astmagan), n = 20 (3)	
		baseline	after completion of treatment	baseline	after completion of treatment
IgA, mg/ml	1.57±0.18	3.1±0.43	2.62±0.35	3.14±0.5	2.41±0.4
	p_{1-2}=0.001 p_{1-3}=0.001	p=0.0008		p=0.0003	
		p_{2-3}=0.08			
IgM, mg/ml	1.11±0.25	2.3±0.4	1.98±0.25	2.44±0.22	1.68±0.4
	p_{1-2}=0.001 p_{1-3}=0.001	p=0.0005		p=0.000001	
		p_{2-3}=0.008			
IgG, mg/ml	13.3±1.8	16.5±2.0	15.6±1.3	16.4±1.2	14.3±2.0
	p_{1-2}=0.001 p_{1-3}=0.001	p=0.07		p=0.0004	
		p_{2-3}=0.016			
IgE, pg/ml	116.7±6.7	280.2±12.0	257.0±9.2	279.0 ±11.0	210.8±24.1
	p_{1-2}=0.001 p_{1-3}=0.001	p=0.0001		p=0.0001	
		p_{2-3}=0.0001			

Note: n, number of patients; p, significance of difference; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis

Table 2. Serum levels of pro-inflammatory cytokine IL4 and anti-inflammatory cytokine IFN-γ in patients with allergic rhinitis and allergic rhinoconjunctivitis before and after completion of treatment and healthy controls

Characteristics pg/ml	Healthy controls (1)	Patients with AR and ARC (basic therapy only) (2)		Patients with AR and ARC (basic therapy plus Astmagan) (3)	
		Baseline	After completion of treatment	Baseline	After completion of treatment
IL4	22.1±2.5	78.1±4.1	37.6±1.7	77.0±2.98	32.6±1.94
	p_{1-2}=0.0001 p_{1-3}=0.0001	p=0.0001		p=0.0001	
		p_{2-3}=0.001			
IFN-γ	38.0±2.1	15.6±1.7	31.3±3.4	15.7±1.27	34.1±3.2
	p_{1-2}=0.0001 p_{1-3}=0.0001	p=0.0001		p=0.0001	
		p_{2-3}=0.01			

Note: n, number of patients; p, significance of difference; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis

patients exhibited reduced Th1 function as indicated by reduced IFN-γ production. The proinflammatory mediator IFN-γ activates differentiation of native T cells towards a Th1 phenotype and inhibits Th2 cells, whereas negative regulation of IFN-γ production is promoted by IL4 and IL10 [7]. This is in agreement with our findings (e.g., the baseline serum IL levels were almost 3.5 times higher in patients with AR and ARC than in healthy controls). It is well known that it is IL4, also named as B-cell stimulating factor 1 that is a major cytokine in the development of allergic inflammation and is secreted by activated Th2 lymphocytes. Its major function consists in switching from IgG1 production to IgG4 production [8]. IL4 secreted by Th2 lymphocytes is capable of inhibiting IFN-γ secretion by T cells and thus increases the shift in the differentiation of Th2 precursors which also secrete IL2.

IgE secretion is T dependent. IL4 secreted by Th2 cells is a major interleukin involved in plasma cell switching from IgM production to IgE production. Plasma cell switching from IgM production to IgE production requires also a direct contact between the plasma cell and the Th2 cell (that is, the interaction between CD40 and CD40L).

In patients with AR and ARC, increased IL4 expression results in the infiltration of the affected mucous with Th2 lymphocytes and eosinophils and increased IgE and IgG production. IL4 can inhibit not only IFN-γ secretion, but also macrophage production of the anti-inflammatory cytokines and chemokines whose synthesis is induced and stimulated by IFN-γ. Therefore, the presence of both increased serum IgE levels and eosinophilia may indicate hyperreactivity of the IgE system and increased IL4 production [9]. IFN-γ usually inhibits IL4 production and promotes cell differentiation to Th1 lymphocytes.

Th2 cells produce not only IL4, but also IL3, IL5 and IL10. IL3 and IL5 are required to enable the development and activation of mast cells and eosinophils, which in turn enable the implementation of the pathochemical and pathophysiological stages of the allergic reaction [10]. In addition, IFN-γ secreted by Th1 has been shown to inhibit IgE production and promote IgG production (that is, IFN-γ and IL4 act as antagonists). The support of IgE production also depends on the effects of other interleukins (such as IL4 and IL6). There is a growing body of evidence supporting the prominent role that almost all immunocompetent cells and their mediators play in the course of AR and ARC. Therefore, further research is required to investigate the essence of allergic inflammation in order to develop a well-grounded approach to the use of available methods of treatment and search of new pharmacological medications. Because ARC is an immune-related disease of high social importance, introducing new pharmacological medications in the treatment of the disease is deemed to be highly relevant.

A recent clinical study [7] by British researchers demonstrated that nutritional supplementation with the mushroom Coriolus versicolor can improve the imbalance between Th1 and Th2 and thus reverse a TH1 to TH2 shift. The current study demonstrated that the patients treated with the basic therapy plus Astmagan exhibited statistically significant improvements in serum levels of IgA, IgM, IgG, IgE, anti-inflammatory cytokine IFN-γ and pro-inflammatory cytokine IL4 compared to the patients treated with the basic therapy only. Therefore, the use of biomass of the macromycetes Ganoderma lucidum and Ganoderma applanatum as a supplement to the treatment of AR and ARC is believed to be pathogenetically grounded and clinically promising.

Conclusion

First, at baseline, serum levels of IgA, IgM and IgE were almost twice as high (p = 0.0008; 0.0005; and 0.0001, respectively); IgG, 1.2 times higher (p = 0.001); pro-inflammatory cytokine IL4, 3.5 times higher (p = 0.0001); and anti-inflammatory cytokine IFN-γ, 2.4 times

lower ($p = 0.0001$) in patients with AR and ARC compared to controls, and these differences were significant.

Second, Astmagan, when used as an adjunct to the basic treatment of AR and ARC, contributed to 8%, 17%, 16.2%, 7.3% and 6.0% greater decreases in the serum levels of IgA, IgM, IgE, IgG and IL4, respectively, and a 16.6% greater increase in the serum level of IFN-γ compared to the basic treatment only, and these differences were significant, with an improvement in immune response to the proposed therapy.

References

1. Savouré M, Bousquet J, Jaakkola J, Jaakkola MS, Nadif R. Worldwide prevalence of rhinitis in adults: A review of definitions and temporal evolution. Clin Transl Allergy. 2022 Mar;12(3):e12130. doi: 10.1002/clt2.12130.
2. Weaver-Agostini J, Kozak Z, Bartlett S. Allergic. Rhinitis: Rapid Evidence Review. Am Fam Physician. 2023;107(5):466-473. https://europepmc.org/article/MED/37192071https://pubmed.ncbi.nlm.nih.gov/37192071/
3. Gani F, Lombardi C, Barrocu L. The control of allergic rhinitis in real life: a multicenter cross-sectional Italian study. Clin Mol Allergy. 2018 Feb 2:16:4. doi: 10.1186/s12948-018-0082-y.
4. Melnikov OF, Gavrylenko IuV. [Clinical and immunological efficacy of allergic rhinitis treatment in children]. Klinychna imunologiia. Alergologiia. Infektologiia. 2018; 111(6):29-32. Ukrainian.
5. Kenyon-Dove K. Observational Non-Controlled Study of the use of coriolus versicolor supplementation in 30 Cancer Patients. Mycology News. 2003; 1[7]. Available from www.mycologyresearch.com
6. Kuznetsova LV, Babadzhan VD, Kharchenko NV, editors. [Immunology: A Textbook]. Vinnytsia: Merkuri Pofillia LTD; 2013. Ukrainian.
7. Monro J. Reversing Th1 and Th2 shifts with Coriolus versicolor Supplementation in Chronic Fatigue Syndrome Patients. Mycology News. 2004; 1[8]. www.mycologyresearch.com
8. Kazymirchuk VE, Kovalchuk LV, Maltsev DV. [Clinical immunology and allergology with age-related features]. Kyiv: Medytsyna;2012. Ukrainian.
9. Bachaer LB, Geha RS. Molecular mechanisms of IgE regulation. Allergy Clin Immunol. 2000 Feb;105(2 Pt 2):S547-58. doi: 10.1016/s0091-6749(00)90059-9.
10. Jayaprakasam B, Yang N, Wen MC, et al. Constituents of the anti-asthma herbal formula ASHMI(TM) synergistically inhibit IL-4 and IL-5 secretion by murine Th2 memory cells, and eotaxin by human lung fibroblasts in vitro. J Integr Med. 2013 May;11(3):195-205. doi: 10.3736/jintegrmed2013029.

Disclosures

Received 29.09.2023

Accepted 10.10.2023

Corresponding Author: *Viktor K. Bohdanov. E-mail: blackmoresvictor5@gmail.com*

Author Contributions: *SMP: Conceptualization, Methodology; VKB: Literature Collection and Review, Writing – original draft; MBM: Immunological Studies; SIP: Ophthalmological Studies; KGB: Writing – review & editing. All authors reviewed the results and approved the final version of the manuscript.*

Conflict of interest: *All authors declare no conflict of interest.*

Abbreviations: *AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; IFN, interferon; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; IL, interleukin; Th, T helpers.*

Funding Information: *This research received no special grant from any funding agency.*