Effects of long-term low-dose treatment by clarithromycin on Th1 cytokine levels in nasal discharge of patients with nasal polyposis

Učinci dugotrajnog, niskodoziranog liječenja klaritromicinom na koncentracije Th1 citokina u nosnome sekretu u bolesnika s nosnom polipozom

Aleksandar Perić[1*], Danilo Vojvodić[2], Vladimir Jakovljević[3], Nenad Baletić[1], Ivan Stanojević[2]

[1]Department of Otorhinolaryngology, Military Medical Academy, Belgrade, Serbia

[2]Institute of Medical Research, Division of Clinical and Experimental Immunology, Military Medical Academy, Belgrade, Serbia

[3]Institute of Physiology, Medical Faculty Kragujevac, Serbia

Primljeno: 12. 11. 2011.
Prihvaćeno: 28. 2. 2012.

Abstract. ***Aim:*** Inflammatory processes in nasal mucosa are reflected in various local mediators, found both in mucosal tissue and nasal discharge. In this prospective study, we assessed the effects of long-term low-dose oral administration of clarithromycin (CAM) on Th1 cytokines in nasal secretions and on clinical parameters of severity of nasal polyposis. ***Methods:*** A total of forty nasal polyp patients (22 nonallergic and 18 allergic) received 500 mg/day single oral dose of CAM for eight weeks. We measured the levels of proinflammatory Th1 cytokines TNF-α, TNF-β, IL-1β, IL-2, IL-12, and IFN-γ in the nasal fluid samples, before and after treatment by CAM, using flow cytometric method. Before and after therapy, we scored each of the 40 patients according to nasal symptom score and endoscopic score. ***Results:*** Following treatment, we found significantly reduced levels of TNF-α (p=0.006) in nasal secretions of nonallergic patients, and of IL-1β (p=0.008) in nasal fluid of allergic patients. Our results suggest an association between the reduction of nasal polyp size and reduction of TNF-α levels in nasal fluid of nonatopic patients and an association between the reduction of nasal polyp size and reduction of IL-12 levels in nasal discharge of atopic patients. Macrolide therapy decreased the size of polyps in 10/22 nonatopic and in 9/18 atopic patients. After macrolide therapy, we found 67.83% nonallergic subjects and 55.55% allergic subjects with improved nasal symptomatology. ***Conclusion:*** Long-term low-dose treatment with CAM is effective in the management of nasal polyposis, because of its antiinflammatory and immunomodulatory actions.

Key words: chronic inflammation, clarithromycin, nasal polyposis, nasal secretions, Th1 cytokines

Sažetak. ***Cilj:*** Upalni procesi u nosnoj sluznici očituju se u različitim lokalnim medijatorima, u tkivu sluznice i u nosnom sekretu. U ovoj prospektivnoj studiji procijenili smo učinke dugotrajne, niskodozirane terapije klaritromicinom (CAM) na Th1 citokine u nosnome sekretu, kao i na kliničke parametre očitovanja nosne polipoze. ***Metode:*** Četrdesetero (22 nealergičnih i 18 alergičnih) bolesnika s nosnom polipozom dobivalo je po jednu dnevnu dozu od 500 mg CAM-a tijekom osam tjedana. Mjerili smo koncentracije proupalnih Th1 citokina TNF-α, TNF-β, IL-1β, IL-2, IL-12 i IFN-γ u uzorcima nosnoga sekreta, prije i nakon terapije CAM-om, primjenom protočne citometrije. Prije i nakon liječenja klinički smo klasificirali svakoga od četrdesetero bolesnika prema nosnome simptom rezultatu i endoskopskom rezultatu. ***Rezultati:*** Nakon liječenja detektirali smo značajno niže koncentracije TNF-α (p = 0,006) u nosnome sekretu nealergičnih bolesnika i IL-1β (p = 0,008) u nosnome sekretu alergičnih bolesnika. Naši rezultati sugeriraju povezanost između smanjenja veličine nosnih polipa i snižavanja koncentracije TNF-α u nosnom sekretu u neatopičnih bolesnika, kao i povezanost između smanjenja veličine nosnih polipa i snižavanja koncentracije IL-12 u nosnome sekretu u atopičnih ispitanika. Terapija makrolidnim antibiotikom smanjila je veličinu polipa u 10/22 nealergičnih i u 9/18 alergičnih bolesnika. Nakon makrolidne terapije našli smo 67,83 % nealergičnih i 55,55 % alergičnih ispitanika s poboljšanim nosnim simptomima. ***Zaključak:*** Zbog protuupalnih i imunomodulacijskih djelovanja, dugotrajna niskodozirana primjena CAM-a korisna je u liječenju nosne polipoze.

Ključne riječi: klaritromicin, kronična upala, nosna polipoza, nosni sekret, Th1 citokini

Corresponding author:
[*] **Dr. Aleksandar Perić**
Clinic of Otorhinolaryngology,
Rhinology Unit
Military Medical Academy
Crnotravska 17, 11 040 Belgrade, Serbia
e-mail: alexneta@sezampro.rs

http://hrcak.srce.hr/medicina

INTRODUCTION

Macrolide antibiotics, such as erythromycin (EM), clarithromycin (CAM), and roxithromycin (RXM), are important chemotherapeutic agents in treatment of infections. Over the last decade, there has been a growing interest of various investigators in the immunomodulatory and anti-inflammatory action of 14-membered and 15-membered macrolides in the long-term low-dose treatment of chronic rhinosinusitis and nasal polyposis. Nasal polyps develop usually in the anterior ethmoidal area and appear as grape-like structures, often in relation to inflammatory condition, but the exact etiology is still under debate. Oxidative stress and chronic persistent inflammation are the main factors in the development of nasal polyps and inflammation triggers include bacterial, fungal and viral infection, allergy, and environmental pollution[1,2].

Macrolide antibiotics are known to have anti-intlammatory and immunomodulatory effects in treatment of chronic rhinosinusitis and nasal polyposis. In this prospective study, we investigated the effects of long-term low-dose treatment by clarithromycin (CAM) on Th1 cytokine levels in nasal fluid of non-atopic and atopic patients with nasal polyposis.

Nasal polyps consist of loose connective tissue, oedema, inflammatory cells and some glands and capillaries, and are covered with various types of epithelium, but mostly pseudostratified epithelium with ciliary cells and goblet cells[3,4]. Additionally, studies have found higher numbers of inflammatory cells, especially eosinophils, neutrophils and lymphocytes in nasal polyp lamina propria compared to healthy nasal mucosa[3,4]. There have been many reports regarding the pharmacological actions of macrolides in treatment of chronic rhinosinusitis and its complicated form-nasal polyposis[5-7]. Those actions include suppression of proliferation of nasal polyp fibroblasts, shrinkage of nasal polyps, suppression of production of chemokines IL-8 and RANTES, etc[5-7]. To our knowledge, there has been a little studies about the role of T helper 1 (Th1) cytokines in etiology of nasal polyposis and there has been a little description of the influence of macrolide treatment on the production of these cytokines.

Nasal secretion contains small amounts of cytokines, potent biologic factors involved in the regulation of inflammation and immune defense, and other inflammatory mediators expressed by various epithelial and nonepithelial cells[8]. These mediators have a dominant role in the patophysiology of airway disease. Thus, the cytokine profile in nasal fluid may help to recognize mechanisms underlying nasal polyposis and the immunomodulatory effects of treatment by antibiotics. In contrast to biopsy, sampling of nasal discharge is easy, non-invasive and reproducibly accessible method.

In the present prospective, non-placebo controlled study, we analysed the effects of long-term low-dose administration of macrolide antibiotic clarithromycin on the clinical parameters of nasal polyposis and Th1 cytokine levels measured in nasal secretions. Our aim was also to investigate whether allergic and nonallergic patients with nasal polyposis have different outcome regarding the production of these cytokines during macrolide treatment.

MATERIALS AND METHODS

Patients

The study population included forty (n = 40) patients with nasal polyposis, 22 nonatopic and 18 atopic. Written informed consent was obtained from all subjects. This prospective study was performed according to the declaration of Helsinki and was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia. The diagnosis of nasal polyposis was based on a documented medical history and on the results of physical examination, nasal endoscopy and computerised tomography (CT) of the paranasal sinuses, according to the current European Guidelines[9]. Nasal polyposis, which is considered to be a complicated form of chronic rhinosinusitis, is defined as inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal disc-

harge (anterior, posterior nasal drip) ± facial pain/pressure, ± reduction or loss of sensation of smell and either endoscopic signs of polyps and/or mucopurulent discharge primarily from middle meatus and/or oedema/mucosal obstruction primarily in middle meatus, and/or CT changes showing mucosal changes within the ostiomeatal complex and/or sinuses for more than 12 weeks[9]. The exclusion criteria were diagnosis of cystic fibrosis, primary ciliary dyskinesia, the presence of lower airways obstruction symptoms, bronchial asthma, aspirin sensitivity, antrochoanal and sphenochoanal polyps. All subjects included in this investigation had no acute respiratory tract infection. Glucocorticosteroid, antibiotic and antihistamine treatment was not allowed and any such treatment was withdrawn at least three weeks before study entry.

Allergy determination

The atopic status was evaluated in all subjects, on the basis of medical history of allergy and positive skin-prick tests. Skin-prick tests were performed on the volar part of the forearm with a standard battery of common aeroallergens: birch, timothy, mugwort (lat. *Artemisia vulgaris*), dog, cat, horse, mite (lat. *Dermatophagoides farinae, Dermatophagoides pteronyssinus*), moulds (lat. *Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum*, *Olea europaea, Parietaria judaica, Plantago lanceolata, Platanus acerifolia*). Negative (0.9% natrium-chloridum solution) and positive (1 mg/ml histamine dihydrochloride solution) controls were also included with each skin-prick tests. Reactions were read after 15 min and a test was considered positive if the diameter of wheal was greater than 3 mm with respect to the negative control.

Drug treatment

Forty (n = 40) patients with nasal polyps, 22 nonallergic and 18 allergic, received 500 mg/day (single oral dose) of the 14-membered ring macrolide antibiotic clarithromycin (CAM) for 8 weeks. There was no concomitant medication used during the macrolide therapy. The exclusion criteria for long-term low-dose macrolide treatment were: pregnancy, macrolide hypersensitivity, age under 18 years, liver dysfunction or gastrointestinal dysfunction.

Clinical score

To investigate the effect of CAM, the patients were asked to assess their symptoms associated with nasal polyposis (obstruction, anosmia, sneezing, rhinorrhea, and itching) on the day of the enrollment in the study and after macrolide treatment and to score their symptoms from 0 to 3: 0 for no symptoms, 1 for mild symptoms, 2 for moderate symptoms, and 3 tor severe symptoms, so that the maximal nasal symptom score is 15.
Nasal endoscopy was performed in a sitting position with a rigid endoscope 0° and 30° (Storz, Tuttlingen, Germany). Neither topical anaesthesia nor decongestants were used. Before the CAM administration and within seven days after it, endoscopic physical findings were scored according to Lildholtd et al.[10]. The degree of nasal polyps is classified in relation to fixed anatomical landmarks in four steps: 0 = “no polyposis”, 1 = “mild polyposis (small polyps not reaching the upper edge of the inferior turbinate)”, 2 = “moderate polyposis (medium sized polyps reaching between the upper and lower edges of the inferior turbinate)”, 3 = “severe polyposis (large polyps reaching below the lower edge of the inferior turbinate)”. The maximal endoscopic score is 6, bilaterally. Treatment results were divided into the following two categories: *improvement* and *no improvement*. We have defined *improvement* as observation of shrinkage of nasal polyps by more than one grade after the CAM administration.

Sampling of nasal fluid and Th1 cytokine determination

Nasal discharge samples were collected from nasal cavities of all 40 subjects (22 nonatopic and 18 atopic nasal polyp patients) before and after treatment with CAM using modified absorption technique. This was done by placing cotton-wool sticks (Institute of Virrology, Vaccines and Sera, Torlak, Belgrade, Serbia) into the nasal cavity posterior to the muco-cutaneous junction, near by the middle nasal meatus for 60 seconds, as previously described[11-13]. All samples were placed in a 2 ml Eppendorf tube containing 1 ml of transfer

medium (phosphate-buffered saline with gentamycin 50 µg/ml, penicillin G 340 U/ml, fungizone 500 µg/ml) for 30 minutes to allow diffusion of cytokines into the medium and then stored at 4°C for a maximum of 2 h until processed. Nasal discharge was centrifuged at 1000 *g* for 10 minutes to separate the cellular components. After centrifugation, supernatants were portioned and stored at -70°C untill cytokine determination. The levels of Th1 cytokines (TNF-α, TNF-β, IL-1β, IL-2, IL-12 and IFN-γ) were measured in each of the 80 samples using commercial flow cytometric kit (Flow Cytomix, Bender MedSystems, USA) on the flow cytofluorimeter (Beckman Coulter XL-MCL, USA), which was connected with BMS Flow Cytomix Pro 2.2 Software, according to the manufacturer's instruction. The sensitivity of detection was as follows: 22 pg/ml for TNF-α; 32 pg/ml for TNF-β; 17 pg/ml for IL-1β; 28 pg/ml for IL-2; 5 pg/ml for IL-12; 8 pg/ml for IFN-γ.

Statistical analysis

Data was presented as means ± standard deviation (SD), according to the normal distribution test. Differences in levels of Th1 cytokines, as well as differences in nasal symptom score and in nasal polyp size were examined using Wilcoxon's signed rank test. Between-group comparisons were analyzed using the nonparametric Chi square-test. A *p* value of 0.05 or less was considered to be statistically significant. All statistical calculations were performed with SPSS software (Statistical Package for the Social Sciences, version 11.0, SPSS Inc, Chicago, IL).

RESULTS

Six female and 16 male nonatopic patients with nasal polyps (mean age 42 (25-72) years) and 8 female and 10 male atopic patients (mean age 45 (19-65) years) received CAM. In nonallergic subjects, the average nasal symptom score improved from 12±2 before treatment with CAM to 9±4 after treatment (p=0.041) (Figure 1). In allergic patients, the average nasal symptom score decreased after therapy by CAM from 12±2 to 10±3 (p=0.046) (Figure 1). After macrolide treatment, we found 67.83% patients in the nonallergic group and 55,55% patients in the allergic group with improved nasal symptoms. These differences were not statistically significant (chi square-test).

In nonallergic patients, we found a significant difference in the endoscopic score before and after treatment (5±1 *vs* 4±2) (p=0.043) (Figure 2). The average size of allergic patients' polyps was smaller after therapy (5±1 *vs* 4±1) (p=0.042) (Figure 2). The size of nasal polyps decreased in 45.45% (10 of 22 cases) of patients without allergy and in 50% allergic patients (9 of 18 cases) but

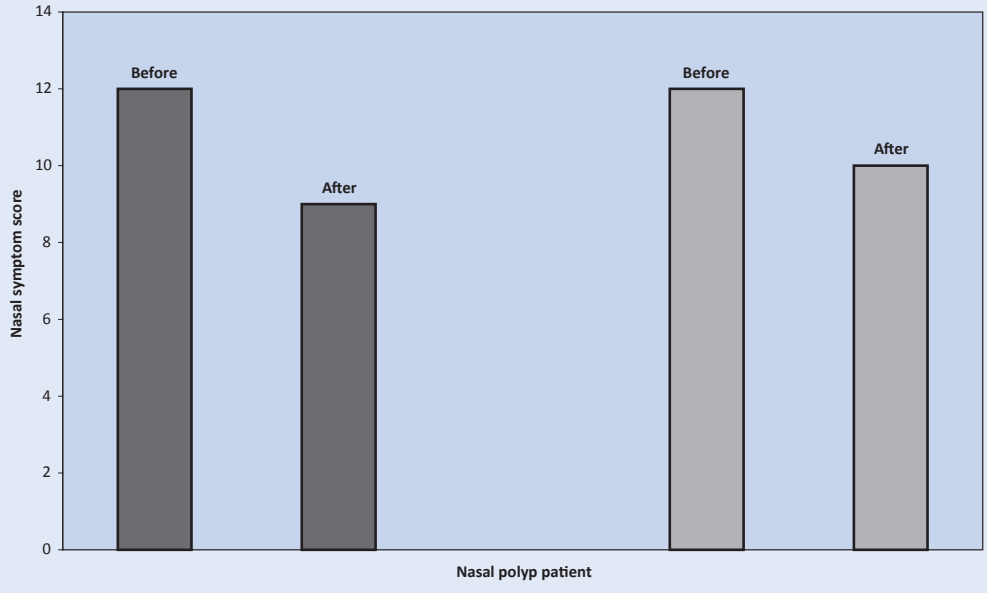

Figure 1. Nasal symptom score of nonallergic patients (dark) and allergic patients (grey) with nasal polyposis, before and after CAM administration.

the difference was not statistically significant (chi square-test). In the *improvement* group, comparing the endoscopic findings before and after CAM therapy, there was a higher statistically significant difference in the nasal polyp size. In nonallergic patients, the size of the polyps decreased from 5±1 to 3±1 (p=0.007) (Figure 3). In allergic subjects, the average endoscopic score improved from 4±1 to 2±1 (p=0.006) (Figure 3).

We found no significant differences in the levels of TNF-β, IL-2, IL-12 and IFN-γ in the nasal secretions before and after macrolide treatment (Table 1). Only the concentration of proinflammatory Th1 cytokine TNF-α in the nasal discharge of nonatopic patients was the highly statistically lower after CAM treatment (p=0.006) (Table 1). In the group of allergic patients with nasal polyposis, we found significantly lower concentration of IL-1β (p=0.008) after therapy with CAM (Table 1). When we divided our patients into the *improvement* and *no improvement* group, we found new interesting details. In nonatopic patients, the levels of TNF-α significantly decreased in the *improvement* group (p=0.003). However, in the *no improvement* group, there was no difference in the mean level of TNF-α before and after treatment (p=0.067) (Table 2). In subjects with allergic rhinitis, comparing the post macrolide treatment outcomes for levels of IL-1β, we found no differences between the *improvement* (p=0.007) and *no improvement* group (p=0.006). However, we found significantly lower levels of IL-12 in the *improvement* group of atopic patients after CAM administration (p=0.037) (Table 2).

DISCUSSION

Ichimura et al[14] found that roxithromycin (RXM) administered at 150 mg/day for at least 8 weeks shrank the nasal polyp size in 52% of twenty investigated patients. They reported that the efficacy of macrolide therapy is not related to allergic symptoms. Our results also showed that there was no relationship between the presence of atopy and clinical efficacy of macrolide treatment. The results of the bacterial cultures suggest that the risk of selecting resistant bacteria is low[15]. In a small number of patients the cultures were positive, but this was not always linked with an increase in symptoms, which could be due to the fact that in addition to the direct bacteriostatic effect of macrolides, they may in some cases reduce the virulence of bacteria without eradicating them[15].

However, the mechanisms of polyp shrinkage during macrolide treatment are not well known. Nonaka et al[16] demonstrated that in vivo RXM treatment directly suppressed nasal polyp fibroblasts (NPFs) proliferation, and that this effect of RXM on fibroblast growth was persistent, indicating that RXM may prevent the progression of nasal polyps by inhibiting the development of fibrosis.

Nasal polyposis is an example of an extreme immune dysregulation. Clinical, as well as experimental studies indicate that nasal polyp formation and growth are activated and perpetuated by an integrated process of mucosal epithelium, lamina propria and inflammatory cells, which, in turn, may be initiated by both infectious and non-infectious inflammation[4]. Various toxic and infectious agents, as well as allergens, encountered at the level of nasal/paranasal mucosa, may activate innate immune mechanisms and lead to induction of pro-inflammatory cytokines. Results presented by Fundová et al[17] suggest that dysregulations in innate immune mechanisms (for example signaling through toll-like receptors and induction of nuclear factor kappa B (NF-κB) and therefore cytokine production) and a defect in homeostasis of epithelial cells and prolonged cell survival, may play a role in pathogenesis and growth of nasal polyps. However, in the world literature, we can find small number of studies regarding the role of Th1 cytokines in pathogenesis of this disease. The Th1 responses are dominated by phagocytic cell-mediated immune responses, with a marked increase in production of Th1 cytokines[18].

IL-2 is an essential growth factor for T-cells and it acts in an autocrine fashion to stimulate T-cell proliferation and also serves to regulate immunoglobulin production and the growth of cytotoxic T-lymphocytes and natural-killer (NK)-cells[18]. IL-12 is a heterodimeric cytokine consisting of p40 and p35 sub-units, that is secreted by macrophages/monocytes, B cells and other antigen-pre-

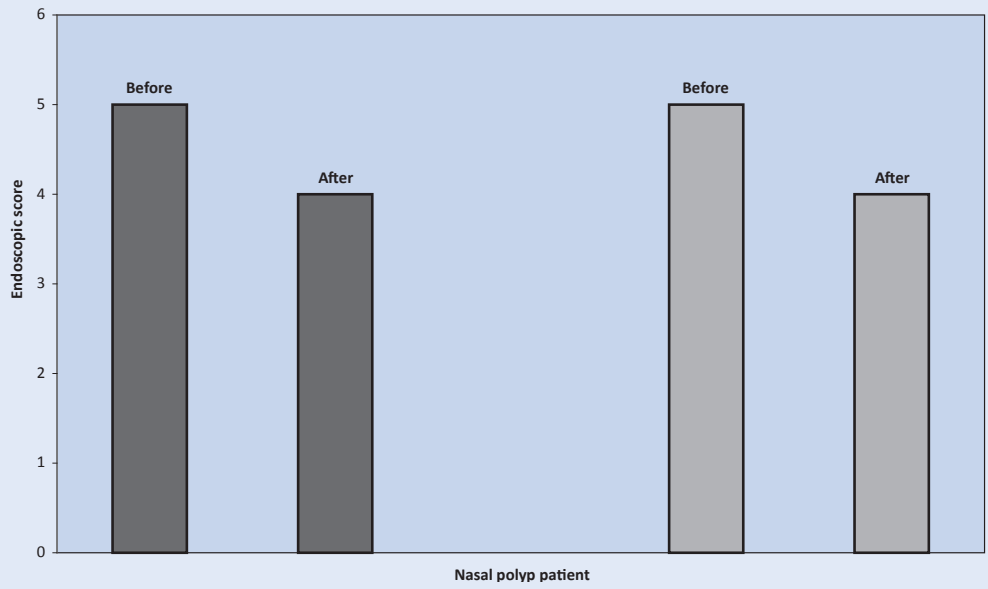

Figure 2. Endoscopic score of nonallergic patients (dark) and allergic patients (grey) with nasal polyposis, before and after treatment by CAM.

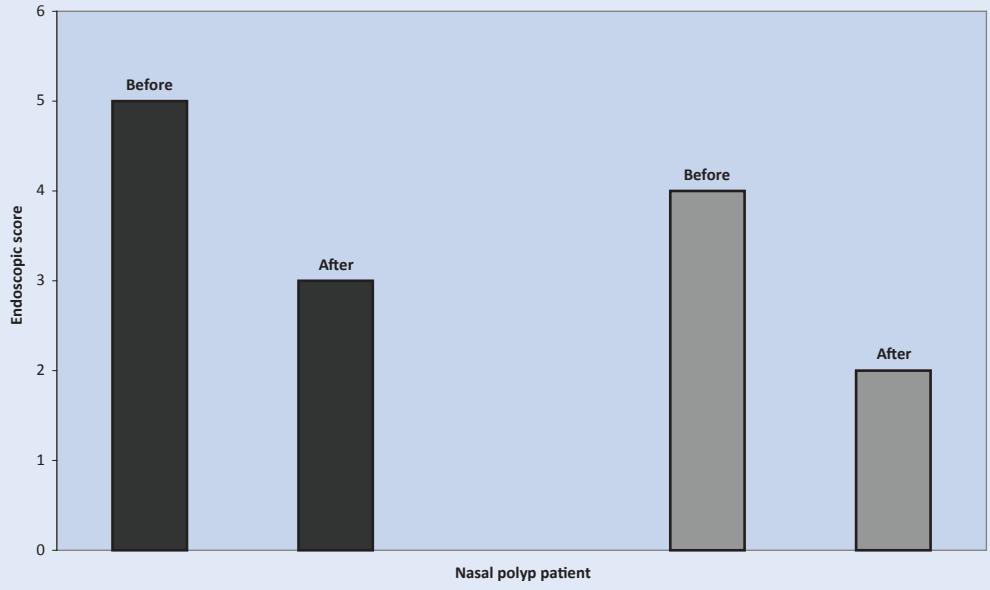

Figure 3. Endoscopic score of nonallergic patients (dark) and allergic patients (grey) in the *improvement* group, before and after CAM administration.

senting cells (APCs), and has multiple immunoregulatory effects on various cell population in different inflammatory conditions[19,20]. IL-12 also plays an important role in the proliferation, cytolytic activity and IFN-γ production by T cells and NK cells[19,20]. According to our results, long-term low-dose CAM administration didn't reduce the level of this cytokine in nasal discharge. However, when we divided our patients into the *improvement* and *no improvement* group, we found reduced levels of IL-12 in the *improvement* group of allergic patients. This suggests a possible association between the reduction of nasal polyp size and reduction of IL-12 levels in nasal secretions only in atopic subjects. IFN-γ is a Th1 cytokine which leads via macrophage activation to extensive inflammatory processes that also enable the killing of intracellular pathogens[18]. Dellacono et al[21] hypothesize that elevated levels of IFN-γ activate lymphocytes and eosinophils within the na-

Table 1. Th1 cytokine levels in nasal discharge of treated patients, before and after macrolide administration.

	Nonallergic patients			Allergic patients		
Cytokine	**Before**	**After**	**p**	**Before**	**After**	**p**
TNF-α	153.05±123.29*	44.22±47.74*	p=0.006	109.88±82.95*	113.31±98.91*	p=0.965
TNF-β	89.39±132.25*	84.88±102.54*	p=0.614	78.66±84.09*	76.99±70.00*	p=0.687
IL-1β	50.39±48.44*	42.85±43.93*	p=0.733	130.00±94.01*	34.31±18.23*	p=0.008
IL-2	148.76±142.87*	132.65±115.05*	p=0.200	122.22±96.36*	134.11±135.82*	p=0.913
IL-12	15.28±14.08*	19.04±18.33*	p=0.287	21.94±15.73*	12.61±14.79*	p=0.057
IFN-γ	19.81±18.56*	22.56±24.06*	p=0.223	39.27±31.91*	30.22±36.36*	p=0.061

*All cytokine levels are expressed in picograms/mililitres (pg/ml) and presented as means ± standard deviation (SD)

Table 2. The relationship between cytokine levels in nasal fluid and change of nasal polyp size: TNF-α levels in nasal secretions of nonallergic patients and IL-1β and IL-12 levels in nasal fluid of allergic patients in *improvement* and *no improvement* group.

	***Improvement* group**			***No improvement* group**		
Cytokine	**Before**	**After**	**p**	**Before**	**After**	**p**
TNF-α	224.38±174.68*	42.86±47.81*	p=0.003	92.13±86.17*	78.86±81.62*	p=0.067
IL-1β	143.28±128.37*	47.36±28.25*	p=0.007	121.15±87.63*	32.26±19.87*	p=0.006
IL-12	31.87±25.58*	11.73±12.06*	p=0.037	26.71±19.22*	21.96±23.13*	p=0.097

*All cytokine levels are expressed in pg/ml and presented as means ± standard deviation (SD).

sal polyp tissue. They found a positive correlation between the increased IFN-γ levels and presence of allergy and asthma in patients with nasal polyps[21].

The results of our investigation showed that the TNF-α concentrations in nasal discharge of nonallergic patients was significantly reduced after treatment with CAM. Therefore, the decreased TNF-α levels in nasal secretions were associated with reduction of polyp size only in nonatopic patients. TNF-α is the main proinflammatory cytokine of Th1 immune response[22]. It is secreted by macrophages, monocytes and natural killer (NK) cells and many other cell types, especially by activated eosinophils[22,23]. TNF-α, among other cytokines can regulate fibroblast activity and collagen formation through modulation of collagenase activity[23]. Relationship between decreased levels of TNF-α in nasal fluid and shrinkage of polyps can be explained by several recently published findings. Eosinophil infiltration is regulated by numerous chemokines and adhesion molecules such as eotaxin, regulated on activation of normal T cell expressed and secreted (RANTES), and vascular cell adhesion molecule (VCAM)-1[24]. To infiltrate sites of inflammation, eosinophils leave the bloodstream and pass through the endothelium in four steps, namely rolling, adhesion, transendothelial migration, and chemotaxis[24]. Adhesion molecules, such as VCAM-1 play an important role during adhesion to endothelial cells[24]. Experiments performed by Ohori et al[24] demonstrated that TNF-α stimulation induces VCAM-1 protein production and mRNA expression in human nasal polyp fibroblasts. Epithelial and immunocompetent cells such as macrophages, mast cells and, especially, eosinophils produce TNF-α. These findings suggest that TNF-α increases VCAM-1 production in nasal fibroblasts and activates the transmigration of eosinophils which induce further production of TNF-α and accelerate the accumulation of eosinophils in nasal polyps. Saji et al[25] demonstrated that NPFs produced RANTES by stimulation with TNF-α and IL-1β. Therefore, results published by Yoshifuku et al[26] showed that eotaxin secretion from fibroblasts was induced by stimulation with IL-4 and synergistically enhanced by simultaneous stimulation with TNF-α and IL-4. Iino et al[27] demonstrated that long-term

low-dose administration of erythromycin inhibits the production of TNF-α by human monocytes *in vitro.* These results showed that treatment with macrolide antibiotic could suppresse TNF-α production and the progression of nasal polyps due to inhibition of fibroblast and monocyte activity in nasal polyp tissue.

Our results also showed decreased levels of IL-1β in nasal secretions in atopic patients after treatment by CAM. IL-1β plays a crucial role in the pathogenesis of chronic rhinosinusitis and nasal polyps. This strong Th1 proinflammatory cytokine secreted by epithelial cells, monocytes, macrophages and fibroblasts upregulates the expression of E-selectin and intercellular adhesion molecule-1 (ICAM-1) in vascular endothelial cells, and thereby induces the extravascular transmigration of neutrophils[28]. The emigrated neutrophils then secrete IL-1β, which amplifies the expression of E-selectin and ICAM-1, resulting in further neutrophil infiltration[28]. Miyanohara et al[29] revealed that clarithromycin suppressed IL-1β gene expression in human nasal epithelial cells *in vitro*. We demonstrated that macrolide treatment of nasal polyposis have different immunomodulatory and similar clinical effects in allergic and nonallergic patients. On the other hand, these results may be due to the fact that atopic and nonatopic patients have different mediator profiles in their nasal secretions[30], implying clear differences in pathogenesis of their nasal polyps.

Treatment by CAM decreased TNF-α levels in non-allergic and IL-1β levels in allergic patients and showed a strong anti-inflammatory and different immunomodulatory effects in non-atopic and atopic patients.

CONCLUSION

Evaluation of the cytokine levels in nasal discharge could be an valuable path in monitoring nasal polyp patients, as well as sensitive way to assess new therapies for nasal polyposis and to study the pathogenesis of this disease. Low-dose macrolide treatment proved effective in the management of nasal polyposis in both atopics and noatopics. These results indicate the importance of TNF-α and IL-1β regarding their influence on eosinophils and neutrophils in the pathogenesis of atopic and nonatopic form of nasal polyposis. Macrolide treatment may help minimize surgical treatment. We suggest that macrolides can be an alternative to topical and systemic glucocorticoids in the management of nasal polyposis.

AKNOWLEDGEMENTS

This study was supported by grants from the Military Medical Academy Research Fund. It was performed as a part of project of the Institute of Medical Research, Military Medical Academy, Belgrade, Serbia (VMA/11-14/B-6). The authors declare that they have no conflict of interest.

REFERENCES

1. Veyseller B, Aksoy F, Ertaş B, Keskin M, Özturan O, Yildirim YS. A new oxidative stress marker in patients with nasal polyposis: advanced oxidative protein products (AOPP). B-ENT 2010;6:105-9.
2. Bonfils P, Avan P, Malinvaud D. Influence of allergy on the symptoms and treatment of nasal polyposis. Acta Otolaryngol 2006;126:839-44.
3. Bachert C, Robillard T. Management of nasal polyposis. B-ENT 2005;Suppl 1:77-86.
4. Norlander T, Brönnegård M, Stierna P. The relationship of nasal polyps, infection, and inflammation. Am J Rhinol 1999;13:349-55.
5. Cervin A, Wallwork B. Macrolide therapy of chronic rhinosinusitis. Rhinology 2007;45:259-67.
6. Nonaka M, Pawankar R, Saji F, Yagi T. Effect of roxithromycin on IL-8 synthesis and proliferation of nasal polyp fibroblasts. Acta Otolaryngol Suppl 1998;539:71-5.
7. Suzaki H, Asano K, Yu M, Hisamitsu T. Influence of roxithromycin on inflammatory cytokine production from nasal polyp fibroblasts *in vitro*. Acta Otolaryngol 2003;123:637-42.
8. Riechelmann H, Deutschle T, Friemel E, Gross HJ, Bachem M. Biological markers in nasal secretions. Eur Respir J 2003;21:600-5.
9. Fokkens W, Lund V, Mullol J. European Position Paper on Rhinosinusitis and Nasal Polyps group. European position paper on rhinosinusitis and nasal polyps 2007. Rhinol Suppl 2007;45(Suppl 20):1-136.
10. Lildholdt T, Rundcrantz H, Bende M, Larsen K. Glucocorticoid treatment for nasal polyps: the use of topical budesonide powder, intramuscular betamethasone, and surgical treatment. Arch Otolaryngol Head Neck Surg 1997;123:595-600.
11. Bachert C, Van Kempen M, Van Cauwenberge P. Regulation of proinflammatory cytokines in seasonal allergic rhinitis. Int Arch Allergy Immunol 1999;118:375-9.
12. Perić A, Vojvodić D, Radulović V, Vukomanović-Đurđević B, Miljanović O. Correlation between cytokine levels in nasal fluid and eosinophil counts in nasal polyp tissue in asthmatic and nonasthmatic patients. Allergol Immunopathol (Madr) 2010;39:133-9.

13. Perić A, Vojvodić D, Radulović V, Vukomanović-Đurđević B, Perić AV, Miljanović O. Proinflammatory cytokine levels in nasal fluid as indicators of severity of nasal polyposis. Acta Clin Croat 2010;49:395-403.
14. Ichimura K, Shimazaki Y, Ishibashi T, Higo R. Effect of new macrolide roxithromycin upon nasal polyps associated with chronic sinusitis. Auris Nasus Larynx 1996;23:48-56.
15. Cervin A, Kalm O, Sandkull P, Lindberg S. One-year low-dose erythromycin treatment of persistent chronic sinusitis after sinus surgery: clinical outcome and effects on mucoliliary parameters and nasal nitric oxide. Otolaryngol Head Neck Surg 2002;126:481-9.
16. Nonaka M, Pawankar R, Tomiyama S, Yagi T. A macrolide antibiotic, roxithromycin, inhibits the growth of nasal polyp fibroblasts. Am J Rhinol 1999;13:267-72.
17. Fundová P, Filipovský T, Funda DP, Hovorka O, Holý R, Navara M et al. Expression of IGF-1R and iNOS in nasal polyps; Epithelial cell homeostasis and innate immune mechanisms in pathogenesis of nasal polyposis. Folia Microbiol 2008;53:558-62.
18. Král B, Krejsek J, Paráková Z, Kopecký O, Vokurková D, Derner V et al. Some serum activity markers of airways inflammation in difficult-to-control asthma patients. Acta Medica (Hradec Králové) 1997;40:61-70.
19. Davidsson Å, Danielsen A, Viale G, Olofsson J, Dell Orto P, Pellegrini C et al. Positive identification in situ of mRNA expression of IL-6, and IL-12, and the chemotactic cytokine RANTES in patients with chronic sinusitis and polypoid disease. Acta Otolaryngol 1996;116:604-10.
20. Shikano H, Kato Z, Kaneko H, Watanabe M, Inoue R, Kasahara K et al. IFN-γ production in response to IL-18 or IL-12 stimulation by peripheral blood mononuclear cells of atopic patients. Clin Exp Allergy 2001;31:1263-70.
21. Dellacono FR, Eisma R, Lafreniere D, Leonard G, Kreutzer D. Interferon gamma expression in human nasal polyps. Laryngoscope 1997;107:626-30.
22. Jurišić V, Čolić S, Jurišić M. The inflammatory radicular cysts have higher concentration of TNF-α in comparison to odontogenic keratocysts (odontogenic tumour). Acta Medica (Hradec Králové) 2007;50:233-8.
23. Đorđević V, Zvezdanović L, Ćosić V, Vlahović P, Kundalić S, Jevtović-Stoimenov T et al. Serum levels and in vitro production of Th1- and Th2-type cytokines by peripheral blood mononuclear cells in patients suffering from systemic lupus erythematosus. Journal of Medical Biochemistry 2010;29:19-27.
24. Ohori J, Ushikai M, Sun D, Nishimoto K, Sagara Y, Fukuiwa T et al. TNF-α upregulates VCAM-1 and NF-κB in fibroblasts from nasal polyps. Auris Nasus Larynx 2007;34:177-83.
25. Saji F, Nonaka M, Pawankar R. Expression of RANTES by IL-1β and TNF-α stimulated nasal polyp fibroblasts. Auris Nasus Larynx 2000;27:247-52.
26. Yoshifuku K, Matsune S, Ohori J, Sagara Y, Fukuiwa T, Kurono Y. IL-4 and TNF-α increased the secretion of eotaxin from cultured fibroblasts of nasal polyps with eosinophil infiltration. Rhinology 2007;45:235-41.
27. Iino Y, Toriyama M, Kudo K, Natori Y, Yuo A. Erythromycin inhibition of lipopolysaccharide-stimulatied tumor necrosis factor alpha production by human monocytes *in vitro*. Ann Otol Rhinol Laryngol Suppl 1992;101(suppl 157):16-20.
28. Tokushige E, Itoh K, Ushikai M, Katahira S, Fukuda K. Localization of IL-1 beta mRNA and cell adhesion molecules in the maxillary sinus mucosa of patients with chronic sinusitis. Laryngoscope 1994;104:1245-50.
29. Miyanohara T, Ushikai M, Matsune S, Ueno K, Katahira S, Kurono Y. Effects of clarithromycin on cultured human nasal epithelial cells and fibroblasts. Laryngoscope 2000;110:126-31.
30. Perić A, Vojvodić D, Miljanović O. Influence of allergy on cytokine level in nasal discharge of patients with nasal polyps. Acta Medica Medianae 2010;49:40-4.