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INFLUENCE OF ALLERGY ON THE IMMUNOMODULATORY AND CLINICAL EFFECTS OF LONG-TERM LOW-DOSE MACROLIDE TREATMENT OF NASAL POLYPOSIS

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Aims. Cytokine levels in nasal secretions reflect the inflammatory status of the nasal and paranasal sinus mucosa and the development of mucosal disease. The results of previous investigations suggest that macrolide antibiotics can be effective in treatment of chronic rhinosinusitis and nasal polyposis. The aim of this prospective study was to compare the immunomodulatory and clinical effects of long-term low-dose macrolide treatment of nonatopic and atopic patients with nasal polyposis.

Methods. Forty (n = 40) patients with nasal polyposis, 22 allergic and 18 nonallergic were administered clarithromycin (CAM) 500 mg/day single oral dose for eight weeks. We measured the levels of proinflammatory Th1 cytokines TNF-α and IL-1β, Th2 cytokines IL-4, IL-5 and IL-6, and chemokine IL-8 in the nasal fluid samples, before and after treatment, using flow cytometric method. We also scored each of the 40 patients before and after therapy according to nasal symptom score and endoscopic score.

Results. Following treatment, we found significantly reduced levels of IL-8 ($p<0.01$) and TNF-α ($p<0.01$) in nasal secretions in nonallergic patients. In subjects with nasal polyposis and allergy, we found decreased levels of IL-8 ($p<0.01$), IL-6 ($p<0.05$) and IL-1β ($p<0.01$). Macrolide therapy decreased the size of polyps in 45.45% of nonatopic and in 50% of atopic patients. After macrolide treatment, we found 67.83% patients in nonallergic group and 55.55% patients in allergic group with improved nasal symptoms.

Conclusions. Long-term low-dose treatment with CAM was effective in the management of nasal polyposis. Our results showed that macrolide treatment of nasal polyposis have different immunomodulatory and similar clinical effects in allergic and nonallergic patients.

INTRODUCTION

Nasal polyposis is a chronic inflammatory disease of the nose and paranasal sinuses mucosa. The condition is characterized by protrusion of benign oedematous polyps from the meatus into the nasal cavities. Several mechanisms have been proposed for the formation of nasal polyps. Histopathological studies of the paranasal sinus mucosa in patients with nasal polyposis has demonstrated eosinophilic tissue infiltration. In addition to increased eosinophilic cell infiltration, increased production and expression of a variety of proinflammatory cytokines and chemokines have been demonstrated in nasal polyp epithelium and lamina propria[1]. 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 noninfectious inflammation[1]. Nasal polyposis is an example of an extreme immune dysregulation[2]. The mechanisms responsible for selective accumulation of eosinophils and neutrophils in polyps are unknown. Nasal polyp fibroblasts could play a role in the recruitment of eosinophils and neutrophils through the release of RANTES (regulated on activation, normal T cell expressed and secreted) and GM-CSF (granulocyte-macrophage colony-stimulating factor)[3]. Several cytokines (interleukin (IL)-4, IL-5, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), RANTES, GM-CSF) have been shown to be upregulated in nasal polyposis, suggesting that resident structural cells can produce a number of molecules to attract inflammatory cells and prolong their survival. These inflammatory cells themselves can also produce cytokines which recruit more inflammatory cells in an autocrine fashion[3].

Although surgery has been the preferred treatment for nasal polyposis for a long time, a change in the treatment strategy in recent years has lead to greater use of medica-

ABBREVIATIONS

IL – Interleukin
TNF – Tumor necrosis factor

tions, especially topical corticosteroids and antibiotics. Recently, there has been considerable interest of Japanese and other investigators in the immunomodulatory and anti-inflammatory action of macrolide antibiotics erythromycin (EM), clarithromycin (CAM) and roxithromycin (RXM) in the long-term low-dose treatment of chronic rhinosinusitis and nasal polyposis.

The prevalence of nasal polyposis in Europe is around 2.7% (ref.[4]). On the other hand, allergic rhinitis is a highly prevalent disease in developed countries, affecting about 10–20% of the general population[4]. The incidence of allergy in patients with nasal polyps has been found to be very differant, depending on the study. However, the relationship between nasal polyposis and allergy remains incompletely defined. The interaction of nasal polyposis and allergic rhinitis has mainly been studied in order to determine whether nasal polyps in allergic individuals are different from those in nonallergic ones. It is widely accepted that the efficacy of macrolide treatment in asthmatic patients with nasal polyps is poor. However, it is not clear whether allergic and nonallergic nasal polyp patients have a different outcome regarding the production of these cytokines during macrolide treatment.

Nasal secretions contain 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[5]. As cytokines play a dominant role in the pathophysiology of airway disease, the cytokine profile in nasal fluid may help to recognize mechanisms underlying nasal polyposis and the immunomodulatory effects of treatment by antibiotics.

In this prospective study, we compared the immunomodulatory and clinical effects of long-term low-dose macrolide treatment of nonatopic and atopic patients with nasal polyposis. We, also assessed the relationships between the changes in cytokine levels in nasal secretions and in the clinical characteristics of nasal polyps.

MATERIALS AND METHODS

Study population

Forty (n = 40) patients with nasal polyposis, 22 nonatopic and 18 atopic, were included in this prospective study, which was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia. The diagnosis of nasal polyposis was based on documented medical history and on the results of clinical examination, nasal endoscopy (rigid optic 0° and 30° endoscope; Storz, Tuttlingen, Germany) and computed tomography (CT) of the paranasal sinuses according to the current European Guidelines[6]. Nasal polyposis, which is considered to be a subgroup 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 discharge (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 from more than 12 weeks[6].

The exclusion criteria were the presence of lower airway obstruction symptoms, bronchial asthma, aspirin sensitivity, antrochoanal and sphenochoanal polyps, cystic fibrosis, and primary ciliary dyskinesia. All subjects included in this investigation had no acute respiratory tract infection and none were treated with oral and topical corticosteroids, antibiotics and antihistamines for at least three weeks before the enrollment.

Diagnosis of allergy

Skin prick tests were performed on all patients for sensitivity to 18 commonly inhaled allergens: *Alternaria alternata, Artemisia vulgaris, Aspergillus fumigatus, Candida albicans,* cat dander, *Cladosporium herbarum,* cockroach, *Cupressus arizonica, Dermatophagoides farinae, Dermatophagoides pteronyssinus,* dog dander, grasses (group), *Olea europaea, Parietaria judaica, Penicillium notatum, Plantago lanceolata, Platanus acerifolia, Salsola kali*). A test result was considered positive when at least one of the induration diameter was 3 mm higher than that in the negative control. Total serum IgE level was measured by ELISA kit (Elitech Diagnostics, France). Subjects were considered allergic if they had a serum IgE level > 160 IU/ml.

Treatment by clarithromycin

Patients received 500 mg/day oral single dose of 14-membered rings 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, younger than 18 years, liver and gastrointestinal dysfunction.

Clinical score

All patients were examined, treated and followed up by the same otorhinolaryngologist for the entire duration of the study. To investigate the effects 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 within the seven days after the macrolide treatment and to score their symptoms according to Tsicopoulos *et al.*[7] 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. The subjects were categorized into *responders* and *nonresponders* to macrolide treatment. Patients with a total score improvement rate of 1 or more after macrolide therapy were considered *responders.*

Nasal endoscopy was performed in a sitting position with a rigid endoscope 0° and 30°; Storz, Tuttlingen, Germany). Topical anaesthesia or decongestion were not

used. Endoscopic physical findings before and within the seven days after the CAM administration were scored according to Lildholdt *et al.*[8]. 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 macrolide treatment. Enabling to compare treatment results in patient groups with different extent of nasal polyposis, the patients were stratified by endoscopic score into P0 (no polyps), P1 group (score 1–2), P2 group (score 3–4), and P3 group (score 5–6).

Sampling of nasal fluid and cytokine determination

Nasal fluid samples were collected from nasal cavities of all 40 subjects (22 patients with nasal polyposis, and 18 patients with nasal polyposis and allergic rhinitis) before and within the seven days after the CAM treatment using modified absorption technique by placing cotton-wool sticks (length 10 millimetres, diameter 4 millimetres; Institute of Virology, Vaccines and Sera, "Torlak", Belgrade, Serbia), into the nasal cavity posterior to the mucocutaneous junction for 60 seconds, as previously described[9,10]. 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 min to allow diffusion of cytokines into the medium and then stored at 4 °C for a maximum of 2 h until processed. Nasal fluid were centrifuged at 1000 *g* for 10 min to separate the cellular components. After centrifugation, supernatants were portioned and stored at –70 °C until cytokine determination. The levels of proinflammatory Th1 cytokines (TNF-α and IL-1β), Th2 cytokines (IL-4, IL-5 and IL-6), and chemokine IL-8 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, lower and upper limits of detection were as follows: from 11 to 17,000 pg/ml for TNF-α; from 12 to 11,000 pg/ml for IL-1β; from 1 to 11,000 pg/ml for IL-4; from 12 to 12,000 pg/ml for IL-5; from 11 to 11,000 pg/ml for IL-6; from 13 to 10,500 pg/ml for IL-8. The examination was performed twice on the same sample and the obtained concentrations were then averaged. Reproducibility within the assay was evaluated in independent experiments, as follows for cytokine determination in the same sample: the coefficients of variation were: for IL-1β 5%, for IL-4 7%, for IL-5 3%, for IL-6 2%, for IL-8 2%, and for TNF-α 8%. According to producer's declaration (Bender MedSystems, USA, Flow Cytomix BMS810FF), the overall intra assay coefficient of variation should not exceed 10%.

Statistical analysis

Data were expressed as means ± standard deviation (± SD). Paired comparisons within a group were performed using the nonparametric Wilcoxon signed rank test. Between group comparisons were analyzed using the nonparametric Chi square-test. A p value less than 0.05 was considered to be statistically significant. For statistical analysis the SPSS software was used.

RESULTS

We included in our study 6 female and 16 male nonatopic patients with nasal polyps (mean age 44.36 ± 14.15 years, aged from 25 to 72 years) and 8 female and 10 male atopic patients (mean age 46.22 ± 12.37, aged from 19 to 65 years). In nonallergic subjects, the average nasal symptom score improved from 12.32 ± 2.34 before treatment with CAM to 9.13 ± 4.09 after treatment (mean, before to after, $p < 0.05$) (Fig. 1a). In allergic patients, the average nasal symptom score decreased after therapy by CAM from 12.77 ± 2.76 to 10.16 ± 3.47 ($p < 0.05$) (Fig. 1b). After macrolide treatment, we found 67.83% *responders* in the nonallergic group and 55.55% patients with improved

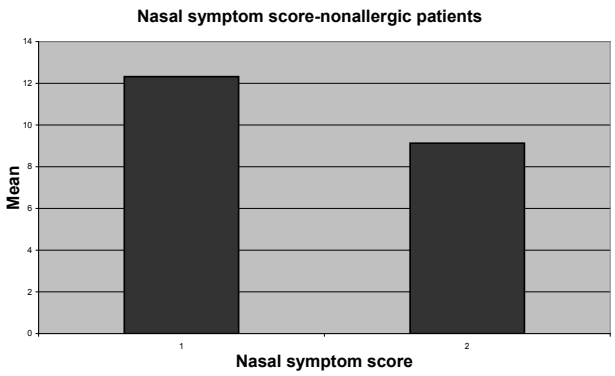

Fig. 1a. In nonatopic patients with nasal polyps, the average nasal symptom score improved after treatment by clarithromycin ($p < 0.05$).

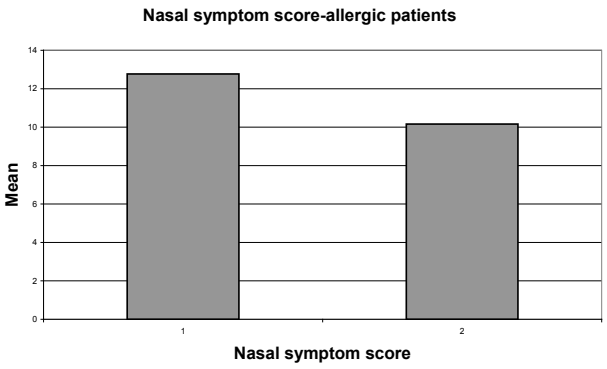

Fig. 1b. In atopic patients with nasal polyps, the average nasal symptom score improved after treatment by clarithromycin ($p < 0.05$).

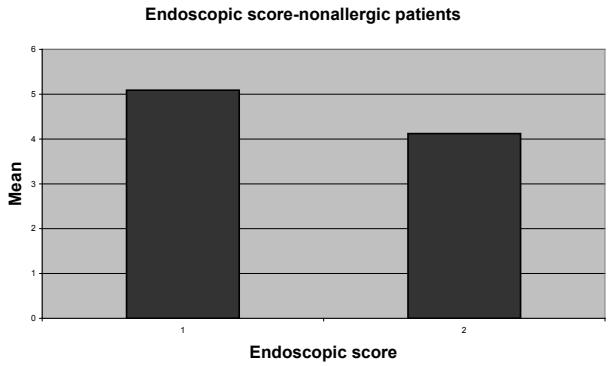

Fig. 2a. In nonallergic subjects, the average polyp size decreased after macrolide treatment ($p < 0.05$).

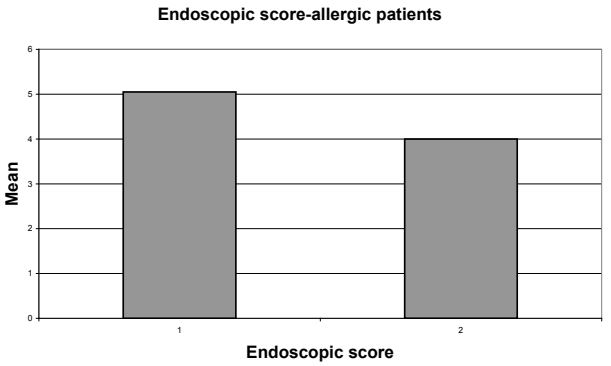

Fig. 2b. In allergic subjects, the average polyp size decreased after macrolide treatment ($p < 0.05$).

nasal symptoms in group with allergic rhinitis. These differences were not statistically significant (Chi square-test).

In nonallergic patients, we found significant difference in the endoscopic score before and after treatment (5.09 ± 1.02 *vs* 4.12 ± 1.78) ($p < 0.05$) (Fig. 2a). The average size of allergic patients' polyps was smaller after therapy (5.05 ± 1.01 *vs* 4.00 ± 1.00) (Fig. 2b). 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 the difference was not statistically significant (Chi squared-test). In one nonallergic patient, polyp size decreased from grade P3 to grade P1; in three patients, polyp size decreased from grade P3 to grade P2; in six patients, polyp size decreased from grade P2 to grade P1. In allergic patients, in one case polyp size decreased from grade P3 to grade P1; in two patients, polyp size decreased from grade P3 to grade P2; in six patients, polyp size decreased from grade P2 to grade P1 (Table 1). We found that the macrolides were more effective in reducing the size of smaller polyps compared with bigger ones.

Table 1. Results of macrolide treatment in nonallergic and allergic patients regarding the endoscopic score.

Nonallergic patients			Allergic patients		
Case	(Before)	(After)	Case	(Before)	(After)
1	6	2	**1**	6	4
2	6	6	**2**	4	2
3	4	2	**3**	4	2
4	6	4	**4**	6	6
5	6	6	**5**	6	6
6	4	2	**6**	4	2
7	4	4	**7**	5	4
8	6	5	**8**	5	2
9	6	4	**9**	4	2
10	6	6	**10**	6	6
11	4	2	**11**	6	4
12	6	6	**12**	4	2
13	4	4	**13**	4	2
14	6	6	**14**	6	6
15	6	6	**15**	6	6
16	4	2	**16**	6	6
17	4	2	**17**	4	4
18	4	4	**18**	5	6
19	4	2			
20	6	6			
21	6	4			
22	4	4			

We found no significant differences in the levels of IL-4 and IL-5 in the nasal secretions before and after macrolide treatment. Only the concentrations of chemokine IL-8 and proinflammatory cytokine TNF-α in the nasal fluid of nonatopic patients were the highly statistically different, much lower after CAM treatment (from 293.59 ± 375.35 pg/ml to 75.30 ± 80.28 pg/ml, mean, before to after, $p < 0.01$ for IL-8; from 153.05 ± 123.29 pg/ml to 44.22 ± 47.74 pg/ml, $p < 0.01$ for TNF-α) (Table 2). In the group of allergic patients with nasal polyposis, we found significantly lower concentrations of IL-8 (from 226.28 ± 111.07 pg/ml to 55.07 ± 17.44 pg/ml; $p < 0.01$), IL-6 (from 282.28 ± 169.62 pg/ml to 75.31 ± 36.79 pg/ml; $p < 0.05$) and IL-1β (from 130.00 ± 94.01 pg/ml to 34.32 ± 18.23 pg/ml; $p < 0.01$) after therapy with CAM (Table 3).

In nonatopic patients, in the *improvement* group, IL-8 levels dramatically decreased from 385.68 ± 227.36 pg/ml to 48.13 ± 29.86 pg/ml ($p < 0.01$), whereas in the *no improvement* group, this difference was statistically lower (from 118.75 ± 96.15 pg/ml to 61.36 ± 56.38 pg/ml; $p < 0.05$). The levels of TNF-α also significantly decreased in the *improvement* group from 224.38 ± 174.68 pg/ml to 42.86 ± 47.81 pg/ml ($p < 0.01$). However, in the *no improvement* group, there was no difference in the mean level of TNF-α before and after treatment (92.13 ± 86.17 pg/ml *vs* 78.86 ± 81.62 pg/ml) (Table 4a).

In subjects with allergic rhinitis, IL-8 levels significantly decreased in the *improvement* group from 276.24 ± 265.34 pg/ml to 58.93 ± 62.34 pg/ml ($p < 0.01$). In the *no improvement* group, we found no significant difference regarding the levels of IL-8 before and after treatment (128.34 ± 103.38 pg/ml *vs* 87.56 ± 77.15 pg/ml). Comparing the post macrolide treatment outcomes for levels of IL-6 and IL-1β, we found no differences between the *improvement* and *no improvement* group in allergic patients (Table 4b).

Table 2. Cytokine levels in nasal fluid in nonallergic patients.

Cytokine	Before treatment Mean ± SD	After treatment Mean ± SD	*p* value
IL-8	293.59 ± 375.35	75.30 ± 80.28	$p < 0.01$
IL-6	116.63 ± 123.21	113.76 ± 131.19	$p > 0.05$
IL-4	227.82 ± 295.70	248.74 ± 287.44	$p > 0.05$
IL-5	437.21 ± 412.67	424.49 ± 341.94	$p > 0.05$
IL-1β	50.39 ± 48.46	42.85 ± 43.93	$p > 0.05$
TNF-α	153.05 ± 123.29	44.22 ± 47.74	$p < 0.01$

All results of cytokine levels were expressed as pg/ml.

Table 3. Cytokine levels in nasal fluid in allergic patients.

Cytokine	Before treatment Mean ± SD	After treatment Mean ± SD	*p* value
IL-8	226.28 ± 111.07	55.06 ± 17.44	$p < 0.01$
IL-6	282.28 ± 169.62	75.31 ± 36.79	$p < 0.05$
IL-4	936.17 ± 414.13	921.60 ± 360.51	$p > 0.05$
IL-5	1190.33 ± 743.38	1281.88 ± 656.14	$p > 0.05$
IL-1β	130.00 ± 94.01	34.31 ± 18.23	$p < 0.01$
TNF-α	109.88 ± 82.95	113.31 ± 98.91	$p > 0.05$

All results of cytokine levels were expressed as pg/ml.

Table 4a. The relationship between cytokine levels in nasal fluid and change of nasal poyp size: cytokine levels in nasal secretions in *improvement* and *no improvement* group of nonallergic patients.

Cytokine	*Improvement* group			*No improvement* group		
	Before	After	p	Before	After	p
IL-8	385.68±227.36	48.13±29.86	p<0.01	118.75±96.15	61.36±56.38	p<0.05
TNF-α	224.38±174.68	42.86±47.81	p<0.01	92.13±86.17	78.86±81.62	p>0.05

Table 4b. The relationship between cytokine levels in nasal fluid and change of nasal polyp size: cytokine levels in nasal secretions in *improvement* and *no improvement* group of allergic patients.

Cytokine	*Improvement* group			*No improvement* group		
	Before	After	p	Before	After	p
IL-8	276.24±265.34	58.93±62.38	p<0.01	128.34±103.38	87.56±77.15	p>0.05
IL-6	323.85±185.92	74.83±47.81	p<0.05	247.83±195.12	87.93±56.31	p<0.05
IL-1β	143.28±128.37	47.36±28.25	p<0.01	121.15±87.63	32.26±19.87	p<0.01

DISCUSSION AND CONCLUSION

Macrolide therapy, that is, long-term, low dose administration of 14-membered lactone ring antibiotics, has recently been reported to be very effective for patients with chronic upper respiratory tract infection. Ichimura *et al.*[11] 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. Yamada *et al.*[12] showed that CAM administered at 400 mg/day for 8–12 weeks resulted in marked shrinkage of polyps in 40% of twenty patients.

The results of the bacterial cultures suggest that the risk of selecting resistant bacteria is low[13]. 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[13].

Ichimura *et al.*[11] reported that the efficacy of macrolide therapy is not related to allergic symptoms. Haruna *et al.*[14] performed an investigation which included 68 patients with chronic rhinosinusitis and nasal polyps. The comparison of the findings of allergic examinations and the therapeutic efficacy found no correlation between the presence of nasal allergies and the efficacy, but there was a statistically significant increase in the percentage of asthma patients who failed to improve[14]. Our results also showed that there was no relationship between the presence of atopy and clinical efficacy of macrolide treatment.

However, the mechanisms of polyp shrinkage during macrolide treatment are not well known. Nonaka *et al.*[15] 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. Interleukin-8 (IL-8), a potent neutrophil and also eosinophil chemoattractant and activating factor, is known to be released by monocytes, macrophages and airway epithelial cells[16]. Recent data have shown that airway fibroblasts are also important sources of this chemokine[16]. Results published by Nonaka *et al.*[16] showed that although RXM did not directly suppress IL-8 production from NPFs, the reduction in the proliferation of fibroblasts suggests that RXM can indirectly reduce the total levels of IL-8 in the nasal polyps and thereby play a role in regulating inflammatory cell recruitment. The results of our investigation showed that the IL-8 and tumor necrosis factor-alpha (TNF-α) concentrations in the nasal fluid of patients with nasal polyposis were significantly reduced after treatment with CAM. The decreased IL-8 and TNF-α levels in nasal secretions were associated with reduction in polyp size in nonallergic patients, whereas in allergic ones only reduction of IL-8 levels relates with polyp shrinkage. TNF-α is secreted by macrophages, monocytes and NK cells and many other cell types[17,18]. TNF-α, among other cytokines can regulate fibroblast activity and collagen formation through modulation of collagenase activity[18]. 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[19]. To infiltrate sites of inflammation, eosinophils leave the bloodstream and pass through the endothelium in four steps, namely rolling, adhesion, transendothelial migration, and chemotaxis[19]. Adhesion molecules, such as VCAM-1 play an important role during adhesion to endothelial cells[19]. Experiments performed by Ohori *et al.*[19] 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.*[20] demonstrated that NPFs produced RANTES by stimulation with TNF-α and IL-1β. Therefore, results published by Yoshifuku *et al.*[21] 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.*[22] 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 fibroblasts and monocytes in nasal polyp tissue.

Our results also showed decreased levels of IL-6 and IL-1β in nasal secretions in atopic patients after treatment by CAM. IL-6 is an important proinflammatory Th2 type citokine involved in the induction of IgE synthesis as well as in mast cell proliferation and maturation[23]. IL-6 also stimulates fibroblast proliferation and collagen synthesis[23]. Immunohistochemical staining and in situ hybridization have shown that the main sources of IL-6 are macrophages, T cells, mast cells, and, especially, eosinophils and fibroblasts because of their autocrine activity[23]. IL-1β play 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[24]. The emigrated neutrophils then secrete IL-1β, which amplifies the expression of E-selectin and ICAM-1, resulting in further neutrophil infiltration[24]. Miyanohara *et al.*[25] revealed that clarithromycin suppressed IL-1β gene expression in human nasal epithelial cells *in vitro*.

Our results 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 these atopic and nonatopic patients with similar clinical findings had different mediator profiles in their nasal secretions, implying clear differences in pathogenesis of their polyps. Evaluation of the cytokine levels in nasal fluid could be an accessible and valuable path in monitoring these patients, as well as sensitive way to evaluate 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. The decreases in IL-8 levels in nasal secretions were associated with reduction in polyp size both in nonallergic and allergic patients. These results indicate the importance of IL-8 regarding its chemotactic activity for neutrophils and eosinophils in the patho-

genesis 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 corticosteroids in the management of nasal polyposis, especially in patients in whom steroid use is contraindicated.

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