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MAIN ARTICLE

Effect of glucocorticoids on nasal polyposis, with detection of inflammatory response by measurement of nitric oxide levels in nasal polyp tissue

T BÜLBÜL[1], Ö GÜMÜŞTEKIN BÜLBÜL[2], O GÜÇLÜ[3], A SERPİL BİLSEL[2], S ÖNAL GÜRSAN[1]

[1]*Department of Otorhinolaryngology, Haydarpaşa Numune Education and Research Hospital, Istanbul,* [2]*Department of Biochemistry, School of Medicine, Marmara University, Istanbul, and* [3]*Department of Otorhinolaryngology, Çanakkale Onsekiz Mart University Faculty of Medicine, Turkey*

Abstract

Objective: To investigate endoscopic staging, and nitric oxide levels in the polyp tissue, in patients with nasal polyposis undergoing glucocorticoid therapy.

Methods: Nasal polyposis was evaluated using endoscopic staging and measurement of polyp tissue nitric oxide levels (chemiluminescence method). Forty-five nasal polyposis patients received either nasal therapy ($n = 15$), oral therapy ($n = 15$) or combined therapy ($n = 15$). Pre-treatment and post-treatment staging and nitric oxide levels were evaluated.

Results: Endoscopic grading indicated significant post-treatment staging improvements in the oral ($p = 0.016$) and combined ($p = 0.016$) groups. Post-treatment staging differed significantly between the three groups ($p = 0.041$), with greater improvements in the oral and combined groups. All groups showed significantly lower post-treatment nitric oxide levels, compared with baseline, but post-treatment levels did not differ significantly between groups. A significant association was found between treatment response and nitric oxide level alteration.

Conclusion: This study demonstrates the favourable effects of glucocorticoids on nasal polyposis, and alteration in nitric oxide tissue levels post-treatment. Nitric oxide level in nasal polyp tissue could be an indicator of treatment response, and may aid surgical decision-making by detecting cases that probably will not respond to medical treatment.

Key words: Nasal Polyps; Nitric Oxide; Glucocorticoids; Corticosteroids; Chemiluminescence

Introduction

Glucocorticoids are steroidal hormones produced by cholesterol metabolism. In the human body, they are produced by the adrenal glands under the control of the hypothalamic-pituitary-adrenal axis. These hormones have immunosuppressant and anti-inflammatory effects. Glucocorticoids began to be used in routine practice after the successful introduction of hydrocortisone in 1948.[1] For the treatment of nasal polyposis, they are used via the oral, intranasal and systemic routes. At the 2002 International Consensus on Nasal Polyposis and the 2004 European Consensus meetings, glucocorticoids were accepted as first-line therapy for nasal polyposis.[1,2]

Nasal polyposis is a chronic inflammatory disease of the upper respiratory tract.[3] Its incidence is higher in men than women, with a reported gender ratio of between 2:1 to 4:1.[4,5] It is generally observed in adulthood (i.e. past 20 years of age) and is rarely seen in children.[4,5] It is generally characterised by mucosal, eosinophilic, oedematous inflammation.[4,5] During this chronic inflammatory process, the interaction of many inflammatory molecules has been observed, within a complex mechanism.

Glucocorticoids are the preferred treatment for nasal polyposis because of their anti-inflammatory efficacy. Although they are not effective in all nasal polyposis patients, a therapeutic effect is seen in 60–80 per cent of cases.[1] Apart from glucocorticoids, intranasal capsaicin, macrolides, amphotericin B nasal spray and intranasal lysine-acetylsalicylic acid have all been used as medical therapy.[6] Surgical treatment is also used.

Nitric oxide (NO) is involved in many biological activities, such as blood circulation, thrombocyte

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function, immunity and neurotransmission. It is synthesised from L-arginine, an amino acid, by nitric oxide synthase. Nitric oxide is exhaled as gas in expired air, being generated mostly by the mucosa of the upper respiratory tract.[7,8] It is believed to play a role in defence against infection in this region. In conditions of inflammation, the amount of NO is markedly increased.[9] Nitric oxide synthase is classified into three subtypes: endothelial, constitutive and inducible.[7,10,11]

In the current study, we investigated the therapeutic efficacy of glucocorticoids in patients with nasal polyposis. Methyl-prednisolone was used as oral therapy and budesonide as intranasal therapy. The efficacy of these drugs was compared after administration alone or in combination. Nitric oxide synthesis is known to vary according to the inflammatory response.[9] In the current study, the effect of glucocorticoids on the nasal polyposis inflammatory response was assessed by direct measurement of NO levels in polyp tissue. Recent studies have demonstrated increased NO synthesis by detecting either altered levels of exhaled NO or increased nitric oxide synthase expression. A review of the literature indicates that determination of NO levels in target tissue is not a commonly used method. In the present study, we investigated the therapeutic response to glucocorticoids in nasal polyposis tissue by determining the levels of NO and its products.

Materials and methods

The study included volunteers who presented to the ENT department of Haydarpaşa Numune Education and Research Hospital, Istanbul, and who received a diagnosis of nasal polyposis. The study was approved by the local ethics board. Patients in whom corticosteroid therapy was contraindicated (i.e. those with diabetes mellitus, hypertension, glaucoma, a history of tuberculosis or emotional instability), as well as those who had received corticosteroids within the last month, were excluded from the study prior to glucocorticoid administration.

A total of 45 patients were enrolled in the study. Prior to medical therapy, patients underwent endoscopic examination and staging according to the Rasp classification, as well as a punch biopsy of polyp tissue and an allergen skin prick test. Three types of medical therapy were tested: oral glucocorticoid (methyl-prednisolone), intranasal glucocorticoid (budesonide) and a combination of the two. Patients were allocated in turn to either the first, second or third treatment group, depending on their order of presentation.

In the oral methyl-prednisolone group ($n = 15$), dosing was commenced at 1 mg/kg and reduced progressively over a 21-day treatment course. Sixteen milligram methyl-prednisolone oral tablets were used, and the dose was reduced by one tablet every four or five days (depending upon the initial dose).

In the intranasal budesonide group ($n = 15$), a dose of 400 μg/day was administered over the 21-day treatment course.

The combined treatment group ($n = 15$) received both intranasal and oral therapy, using the doses described above.

At the end of medical therapy, all patients underwent repeated nasal polyposis staging and punch biopsy of polyp tissue.

Nitric oxide measurement

Nitric oxide levels were measured using the Sievers Nitric Oxide Analyzer (NOA 280i; GE Analytical Instruments, Boulder, Colorado, USA), which contains a highly sensitive detector which measures the reaction between nitric oxide and ozone using a technique based on gas-phase chemiluminescence. As a result of the reaction between NO and ozone, excited-state nitrogen dioxide is formed. When the excited NO_2 returns to its normal form, photon rays are emitted, as follows:

$$NO + O_3 \rightarrow NO_2^- + O_2$$

$$NO_2^- \rightarrow NO_2 + \text{light } (>600\,\text{nm})$$

These emitted photon rays are observed near the infrared end of the spectrum, and may be measured using the chemiluminescence method. The photons are detected by a thermoelectrically cooled, photomultiplier tube sensitive to red. For liquid samples, the detection limit is approximately 1 pM.

In our study, NO levels were calculated as micromoles of NO per gram of tissue.

Measurements were performed in the laboratory of the biochemistry department, School of Medicine, Marmara University. Biopsy samples were stored at a temperature of −80°C. Each sample was mixed with a quantity of phosphate buffer five times the weight of the sample and the resultant mixture homogenised. Homogenates were centrifuged at 2000 rpm for 20 minutes at a temperature of +4°C. Liquid supernatant was taken from the surface of the precipitate and then deproteinised. For deproteinisation, 1 ml of ethanol at 0°C was added to 0.5 ml of supernatant and mixed using a vortex mixer. After a 30 minute incubation at 0°C, the mixture was centrifuged at 14 000 rpm for 5 minutes at a temperature of 0°C. The supernatant was then collected and used to measure NO. The NO concentration was measured in a volume of 5 μl using the chemiluminescence method discussed above.

The study also measured nitrate, the major oxidation product of NO, utilising the ability of vanadium(III) chloride to convert nitrate to nitric oxide in hydrogen chloride.

Statistical analysis

Statistical analysis was performed using GraphPad Prism V.3 software. In addition to descriptive statistics, the Kruskal–Wallis test was used for intergroup

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comparisons, Dunn's multiple comparison test was used for subgroup comparisons, the Wilcoxon test was used to compare pre- and post-treatment group values, the chi-square test was used to compare qualitative data, and McNemar's test was used for recurrent measurements of qualitative data. Results were evaluated at a significance level of $p < 0.05$.

Results and analysis

The study included a total of 45 volunteers who presented to the ENT department of Haydarpaşa Numune Education and Research Hospital and who received a diagnosis of nasal polyposis. Of these patients, 25 (55.6 per cent) were male and 20 (44.4 per cent) female. The mean patient age ± standard deviation was 34.73 ± 16.72 years. Fifteen patients each were assigned to the nasal therapy, oral therapy and combined therapy groups.

The skin prick test was positive in 22 patients: 9 (60 per cent) in the oral group, 7 (46.7 per cent) in the intranasal group and 6 (40 per cent) in the combined group. The differences between groups were not statistically significant ($p = 0.537$).

Results for pre- and post-treatment endoscopic staging are shown in Table I. There were no significant differences between the three treatment groups as regards pre-treatment staging; however, following treatment a statistically significant difference was noted between the groups ($p = 0.041$). A statistically significant improvement was observed in the oral group ($p = 0.016$) and the combined group ($p = 0.016$), compared with baseline values. Overall, 18 patients (40 per cent) showed a favourable change in staging.

Within each treatment group, mean pre- and post-treatment NO tissue concentrations (μmol/g) were compared. In all groups, NO levels showed a statistically significant decrease compared with baseline values (Table II and Figure 1). No statistically significant difference was found between the post-treatment NO levels in the three groups.

The mean change in NO level was calculated separately for patients with and without a therapeutic response. A statistically significant correlation was found between therapeutic response and NO level change (Table III).

TABLE I
PATIENTS' PRE- AND POST-TREATMENT STAGING

Stage	Treatment group (*n* (%))			*p*
	Oral	Nasal	Comb	
Pre-GC				
1	3 (20.0)	2 (13.3)	1 (6.7)	0.446
2	8 (53.0)	5 (33.3)	9 (60.0)	
3	4 (26.7)	8 (53.3)	5 (33.3)	
Post-GC				
1	9 (60.0)	2 (13.3)	4 (26.7)	0.041*
2	5 (33.3)	9 (60.0)	10 (66.7)	
3	1 (6.7)	4 (26.7)	1 (6.7)	

*Statistically significant. Pts = patients; Comb = combined oral and nasal treatment; GC = glucocorticoid treatment

Discussion

Nasal polyposis results from a chronic inflammatory process. Polyp tissues are oedematous, submucosal lesions that may be easily detected during physical examination. Nasal polyposis has been known since the time of Hippocrates. It manifests clinically as nasal congestion, olfactory problems and nasal discharge, together with symptoms associated with sinusitis or coexistent allergy. Upon histopathological examination, various inflammatory cells are observed, with eosinophils predominant, within an oedematous stroma.[5] At the molecular level, the development of chronic nasal polyposis involves a complex interaction between cytokines and structural and inflammatory cells.[1]

We have mentioned above that corticosteroids are often the drugs of choice in the medical therapy of nasal polyposis. A review of studies on the mechanism of action of corticosteroids indicates that corticosteroids have suppressive effects on proinflammatory cytokines (i.e. interleukins (ILs) 1, 2, 3, 4, 5, 6, 11 and 13, tumour necrosis factor α, and granulocyte-macrophage colony-stimulating factor), chemokines (IL-8, the 'regulated on action, normal T cell expressed and secreted' protein (also termed 'RANTES'), monocyte chemoattractant proteins 1, 3 and 4, and eotaxin), adhesion molecules (intercellular adhesion molecule-1 and E-selectin) and mediator synthesising enzymes (I-nitric oxide synthase, cyclo-oxygenase-2 and phospholipase A2). Corticosteroids also enhance anti-inflammatory agents. As a result of all these effects, corticosteroids are used medically to reduce inflammation and to treat clinical symptoms.[12]

The therapeutic efficacy of corticosteroids varies from patient to patient, and some individuals do not show a therapeutic response. Fernandes *et al.* reported a therapeutic response ranging from 60 to 80 per cent.[1] Benson stated that this variation may result from differences in the expression of glucocorticoid receptors.[12] These receptors have two isoforms in humans, α and β. Increased expression of the β isoform has been demonstrated in individuals with corticosteroid-resistant asthma and nasal polyposis.[1,12–14] In our study, 27 patients (60 per cent) did not respond to therapy, as indicated by clinical staging. This is a higher proportion than that reported in recent studies. However, we believe this difference may be incidental, due to the low number of patients in our study, or may have arisen due to genetic differences between our patients and those included in previous studies.

Complete recovery was not observed in our study groups, in contrast to earlier studies. For example, Karataş *et al.* reported a complete response to systemic corticosteroid therapy in 28.1 per cent of cases, while Özcan *et al.* reported a 5.7 per cent complete response rate.[15,16] Therefore, glucocorticoid receptor expression

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TABLE II
PATIENTS' PRE- AND POST-TREATMENT NITRIC OXIDE LEVELS

Parameter	Treatment group			p^*
	Oral	Nasal	Comb	
Pre-GC NO (mean ± SD; μmol/g)	0.35 ± 0.25	0.27 ± 0.19	0.32 ± 0.21	0.610
Post-GC NO (mean ± SD; μmol/g)	0.20 ± 0.15	0.18 ± 0.13	0.22 ± 0.19	0.980
Z	3.40	3.35	2.55	
$p^\dagger$	0.001‡	0.001‡	0.011‡	

*Comparing different treatment groups; †comparing pre- and post-treatment values. ‡Statistically significant. Comb = combined oral and nasal treatment; GC = glucocorticoid treatment; NO = nitric oxide; SD = standard deviation

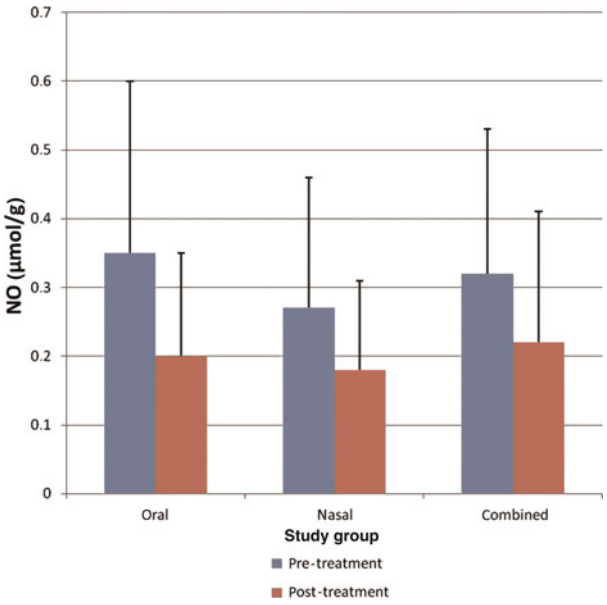

FIG. 1
Tissue nitric oxide (NO) levels in groups before and after glucocorticoid treatment.

may play a key role in the therapeutic response, but the mechanism is quite complex and has not yet been fully elucidated.

In addition to these differences in therapeutic response, there are also differences regarding administration routes and active constituents. In our study, our three treatment groups received different therapeutic modalities, enabling us to investigate the effect of oral, intranasal and combined corticosteroid delivery on therapeutic outcomes. Nasal polyposis and allergy may be seen concomitantly. In our study, skin prick test results did not differ between study groups. Pre-treatment endoscopic staging and anterior rhinoscopy established that 17 (37.8 per cent) patients had stage 3 disease, 22 (48.9 per cent) had stage 2 and 6 (13.3 per cent) had stage 1. After treatment, six patients (13.3 per cent) had stage 3 disease, 24 (53.3 per cent) had stage 2 and 15 (33.3 per cent) had stage 1. Thus, an objective regression of disease was seen in response to corticosteroid therapy. There was a statistically significantly improvement in patients treated with oral and combined corticosteroid, compared with baseline values. We did not detect a significant improvement in patients treated with intranasal steroids alone. Joe and colleagues' meta-analysis of the effect of intranasal steroids on nasal polyposis reported that these drugs were beneficial; however, the present study showed no statistically significant effect on polyp size.[17] Local corticosteroid administration may result in higher doses and better response, without side effects. However, we believe that, in the absence of any contraindications, oral therapy should be used for initial treatment or for preoperative preparation. The literature reports that combined steroid therapy gives similar results to oral therapy alone.[18,19] Tuncer *et al.* observed a significant improvement in symptom scores and nasal polyp size in patients receiving combined steroid therapy; they reported complete recovery in 12 per cent and significant recovery in 76 per cent of their patients.[19]

The second part of our study assessed changes in inflammatory response by measuring NO levels in nasal polyposis tissue. Nitric oxide has been studied for the last 30 years and has various reported functions. Earlier studies found that the source of NO in exhaled air was the upper respiratory tract.[10,11] In the presence of inflammation or nasal polyposis, NO synthesis is markedly increased.[9,20] Our study measured NO level as an indicator of inflammation at the molecular level.

TABLE III
ASSOCIATION BETWEEN STAGING AND NITRIC OXIDE RESPONSES TO TREATMENT

	Staging response		MW	p
	−	+		
NO response (mean ± SD; μmol/g)	0.06 ± 0.12	0.19 ± 0.14	115	0.003*

*Statistically significant. MW = Mann–Whitney U test; − = reduced stage; + = increased stage; NO = nitric oxide; SD = standard deviation

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In the literature, direct and indirect methods have been reported for NO measurement. Nasal NO measurement generally utilises mass spectroscopy of exhaled air or the determination of nitric oxide synthase expression via gas chromatography mass spectrometry or immunohistochemistry.[21] We used chemiluminescence, a direct measurement method which allowed us to directly determine NO levels in biopsy tissue at baseline and after therapy. When mean values were compared, all three study groups showed a statistically significant decrease in NO levels following corticosteroid therapy. There was no statistically significant change in tissue NO levels in patients with no clinical response to therapy. Based on this, we believe that patients without a therapeutic response to corticosteroids show no reduction in NO levels. Furthermore, we suggest that lack of alteration in NO level can be used as an indicator to identify patients who will not respond to steroid therapy. Thus, assessment of NO level alteration could be useful when deciding whether to recommend surgery for nasal polyposis patients, and could reduce the duration of unnecessary, lengthy medical therapy in such patients.

- **Nasal polyposis has a complex mechanism of development**
- **It is commonly treated with glucocorticoids, exerting an anti-inflammatory effect**
- **This study assessed nasal polyposis patients' response to glucocorticoids (oral, nasal and combined)**
- **Endoscopic staging and nitric oxide (NO) levels (in biopsy tissue) improved post-treatment**
- **Changes in NO levels may indicate inflammatory response and help guide surgical decisions**

Oh *et al.* considered NO to be a potential indicator of respiratory tract inflammation.[9] They reported that, in asthmatic patients, NO concentration decreased in response to corticosteroid therapy, and suggested that this may be useful for monitoring drug dosages. In our study of nasal polyposis patients, we evaluated therapeutic response using NO as an indicator of inflammation; however, we determined NO levels in the target tissue rather than in exhaled air. Studies of nasal polyposis patients have found a low concentration of exhaled NO; it has been suggested that this is commonly caused by obstruction of the maxillary sinus ostium and upper respiratory tract.[9,22] For this reason, we preferred direct evaluation of NO level in nasal polyposis biopsy tissue, as we believed that this constituted a more reliable measurement.

Conclusion

The mechanism of nasal polyposis, the mechanism of corticosteroid action and the optimum therapeutic course are still conflicting and unclear issues. Corticosteroids are reasonably successful drugs which markedly improve patients' quality of life. Combined oral and nasal corticosteroid therapy is not suitable for long-term use; however, despite this limitation, it is more likely to produce a positive clinical response. On the other hand, some nasal polyposis patients do not respond to medical therapy. Assessment of tissue NO levels could indicate nasal polyposis patients' capacity to respond to steroid therapy. More studies on this topic are warranted.

References

1 Fernandes AM, Valera FC, Anselmo-Lima WT. Mechanism of action of glucocorticoids in nasal polyposis. *Braz J Otorhinolaryngol* 2008;**74**:279–83
2 Mladina R, Clement P, Lopadin A, Mann W, Passali D. International Consensus on Nasal Polyposis 2002–2004. *Eur Arch Otorhinolaryngol* 2005;**262**:519–21
3 Seethala RR, Pant H. Pathology of nasal polyps. In: Onerci TM, Ferguson BJ, eds. *Nasal Polyposis*. Berlin, Heidelberg: Springer Verlag, 2010;17–26
4 Pearlman AN, Chandra RK, Conley DB, Kern RC. Epidemiology of nasal polyps. In: Onerci TM, Ferguson BJ, eds. *Nasal Polyposis*. Berlin, Heidelberg: Springer Verlag, 2010;9–16
5 Patiar S, Reece P. Oral steroids for nasal polyps. *Cochrane Database Syst Rev* 2007;(1):CD005232
6 Becker SS, Duncavage JA. The role of steroid injection in the management of sinonasal polyps. *Curr Opin Otolaryngol Head Neck Surg* 2008;**16**:38–43
7 Watkins DN, Lewis RH, Basclain KA, Fisher PH, Peroni DJ, Garlepp MJ *et al*. Expression and localization of the inducible isoform of nitric oxide synthase in nasal polyp epithelium. *Clin Exp Allergy* 1998;**28**:211–9
8 Kimberly B, Nejadnik B, Giraud GD, Holden WE. Nasal contribution to exhaled nitric oxide at rest and during breathholding in humans. *Am J Respir Crit Care Med* 1996;**153**:829–36
9 Oh CK, Miman MC, Duncavage JA. Current value of nasal nitric oxide measurement in rhinology. *Curr Opin Otolaryngol Head Neck Surg* 2004;**12**:30–3
10 Lundberg JO. Nitric oxide and the paranasal sinuses. *Anat Rec (Hoboken)* 2008;**291**:1479–84
11 Lundberg JO, Weitzberg E. Nasal nitric oxide in man. *Thorax* 1999;**54**:947–52
12 Benson M. Pathophysiological effects of glucocorticoids on nasal polyps: an update. *Curr Opin Allergy Clin Immunol* 2005;**5**:31–5
13 Hamilos DL, Leung DY, Muro S, Kahn AM, Hamilos SS, Thawley SE *et al*. GRbeta expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone. *J Allergy Clin Immunol* 2001;**108**:59–68
14 Pujols L, Alobid I, Benítez P, Martínez-Antón A, Roca-Ferrer J, Fokkens WJ *et al*. Regulation of glucocorticoid receptor in nasal polyps by systemic and intranasal glucocorticoids. *Allergy* 2008;**63**:1377–86
15 Karataş A, Koç A, Bilgili M. Preoperative systemic steroid therapy and results in sinonasal polyposis [in Turkish]. *Türkiye Klinikleri J ENT* 2004;**4**:90–4
16 Özcan C, Apa DD, Pata YS, Görür K, Akbaş Y. Expression of inducible nitric oxide synthase in antrochoanal polyps. *Int J Pediatr Otorhinolaryngol* 2003;**67**:383–8
17 Joe SA, Thambi R, Huang J. A systematic review of the use of intranasal steroids in the treatment of chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 2008;**139**:340–7
18 Damm M, Jungehülsing M, Eckel HE, Schmidt M, Theissen P. Effects of systemic steroid treatment in chronic polypoid rhinosinusitis evaluated with magnetic resonance imaging. *Otolaryngol Head Neck Surg* 1999;**120**:517–23
19 Tuncer U, Soylu L, Aydogan B, Karakus F, Akcali C. The effectiveness of steroid treatment in nasal polyposis. *Auris Nasus Larynx* 2003;**30**:263–8
20 Alobid I, Benítez P, Bernal-Sprekelsen M, Roca J, Alonso J, Picado C *et al*. Nasal polyposis and its impact on quality of

https://doi.org/10.1017/S002221511300073X Published online by Cambridge University Press

life: comparison between the effects of medical and surgical treatments. *Allergy* 2005;**60**:452–8
21 Ramis I, Lorente J, Roselló-Catafau J, Quesada P, Gelpí E, Bulbena O. Differential activity of nitric oxide synthase in human nasal mucosa and polyps. *Eur Respir J* 1996;**9**:202–6
22 Maniscalco M, Sofia M, Pelaia G. Nitric oxide in upper airways inflammatory diseases. *Inflamm Res* 2007;**56**:58–69

Address for correspondence:
Assist Prof Dr Oğuz Güçlü,
Department of Otorhinolaryngology,
Çanakkale Onsekiz Mart University Faculty of Medicine,
17100 Çanakkale, Turkey,

Fax: +90 286 2180393
E-mail: droguzguclu@hotmail.com

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