NON-ALLERGIC RHINITIS WITH EOSINOPHILS AND MAST CELLS CONSTITUTES A NEW SEVERE NASAL DISORDER

M. GELARDI, A. MASELLI DEL GIUDICE, M.L. FIORELLA, R. FIORELLA, C. RUSSO P. SOLETI[1], M. DI GIOACCHINO[2] and G. CIPRANDI[3]

Department of Ophthalmology and ENT, Faculty of Medicine and [1]Department of Statistic Sciences, Faculty of Economics, University of Bari; [2]Allergy Related Diseases Unit, G. d'Annunzio University Foundation, Chieti; [3]Department of Internal Medicine, Azienda Ospedaliera Universitaria San Martino, Genova, Italy

Received April 30, 2007 – Accepted September 29, 2007

Three main types of inflammatory Non-Allergic Rhinitis (NAR) have been defined: NAR infiltrated by eosinophils (NARES), by mast cells (NARMA), and by neutrophils (NARNE). A new particular type has been characterized with current infiltration by eosinophils and mast cells (NARESMA). The aim of this study is to evaluate the clinical and functional characteristics in patients with NARES, NARMA, NARNE, and NARESMA and to define the latter. One hundred and seventy-six NAR patients were prospectively and consecutively evaluated: 52 patients with NARES, 38 with NARMA, 36 with NARNE, and 50 with NARESMA. Clinical features, Quality of Life (QoL), and rhinomanometry were evaluated in all of them. QoL was significantly different in the 4 groups. NARESMA patients had the worst QoL. Nasal function and QoL in NARESMA patients were significantly correlated. Significant associations were shown with both nasal polyps and asthma in NARESMA patients. This study provides the first evidence that NARESMA constitutes a new type of NAR and is a particularly severe disorder.

Non-Allergic Rhinitis (NAR) is a heterogeneous disease, characterized by nasal hyperreactivity that results in typical symptoms due to irritation, such as rhinorrhoea and sneezing, and/or due to vasodilatation obstruction. Diagnosis of NAR is based on persistent symptoms throughout the year after exclusion of infection, any anatomical or medical disorder, and absence of serum IgE specific to relevant aeroallergens (1-4).

The aetiology of NAR is largely unknown for a majority of about 75–80% of the patients and NAR is classified as idiopathic or vasomotor rhinitis. This high percentage of idiopathic aetiology is, at least in part, due to the fact that nasal cytology is very rarely performed. Indeed, nasal cytology only allows to recognize and identify the different NAR types on the basis of the particular inflammatory cell infiltrate (5). In this regard, three main types of NAR characterized by distinct inflammatory cell infiltrate have been defined: NAR infiltrated by eosinophils (i.e. non-allergic rhinitis with eosinophils, NARES), by mast cells (i.e. non- allergic rhinitis with mast cells, NARMA), and by neutrophils (i.e. non-allergic rhinitis with neutrophils, NARNE). These forms of NAR are characterized by a local inflammation that appears to be the main underlying pathogenetic

Key words: non-allergic rhinitis, nasal cytology, eosinophils, mast cells, quality of life, resistance, polyps, asthma

Mailing address: Giorgio Ciprandi, M.D.
Semeiotica Medica I
Padiglione 3,
A.O.U. San Martino,
Largo R. Benzi 10, 16132 Genova, Italy
Tel: ++39 10 35331820 Fax: ++39 10 3537573
e-mail: gio.cip@libero.it

0394-6320 (2008)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder. Unauthorized reproduction may result in financial and other penalties

mechanism (6).

NARES is characterized by a profound nasal eosinophilia (>20% of the cells present in the mucosa), not associated with allergic disease (7). NARES may be prevalent in up to one-third of adults, and in severe cases may be associated with non-IgE-mediated asthma, aspirin intolerance and nasal polyps (8). NARMA was initially described by Connell and is characterized by a predominant mast cell infiltration (9). NARNE is characterized by predominant neutrophilic inflammation without infections; its aetiology is multi-factorial, including cystic fibrosis, antro-choanal polyp, pollution, tobacco smoke, etc. (10-12).

A fourth type of NAR is characterized by contemporaneous infiltration of eosinophils and mast cells: non-allergic rhinitis with eosinophils and mast cells, NARESMA. The aim of this study is to define the clinical and functional features of this new particular type of NAR, comparing it with the well-known types, such as NARES, NARMA, and NARNE.

MATERIALS AND METHODS

Study design

This prospective study included patients with NAR consecutively visiting the ENT Clinic of Bari (Italy). Subjects with acute upper respiratory infections, anatomic nasal defects (i.e. septum deviation), using nasal or oral corticosteroids, nasal or oral decongestants, antileukotrienes, and antihistamines during the previous 4 weeks were excluded. The diagnosis of NAR was made on the basis of a history of nasal symptoms (including sneezing, rhinorrhea, and nasal obstruction typically dependent on exposure to triggers such as odors, irritants, weather changes), presence of inflammatory cells on nasal smear, and negative skin prick test according to validated criteria (2-3).

Skin prick tests, nasal endoscopy, rhinomanometry, and Quality of Life (QoL) evaluation by questionnaire were carried out on all subjects. The study was approved by the Institutional Review Board of the University of Bari and informed consent was obtained from all patients.

Skin prick test

Allergy was assessed by the presence of sensitization to the most common classes of aeroallergens by performing skin prick tests. They were performed as stated by the European Academy of Allergy and Clinical Immunology: sensitization was considered when the wheal diameter was equal or greater than 3 mm (13). The allergen panel consisted of the following: house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), cat, dog, grasses mix, *Compositae* mix, *Parietaria judaica*, birch, hazel tree, olive tree, *Alternaria tenuis*, *Cladosporium*, and *Aspergilli* mix; the concentration of allergen extracts was 100 I.R./mL (Immunologic Reactivity) (Stallergenes, Milan, Italy).

Nasal cytology

Cytological samples were obtained by scraping with a Rhino-Probe™. The samples were collected from the medial portion of the inferior middle turbinate. After fixing with absolute alcohol for 3 minutes and drying, the samples were stained using the May-Grünwald-Giemsa (Carlo Erba, Milan, Italy) method, then mounted on covered slides and examined under microscopy (Nikon E600, Nikon Italy). Cell count was performed on 10 microscopic samples at high-power magnification (x1000) in immersion (14). Samples were examined blindly by two different investigators.

NARES (Fig. 1A) was diagnosed if nasal eosinophils were >20% of total cells recovered from nasal scraping, including both inflammatory and epithelial cells; NARMA (Fig. 1B) was diagnosed if nasal mast cells were >10% of total cells; NARNE (Fig. 1C) was diagnosed if nasal neutrophils were >50% of total cells; NARESMA (Fig. 1D) was diagnosed if concurrent nasal eosinophils were >20% and mast cells >10% of total cells.

Rhinomanometry

Nasal airflow resistance was measured by active anterior electronic rhinomanometry. Patients wore a tight-fitting facemask, and breathed through one nostril with their mouth closed. A sensor, placed in the controlateral nostril, recorded data on pre- and post-nasal pressures via airflow and pressure transducers. The instrument (Rhinomanometer Menfis, Amplifon Italy) was connected to a personal computer. The signals of transnasal airflow and pressure were amplified, digitalized, and saved for statistical analysis.

Nasal resistance is measured as Pa/mL/sec (Pascal) as the sum of the recorded airflow in milliliter per second through the right and left nostrils at a pressure difference of 150 Pa across the nasal passage. Four or more airflow measurements were performed for each patient and the mean value was recorded when reproducible values were achieved. Normal values are < 0.50 Pa/mL/sec (16).

Quality of Life

QoL assessment was performed by a disease-specific instrument. It was the Health-Related Quality of Life in Rhino Surgery (HRQL-RS), validated (17), translated

and adapted for use in the Italian population. This specific health profile HRQL-RS questionnaire consists of 25 items summarized in 6 dimensions: nasal and non-nasal symptoms, sleep, emotional symptoms, headache, and practical problems, these last three were summarised in a single dimension: social problems. Responses to the items are scored on a 4-point scale, the lower the score, the better the QoL. Most items are the same used in the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) provided by Juniper and colleagues (18). Moreover, a visual analogue scale for QoL assessing(from 0=worst QoL to 10=best QoL) was given to measure the patients' general feelings related to their nasal disease.

Statistical analysis

Data were described as mean and standard deviation. Continuous variables and categorical variables were compared by means of the Kruskall Wallis test (non-parametric analysis of variance) and the Mann Whitney U test for post-hoc comparisons. Kolmogorov-Smirnov test was used to compare nasal resistance values. Correlations were evaluated by the Spearman test. Q^2 and G^2 tests were used to compare the frequency and onset age of polyps to NAR subsets. SPSS software was used for computation. A p-value<0.05 was considered statistically significant.

RESULTS

A total of 176 patients (92 males and 84 females) with NAR were consecutively evaluated: their characteristics are reported in Table I. All were negative to skin prick tests. They were grouped according to diagnosis, such as the type of infiltrating inflammatory cells: 52 patients with NARES, 38 with NARMA, 36 with NARNE, and 50 with NARESMA (Fig. 1).

Quality of Life

Globally there was a significant difference (p<0.001) between groups concerning the four dimensions as reported in Table IIa. Both NARESMA and NARES patients had the worst QoL. The inter-group analysis indeed demonstrated that NARES compared with both NARNE and NARMA determined the worst QoL for all dimensions (p<0.001) as well as NARESMA compared with NARNE (p<0.001) and NARMA (Table IIb). The comparison between NARES and NARESMA showed that NARESMA patients had the worst QoL for sleep dimension (p=0.017) (Table IIb).

The analysis of single items of the nasal symptom dimensions revealed that NARESMA patients had a more intense impairment for dry nose, snoring and sore throat (p<0.001, p=0.005, and p=0.026, respectively), whereas NARES patients showed more severe post nasal drip (p=0.004) (Table IIc). Moreover, there was a significant relationship between nasal obstruction and snoring, and between nasal obstruction and sore throat only in NARESMA and NARES patients (Table IIIa). In addition, a relationship between Qol assessed by VAS and all dimensions existed only for NARESMA and between QoL-VAS and social problems in NARMA (Table IIIb). Snoring and nocturnal awakening were more frequent in NARESMA patients compared to other groups, as reported in Fig. 2.

Clinical features

The association with nasal polyposis was more frequent in NARESMA and NARES patients in comparison with other groups (Table IVa), however the association with asthma was more evident in NARESMA patients (Table IVb).

Relationship between QoL and rhinomanometry

A significant association was noted only for

Table I. *Characteristics of patients.*

	NARES (n=52)	NARMA (n=38)	NARNE (n=36)	NARESMA (n=50)
Males				
Number	31	20	21	20
Age mean (years)	40	32.2	34.9	42.6
Range	14-72	16-62	21-58	18-72
Females				
Number	21	18	15	30
Age mean (years)	42	38.8	38.5	40.5
Range	14-66	16-62	21-58	18-72

Table II. *Scores of the single QoL dimensions (data are expressed as median and 25th-75th percentile) for each type of NAR (a); comparisons between the single QoL dimensions and each type of NAR (b); and between each item of nasal symptoms dimension and each type of NAR (c). Globally there was a significant difference (p<0.001) between groups concerning the four dimensions. NARESMA and NARES patients had the worst QoL.*

(a)	NARES (n=52)	NARMA (n=38)	NARNE (n=36)	NARESMA (n=50)	Kruskall-Wallis
Social problems	2.80 (2.40-3.00)	1.60 (1.25-1.80)	1.60 (1.40-1.80)	2.60 (2.20-3.00)	**<0.001**
Sleep	2.33 (2.00-3.00)	1.33 (1.00-2.00)	1.67 (1.33-1.67)	2.67 (2.33-3.33)	**<0.001**
Non-nasal symptoms	2.50 (1.75-2.75)	1.25 (1.00-1.50)	1.75 (1.31-1.94)	2.50 (2.00-3.00)	**<0.001**
Nasal symptoms	2.58 (2.33-2.83)	1.50 (1.33-1.67)	1.67 (1.50-1.83)	2.33 (2.17-3.00)	**<0.001**

(b)	Mann-Whitney p-value				
Types of NAR	**Social problems**	**Sleep**	**Non-nasal symptoms**	**Nasal symptoms**	**QoL-VAS**
NARNE vs NARES	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
NARMA vs NARES	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
NARNE vs NARMA	0.83	0.625	**<0.01**	**0.04**	**<0.01**
NARNE vs NARESMA	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
NARES vs NARESMA	0.180	**0.017**	0.235	0.317	0.285
NARMA vs NARESMA	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

(c)	Mann-Whitney				
Types of NAR	Snoring	Sore throat	Nocturnal awakening	Dry nose	Post nasal drip
NARNE vs NARES	**0.000**	0.957	0.067	**<0.001**	**<0.001**
NARMA vs NARES	**<0.001**	**<0.001**	**<0.001**	**0.026**	**<0.001**
NARNE vs NARMA	0.271	**<0.001**	**0.002**	**<0.001**	**0.013**
NARNE vs NARESMA	**<0.001**	**0.043**	**0.001**	0.289	0.090
NARES vs NARESMA	**0.005**	**0.026**	0.125	0.001	0.004
NARMA vs NARESMA	**<0.001**	**<0.001**	**<0.001**	<0.001	<0.001

Table III. *Relationships between QoL-VAS and the single QoL-dimensions for each type of NAR (a) and between single items (nasal obstruction with snoring; nasal obstruction with sore throat) in the different types of NAR (b). NARESMA patients had a more intense impairment for dry nose, snoring and sore throat, whereas NARES patients showed more severe post nasal drip. Correlations were evaluated by the Spearman (Sp) test.*

(a)	NARESMA		NARNE		NARES		NARMA	
	R-Sp	p	R Sp	p	R Sp	p	R Sp	p
Nasal obstruction and snoring	0.334	**0.029**	-0.045	0.795	0.375	**0.006**	0.141	0.404
Nasal obstruction and sore throat	0.363	**0.017**	0.106	0.538	0.343	**0.013**	0.284	0.088
(b)	**NARESMA**		**NARNE**		**NARES**		**NARMA**	
	R-Sp	P	R Sp	p	R Sp	p	R Sp	p
Social problems-QoL	-0.412	**0.007**	0.013	0.938	-0.096	0.496	-0.417	**0.009**
Sleep-QoL	-0.427	**0.005**	-0.232	0.174	-0.262	0.061	-0.132	0.431
Non-nasal Symptoms/QoL	-0.458	**0.002**	-0.144	0.402	-0.238	0.090	-0.247	0.135
Nasal symptoms-QoL	-0.483	**0.001**	-0.293	0.083	-0.225	0.109	-0.098	0.560

Table IV. *Association of each single type of NAR with nasal polyposis (a) and with asthma (b). NARESMA is more frequently associated to nasal polyps and asthma than the other groups of patients.*

(a)	Fisher's exact Test
Types of NAR	Association with nasal polyps
NARES vs NARESMA	0.082
NARES vs NARNE	**<0.001**
NARES vs NARMA	**0.003**
NARESMA vs NARNE	**<0.001**
NARESMA vs NARMA	0.096
NARMA vs NARNE	**0.004**
(b)	Fisher's exact Test
Types of NAR	Association with asthma
NARESMA vs NARES	**0.003**
NARESMA vs NARMA	0.089
NARESMA vs NARNE	**0.006**
NARES vs NARMA	0.342
NARES vs NARNE	0.625
NARMA vs NARNE	0.338

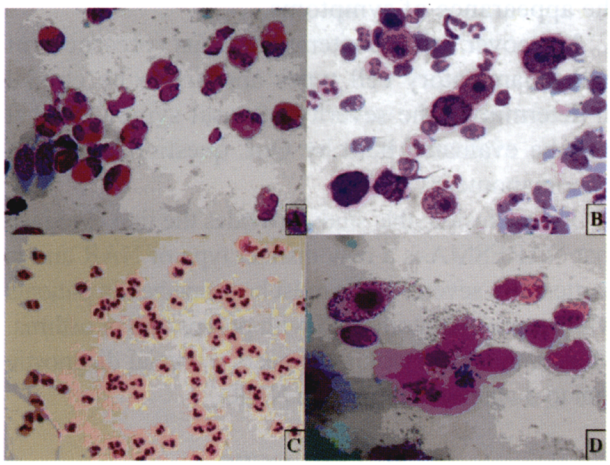

Fig. 1. *Nasal cytology in NARES, NARMA, NARNE, and NARESMA. Typical inflammatory infiltrate is evident; eosinophils (A), mastcells (B), neutrophils (C), and mast cells associated with eosinophils (D). Magnification 1000x*

NARESMA concerning social problems (p=0.002), sleep (p=0.006), and non-nasal symptoms (p=0.009) (Fig. 3).

DISCUSSION

NAR diagnosis is based on two main features in patients with nasal symptoms consequent to exposure to irritants: negative skin prick test and cytological

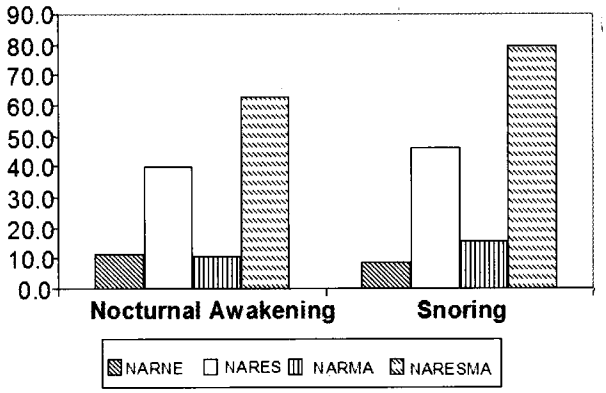

Fig. 2. *Patients reporting score 3-4 concerning nocturnal awakening and snoring reported more frequently by NARESMA patients.*

assessment. Nasal cytology is a crucial step in managing patients with NAR. Indeed, cytology alone allows to correctly diagnose the different types of NAR. On the basis of prevalent inflammatory cell type, four main forms of NAR may be considered: NARESMA, NARES, NARNE and NARMA.

Previously, we demonstrated that there is a different clinical and functional feature between NARES, NARNE, and NARMA (manuscript submitted). Therefore, the type of the predominant infiltrating cell is relevant to differentiate the severity of different NARs.

This study confirms previous findings and provides clear evidence that NARESMA constitutes a new particular type of NAR.

Patients with NARESMA had the worst QoL for most dimensions, thus underlining the severity of this form. Moreover, this clinical aspect is confirmed by functional findings: the nasal resistance is higher in this group compared to the others. This issue highlights the importance of the nasal obstruction in determining the severity of NARs. Indeed, nasal obstruction clearly determines the occurrence of snoring, nocturnal awakening, sore throat, and dry nose as oral respiration arises.

In addition, NARESMA is frequently associated with nasal polyposis: this phenomenon underlines the severity of this form as polyps are a typical consequence of mucosal inflammation. Finally, NARESMA alone is significantly associated with asthma. This issue allows to understand

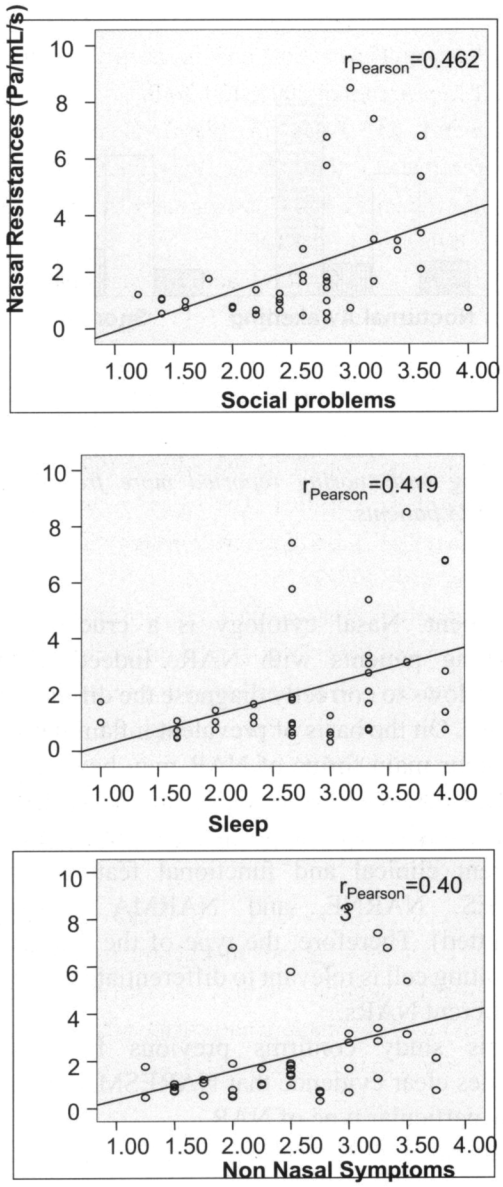

Fig. 3. *Relationship between nasal resistance and social problems (upper quadrant), sleep (centre quadrant), and non-nasal symptoms (lower quadrant). There was a significant association only for NARESMA patients.*

the close relationship between upper and lower airways, as recently reported (19). In this regard, the concurrent presence of NARESMA, nasal polyps, and asthma may represent a prognostic factor of relapse after polypectomy (manuscript in preparation). Therefore, this study provides the first evidence that NARESMA is a distinct type of NAR and is more severe than others as demonstrated by worst QoL, increased nasal resistances, frequent association with nasal polyps and asthma. Eosinophilic infiltrate consequently induces severe inflammation that causes the most severe symptoms. Indeed, eosinophil inflammation is the best marker of allergic inflammation (20). Moreover, eosinophils are also the main inflammatory cells that infiltrate nasal polyps (21). Inflammatory oedema associated with polyp formation contributes to the impairment of nasal airflow. In this regard, nasal obstruction is a crucial symptom that allows the occurrence of several complications (22). In addition, mast cells represent a relevant source for mediator release, mainly histamine and leukotrienes, both of which cause vasodilatation, increased vessel permeability, and stimulation of nerves causing the appearance of symptoms.

Moreover, the contemporaneous presence of two inflammatory cell types appears to be crucial in determining the severity of nasal symptoms that may be evaluated both by QoL questionnaires and rhinomanometry.

In conclusion, this study provides the first evidence that NARESMA is a new distinct type of NAR and is particularly severe, and it must be suspected in patients with severe symptoms and with polyps and/or asthma. Moreover, nasal cytology appears a crucial diagnostic tool to diagnose and manage NARs.

REFERENCES

1. Settipane RA, Charnock DR. Epidemiology of rhinitis: allergic and non-allergic. Clin Allergy Immunol 2007; 19:23-7.
2. Dykewicz MS, Fineman S, Skoner DP et al. Diagnosis and management of rhinitis. Complete guidelines of the Joint Task Force on practice parameter in allergy, asthma, and immunology. Ann Allergy Asthma Immunol 1998; 81:478-81.
3. Bousquet J, Van Cauwenberge P, Khaltaev N. The ARIA Workshop Group. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001; 108:147-52.
4. Fokkens WJ. Thoughts on the pathophysiology of non-allergic rhinitis. Curr Allergy Asthma Reports

2002; 2:203-8.

5. Gelardi M, Cassano P, Cassano M, Fiorella ML. Nasal cytology: description of a hyperchromatic supranuclear stria as a possible marker for the anatomical and functional integrity of the ciliated cell. Am J Rhinol 2003; 17:263-9.
6. Bachert C. Persistent rhinitis - allergic or non-allergic? Allergy 2004; 59:11-7.
7. Ellis AK, Keith PK. Non-allergic rhinitis with eosinophilia syndrome and related disorders. Clin Allergy Immunol 2007; 19:87-91.
8. Lund VJ, Aaronson A, Bousquet J. International Consensus Report on the diagnosis and management of rhinitis. Allergy 1994; 19:5-9.
9. Connell JT. Allergic rhinitis. Human experimental model. N.Y. State J Med 1970; 70:1751-8.
10. Hellgren J, Toren K. Non-allergic occupational rhinitis. Clin Allergy Immunol 2007; 19:241-6.
11. Gluck U, Gebbers JO. Cytopathology of the nasal mucosa in smokers: a possible biomarker of air pollution? Am J Rhinol 1996; 10:55-60.
12. Zeiger RS, Schatz M. Chronic rhinitis: A practical approach to diagnosis and treatment. Immun Allergy Practice 1982; 4:63-6.
13. Dreborg S. EAACI Subcommittee on Skin Tests. Skin tests used in type I allergy testing. Position Paper. Allergy 1989; 44(S):22-7.
14. Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA. Nasal obstruction in patients with seasonal allergic rhinitis: relationships between allergic inflammation and nasal airflow. Int Arch Allergy Immunol 2004; 134:34-8.
15. Gelardi M. Atlas of Nasal Cytology. Centro Scientifico Editore, Torino, Italy, 2006; p.1.
16. Cole P, Fenton RS. Contemporary rhinomanometry. J. Otolaryngol. 35:83-8.
17. Kramer M.F., G. Rasp and E. Kastenbauer. 2003. Health-Related Quality of Life in rhino surgery. Am J Otolaryngol 2006; 24:97-9.
18. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clin Exp Allergy 1991; 21:77-82.
19. Ciprandi G, Cirillo I. The lower airway pathology of rhinitis. Update Review. J Allergy Clin Immunol 2006; 118:1105-9.
20. Ciprandi G, Vizzaccaro A, Cirillo I, Tosca M, Massolo A, Passalacqua G. Nasal eosinophils display the best correlation with symptoms, pulmonary function and inflammation in allergic rhinitis. Int Arch Allergy Immunol 2005; 136:266-71.
21. Bernstein JM. Update on the molecular biology of nasal polyposis. Otolaryngol Clin North Am 2005; 38:1243-8.
22. Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA. Nasal obstruction in patients with seasonal allergic rhinitis: relationships between allergic inflammation and nasal airflow. Int Arch Allergy Immunol 2004; 134:34-9.