

Examination of the distribution of mast cells in the nasal mucosa of patients with seasonal allergic rhinitis

Nihal Kilinc, MD, Sebahattin Cureoglu, MD, Faruk Oktay, MD.

ABSTRACT

Objective: It is known that mast cells play an important role in pathogenesis of allergic rhinitis. In this study, we investigated the distribution of mast cells in the nasal mucosa of patients with seasonal allergic rhinitis during the pollen-season.

Methods: This study was carried out at the Faculty of Medicine, Dicle University, Turkey, during the grass-pollen season between, March and July in 2002. Twenty patients with seasonal allergic rhinitis (12 females and 8 males) and 20 healthy (10 females and 10 males) non-allergic controls were examined for the distribution and abundance of mast cells in nasal biopsies. Biopsies were performed in all patients and controls, once during natural provocation in the spring and were taken from the lower edge of the inferior turbinate using a forceps. The samples of nasal mucosa

were fixed in 10% neutral buffered formaline, stained with 0.5% aqueous toluidine blue and hematoxylin and eosin were examined under a light microscope.

Results: Mast cells were observed in the nasal mucosa obtained from 12 patients (60%) and 5 patients (25%) controls cases ($p=0.025$). It was found out that intrapitelial mast cells are present in nasal mucosa samples of patients with SAR (seasonal allergic rhinitis) but not in the epithelium of non-allergic controls.

Conclusion: The number of submucosal mast cells has considerably increased in the nasal mucosa samples of patients with SAR. Besides this, these cells are determined in great amounts in non-allergic individuals.

Saudi Med J 2004; Vol. 25 (5): 621-624

Metachromatic cells in atopic patients, namely, mast cells and basophils are present in nasal secretions, nasal mucosa, sputum and bronchial lavage. The structural morphology of human mast cells has been studied extensively and well documented.¹ The changes in mast cell numbers during allergen provocation have not been conclusive. Wihl and Mygind² found no significant change after allergen challenge. However, Pipkorn and Enerbäck³ reported a decrease in the number of mast cells in epithelium and lamina propria. Borres reported the temporary redistribution of metachromatic cells towards the mucosal surface after allergen challenge.⁴ We investigated the

distribution of mast cells in the nasal mucosa of patients with seasonal allergic rhinitis (SAR) during the pollen-season.

Methods. Study design. The study was performed with the subjects' informed consent. Biopsy specimens of nasal mucosa were taken during the grass-pollen season between March and July in 2002. These biopsies were compared with those of controls. Twenty patients with allergic rhinitis (12 females and 8 males) ranging in age from 18-34-years (mean, 23.4 ± 4.7) were examined for the distribution and abundance of mast cells in

From the Department of Pathology, Dicle University Medical School, Diyarbakir, Turkey.

Received 9th September 2003. Accepted for publication in final form 18th January 2004.

Address correspondence and reprint request to: Dr. Nihal Kilinc, Assistant Professor in Pathology, Dicle University Medical School, 21280 Diyarbakir, Turkey. Tel. +90 (412) 2488001/4227. Fax. +90 (412) 2488440. E-mail: nhl@dic.edu.tr

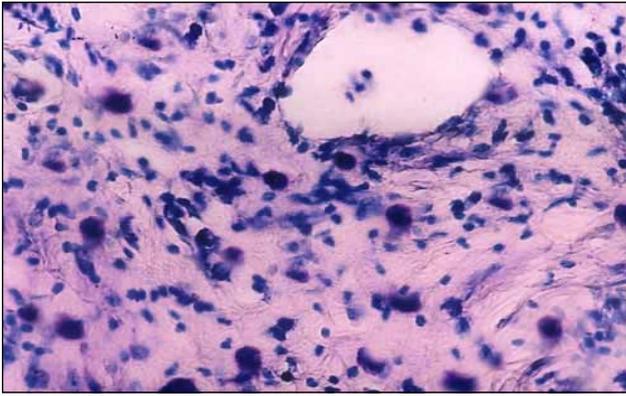


Figure 1 - Mast cells around the glands and in the stroma of nasal mucosa (Toluidin blue, original magnification x 200).



Figure 2 - Mast cells in the epithelial layer and the lamina propria of nasal mucosa are shown (Toluidin blue, original magnification x 200).

nasal biopsies. At the time of study, all the patients were symptomatic. Diagnosis of allergic rhinitis was made on the basis of a typical history of seasonal allergic rhinitis, eosinophilia in nasal smears and positive skin-prick tests to grass-pollen allergens. According to criteria reported in the position paper of European Academy of Allergy and Clinical Immunology (EAACI)⁵ the results of skin-prick tests were scored as follows: 0=negative, + = less or equal to one quarter of histamine, ++ = from one quarter to one half of histamine, +++ = from one half to equal to histamine, ++++ = larger than histamine control. Patients who had a background of perennial allergy, had previously received immunotherapy, acute respiratory tract infection, or had taken topical or oral medication within one month were excluded from the study. The biopsies were performed in all patients with isolated grass-pollen allergy and controls, once during natural provocation in the spring. Twenty control subjects had no background of allergy, and had negative skin prick tests to a panel of common aeroallergens. The biopsies of nasal mucosa were taken from the lower edge of the inferior turbinate, 2 cm posterior to the anterior edge using a forceps with a cup diameter of 2-5 mm. Local anesthesia was obtained by placing a cotton-wool carrier with 50-100 mg lidocaine and 3 drops of adrenaline (1:1000) under the inferior turbinate without touching the place where the biopsy would be taken.

Histopathologic analysis. The samples of nasal mucosa were fixed in 10% neutral buffered formaline for 24-hours. Each sample was then embedded in paraffin block by a standard method and cut with microtome of a section thickness of 4 μ m. The samples were stained with 0.5% aqueous toluidine blue and hematoxylin and eosin were examined under a light microscope.

Statistical analysis. Statistical analysis was evaluated by statistical package for social sciences

7.5 computer program. Categorical variables were compared with χ^2 test. Statistically $p < 0.05$ was accepted significantly. Data were demonstrated by the means \pm SD.

Results. Nasal mast cells were present in 12 patients with SAR and in 5 controls. In patients with SAR the presence of mast cells in the submucosal layer 12 patients (60%) was more than in normal subjects 5 patients (25%) ($\chi^2 = 5.013$, $p = 0.025$). Thinned basal membrane, infiltration of mononuclear cells and polymorphonuclear cells in the stroma, the increase of capillary vessels and edema were observed in the samples of the nasal mucosa of patients' group. The patients with SAR showed an increase in the numbers of mast cells in the lamina propria (mean, 8.8/ mm^2) during the season (**Figure 1**). The mean number of mast cells in the lamina propria was 3.5 in the 5 controls. However, intraepithelial mast cells were observed in only 3 of these 12 patients with SAR (**Figure 2**). During natural provocation, almost all the mast cells in the epithelium and half of those in the lamina propria were degranulated. The mast cells are present in the lamina propria but not in the epithelium of non-allergic controls.

Discussion. The mast cells play an important role in allergic rhinitis. Symptoms of allergic rhinitis are accompanied by infiltration of inflammatory cells, predominantly eosinophils and metachromatic cells (basophils and mast cells) in the nasal mucosa. The allergic reaction of the mucosa is induced by an interaction between allergen and immunoglobulin (Ig) E antibodies on the surface of the mast cell. Most of the studies performed on the role of metachromatically staining cells in the nasal mucosa have been performed in nasal smears or scraping material.⁶ Few quantitative

studies have been carried out in biopsies of nasal mucosa. In the present study, we used the method of punch biopsy. The studies on mast cells have employed a variety of different tissue fixatives and staining methods, which make it difficult to compare the results reported by different authors. The common method in recent literatures is toluidine blue staining (pH 0.5) after fixation with 10% formaldehyde. In our study, we used the same method. The mast cell degranulation is important in the pathogenesis of allergic rhinitis. The results of the available quantitative studies on the mast cells in the nasal mucosa differ widely, ranging from an overall decrease via redistribution to an overall increase after allergen exposure.^{7,8} These differences could be due to the use of different staining techniques, which do not always guarantee that all mast cells, including the degranulated ones, are counted. It is conceivable that some of the literature cited refers to different stages of an ongoing process. To study changes in the number of degranulation of mast cells, we investigated these cells in the epithelium and lamina propria of the nasal mucosa of the lower inferior turbinate in patients with isolated grass-pollen allergy and normal controls during natural provocation. More studies have been carried out on mast cells in allergic nasal mucosa and on the numerical changes, shown by mast cells in epithelium and lamina propria during (natural) allergen provocation. The changes in mast cells numbers during allergen provocation have not been conclusive. Okuda et al⁹ found an increased number of mast cells in the epithelium of the allergic patients but not in the lamina propria. Wihl and Mygind² did not find any change after allergen challenge at all. Other authors reported no changes in the total number of mast cells in patients with allergic rhinitis^{10,11} Pipcorn et al¹² reported a decrease in the number of mast cells in epithelium and lamina propria. A temporary redistribution of metachromatic cells towards the mucosal surface after allergen challenge was reported.⁴ Some comparative studies revealed that the numbers of nasal mucosal mast cells increased during natural provocation.^{7,13,14} Gomez et al¹⁵ demonstrated a small increase of nasal mucosal mast cells during provocation after use of nasal corticosteroid spray. This suggests that the use of nasal corticosteroids has led to an underestimation of the increase of mast cells found in the natural provocation study. In Drake-Lee's et al¹¹ the number of mast cells in the inferior turbinate from patients with perennial allergy due to house dust mite was compared with that from normal controls, and no statistical differences were found. The lack of increase in mast cell numbers was attributed to degranulation since numbers have been shown increased in perennial allergy when sections are examined ultrastructurally.¹¹ In this study, we found

that the number of mast cells in the lamina propria increased during seasonal grass pollen-induced allergic rhinitis. This is in agreement with the finding of Lozewicz et al¹³ who, although they did not indicate results concerning epithelium and lamina propria separately, found an increase during natural provocation. However, in this study, intraepithelial mast cells were seen in only 3 patients. The present results show that mast cells exist in the lamina propria but not in the epithelium of non-allergic controls. This finding is in agreement with results obtained by other authors.^{8,14} Specific IT (immunotherapy) reduces mucosal eosinophilia and numbers of metachromatic cells in the epithelium. IT has been successfully used in the treatment of allergic rhinitis for years. Several mechanisms have been postulated for the clinical efficacy of IT.^{6,16} Cengizlier et al¹⁶ also reported that the reduction in the number of metachromatic cells could be at least partially responsible for the clinical efficacy of IT in children with perennial allergic rhinitis. Nasal cytologic examination for inflammatory cellular infiltrates of eosinophils and basophilic metachromatic cells in allergic rhinitis not only establishes the diagnosis of allergic rhinitis but also useful in the follow up of patients with this condition.¹⁷ Finally, although intraepithelial mast cells were observed in nasal mucosa samples of patients with SAR, this can also be determined in non-allergic individuals.

Acknowledgment. We would like to express our thanks to Mr. Faruk Bozgöz, for his unique contribution of translating the manuscript to Arabic.

References

1. Galli S, Dvorak AM, Dvorak HF. Basophils and mast cells morphologic insights into their biology, secretory patterns, and function. *Prog Allergy* 1984; 34: 50-141.
2. Wihl JA, Mygind N. Studies on the allergen-challenged human nasal mucosa. *Acta Otolaryngol* 1977; 84: 281-286.
3. Pipcorn U, Enerbäck L. Nasal mucosal mast cells and histamine in hay fever: effect of topical glucocorticoid treatment. *Int Arch Allergy Appl Immunol* 1987; 84: 123-128.
4. Borres MP, Irander K, Björkstén B. Metachromatic cells in nasal mucosa after allergen challenge. *Allergy* 1990; 45: 98-103.
5. EAACI. Subcommittee for Skin Test: 'Skin test used in type I allergy testing' Position Paper. *Allergy* 1990; 44: 22-30.
6. Wilson DR, Irani AM, Walker SM, Jacobson MR, Mackay IS, Schwartz LB et al. Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. *Clin Exp Allergy* 2001; 31: 1705-1713.
7. Slater A, Smallman LA, Drake-Lee AB. Increase in epithelial mast cell numbers in the nasal mucosa of patients with perennial allergic rhinitis. *J Laryngol Otol* 1996; 110: 929-933.
8. Igarashi Y, Goldric MS, Kaliner MA, Irani AM, Schwartz LB, White MV. Quantitation of inflammatory cells in the nasal mucosa of patients with allergic rhinitis and normal subjects. *J Allergy Clin Immunol* 1995; 95: 716-725.

9. Fokkens WJ, Godthelp T, Holm AF, Blom H, Mulder PGH, Vroom M et al. Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study. *Clin Exp Allergy* 1992; 22: 701-710.
10. Okuda M, Otsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. *Eur J Respir Dis* 1983; 128: 7-14.
11. Drake-Lee AB, Moriarty B, Smallman LA. Mast cell numbers in the mucosa of the inferior turbinate in patients with perennial allergic rhinitis: a light microscopic study. *J Laryngol Otol* 1991; 105: 736-738.
12. Pipkorn U, Karlsson G, Enerback L. Secretory activity of nasal mucosal mast cells and histamine release in hay fever. *Int Arch Allergy Appl Immunol* 1988; 87: 349-360.
13. Lozewicz S, Gomez E, Claque J, Gatland D, Davies RJ. Allergen-induced changes in the nasal mucous membrane in seasonal allergic rhinitis: effect of nedocromil sodium. *J Allergy Clin Immunol* 1990; 85: 125-131.
14. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: Increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992; 89: 877-883.
15. Gomez E, Claque JE, Gatland D, Davies RJ. Effect of topical corticosteroids on seasonally induced increases in nasal mast cells. *Br Med J (Clin Res Ed)* 1988; 296: 1572-1573.
16. Cengizlier R, Saraclar Y, Adaloglu C, Tuncer A. Changes in nasal metachromatic cells during allergen immunotherapy in children. *Allergol Immunopathol* 1995; 23: 111-116.
17. Jirapongsananuruk O, Vichyanond P. Nasal cytology in the diagnosis of allergic rhinitis in children. *Ann Allergy Asthma Immunol* 1998; 80: 165-170.

NEUROSCIENCES JOURNAL SUPPLEMENTS

- **ADVANCES IN MANAGEMENT OF NEUROVASCULAR DISEASE**
(Vol. 9 Supplement 2 January 2004)
- **12th ANNUAL SAUDI NEUROSCIENCES SYMPOSIUM
4TH ANNUAL MEETING OF THE SAUDI CHAPTER OF EPILEPSY
2ND ANNUAL JEDDAH NEUROSCIENCE UPDATE**
(Vol. 9 Supplement 1 January 2004)
- **EEG & EPILEPSY**
(Vol. 8 Supplement 2 April 2003)
- **9th PAN ARAB UNION OF NEUROLOGICAL SCIENCES**
(Vol. 8 Supplement 1 January 2003)

TO ORDER:

Call: +966 1 477 7714 ext. 6577, 6570, 6596, 6579 Fax: +966 1 476 1810. 477 7194
e-mail: info@smj.org.sa