

ORIGINAL ARTICLE

Olfactory dysfunction in seasonal and perennial allergic rhinitis

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Abstract

Conclusions: The limitation in olfactory function in patients with seasonal allergic rhinitis (AR) can be ascribed to an increase in eosinophilic and mast cell activity in the olfactory cleft. Therefore, the decrease in olfactory functions seems to be predominantly caused by the inflammation of the epithelium and not by the obstruction of the nose caused by the inflammation. **Objective:** Olfactory dysfunction is frequently seen in patients with AR; however, little is known about the underlying pathophysiological mechanisms. Therefore, the aim of the present study was to examine the olfactory function in patients with seasonal or perennial AR, and to correlate the results with data obtained by analysis of nasal secretion and obstruction. **Methods:** Olfactory function was tested using the SniffinSticks test in patients with seasonal or perennial AR and in a control group. Nasal secretion analysis included eosinophilic cationic protein (ECP) and tryptase testing. Nasal obstruction was evaluated by rhinomanometry. **Results:** Patients with AR (seasonal and perennial) showed impaired olfactory functions in comparison with the control group. Nasal secretion analysis showed increased values of ECP and tryptase in the seasonal group in comparison with controls. Rhinomanometry showed no differences in nasal flow between the three groups.

Keywords: Olfactory function, eosinophilic cationic protein, ECP, tryptase, rhinomanometry

Introduction

Allergic rhinitis (AR) is a common inflammatory disease with a relevant impact on the quality of life affecting between 10 and 25% of the worldwide general population [1]. Olfactory dysfunction, especially hyposmia, is a frequent symptom of AR [2]. Previously, accurate evaluation of olfactory function for seasonal and perennial AR was analyzed by Moll and co-workers [3]. They found decreased scores using the Connecticut Chemosensory Clinical Research Center Test (CCCRC) for olfactory threshold, identification, and discrimination for patients with perennial AR and even worse for patients with seasonal AR measured during the season. In further studies by the same research group, nasal eosinophilic inflammation was found to be the main reason for olfactory distortion in patients with seasonal AR [4].

The inflammatory milieu in the olfactory cleft directly impacts olfactory receptor cells, but the question as to which kind of inflammatory cells and even more which kind of released inflammatory mediators are responsible for the decrease in olfactory function still has not been answered. Most of the previous studies dealt with eosinophilic inflammation for loss in olfaction. On the other hand, little is known about other inflammatory mediators like tryptase or interleukins, which play an important role in the development of AR [5]. Zweimann et al. found a significant increase in nasal histamine, tryptase, leukotriene C₄, and albumin after allergen challenge in comparison with a nasal challenge with buffer solution within the same individual. No significant decrease in olfactory acuity occurred but a decrease in nasal minimal cross-sectional area was seen [6]. Nasal obstruction as one potential cause of olfactory

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Table I. Patient demographics and study results.

Characteristic	No. of patients	%
All patients	72	100
Mean age, years (range)	33 (19–69)	
Gender		
Male	28	39
Female	44	61
Seasonal allergic rhinitis	23	32
Perennial allergic rhinitis	16	22
No allergy	33	46

distortion in AR patients is still under controversial discussion [7].

Therefore we performed a prospective study on olfactory function in patients with seasonal and perennial AR in comparison with a control group. As well as measurement of eosinophilic cationic protein (ECP) for the analysis of eosinophilic inflammation, particular attention was paid to tryptase in nasal secretion analysis as a marker for early-phase mast cell activation. Taking into account current results from olfactory research on odorant-binding proteins (OBPs), tryptase could potentially be one important further mediator responsible for olfactory loss in patients with AR [8]. The SniffinSticks test recommended by the German Society of Otorhinolaryngology, Head and Neck Surgery was used for olfactory testing. In comparison with the CCCRC the SniffinSticks test battery gives a more precise impression of olfactory function, as it is well adapted to the German-speaking region and the subtests are more comprehensive. In particular, the discrimination subtest in the CCCRC that was used in former studies consists of a limited number of triplets – only 4 triplets in comparison with 16 triplets in the SniffinSticks subtest. Also the two other subtests are more detailed (12 vs 16 items in the identification test, 12 vs 16 threshold levels in the threshold test).

Material and methods

Patients who presented symptoms of AR at the university outpatient clinic of the Department of Otorhinolaryngology-Head and Neck Surgery of Ludwig-Maximilians University of Munich between January 2007 and May 2011 were consecutively included in the study. Investigations were performed according to the Declaration of Helsinki on Biomedical Studies Involving Human Subjects. The study design was approved by the local ethics committee. All subjects were informed about the procedures and aims of the study, and provided written informed consent.

Seventy-two patients were included in the study, with a gender distribution of 61% female, 39% male (Table I). Twenty-three patients with proven seasonal AR and no sensitization to perennial allergens could be measured intra-seasonally (mean age 36 years, range 23–69 years, standard deviation (SD) 11.5). Relevant allergens were grass, rye, birch, ragweed, and mugwort or a combination of them. Sixteen patients were diagnosed as having a single perennial AR and no further sensitization to seasonal allergens (mean age 35 years, range 19–55 years, SD 11.9). Another 33 healthy volunteers were measured as controls (mean age 30 years, range 20–52 years, SD 7.5). The main perennial allergen was house dust mite; two patients showed further allergies to cat. The ages of the participants were not significantly different in the three groups ($H = 5.806$, $p = 0.055$).

Study design

Patients underwent detailed evaluation of medical history and an otorhinolaryngological examination including inspection of the mouth, anterior rhinoscopy followed by endoscopic posterior rhinoscopy and laryngoscopy. Patients were diagnosed as having seasonal or perennial AR according to guideline specifications (Guideline of the German Society for Allergology and Clinical Immunology). Inclusion criteria for the patients were a history of nasal allergic symptoms for at least 2 years (recurrent seasonal symptoms or persistent perennial symptoms), a positive skin prick test (mean wheal diameter ≥ 3 mm; ALK-Abelló, Wedel, Germany) to either seasonal or perennial relevant aeroallergens, and a positive evaluation of allergen-specific IgE (CAP class ≥ 1 ; UniCAP-FEIA, Phadia Diagnostics, Freiburg, Germany). Exclusion criteria were the existence of nasal polyps, a history of chronic or acute rhinosinusitis, or specific immunotherapy to one of the rhinitis-causing allergens. Patients who were receiving symptomatic medication (topic or systemic steroids, antihistamines, decongestant nasal spray) in the 2 weeks before examination were also excluded from the study.

To analyze the degree of nasal obstruction, rhinomanometry (RhinoSys, Happersberger otopront GmbH, Hohenstein/Breithardt, Germany) was performed separately for each nostril at a pressure of 150 Pa. Results of the right and left nostril were added together and given as inspiratory flow in milliliters per second.

SniffinSticks

Olfactory function was evaluated using the SniffinSticks test battery (Burghart Messtechnik GmbH, Wedel, Germany) consisting of a threshold, a discrimination,

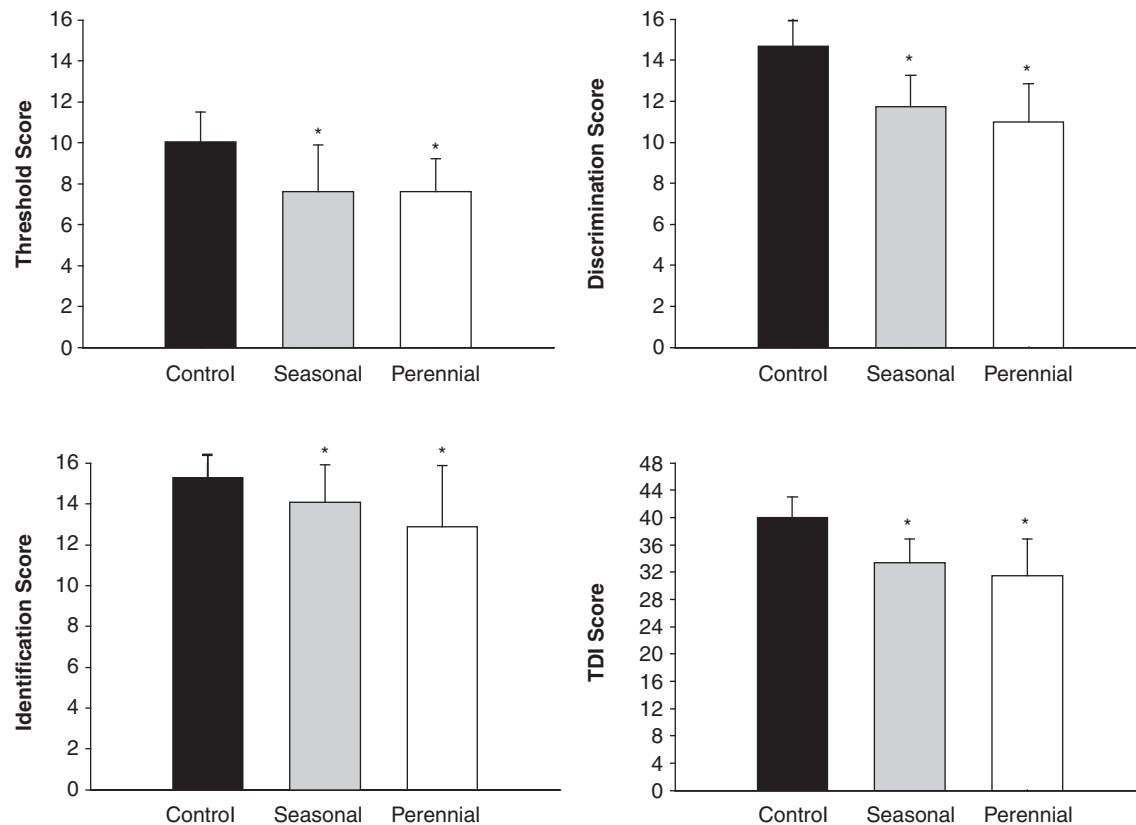


Figure 1. Results of the SniffinSticks test. Results of the three subtests and the TDI score are given as mean + SD. * $p < 0.05$ vs control.

and an identification test [9]. For odor presentation, the cap of the pen was removed by the investigator for approximately 3 s and the odorized tip was placed approximately 2 cm in front of the subject's nostrils. All olfactory tests were performed birhinally.

n-Butanol odor thresholds were assessed using a single-staircase, three alternative, forced choice procedure as described previously [9]. Sixteen dilutions were used in a series starting with 4% n-butanol diluted in aqua conservans. The dilution ratio for the other 15 pens was 1:2. At each trial, three pens were presented in randomized order. Two pens contained the solvent only and the third one the odorant in a certain dilution. The task for the blindfolded subject was to detect the odor-containing pen. Triplets were presented at intervals of approximately 20 s, starting with the lowest concentrations and moving stepwise towards higher concentrations. Reversals of the staircase towards higher dilutions were triggered by correct odor identification in two successive trials. Staircase reversals in the opposite direction, with stepwise increasing concentrations, were triggered by failed trial. The dilutions marking reversals were assessed, and trials continued until seven reversals had occurred. The threshold for n-butanol was defined as the mean of the last four of

seven staircase reversals. Scores between 1 and 16 were possible.

To assess odor discrimination, triplets of pens were presented in randomized order. Two of the pens in a triplet contained the same odorant, while the third one contained a different odorant. The blindfolded subjects had to detect the single odor among the three smells in each of the 16 trials. The intervals between the different pens as well as between the different triplets were already mentioned above. The score for discrimination was the sum of correctly discriminated triplets, ranging from 0 to 16.

A forced choice method was used for assessment of odor identification. Subjects had to identify 16 different odorants by means of 4 given possibilities for each pen. Correct answers were summed up to form the identification score, ranging from 0 to 16. The sum of threshold, discrimination, and identification scores is considered the TDI score, ranging from 1 to 48.

Nasal secretion analysis

Nasal secretions were obtained by placing small cone-shaped cotton wool pieces (absorbent cotton, Hartmann Inc., Heidenheim/Brenz, Germany) with a length of about 4 cm and a diameter of about 6 mm

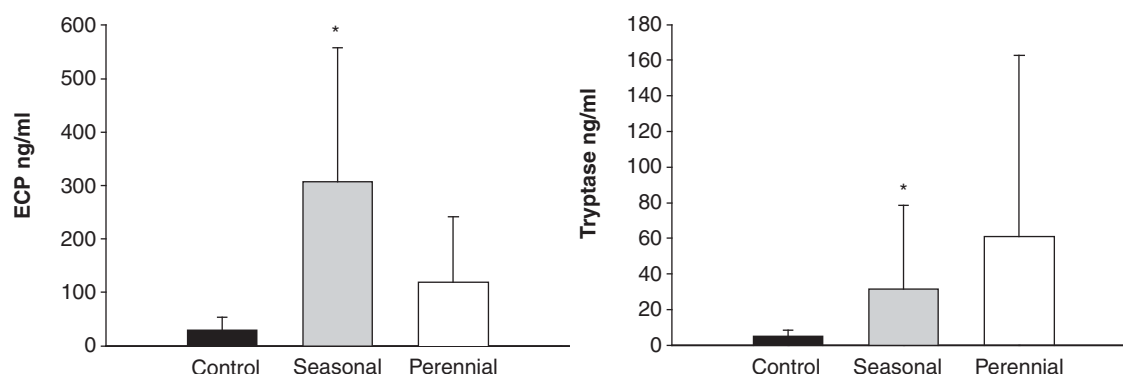


Figure 2. Results of nasal secretion analysis. Eosinophilic cationic protein (ECP) and tryptase are given as mean + SD. * $p < 0.05$ vs control.

into the middle meatus of the nose under anterior rhinoscopic view for 10 min, followed by centrifugation ($+8^{\circ}\text{C}$, 2000 g) of the cotton wool pieces for 10 min [10]. ECP and tryptase concentrations were measured by ELISA (Phadia Diagnostics). Thresholds for detection were < 10 ng/ml for ECP and 5 ng/ml for tryptase.

Statistical analysis

Statistical analysis was performed using SigmaStat (Jandel Corp., San Rafael, CA, USA) on a Lenovo ThinkPad X200s Notebook. For descriptive statistics we used mean values with SD. For the comparison of groups and normally distributed data we used a one-way analysis of variance (ANOVA) (F value/ p value) followed by the Student-Newman-Keuls method. For data that were not normally distributed, we used the Kruskal-Wallis one-way analysis of variance on ranks (H -value with two degrees of freedom/ p value). For correlations the Pearson's moment product correlation was used.

Results

Inspiratory nasal flow, which was measured by rhinomanometry, yielded no significant differences between the three groups ($H = 0.451/p = 0.798$). There was a slight decrease in the seasonal (462.7 ml/s ± 223.3 SD) and the perennial group (474.7 ml/s ± 349.0 SD) in comparison with the control group (515.7 ml/s ± 142.6 SD).

Patients with seasonal or perennial AR performed significantly worse than controls in olfactory threshold (control, 10.0 ± 1.5 SD; seasonal, 7.6 ± 2.3 SD; perennial, 7.6 ± 1.6 SD; $F = 12.462$; $p < 0.001$), discrimination (control, 14.7 ± 1.6 SD; seasonal, 11.7 ± 1.5 SD; perennial, 11.0 ± 1.8 SD; $F = 29.915$; $p < 0.001$), and identification tests (control, 15.3 ± 1.0 SD; seasonal, 14.1 ± 1.8 SD; perennial,

12.9 ± 3.0 SD; $H = 12.939$; $p = 0.002$). Graphical illustrations can be found in Figure 1. The TDI score for overall olfactory function ($H = 33.062$, $p < 0.001$) resulted in 40.0 ± 3.0 SD for the control group in comparison with 33.4 ± 3.5 SD for the seasonal group and 31.5 ± 5.4 SD for the perennial group. Also, there was no significant difference in the TDI score as in the subtests between the two allergic groups.

Patients with seasonal AR showed significantly increased levels for ECP (307.6 ng/ml ± 250.3 SD) in comparison with controls (29.7 ng/ml ± 23.9 SD) but not in comparison to the perennial group (119.1 ng/ml ± 122.5 SD), $H = 14.110$ and $p < 0.001$ (Figure 2). The examination of tryptase levels revealed similar results with a significant increase of tryptase in the seasonal group in comparison with the control group but not the perennial group (control, 5.1 ng/ml ± 3.7 SD; seasonal, 31.5 ng/ml ± 46.9 SD; perennial, 60.9 ng/ml ± 102.0 SD; $H = 7.516$; $p = 0.023$).

There were no correlations between ECP or tryptase with olfactory threshold, discrimination, identification, or the TDI score.

Discussion

This is the first study to use the SniffinSticks test battery for evaluation of olfactory function in patients with seasonal or perennial AR. Results were compared with data obtained by nasal secretion analysis for ECP and tryptase. Rhinomanometry was applied to evaluate the influence of nasal obstruction.

Olfactory function was decreased in threshold, discrimination, and identification testing in patients with seasonal or perennial AR, which is in good accordance with other published studies [3,11]. Levels of ECP were significantly elevated in our patients, with seasonal AR being in line with comparable data published by Klimek et al. [4], who showed eosinophilic inflammation of the nose after 3 weeks

after the beginning of allergy season and Kramer et al. [12] who compared ECP levels of different chronic nasal inflammatory diseases. In addition, we found significantly increased levels for tryptase in the seasonal group in comparison with the control group. In the perennial group no significant increase of ECP and tryptase levels in comparison to the control group was seen. Tryptase levels in both groups were lower, as in the study by Zweimann et al., who found tryptase levels of 95 ± 83 ng/ml after allergen challenge [6]. As the study was performed in only five patients out of the allergen season, results must be handled with caution when compared to data collected in patients with a perennial allergy during the allergen season, which lasts for weeks to months.

For a long time obstruction of the olfactory cleft was thought to be the reason for decreased olfactory functions in patients with AR. However, direct inflammatory damage of the olfactory receptor cells or a change in the surrounding milieu now seem to be much more important [4,13,14]. The question as to which inflammatory mediators are responsible for the decrease in sense of smell is still not answered. Is it the whole inflammatory milieu with a mixture of different mediators from different kinds of cells or is it a single mediator leading to this lack of olfactory function? Current studies in humans [11] and in an animal model using allergic mice [15] showed an infiltration of eosinophils, neutrophils, mast cells, plasma cells, and macrophages in the olfactory mucosa. So far the available studies have mainly focused on the role of eosinophilic cells and their main toxic mediator in AR ECP. In the present study we additionally wanted to see if another important group of cells in the development of AR, the mast cell, could also be responsible for the decreased sense of smell. Mast cells are part of the early phase of the allergic reaction. When allergen-specific IgE is attached to the surface of the cell and subsequent allergen exposure on the nasal mucosa takes place, bridging of adjoining IgE antibodies by the allergen leads to a liberation of different mediators such as tryptase, leukotrienes or histamine [16]. Tryptase is an enzyme that is able to split peptide bindings of proteins via serine, a catalytic amino acid. This serine proteinase might interact with small abundant extracellular proteins (odorant-binding proteins, OBPs), which are thought to play a role in perireceptor events of odor detection [17]. OBPs can be found in the nasal mucus of the olfactory cleft and bind to inhaled odorants reaching the corresponding regions [18]. This binding guarantees a quick and safe transport of hydrophobic odorants through aqueous nasal mucus to the olfactory receptor cell [8]. The long-lasting reduction of OBP in the nasal mucus by the

protein-disrupting effect of high amounts of tryptase might lead to a decreased sense of smell.

ECP is released during degranulation of eosinophils in allergic reactions but also in parasitic infections. ECP belongs to the ribonuclease superfamily and has neurotoxic, helmintho-toxic, ribonucleolytic activities [19]. In comparison to the above explained pre-receptor activity of tryptase, ECP can influence olfaction, as described previously [4,13].

Rhinomanometric analysis of nasal flow showed no significant differences between the three groups. Klimek et al. [4] demonstrated a significant reduction of nasal flow at day 3 of the pollen season in pollen allergic patients but no impact on olfactory threshold. A decrease in olfactory function was seen only after a significant increase of ECP in nasal secretion at day 14 after the beginning of the pollen season [4]. Our patients showed slightly decreased but not significantly reduced values for inspiratory nasal flow. The main flux of air (70–80%) tends to flow through the inferior part of the nose around the middle and inferior turbinate, as reported by various research groups using different models [20,21]. This means that an obstruction in the olfactory cleft, the narrowest part of the nose, must not necessarily lead to a decrease in nasal air flow measured by rhinomanometry. As other parameters to assess olfactory cleft consistency (such as direct endoscopic cleft visibility) also failed to explain the loss in olfactory abilities, it seems that olfactory cleft obstruction is an unimportant factor in the development of olfactory loss in patients with AR.

For a deeper understanding of the complex interaction between allergic inflammatory cells on the one hand and olfactory receptor cells on the other hand, it will be essential to investigate further inflammatory mediators like interleukins and histamines, and their influence on olfactory function. The results of the current study might indicate a pre-receptor mechanism caused by tryptase as well as a direct ECP-driven influence on the olfactory cell.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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