

The value and safety of specific nasal provocation in the diagnosis of allergic rhinitis in mild persistent asthma under inhaled steroid therapy

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Summary

Although specific nasal provocation is an objective diagnostic test for allergic rhinitis, it can also increase the lower airway responsiveness in asthmatic patients. Our goal was to determine the value and safety of specific nasal provocation test for the diagnosis of allergic rhinitis in mild persistent asthmatic patients under low-dose inhaled steroid therapy. The study was performed on 32 mild persistent, stable, mite-sensitive allergic asthmatics (group 1), 9 mild persistent nonallergic asthmatics (group 2) and 9 healthy non-smokers (group 3). Nasal symptoms were noted, paranasal sinus computerized tomography (PNCT) and rhinoscopic evaluations were performed. Cases with pathologic-anatomic changes in PNCT and rhinoscopy were excluded. Symptom scoring, flow-volume, peak expiratory flow (PEF), serum and nasal lavage eosinophil cationic protein (ECP) and nasal lavage eosinophil counts were performed before mite specific nasal provocation test and at the 0th, 4th and 24th hours following the test. No adverse effects were observed in all diagnostic procedures. Total diagnostic value of nasal symptoms were found to be at 92%, while being 70% for rhinoscopy and 88% for specific nasal

provocation test respectively in the diagnosis of allergic rhinitis in group 1. Statistically significant differences were found between basal nasal lavage eosinophil values ($p < 0.001$) and ECP levels ($p < 0.05$) when group 1 was compared with both group 2 and group 3. In the remaining measured values between three groups, no statistically significant differences were found. Specific nasal provocation test is a safe method for mild house dust mite allergic asthma cases under low-dose inhaled steroid therapy, but history of rhinitis might be sufficient for the diagnosis of allergic rhinitis. (*Asian Pac J Allergy Immunol 2010;28:115-21*)

Key words: allergic asthma, allergic rhinitis, nasal provocation, rhinomanometry, inhaled steroids

Introduction

Allergic rhinitis and bronchial asthma are chronic inflammatory airway diseases with common immunopathological changes. More than 70% of allergic and 80% of non-allergic asthma cases also suffer from rhinitis.^{1,2} The asthma-rhinitis association is generally recognized by physicians treating asthma in Turkey.³ However, there is a certain need with regards to training in terms of the attitude towards examination and appropriate use of medications. Generally, physicians prescribe only inhaled steroid therapy for mild asthma cases regardless of concomitant presence of rhinitis.⁴

Medical history is the simplest way to diagnose rhinitis, but rhinomanometry with specific nasal provocation tests play a special role in the objective diagnosis of this disease. Although the nasal provocation test is mostly a research tool, it has the potential to be an

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objective method for the diagnosis of allergic rhinitis in asthmatic patients. However, nasal provocation test may also affect pulmonary functions and the nasal and pulmonary immunological parameters in patients with asthma.^{5,6}

Our goal was to determine the diagnostic value of specific nasal provocation test in the diagnosis of allergic rhinitis in mild persistent asthmatic patients under low-dose inhaled steroid therapy, and to explore objectively the effects of specific nasal provocation test on pulmonary function and nasal immunologic parameters.

Methods

Our study was performed according to the Helsinki Declaration. The study was performed in the Department of Pulmonary Diseases and ENT of the Cerrahpasa Medical Faculty following the approval of the study protocol by the Ethics Committee (No: 25113). Written informed consents were obtained from all subjects.

Study Population

One hundred and eighteen patients diagnosed as having mild persistent asthma according to GINA Guideline,¹ who were only house dust mite allergy-positive (*Dermatophagoides pteronyssinus*), under low-dose inhaled steroid therapy, being under follow-up in the Asthma Outpatient Clinics and without therapy for rhinitis at least three months were selected. 62 of them accepted to take part in the study and were accordingly randomized. 22 out of the 62 patients were excluded because of sinusitis or disordered anatomy of the nose or non-compliance to inhaled steroid therapy. Also, 8 other patients were lost to follow-up during the trial. The remaining 32 patients (group 1) completed the trial. 10 were male, 22 were female, with a mean age \pm SD of 33 ± 11 years. All the patients in group 1 had persistent mild to moderate rhinitis.²

Seventy eight patients diagnosed with non-allergic mild persistent asthma according to the National Asthma Diagnosis and Treatment Guideline but without a history of rhinitis were selected. All 78 patients were under low-dose inhaled steroid therapy. 24 of 78 patients who accepted to take part in the trial and who had no disordered anatomy of the nose were used as the asthmatic but non-allergic asthmatic control (group 2). 9 of them completed the study. One

was male and 8 were female, with a mean age \pm SD; 42 ± 9 years.

14 relatives of the patients from outpatients clinic, with no asthma or atopy and who have negative skin tests and no rhinitis symptoms, were used as healthy controls (group 3). 9 of them completed the study. 4 were male, 5 were female, with a mean age \pm SD; 34 ± 13 years.

History, physical findings and pulmonary function tests were used in the differential diagnosis of asthma.¹ Nasal history, examination and rhinoscopy, paranasal sinus computerized tomography (PNCT), rhinomanometry with nasal provocation tests were used in the diagnosis of allergic rhinitis.

Inclusion criteria for groups 1 and 2 were as follows: Mild, persistent asthma; use of low-dose inhaled steroids (200 μ g fluticasone or 400 μ g budesonide daily) and also inhaled short acting beta₂ agonist as rescue medication; no use of H₁-receptor blockers or chromones, intranasal steroids or antihistaminics or chromones; no smoking patients; no other additional chronic disease; stability for at least 6 weeks at the beginning of this study; normal range of rhinomanometric measurements.

Study Design

On first day of the trial, all patients completed a record form and had flow-volume loops, prick tests, and nasal lavages to perform the cytologic examination and determine ECP levels. In addition, PNCT were obtained and a simple nasal examination and rhinoscopic examination were performed. The patients under inhaled low-dose steroid and on-demand short acting beta₂-agonist were asked to monitor their PEF values before and at least 2-hours following the inhalation of these drugs. Patients also recorded their daily symptoms and daily rescue medication use: no asthma symptoms and no rescue medication use were scored as 0; mild asthma symptoms 1-2 times with no rescue medication use were scored as 1; asthma symptoms on 1-2 occasions with rescue medication use 1-2 times were scored as 2; 3 or more asthma symptoms with rescue medication use were scored as 3.

The patients were invited again to perform secondary evaluation tests. In this visit, asthma and rhinitis symptoms were questioned, PEF values were obtained, flow-volume loops were drawn and blood samples were taken for the evaluation of basal serum ECP levels. Nasal



provocation tests were performed between 9-11 a.m. . For the following 4 hours, the patients remained at the clinic and their symptoms, PEF values, flow-volume loops and nasal lavages for cytology and ECP studies were evaluated. In addition, each patient recorded his/her PEF values at the 12th and 24th hours with his/her PEFmeter. All patients were asked to record any additional symptoms and to measure their PEF values before using rescue medications. PEF, symptoms, flow-volume loops, nasal lavage and ECP cytology were re-evaluated at the 24th hour.

Study Tests and Measurements

Pulmonary Function Tests

All of the functional measurements were performed according to the methods of the ERS/ATS Task force.⁷ Vmax22 Sensormedics respiratory function apparatus was used in the pulmonary function tests. Ferraris Pocketpeak (Devilbiss, England) was used during PEFmeter monitoring.

Skin Test

All patients had epicutaneous prick allergy test (ALK, Albello, Denmark) in volar surface of the forearm between wrist and antecubital fossa using 31 different allergen extracts.

ECP Measurement

2-5 ml of venous blood and 2-5 ml of nasal lavage fluid samples were obtained for serum and nasal ECP measurements. The ECP concentrations in these sera were measured in 5 days (after storage in a refrigerator) with fluoroimmunoassay using Pharmacia Unicap 100 instrument. The laboratory normal reference range is $0-11.7 \pm 4.3$ $\mu\text{g/l}$.

Nasal provocation test with rhinomanometry

Prior to the application of the nasal provocation test, patients were requested to discontinue their anticholinergic and beta₂agonist medications for at least 8 hours. First rhinomanometry measurements were taken and in patients with no anatomical defects, oxymethasoline that does not cause mediator release was applied to both nostrils in order to prevent mucosal edema.

Nasal provocation was applied with KoKo Rhinomanometry (Sensor Medics, The Netherlands) in coordination with Vmax22.

ALK Albello, Denmark kit used for provocation: Kit contains lyophilised allergen

extract and diluent of *Dermatophagoides pteronyssinus*. Allergen and diluent mixture comes in 100 SQ/ml, 1000 SQ/ml, 10.000 SQ/ml and 100.000 SQ/ml concentrations in four separate vials which can deliver 100 μl dose per every use. Vials were stored in a refrigerator and taken out to room temperature 1 hour before the testing is performed.

Nasal provocation test with rhinomanometry was performed in three steps (basal measurement, control measurement and provocation) as indicated by the International Committee on Objective Assessment of the Nasal Airway.⁸ First basal measurement was done in both nostrils during inspiration under 150 Pa pressure gradient. Physiologic saline in the same amount as the allergen solution was delivered to the inferior turbinate and control measurement is delivered after 15 minutes. If the nasal flow rate did not decrease more than 15%, then physiologic saline was set to be non-irritant to the patient. Then, allergen solution was applied just as described for the saline solution. At least 15-20 minutes later, tests were repeated with increasing allergen concentrations and the results were recorded. If flow rate with 150 Pa pressure gradient decreases more than 40% ($V_{150} \geq 40\%$), the test was accepted as positive. Nasal reactivity was interpreted according to clinical observation and the decrease in flow rate.^{9, 10} The occurrence of at least two of the following symptoms was interpreted as positive clinical observation: Serous nasal discharge, sneezing, nasal obstruction, dyspnea and lacrimation.¹¹⁻¹³

Nasal Lavage and Cytology

Nasal lavages were performed as described by Naclerio *et al.*⁵ Material was centrifuged in 500g for five minutes. Supernatant was separated for biochemical studies and at least 2 smears were prepared with cellular material. One of them was stained with Papanicolaou method to examine the cellular morphologies and especially to differentiate the polymorphonuclear leucocytes and lymphocytes from respiratory cells and inflammatory cells, while the other smear was stained with May-Grunwald-Giemsa (a neutral dye specific for eosinophils and mast cells). Eosinophilia was especially assessed during this trial. During quantitative determinations, 100 and 200 cells in materials with poor and rich cellularity were counted respectively and reported as percentages.



Table 1. Basal symptoms, pulmonary function test results, serum ECP levels, ECP levels and eosinophil counts of nasal lavage fluids of three groups.

	Symptoms	FVC (l)	FEV ₁ (l)	FEV ₁ /FVC (%)	Serum ECP (µg/l)	Nasal lavage ECP (µg/l)	Nasal lavage Eosinophils (%)
Group 1	1±0,4	3,5±0,8	2,8±0,7	78±10	39±41	3.1±13***	3.16±6.8*
Group 2	0,9±0,3	2,8±0,6	2,5±0,5	74±7	30±38	0.01±0.00	0.02±0.00
Group 3	0±0	3,7±1,1	3,1±0,9	81±7	25±29	0.01±0.00	0.01±0.00

<**p* <0.05, ***p* <0.01, ****p* <0.001

Rhinoscopy

Anterior rhinoscopy was first used to examine the inner surfaces of the nose. Following this, a decongestant and a topical anaesthetic were applied. After allowing sufficient time for the anaesthetic to be effective, endoscopy was performed while the patient was lying in supine position. 30 degree and 2.0 mm thick endoscopes were used. 30 degree allows for straight viewing without touching the mucosal surfaces. It also allows for the examination of deeper and angled structures while turning the endoscope. To examine deeper areas, 2.7 mm endoscopes were used.

Statistical Evaluation

In the analysis of the obtained data, EPIINFO software was used. The results were defined as mean ± standard deviation. A *p* <0.05 was considered statistically significant. ANOVA and/or Kruskal-Wallis test was used for the comparisons of quantitative parameters. Wilcoxon and/or chi-square tests were used for the comparisons of qualitative parameters.

For the diagnostic values which sensitivity, specificity, positive and negative predict, total diagnostic values were also calculated. Kabba test was used for the assesment of the diagnostic values.

Results

Basal asthma symptom scores, pulmonary function test results, serum ECP levels, ECP levels and eosinophil counts of nasal lavage fluids of group 1, 2, and 3 patients are summarized in Table 1.

The mean of symptom scores and PEF values recorded during 2-3 days in the three groups were used as basal values. Basal and post-provocation symptom scores were not >2 in any of the patients.

22 of the 32 (68,75%) patients from group 1 were diagnosed with allergic rhinitis through nasal provocation test with positive nasal reactivity. There were no positive provocation test results in the other two groups. We found positive correlation between positive history and nasal provocation test findings (*p* <0.000). We found that nasal provocation test has 100% specificity, 81.3% sensivity and 55% total diagnostic value in the diagnosis of allergic rhinitis in group 1 patients.

In 22 of the 32 patients from group 1, nasal itching and sneezing were recorded immediately post-provocation (0th hour), which gradually subsided with no symptoms at 20 minutes following the provocation. There were no PEF changes during the whole study period. Also, asthma symptoms did not increase and no patient needed rescue medication.

Basal and post-provocation FEV₁, PEF values and the eosinophil and ECP levels in nasal lavage fluids are shown in Figure 1, 2. In all groups, none of these parameters changed over time according to the Wilcoxon test. Eosinophil counts increased in group 1 at the 24th hour, but this increase had no statistical significance.

Discussion

Epidemiologic studies show that >75% of allergic and non-allergic asthmatic subjects have rhinitis.² Togias has reported that 85-95% of allergic asthma patients have symptoms of concomittant rhinitis.⁶ Kilpelainen *et al*¹⁴ has shown that history findings have high sensitivity, specificity and positive predictive values in both asthma and in allergic diseases such as rhinitis and conjunctivitis. We found positive correlation between positive history and nasal provocation test findings (*p* < 0.000). The value and reliability of nasal provocation test has been reported as



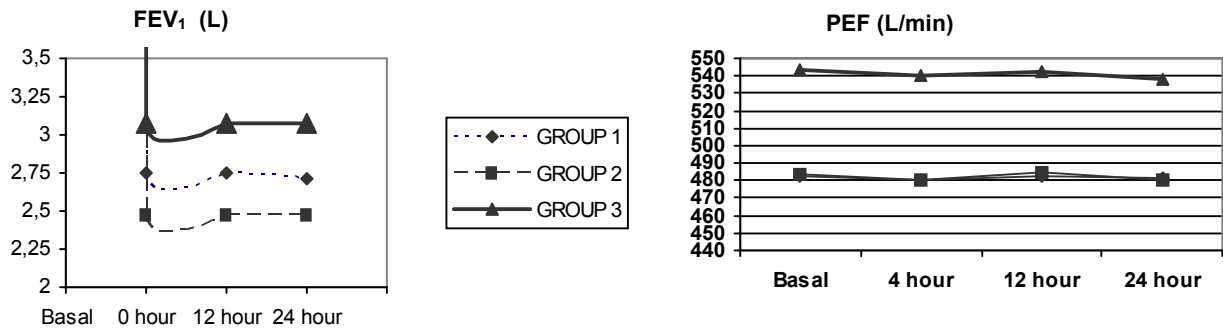


Figure 1. Basal and post-provocation (0th, 12th and 24th hour) FEV₁ values and PEF values in 3 groups of patients.

72-94% in literature.¹⁵⁻¹⁸ We found that nasal provocation test has 100% specificity, 81.3% sensitivity and 55% total diagnostic value in diagnosis. Kurumito *et al* found that the total diagnostic value of nasal provocation test was approximately 87.5 percent and that, especially, the presence of allergy could be demonstrated with these tests.¹⁹ Kus *et al*¹⁰ and Dishoeck *et al*¹⁵ emphasized that nasal provocation test is a valuable, simple and safe method in the diagnosis of atopy. There are some studies in literature indicating that the diagnostic value of nasal provocation test with mite allergens was lower compared to the diagnostic value of nasal provocation test with pollen allergens.^{15,20,21} Although we found the total diagnostic value of nasal provocation test to be high, it was lower compared to some other studies. This can be explained with the protective effects of lower-dose inhaled steroids on the nasal mucosa. We think that history alone might be sufficient

in diagnosing allergic rhinitis in mite-sensitive allergic asthmatics using low-dose inhaled steroids if other diagnoses are eliminated with computerised paranasal tomography and rhinoscopy. Nasal provocation test is an objective diagnostic test in allergic rhinitis without concomitant asthma therapy. However, low-dose inhaled steroids can have a preventive effect on positive nasal provocation test results.

Basal ECP and eosinophil levels of the nasal lavage fluids were higher in group 1 patients than in the patients of the other two groups. There are some reports in the literature supporting this finding.²² In their bronchial and nasal mucosal biopsy studies, Chanez *et al*²³ has shown nasal eosinophilic infiltration, albeit lower in bronchial mucosa. In addition, this findings suggests that inhaled steroid treatment cannot suppress nasal eosinophilia adequately. Indeed, Chanez *et al* found eosinophilia in nasal mucosa of steroidnaive patients but could not find

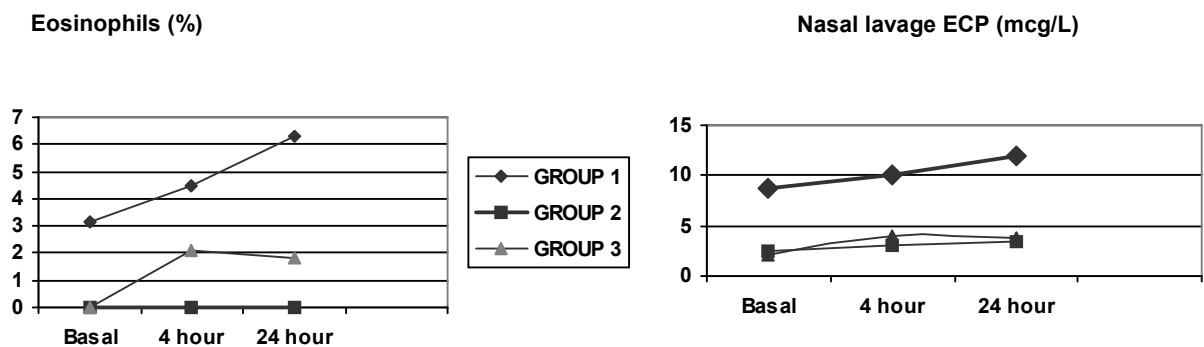


Figure 2. Basal and post-provocation (4th and 24th hour) eosinophil levels and ECP levels in nasal lavage fluid

eosinophils in steroid-dependent, oral steroid users.²³ Kharitonov *et al* also thought that nasal steroids do not suppress the inflammation in both airways and tried to show this through measurements of the amounts of NO in expired air.²⁴ In our study, group 1 consisted of asthmatic patients using inhaled steroids and 87.8% of them also had rhinitis. We found more eosinophils in the nasal mucosa of group 1 patients than in the nasal mucosa of patients from the other groups. This suggests that the importance of eosinophilia in the pathogenesis of allergic rhinitis must be re-evaluated. We think that eosinophilia may be a parameter that must be taken into account during the treatment of rhinitis and allergic asthma. Togias reported in a review on rhinitis and asthma that successful management of the chronic allergic respiratory syndrome requires an integrated view of the airways and an understanding of their interactions.⁶

Specific nasal provocation test was found to be safe in the diagnosis of allergic rhinitis in mild persistent asthmatics using low-dose steroids. We studied in particular the late asthmatic response and found no significant changes in symptom scores, functional parameters and rescue medication (beta₂ agonist) use. In the case of rhinitis, only the expected symptomatic changes were seen immediately after nasal provocation test and these changes were short-lived and did not extend to the late phase. There were no significant changes in findings of nasal lavage (ECP and eosinophil levels) in late stage.

According to literature, late phase reaction is best recorded between the 4th and 12th hours following (post) challenge.²⁵

We monitored the late phase reaction for 24 hours with functional and symptomatic parameters, but no significant finding was noted. Ferreira and Carlos reported symptomatic and functional disorders in 9/20 and 2/20 cases of moderately persistent asthmatics with and without rhinitis, respectively.²⁶ In both studies, subjects were using inhaled steroids. The severity of asthma (mild) and rhinitis (mild-moderate) in our patients might have led to the difference in the results.

In addition, there are studies where symptomatic and functional disorders are not reported. For example, Schumacher *et al* used saline, histamine and pollen provocations in 6 patients with allergic asthma and in 5 controls,

and obtained no significant changes in symptoms and flow-volume loops.²⁷ In the double-blind, placebo controlled study of Corren *et al*, nasal provocation test caused no changes in symptoms, PEF and FEV₁ in patients with allergic asthma and rhinitis. 4 hours following the specific provocation, methacholine response was significantly decreased in the treatment group.²⁸

Reinart *et al* found that grass pollen mono-sensitized subjects have a more severe clinical response to nasal challenge than poly-sensitized subjects.²⁹ As group 1 subjects have allergen sensitivity only to mites, our study population was composed of mono-sensitized subjects.

Kirerleri *et al* also suggests that a nasal provocation test with allergens is unnecessary in children with positive skin prick test and serum IgE specific to house dust mite.³⁰

In our study, late nasal lavages were performed 4 and 24 hours later than provocation and showed no statistically significant increases in ECP or eosinophil levels. But in group 1 patients, eosinophilia had increased at the 24th hour, albeit by a non-significant amount. The lack of statistical significance might be explained by the inhaled steroid usage and suggests that the single treatment (inhaled steroid) suppressed the late phase reaction in the airways.

One of the drawbacks of our study was that the same parameters were not examined in patients that were not under steroid treatment; some of the interpretations might hence be speculative.

In conclusion, specific nasal provocation test is a safe method for mite-sensitive patients with mild asthma and rhinitis under low-dose inhaled steroid therapy, but a history of rhinitis might be sufficient for diagnosis of allergic rhinitis.

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