

Hyposensitization Therapy Reduced Exhaled Nitric Oxide in Asthmatic Children with Corticosteroid Dependency

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*Measurement of exhaled nitric oxide (FeNO) is a simple and noninvasive method for assessment of airway inflammation severity in asthmatic patients. The aim of this study was to investigate whether hyposensitization with extracts of *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) could decrease airway inflammation as determined by FeNO levels. Ten asthmatic children with corticosteroid dependency were recruited for the study. Eight subjects completed a ten months mite hyposensitization regimen. FeNO concentrations, asthma symptom scores and peak expiratory flow rates (PEFR) were measured monthly before and after treatment. FeNO levels from asthmatic patients were found to be higher before treatment (65.34 ± 31.63 ppb). FeNO levels fell significantly after four months' treatment (39.89 ± 21.13 ppb; $p < 0.05$). PEFR increased significantly five months after hyposensitization treatment (214.38 ± 49.38 vs 251.25 ± 28.00 ; $p < 0.05$). Asthma symptom scores decreased 5 months after hyposensitization treatment (6.84 ± 1.22 vs 5.80 ± 0.67 , $p < 0.05$).*

Hyposensitization with extracts of Der p and Der f appears to improve clinical symptoms and reduce airway inflammation as determined by FeNO concentrations in children with mite allergy asthma. (Acta Paediatr Tw 2004; 45:89-93)

Key words: exhaled nitric oxide, asthma, hyposensitization therapy

INTRODUCTION

Hypersensitivity to the house dust mites *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) is common in many countries. Together, Der p and Der f allergens are responsible for about 90% of allergic sensitization in Taiwanese asthmatic children.¹ Hyposensitization therapy with standard extracts of Der p and Der f has been found to be effective for the treatment of asthmatic children.²⁻⁵ Additionally, hyposensitization therapy with Der p antigen has been shown to decrease the late asthmatic reaction (LAR) to bronchial provocation with the antigen in asthmatic children.⁴

Exhaled nitric oxide (FeNO) is a noninvasive marker that can be used to assess airway inflammation.⁶⁻⁸ Nitric oxide (NO) is synthesized from the amino acid, L-arginine, and molecular oxygen through the action of constitutive and inducible NO synthase (NOS) isoforms.⁹ Inducible NOS is expressed in various respiratory

inflammatory cells after exposure to certain cytokines.¹⁰⁻¹² Several studies have demonstrated increased FeNO concentrations in patients with asthma.⁶⁻⁸ This study attempted to determine whether hyposensitization with extracts of Der p and Der f could decrease airway inflammation as determined by FeNO changes and whether changes in FeNO were also correlated with therapeutic effects.

MATERIALS AND METHODS

A total of 10 asthmatic children aged between 6 and 17 years that attended the outpatient department of Tri-Service General Hospital were enrolled in the study after obtaining informed consent from their parents. Asthmatic subjects recruited for the study were those with six or more episodes of asthma in a year and with fluorescent immunoabsorbent assay-proven hypersensitivity to Der p and Der f (Multiple Allergen Simultaneous Test

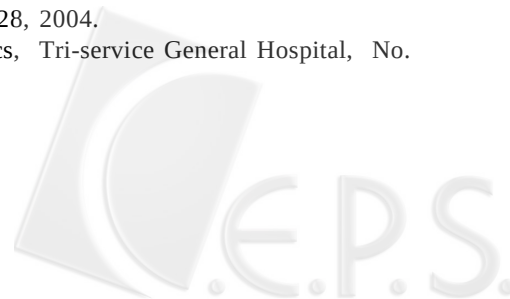
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[MAST], Medical Pharmacy Enterprise, San Jose, CO). Patients with *Der p* and *Der f* hypersensitivity were chosen because *Der p* and *Der f* were responsible for about 90% of asthma hypersensitivity in Taiwan¹ and evidence revealed that FeNO concentrations were elevated in patients with atopic asthma but not in those with nonatopic asthma.¹³

The diagnosis of asthma was based on a clinical history of repeated episodes of wheezing associated with impaired peak expiratory flow rates (PEFR) in addition to detectable IgE specific to *Der p* and *Der f* (MAST).¹⁴ All subjects were receiving high-dose inhaled budesonide more than 800 µg per day. Once they experienced asthmatic attack, they received 5 days' course of oral prednisolone (1 mg/day). Corticosteroid dependency was defined as a requirement for oral prednisolone in doses of > 0.25 mg/kg/d for at least two months prior to the study.¹⁵ The patients' steroid dosage was calculated in milligrams of prednisolone per kilogram per day, averaged over the preceding two months. The average was determined by calculating the patient's cumulative dose of steroids, dividing by 60, and then dividing by the patient's body weight. The study was approved by the institutional review board of the Tri-Service General Hospital.

The study was conducted in an open label fashion. *Der p* and *Der f* antigen injections were administered once weekly over ten months until the maintenance dose was reached. Hyposensitization therapy was started by a protocol consisting of an increase of allergen doses weekly from 10 allergen units (AU) 0.05 ml to 1000 AU 0.5 ml. The standardized *Der p* and *Der f* extracts were purchased from Allermid Laboratories Inc (San Diego, CA). Injections were only given under medical supervision that continued for at least 30 minutes after the injection. FeNO and PEFR values were determined before and 30 minutes after each hyposensitization treatment. The same assessment was performed monthly after the treatment. The parents were instructed to keep a daily diary card of asthma scores during the hyposensitization course. The asthma scores, modified from a previous study,¹⁶ were based on clinical assessment that included coughing, wheezing, chest tightness and difficulty sleeping. In this scoring system, a score of 0 to 3 was assigned to each variable. The final scores ranged from 0 to 12. PEFR values were measured using the peak flow meter supplied by Astech Co. (Port Washington, NY). FeNO was measured using a fast response chemiluminescence analyzer (NOA 280; Seivers Instrument Inc., Boulder, CO.) using the validated single-breath technique previously described.¹⁷ In short, subjects exhaled against a fixed resistance at a constant mouth pressure (15 cm H₂O mmHg) that corresponded to expiratory flow (75 ml/sec), a maneuver

that closed the velum of the posterior nasopharynx and prevented nasal NO contamination. In order to maintain a steady flow, mouth pressure was displayed on a computer screen as a reference for the children.¹⁷ The FeNO values were recorded at the plateau phase of the last part of exhalation. Measurements were only recorded when three values over three measurements showed less than 10% variability. Most patients were able to complete three measures without needing further trials. The final values were then determined by averaging the three values. Ambient NO levels were monitored during the studies to correct for any influence from ambient NO.^{17,18} Patients were required to record PEFR and daily β -agonist consumption on a daily card.

Statistical analysis

Changes in FeNO, asthma scores and PEFR after hyposensitization were analyzed using Wilcoxon signed rank test. Correlations between FeNO changes and PEFR changes were analyzed using Pearson coefficients. Changes in FeNO and PEFR values taken before and 30 minutes after hyposensitization therapy were also analyzed with Wilcoxon signed rank test.

RESULTS

Subjects studied

A total of 10 patients were recruited for the study. Eight subjects (mean age, 11.25 ± 2.96 years) completed the study. Two patients were excluded from the study because of painful injections.

Changes in eNO after hyposensitization therapy

Patients with *Der p* and *Der f* allergic asthma exhibited elevated FeNO (65.34 ± 31.63 ppb) relative to age-matched healthy children (8.51 ± 4.94 ppb). FeNO levels decreased significantly after four months' hyposensitization therapy (39.89 ± 21.13 ppb; $p=0.002$). FeNO levels remained low as the hyposensitization therapy continued for a further 6 months (except 6th month) (Table 1).

Asthma symptom score and PEFR changes after hyposensitization therapy

Patients' PEFR were found to be significantly increased at months 5 (251.25 ± 28.00 ; $p=0.014$), 6 (262.50 ± 49.79 ; $p=0.023$), 7 (267.50 ± 45.28 ; $p=0.004$), 8 (258.75 ± 41.90 ; $p=0.02$), 9 (268.75 ± 38.34 ; $p=0.009$) and 10 (273.13 ± 42.84 ; $p=0.002$) following treatment (Table 1). FeNO (46.03 ± 30.97 vs 48.98 ± 30.70 , $p>0.05$) and PEFR (249.55 ± 47.46 vs 251.25 ± 47.89 , $p>0.05$) were not changed significantly 30 minutes after treatment. Asthma

Table 1. Monthly Changes of FeNO and PEFR After Hyposensitization

month	FeNO (mean $\pm$ sd)	PEFR (mean $\pm$ sd)	Asthma scores (mean $\pm$ sd)
0	65.34 $\pm$ 31.63	214.38 $\pm$ 49.38	6.84 $\pm$ 1.22
1	52.56 $\pm$ 46.44	222.50 $\pm$ 65.41	6.68 $\pm$ 1.11
2	47.50 $\pm$ 40.09	228.75 $\pm$ 56.68	6.65 $\pm$ 1.01
3	53.73 $\pm$ 40.98	232.50 $\pm$ 50.36	6.24 $\pm$ 1.16
4	39.89 $\pm$ 21.13*	247.50 $\pm$ 30.12	6.29 $\pm$ 0.89
5	45.69 $\pm$ 23.81*	251.25 $\pm$ 28.00*	5.80 $\pm$ 0.67*
6	55.66 $\pm$ 24.55	262.50 $\pm$ 49.79*	4.34 $\pm$ 0.85*
7	40.11 $\pm$ 36.35*	267.50 $\pm$ 45.28*	3.93 $\pm$ 0.84*
8	39.26 $\pm$ 28.24*	258.75 $\pm$ 41.90*	3.61 $\pm$ 0.97*
9	32.95 $\pm$ 21.35*	268.75 $\pm$ 38.34*	3.40 $\pm$ 0.73*
10	33.66 $\pm$ 11.51*	273.13 $\pm$ 42.84*	3.03 $\pm$ 0.56*

* $p < 0.05$ in comparison to the initial status.

symptom scores decreased after hyposensitization was completed (6.84 ± 1.22 vs 3.03 ± 0.56 , $p < 0.05$). There was a significant inverse correlation between the changes in FeNO and PEFR ($r = -0.725$, $p = 0.042$) (Fig. 1). In addition to high-dose inhaled budesonide 800 μ g per day, patients' received average oral steroid consumption was oral prednisolone 0.089 mg/kg/d two months after the study was completed. Mean inhaled β -agonist consumption decreased after 10 month hyposensitization therapy (3.56 ± 1.23 puff/day vs 1.45 ± 0.82 puff/day, $P < 0.05$).

DISCUSSION

The efficacy of hyposensitization therapy for patients with asthma has been the subject of some debate.¹⁹ Some studies of *Der p* hyposensitization therapy demonstrated that hyposensitization therapy could reduce the severity of LAR evoked by *Der p* in allergic asthmatic patients.^{4,20,21} LAR is largely due to chronic inflammation and nonspecific bronchial responsiveness.²² Employing noninvasive detection of FeNO levels as an airway inflammation marker, the present study demonstrated that hyposensitization over four months was able to decrease FeNO. Several researchers have demonstrated that increased FeNO concentrations are found in patients with asthma attacks,^{6,7} and FeNO levels have been thought to reflect asthma severity.⁶ Results from the present study suggest that mite hyposensitization therapy over four months can reduce airway inflammation, as demonstrated by reduced levels of FeNO. Van Bever et al.²³ suggested that hyposensitization therapy helps to inhibit the proliferative response, inhibiting not only the degree of the allergen-induced lymphocyte proliferation but also the sensitivity of the proliferating

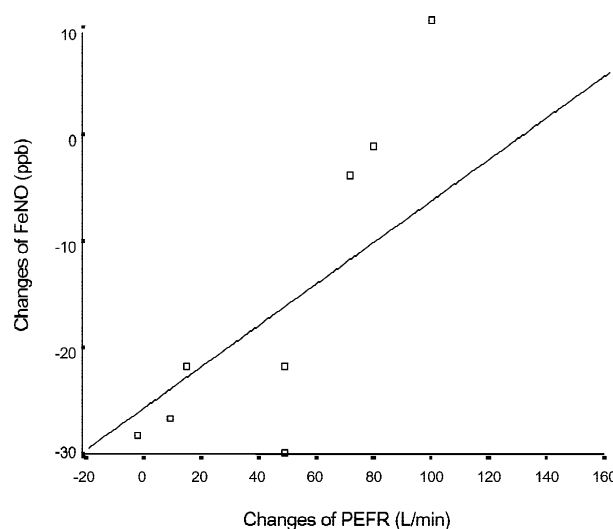


Fig. 1. Correlation between changes of FeNO levels and PEFR after hyposensitization treatment for 5 months ($r = -0.725$, $P = 0.042$).

lymphocytes to interleukin-2. Asthma is an allergen-induced airway inflammation disease that involves T helper 2 (Th2) reactions.^{24,25} Accordingly, the various effects on lymphocytic activation may be responsible for the anti-inflammatory effects of hyposensitization.

House dust mites are the major allergens responsible for asthma throughout the world. Hyposensitization with standardized *Der p* extract has been demonstrated to be effective for the treatment of asthma after short course therapy.² The results of long-term treatment with various extracts suggest that hyposensitization is more effective in children than in adults,⁵ and that the results of treatment are apparent within a few months.⁵ In this study, FeNO was reduced and PEFR improved four to five

months after hyposensitization had begun with standardized *Der p* and *Der f* extracts. This suggests that once allergen-induced airway inflammation has been controlled, pulmonary function as reflected by PEFR can improve.

Various parameters can be used to evaluate the efficacy of hyposensitization for childhood asthma, including bronchial allergen challenge test responses, symptom scores, and medication scores.^{3,26,27} None of these parameters directly reflect airway inflammation, however. On the other hand, FeNO is a sensitive marker for assessing the response to anti-inflammatory treatment in asthmatic children.⁶⁻⁸ Stirling et al.²⁸ proposed that FeNO measurement in combination with symptom scores could be a useful tool for monitoring asthma control in patients with difficult or severe asthma where changes in lung function can have limited sensitivity. In this study, FeNO levels and asthma scores were reduced significantly in asthmatic children after 4-5 months' hyposensitization therapy. PEFR improved significantly after 5-6 months' treatment.

FeNO levels are increased in acute asthma attacks and are reduced by anti-inflammatory treatment, such as steroids and leukotriene receptor antagonists.^{28,29} In this study, mite hyposensitization treatment was also able to reduce FeNO in steroid-dependent asthmatic patients. FeNO levels were elevated in these patient despite steroid use, but decreased nevertheless after mite hyposensitization. However, the effect of corticosteroid on FeNO expression could not be excluded. It is possible that mite hyposensitization treatment may have synergic effect to corticosteroid to reduce FeNO in the treatment of steroid-dependent asthmatic children. It is still hard to reach a conclusion because the case numbers were too small and there was no control group in this study.

The efficacy of hyposensitization for asthma was debated some years ago, but significant progress has been made with the availability of standardized allergen extracts. Hyposensitization represents a viable treatment strategy for allergic asthma, but its use should be restricted to select cases. Further study with greater case numbers is needed to confirm the efficacy of hyposensitization therapy in asthmatic patients with variable asthma severity.

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