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Nasal nitric oxide in the inferior turbinate surface decreases with intranasal steroids in allergic rhinitis: A prospective study

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ABSTRACT

Objective: It remains controversial whether nasal nitric oxide (NO) serves as a reliable parameter to evaluate treatment efficacy in patients with allergic rhinitis (AR). The measurement of local nasal NO levels has been shown to be a sensitive marker for the diagnosis of symptomatic AR patients. Here we assessed the applicability of nasal NO to evaluations of the efficacy of intranasal steroids (INS) in a prospective design.

Methods: We enrolled 25 patients with perennial AR and 10 age-matched healthy participants. The AR patients received fluticasone furoate (FF) once daily for 2 months. Fractional exhaled NO and nasal NO measurements were carried out using an electrochemical analyzer at pretreatment and at 2 weeks and 2 months after treatment. Nasal NO levels were directly measured at two different areas of the nasal cavity: the surface of the inferior turbinate (IT area) and the front of the middle meatus (MM area). Subjective nasal symptoms were also recorded at each visit.

Results: The mean total nasal symptom score in the AR patients decreased significantly after FF treatment ($p < 0.0001$). The mean nasal NO levels in the IT area in the AR patients were significantly higher at pretreatment than those of the healthy participants (109 vs. 62.5 ppb, respectively; $p < 0.001$). After FF administration, the nasal NO levels in the IT area of the AR group showed a significant reduction at both 2 weeks and 2 months (79.1 and 71.9 ppb, respectively; $p < 0.05$ and $p < 0.01$). There was no significant difference in nasal NO levels in the MM area between the controls and the AR group at any visit timepoint. When the ratio of the MM area to the IT area (MM/IT ratio) was plotted for each subject, the untreated AR patients showed a marked decrease in the ratio, whereas after the FF treatment, the AR patients' mean MM/IT ratios showed a significant increase. No significant difference compared to the control group existed at 2 months.

Conclusion: Nasal NO measurement around the inferior turbinate is an objective measure to evaluate allergic conditions and is useful to monitor therapeutic effects of INS.

1. Introduction

Patients with allergic rhinitis (AR) show augmented activity of the nitric oxide (NO) metabolism in the inferior turbinate mucosa, similar to that seen in bronchial asthma [1–4]. In this sense, nasal NO can be used as an objective marker for AR, similar to the situation in lower airways where fractional

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exhaled NO (FeNO) has been used for asthma diagnoses, screening tests, and assessments of steroid treatment efficacy [5,6]. However, the issue of whether nasal NO serves as a reliable index of the clinical efficacy of various therapeutic modalities in AR patients has been controversial. The conflicting results are likely due to the functional complexity of NO as well as to variations in the anatomical structure of the nasal cavity and paranasal sinuses [3,7].

We reported that increased nasal NO levels near the surface of the inferior turbinate can be a sensitive marker for the diagnosis of AR, with the significance being more prominent than FeNO levels [8]. In the present study, we examined the applicability of nasal NO as an objective outcome parameter for clinical intervention trials. For this purpose, we assessed the effect of intranasal steroids (INS) on nasal NO in a group of perennial AR patients in a prospective design.

Treatment with INS is widely recognized to be the first-line anti-inflammatory treatment for AR in current guidelines [9,10]. We also examined the consecutive reproducibility of the nasal NO measurement in normal healthy participants without AR. Our investigation revealed that the untreated AR patients showed higher nasal NO levels in a specific area around the inferior turbinate [8], and these levels showed a significant reduction after INS treatment corresponding to the improvement of subjective symptoms.

2. Methods

2.1. Study design

This was a prospective normal subjects-controlled between-group study conducted at the Department of Otorhinolaryngology—Head and Neck Surgery, Hiroshima University Hospital, Hiroshima, Japan between May 2015 and March 2017. The subjects were 25 patients with perennial AR without bronchial asthma and 10 age-matched healthy participants without nasal symptoms. The exclusion criteria of asthma were based on the absence of a clinical history of episodic symptoms with airflow limitation. The diagnosis of AR was based on the clinical history, the presence of nasal symptoms together with positive nasal eosinophils, and positive allergen-specific IgE antibody or skin tests against house dust mites.

Nasal endoscopy was performed for all subjects before enrollment in order to assess the patency of the middle meatus and to exclude the presence of nasal polyposis or severe septal deviation. We also excluded patients with the presence of airway infection, sinusitis or previous nasal surgery, and those who were treated with allergen-specific immunotherapy. The AR patients did not receive any anti-allergic medication in the 30 days before the study.

The AR patients were treated once daily with 55 μg of fluticasone furoate (FF, Allermist®, GlaxoSmithKline) for 2 months. Use of any other anti-allergic medication was prohibited during the study period. The AR patients' subjective symptoms were recorded at each visit and classified according to the Japanese guideline for allergic rhinitis [10]. The symptoms include paroxysmal sneezing, nose-blowing, and nasal blockage. Each symptom was scored on a scale of 0–4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe), and total nasal symptom scores (TNSS) were calculated to evaluate the severity.

The study protocol was approved by the Institutional Review Board of Hiroshima University (no. 496) and registered at the UMIN Clinical Trials Registry System (ID. UMIN000016536, http://www.umin.ac.jp/ctr/index.htm). Written informed consent was obtained from all participants prior to the study.

2.2. Measurements of NO

Three different parameters of airway NO levels, i.e. oral FeNO, nasal FeNO, and nasal NO were measured in the AR patients at pretreatment and at both 2 weeks and 2 months after treatment. To examine the longitudinal fluctuations in the physiological production of airway NO and to compare those with the changes produced by therapeutic effects, we also measured the NO levels in the healthy participants on the same schedule. They did not receive an intranasal placebo, due to technical difficulties.

The NO levels were measured in the outpatient clinic under constant environmental conditions using a handheld electrochemical analyzer (Nobreath®, Bedfont Scientific, Rochester, UK) according to the ATS/ERS guidelines [6]. For the oral FeNO measurements, the subject exhaled at a flow rate of 50 mL/s through a mouthpiece according to the manufacturer's instructions. For the nasal FeNO measurements, the subject was instructed to exhale transnasally into a nose adaptor (NE-C10-10, OMRON Health Care, Kyoto, Japan) with his or her mouth closed at the same flow rate as described [11].

To examine the local gradients of the NO concentration in different areas in the nasal cavity, we also measured nasal NO levels by directly aspirating air from the nasal cavity. For this purpose, the NO analyzer was connected to a suction catheter and an air-suction pump (MP-Σ300N, Sibata Science, Saitama, Japan) via a silicon tube with a sterile filter as described [8]. The aspiration flow rate was fixed at a constant rate of 50 mL/s. The tip of the catheter was placed inside the subject's nasal cavity under direct vision during the sampling period.

Two different target areas were set based on the anatomical features of the nasal cavity: near the surface of the inferior turbinate (IT area), and the front of the middle meatus (MM area) (Fig. 1). The subject was advised to breathe through the mouth with the soft palate elevated to block the airflow contamination from the lower respiratory tract. Nasal NO levels were measured separately for the right and left sides, leaving the other nostril open, in accord with the ATS/ERS recommendations [12]. Each measurement was performed in triplicate, and the mean value was used for the analyses.

2.3. Data analysis

Power and sample size calculations for the study design were performed based on data from the previous studies using the PS program Ver. 3.0 (http://biostat.mc.vanderbilt.edu/PowerSampleSize). For multiple comparisons, we first carried out a screening of data for differences using an analysis of variance (ANOVA). If the analysis gave a significant result, a

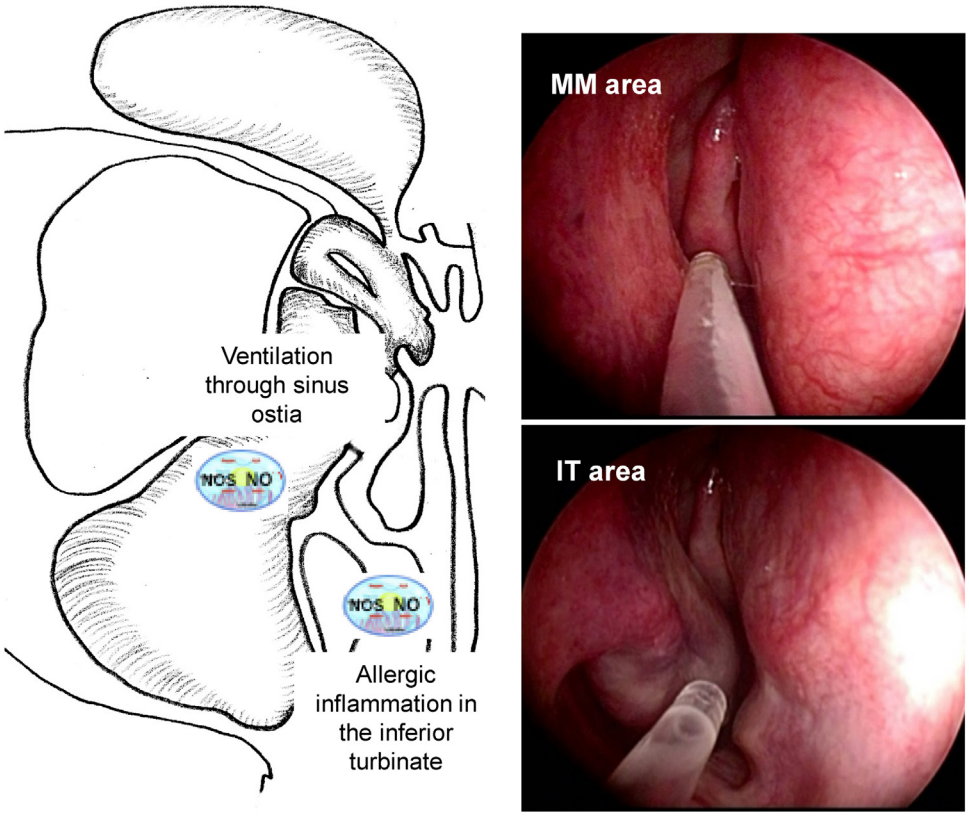

Fig. 1. A schematic diagram and endoscopic views for direct nasal NO measurements. The tip of the catheter was placed in the anterior surface of the inferior turbinate (IT) or in front of the middle meatus (MM) during the aspiration period.

further comparison between each visit was done using Tukey's multiple comparison test. The Mann-Whitney U-test was used for comparisons of two independent samples between the groups. A p-value <0.05 was considered significant.

3. Results

3.1. Nasal symptoms and FeNO levels

The demographics, clinical background and changes of nasal symptoms and FeNO levels of the study population are summarized in Table 1. There was no significant difference between the AR and control groups in the baseline data of age or gender distribution. All subjects were able to complete the study protocol including the three different maneuvers of NO level measurements. None of the subjects had adverse events of any relevance to the study. FeNO levels were measured in the healthy participants at three visits on the same schedule as that of the AR patients. There was no significant difference in either the NO or FeNO level compared with the baseline levels.

The administration of FF for 2 months was associated with an improvement in the clinical symptoms of the AR patients.

Table 1
Demographics, clinical background, and changes in nasal symptoms and FeNO levels of the study population.

		Healthy participants	AR patients with FF
n (Male/female)		10 (8/2)	25 (18/7)
Age		35.9 (11.7)	30.5 (7.8)
Total nasal symptom score (TNSS)	Pre	–	5.24 (1.13)
	2 weeks	–	2.96 (1.41)****
	2 months	–	2.44 (0.84)****
Oral FeNO levels (ppb)	Pre	17.3 (12)	16 (11.3)
	2 weeks	14.7 (7.93)	16.4 (11.1)
	2 months	19.4 (7.26)	19.6 (14.1)
Nasal FeNO levels (ppb)	Pre	32.3 (9.97)	44.9 (13.7)†
	2 weeks	29.2 (7.57)	35.9 (14.4)*
	2 months	29.8 (11.5)	35.1 (9.93)*

Data are shown as mean with standard deviations in parenthesis unless otherwise stated. Pre: pretreatment, FF: fluticasone furoate, TNSS: total nasal symptom score, FeNO: fractional exhaled nitric oxide.

* $p < 0.05$ indicate significant changes after FF treatment compared to pretreatment values.

**** $p < 0.0001$ indicate significant changes after FF treatment compared to pretreatment values.

† $p < 0.05$ indicates difference with the healthy group is significant.

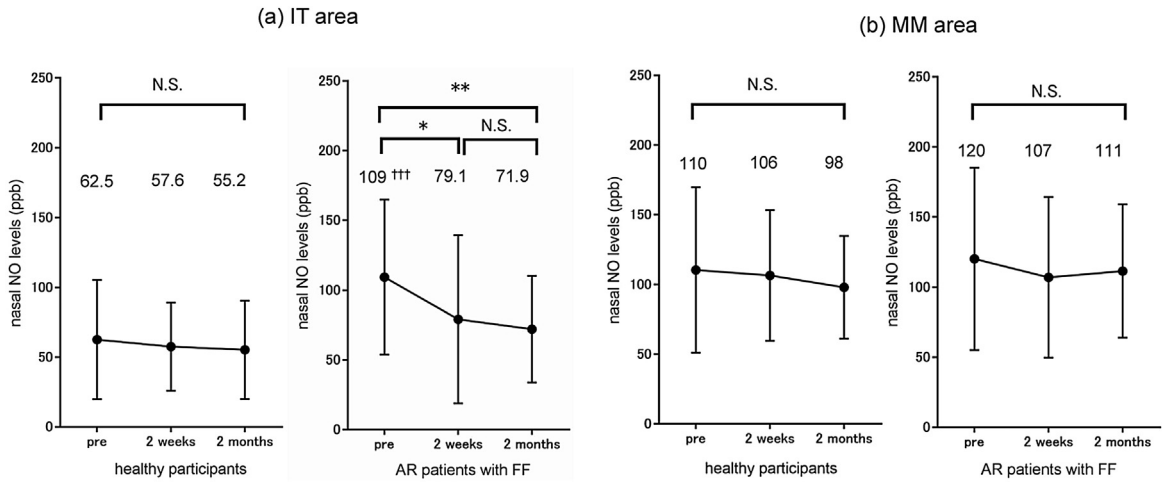

Fig. 2. Time-course changes in the level of nasal NO (a) in the IT area and (b) in the MM area in the healthy participants and the AR patients treated with FF. Error bars: mean values and SD. *$p < 0.05$, **$p < 0.01$ indicate significant changes after FF treatment compared to pretreatment values. $^{\dagger\dagger\dagger}p < 0.001$ vs. the control group. N.S.: no significance.

The TNSS showed a significant decrease at 2 weeks and at 2 months ($p < 0.0001$). No significant difference was found in the baseline data of oral FeNO levels at pretreatment between the control and AR groups. The mean oral FeNO levels in the AR group after treatment did not show a significant difference from the baseline levels. However, the AR patients showed significantly higher nasal FeNO levels at pretreatment compared to the control group. The nasal FeNO levels in the AR group showed a reduction after FF treatment, and the differences at the 2-week and 2-month post-treatment visits were both significant.

3.2. Changes in local gradients of nasal NO levels

The nasal NO levels were directly measured from the IT and the MM areas for all subjects. We found a substantial inter-individual variability in nasal NO levels of both areas in the healthy participants, as was reported in another study [8]. However, there was no significant difference in the baseline nasal NO levels within subjects in this group at the consecutive three visits (Fig. 2).

In the AR patients, the nasal NO levels in the IT area were significantly higher at pretreatment compared to those of the healthy participants ($p < 0.001$). After the FF treatment, the mean nasal NO levels in the IT area showed a marked reduction, and the differences at 2 weeks and 2 months post-treatment were both significant ($p < 0.05$ and $p < 0.01$, respectively) compared to those at pretreatment. No significant difference in nasal NO levels in this area existed between the control and AR groups at these two visits after treatment.

In contrast to the IT area, the MM area showed a different set of NO level gradients. There was no significant difference in nasal NO levels in the MM area between the control and AR groups at any of the visits. The mean nasal NO levels in the MM area of the AR group tended to show a reduction at 2 weeks, but no significant difference was observed among the three visits.

We also plotted the ratio of nasal NO levels of the MM area to the IT area (MM/IT ratio) in the same nasal cavity for each subject (Fig. 3). The mean baseline levels of the ratio in the healthy participants remained unchanged (range 1.99–2.07) during the study period and no significant difference was found among the three visits. In contrast, the AR patients showed a marked decrease in the MM/IT ratio at pretreatment as might primarily reflect higher NO levels in the IT area. The mean ratios were significantly lower than those of the control group at pretreatment and at 2 weeks post-treatment ($p < 0.0001$ and $p < 0.05$, respectively). After FF treatment, the mean MM/IT ratios in the AR group showed a significant increase compared to that at pretreatment. Further, no significant difference existed at 2 months between the AR and control groups.

4. Discussion

Allergic rhinitis has been associated with increased nasal NO levels by an enhanced expression of inducible nitric oxide synthase (iNOS) in the nasal turbinate mucosa [2,3]. However, it has not yet been determined whether nasal NO serves as a valid objective marker for therapeutic efficacy. In the present study, we examined to what extent NO concentrations inside the nose contribute to the pathologies caused by allergic inflammation, and we tried to assess the applicability of nasal NO as a potential objective outcome parameter for clinical intervention trials for AR patients. For this purpose, we sequentially measured the airway NO levels, i.e., oral and nasal FeNO and nasal NO, in different ways.

Our findings demonstrated that the untreated AR patients showed significantly higher nasal NO levels in a specific area around the inferior turbinate. The nasal NO levels in this area showed a marked reduction after 2-month FF treatment in the AR patients, corresponding to the improvement of their subjective symptoms. The discriminative power of nasal NO in the IT area in the present study was higher than that obtained with conventional nasal FeNO measurement techniques.

For nasal NO measurements, the ATS/ERJ guideline recommends aspiration at a constant flow rate from one nostril with gas inflow via the other nostril, whereby nasal air samples can avert the contamination from the lower respiratory tract by the velum closing maneuver [12,13]. We found that the present

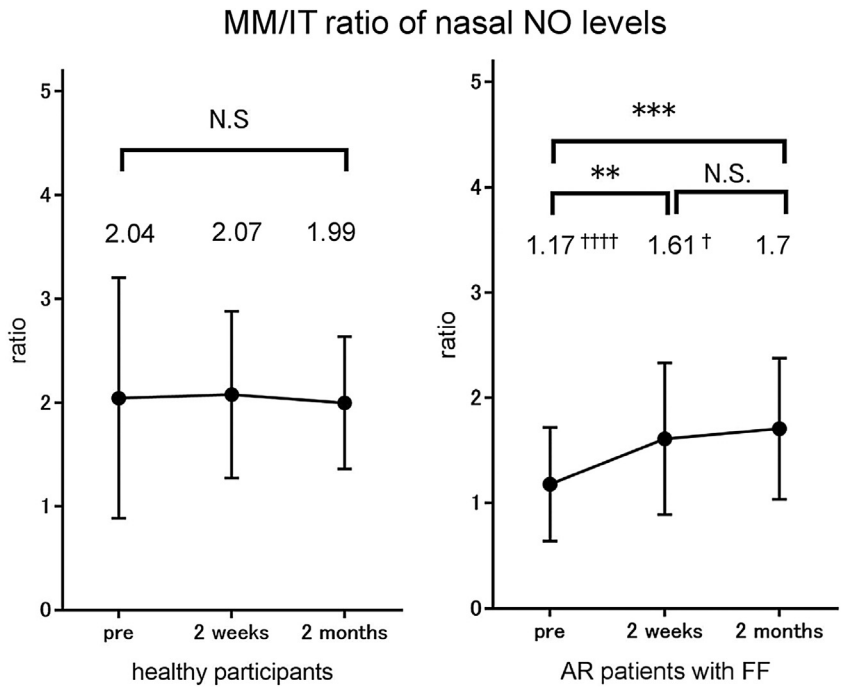

Fig. 3. Time-course changes in the MM/IT ratio in the healthy participants and the AR patients treated with FF. Error bars: mean values and SD. **$p < 0.01$, ***$p < 0.001$ indicate significant changes after FF treatment compared to pretreatment values. †$p < 0.05$, ††††$p < 0.0001$ vs. the control group. N.S.: no significance.

method for nasal NO sampling enabled us to confirm and extend data from our previous study of a different study population [8]. It should be anticipated that a change in nasal NO levels beyond the baseline variation will be necessary to detect a potential drug effect in clinical intervention trials. We found no significant difference within each healthy participant between the consecutive visits, indicating fair reproducibility of the aspiration method. We also consider that a unilateral evaluation of the nasal cavity as a corresponding pair for each side may be favorable to reduce between-subject variability. Good reproducibility of nasal NO levels was also reported in studies using direct sampling methods with different flow rates in patients with perennial AR [14,15].

The significant decrease in the nasal NO levels limited in the IT area is likely to reflect inhibitory effects of INS on the expression of iNOS, leading to diminished local NO production in AR patients. Heterogeneous results regarding whether nasal NO serves as a reliable index of the clinical efficacy of INS treatment have been reported. Kharitonov et al. initially reported that the mean nasal NO levels were approx. 55% lower in patients with AR treated with INS compared to those measured in patients with untreated AR [16]. In addition, children with perennial AR who had higher levels of nasal NO than non-atopic controls showed a significant reduction in nasal NO production after INS therapy [17]. Yamada et al. investigated the efficacy of intranasal mometasone furoate (MF) in perennial AR patients [18]. They reported that 2-week treatment with MF achieved a significant decrease in nasal NO, especially among patients with severe nasal symptoms. In contrast, Wilson et al. found no significant suppression for nasal NO at 2 and 4 weeks in seasonal AR patients who had been treated with INS and antihistamine [19]. They also reported no significant detectable effects of 2-weeks' intranasal MF on nasal NO levels or nasal airways resistance in another crossover study [20]. These observed inconsistent results may reflect different methods and devices for NO measurement and the dynamics of NO distribution in the complex structure of the nasal cavity.

In the present investigation, no significant difference in nasal NO levels was observed in the MM area between the control and AR groups during the study period. Therefore, FF treatment in the AR patients caused considerable shifts of the nasal NO distribution, as indicated by the increase in the MM/IT ratio, similar to that in the healthy participants. This result indicates the paranasal sinuses as another major contributor to NO production in subjects with ostiomeatal complex (OMC) patency [4,7,21,22].

We performed nasal endoscopy in all subjects to exclude OMC obstruction, and we found that 96.7% (58/60) of the nasal cavities tested in the healthy participants showed an MM/IT ratio >1. By emphasizing the MM area in the AR patients, it remains uncertain whether INS treatment may directly affect the NO influx through OMC ventilation [23,24]. In the present study, the nasal NO levels in the MM area showed no significant changes after FF treatment. It remains unclear whether nasal NO production by allergic inflammation affects the NO gradients in the neighboring middle meatus. Further studies including radiological assessments and biological analyses are required to explore these questions.

There are some limitations in this study. An established guideline with normal nasal NO levels has not been available for clinical use. There are confounding factors that may limit the applicability of our findings, including paranasal sinus infection and severe nasal blockage with rhinorrhea. In conclusion, the

nasal NO measurement described here is a reliable parameter to objectively monitor therapeutic effects against AR. Based on the current stream of evidence-based medicine, this sampling and measurement technique is simple and non-invasive, and clinical relevance exists between the biomarker and the pathophysiological events in the disease.

Ethical statement

The study protocol was approved by the Institutional Review Board of Hiroshima University (no. 496), and registered in the UMIN Clinical Trials Registry System (ID. 000016536).

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