Correlation of Nasal Nitric Oxide Measurement with Computed Tomography Findings in Chronic Rhinosinusitis

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Abstract Nitric oxide (NO) is a biological messenger produced by mammalian cells serving various functions including regulation of blood flow, platelet function, immunity, and neurotransmission. The paranasal sinuses and nasal mucosa are a major source of exhaled NO. The aim of the study is to compare the nasal NO (nNO) levels in patients with chronic rhinosinusitis with those of common cold patients and controls and to correlate CT scores with nNO levels. The nasal concentration of NO was measured by electroluminescence in 13 healthy volunteers, in 13 patients suffering from common cold and 13 patients with chronic rhinosinusitis. The concentration of NO was correlated with symptom scores, endoscopic findings and CT findings. The measured levels of NO did not differ between healthy volunteers and common cold patients, but they were significantly lower in patients suffering from chronic rhinosinusitis. As NO is a regulator of mucociliary activity and has bacteriostatic and antiviral effects, the decreased concentration of nNO in patients suffering from sinusitis suggests that lack of NO may contribute to the pathogenesis of this disease. Thus, nNO, which is easily measured, provides a valuable non-invasive objective measure of chronic rhinosinusitis.

Keywords Diagnosis · Nasal polyps · Nitric oxide · Rhinosinusitis

Introduction

Until the 1980s, nitric oxide (NO), a product of fossil fuel combustion, was thought only to play a role in the detrimental effects of air pollution on the respiratory tract [1]. In 1987, experiments with coronary arteries showed that NO was the long sought after endothelium derived relaxing factor (EDRF). After scientists realized that NO played a biological role, its role as a cell signaling molecule and neurotransmitter became clear from abundant studies [2].

NO was first detected in exhaled breath samples in 1991 [3]. In 1992, NO was voted molecule of the year by the scientific journal *Science* [4]. In 1993, researchers from the *Karolinska Institute* in Sweden were the first to report increased eNO in asthmatics [5].

In the airways NO, besides being a mediator of vasodilatation [6], and inflammation [7] also plays an antibacterial role and possibly mediates regulation of blood flow and ciliary beat frequency. Exhaled NO (eNO) has become a standardized, rapid, low cost, objective tool in diagnosis and management of asthma [5]. Measurements of orally fractional ENO (FENO) allows the monitoring of airway inflammation in the lower airways. In patients with lower airway inflammation, particularly of the eosinophilic type, oral FENO is higher than in healthy controls.

Endothelial NO synthase and neuronal NO synthase (nNOS or NOS-1) are constitutive enzymes and act in the locations suggested by their names. These enzymes appear to be calcium dependent (i.e., adequate calcium levels are required for the generation of NO). However, inducible NO synthase (iNOS or NOS-2) is a calcium-independent enzyme. It is present in cells such as macrophages, dendritic cells, and hepatocytes and is activated in response to inflammation. Thus, eNOS, nNOS, and iNOS all refer to an enzymatic system with NO as a common product.

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High levels of NO are produced constitutively in normal individuals within the paranasal sinuses by calcium-independent NO synthase, with levels measured at 20–25 ppb [8].

Additionally, NO is also formed in the nasal mucosa by iNOS in response to inflammation. NO and its metabolites are toxic to micro-organisms and likely form part of the innate defense mechanism of the respiratory tract. NO may also stimulate cilia beat frequency within the epithelium and regulate nasal vascular tone.

In the nasal airways, NO is produced in the paranasal sinuses and, to a lesser extent by the nasal mucosa [9]. In the paranasal sinuses, very high concentrations of NO have been found, ranging between 300 and 23,000 ppb [10, 11].

The biological significance of NO in the nasal region remains unclear. It is believed to take part in the local host defense system keeping the sinuses sterile due to its antiviral and bacteriostatic properties [12]. NO is known to increase the mucociliary beat frequency and hence increase the mucociliary clearance [13, 14]. NO is also a vasodilator and might contribute to mucosal swelling in, for example, rhinitis. NO has also been proposed as an “aerocrine messenger” between the nose and the lungs, in view of its capacity to improve oxygen uptake in the lower airways [15], although subsequent research has shown many other interactions between components of what is now known as the unified airway [16].

NO seems thus to be involved in the inflammatory response, and nasal NO (nNO) might be used for assessing nasal inflammation [17].

Nasal NO as a Diagnostic Tool

Far greater levels of NO are produced in the upper than in the lower respiratory tract, with contributions from the sinuses and to a lesser extent from the nasal mucosa [18]. Measurement of nNO may be a useful tool in the diagnosis and management of patients with chronic rhinosinusitis, nasal polyps, and cystic fibrosis, as well as in the diagnosis of primary ciliary dyskinesia. Measuring both bronchial and nNO may assist the combined management of upper and lower airway.

Aims and Objectives

1. To compare the nNO levels in patients with chronic sinusitis with those of common cold patients and controls.
2. To correlate CT scores with nNO levels.

Materials and Methods

Study Subjects

Three different groups were investigated; (i) for the control group healthy volunteers aged 18 years or older without acute

or chronic nasal disease. The sole inclusion criterion was that the subject should be free of known sinus disease. (ii) Patients seeking treatment for common cold with clinical signs of common cold such as purulent rhinorrhoea, headache and fever without any previous history suggestive of chronic rhinosinusitis. None were on antibiotic treatment, and all symptoms resolved spontaneous within one to 2 weeks. The presented data were in most cases obtained on days 3 or 4 from the onset of the common cold symptoms. (iii) Patients suffering from chronic rhinosinusitis (CRS), with or without nasal polyposis. This group included patients who had persistent symptoms and demonstrated clinical, endoscopic and CT findings of chronic rhinosinusitis. These patients had partial or complete opacification of one or more sinuses on CT. Thirteen patients with chronic rhinosinusitis (CRS) and 13 patients with common cold and 13 healthy controls were enrolled in the study. Exclusion criteria were pregnant females, steroid dependent asthma, acute sinusitis, exacerbations of a common cold within the last 4 weeks, use of oral antibiotics, systemic, inhaled or nasal steroids, or nasal decongestants within 4 weeks prior to the investigation. Informed consent was obtained from all study participants. Local ethical committee approval was obtained. In all 20 male and 19 female patients aged 18 years or older, were screened and their nNO levels measured and CT scan findings scored. Thus the sample size was 39. The nNO measurements were done on the more involved side for the CRS and common cold group. However the nNO measurements were recorded by an assessor who was instructed about the side to be studied but was blinded as to which of the three groups the patient belonged.

Measurements of Nitric Oxide

Study participants had to refrain from eating and drinking at least for 1 h before measurements. The nNO measurements were performed using the NIOX MINO[®] Airway Inflammation Monitor (Aerocrine, Sweden), a hand-held electrochemical NO analyzer, which measures NO by electroluminescence [19]. The nasal air was sampled directly from one nostril [18] using the intrinsic flow of the electroluminescence analyser with a target airflow rate of 0.25 l/min (aspiration/insufflation flows of ~5 ml/s). The probe was connected to a polystyrene nasal olive and gently introduced into the vestibulum of one nostril. To avoid contamination with NO from the lower airway the subject was asked to exhale orally against a resistance in order to close the soft palate [20, 21]. The contralateral nostril was left open. An intranasal NO plateau indicating steady state conditions was then reached after ~20 s.

Scoring of CT-PNS

CT scans of paranasal sinuses were done for each patient and they were assessed and scored in order to define the

surgical anatomy and the extent of the disease [22]. The Lund–Mackay scoring system was used with 0–2 scoring for frontal, maxillary, anterior ethmoid, posterior ethmoid, sphenoid and OMC each. This gives a maximum score of 12 on each side. For the sinusitis group the nNO values were measured for the side with the higher score. The nNO values were correlated with the CT scores for the same side.

Results

In total, 13 healthy volunteers, 13 patients with common cold, and 13 patients with chronic rhinosinusitis were screened for nNO levels. The nNO levels measured in the three groups were tabulated (Table 1).

Statistical Analysis

A statistical analysis of the nNO levels of the three groups was done for comparison using Graph Pad Instat (Table 2). The data are expressed as mean $\pm$ standard error of mean (SEM). Comparisons of data between different groups were made by analysis of variance (ANOVA) and a Tukey–Kramer post hoc multiple comparisons test (Table 3) was used when differences were indicated. Linear regression curves and correlation coefficients were obtained by the least squares method. Values of $P < 0.05$ for comparisons were considered significant.

Results

1. The measured levels of nNO did not differ between healthy volunteers (313.4 ± 106) and common cold

patients (239.8 ± 86), but they were significantly lower in patients suffering from chronic sinusitis (82.6 ± 71.68) as shown in Fig. 1.

2. Baseline absolute NO levels correlated inversely with CT scores $P (<0.0001)$ as shown in Fig. 2. The correlation coefficient was significant -0.8 (Spearman ρ).

Discussion

The results of the present investigation show NO to be easily and reproducibly measured in the nose without any discomfort for the patient with the electroluminescence method. The method is fast, non-invasive and well-suited to investigate children. However the NO analyser has some disadvantages. There is a mandatory external quality control (QC) Procedure for NIOX MINO that includes a Daily QC and a Full QC.

The nNO levels are considerably higher than lower airway levels [23, 24]. In contrast to the lower airways, nNO levels in patients with upper airway inflammation are rather decreased than increased when compared with healthy controls [25–27]. Particularly low nNO levels have been observed in patients with immotile cilia syndrome. [28].

For both upper and lower airway NO measurements, chemiluminescence NO analyzers are the current standard equipment. They are fast responding and highly sensitive, but also rather bulky and expensive. The hand-held NO analyzer used in this study, which works with an NO detecting electrochemical sensor, overcomes these limitations [29]. Comparing this with a standard chemiluminescence device, acceptable accuracy, test–retest reliability, and handling properties have already been demonstrated [30].

In our study the measured nNO levels were significantly lower in the chronic sinusitis group compared to the common cold patients and the control group. Also they were lower in the common cold group than in the control, but this difference was not statistically significant. Lindberg et al. [25] made no distinction between chronic rhino-sinusitis with or without polyps and had nNO significantly lower compared to healthy controls. Whereas in a study by Arnal et al. [31] nNO was not significantly lowered in the chronic sinusitis group, though in the nonallergic polyposis group it was significantly lowered and in both groups there was an inverse correlation of nNO levels with the extent of CT scan alterations. Arnal et al. also reported that in the group with Kartagener's syndrome, the nNO concentration (13.1 ppb) was dramatically decreased compared with all other groups.

Bommarito et al. [32] compared nNO levels and found that levels were only reduced in those with polyposis. Colantonio et al. [33] reported that NO levels were reduced corresponding to the grade of nasal polyposis and that they

Table 1 nNO levels

No.	Control	Common cold	Sinusitis
1	299	321	38
2	100	257	106
3	372	121	20
4	249	249	20
5	432	221	81
6	301	85	90
7	280	128	149
8	206	358	100
9	489	335	275
10	375	217	33
11	268	263	10
12	265	323	47
13	438	240	105
Mean	313.3	239.8	82.6

Table 2 Statistical analysis

nNO	Control	Common cold	Sinusitis
Mean	313.3846153846	239.8461538462	82.6153846154
Standard deviation (SD)	105.97	86.080	71.683
Sample size (<i>N</i>)	13	13	13
SEM	29.391	23.874	19.881

Table 3 Post hoc analysis Turkey–Kramer multiple comparison test

Comparison	Mean difference	q	<i>P</i> value
Control versus common cold	73.538	2.978	>0.05ns
Control versus sinusitis	230.77	9.346	<0.001***
Common cold versus sinusitis	157.23	6.368	<0.001***

If the value of *q* is >3.460 then the *P* value is <0.05. A *P* value of <0.0001 was obtained, which is considered extremely significant

Fig. 1 Comparison of mean **Fig. 2** Correlation of CT scores with nNO levels

rose on therapy. Two studies assessed nNO in the common cold. Lindberg et al. [25] found no significant difference with healthy controls. Ferguson and Eccles [34] included a more comprehensive definition with a number of exclusion criteria and aimed to exclude those with chronic sinusitis;

they found no significant differences when comparing pre- and post infection nNO levels.

We found an inverse correlation between nNO levels and CT scores in our study, and the nNO levels were the lowest in patients with severe sinus pathology as evidenced by complete sinus opacification on CT scans. This inverse correlation which is in accordance with studies by Ragab et al. [27] and Arnal et al. [31] suggests that the nNO levels may be of some value for evaluation of sinus pathology and to screen patients for sinus ostium patency as well as possible defects in mucociliary clearance.

Conclusions

Nasal nitric oxide (nNO), which is easily measured, provides a valuable non-invasive objective measure of chronic rhinosinusitis. It might moreover be used to monitor the course of CRS with polyps, where a decreasing nNO may indicate deterioration and the need for therapeutic intervention. However, in the meanwhile, there is a need for well-conducted prospective trials confirming the preliminary results obtained in this pilot trial.

Summary

The paranasal sinuses seem to be the major source of NO and large amounts of NO are constantly being released in the nasal airways of humans. The novel hand-held NO analyzer NIOX MINO offers a standardized and reliable technique for nNO measurements in the clinical setting and is useful in the diagnosis and treatment monitoring of certain respiratory disorders. Moreover, this simple test may be clinically useful in the diagnosis and treatment monitoring of chronic rhinosinusitis. nNO is a useful measure to alert the clinician to a possible defect in mucociliary clearance (immotile cilia syndrome, cystic fibrosis) and may have some value in the evaluation of the patency of the sinus ostium. We also need to know more about the different factors that affect nNO levels.

Future Directions

Weitzberg and Lundberg [35] found that humming induced a large increase in nNO and that these increases were not detected in patients with nasal polyps and sinus ostium obstruction. Furthermore, they suggest that absence of a normal response to humming during nNO measurement could be used to identify allergic rhinitis with sinus ostium obstruction. Whether this adds significant value to standard testing has yet to be fully appreciated.

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