Nitric oxide metabolites as biomarkers for follow-up after chronic rhinosinusitis surgery

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ABSTRACT

Background: Nitric oxide (NO) has a variety of effects on the pathophysiology of the nasal cavity and seems to play an important role in inflammation. It is increased in the common cold but decreased in acute and chronic rhinosinusitis (CRS). Exhaled NO increases after endoscopic sinus surgery in CRS. In our previous study we showed that NO metabolite (nitrate and nitrite) levels are increased in the sinus cavity of CRS patients. We hypothesized that NO metabolite levels are increased to normal in the nasal lavage of CRS patients after endoscopic sinus surgery and NO metabolites in the nasal lavage can be used as indicators of the disease status after surgery.

Methods: This study was performed on 52 patients with CRS who did not respond to medical therapy and who underwent surgery. NO metabolite levels were measured in nasal lavages of the patients before surgery and 2 months after surgery.

Results: NO metabolite levels (mean ± SEM) were 18.11 ± 3.08 μmol/L and 35.97 ± 4.64 μmol/L in nasal lavages of patients before and after surgery, respectively. The levels of NO metabolites were increased significantly (p < 0.01) after surgery in nasal lavages and patients reported significant improvement based on the visual analog scoring after the operation.

Conclusion: NO metabolite levels were decreased in nasal lavages of CRS patients and were increased to normal levels after surgery along with improvement of the disease. NO metabolite levels may be used as an indicator for the follow-up of patients after endoscopic sinus surgery.

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Key words: Chronic rhinosinusitis, CRS, endoscopic sinus surgery, FESS, nasal lavage, nitric oxide, nitric oxide metabolites, nose, sinus, sinusitis

Chronic rhinosinusitis (CRS) is a rhinosinusitis lasting >12 weeks in adults. There have been many studies regarding the pathophysiology of this disease; however, much is still unknown. Nitric oxide (NO) has been found to play an important role in various physiological and pathophysiological processes in the body including vasoregulation, hemostasis, neurotransmission, immune defense, and respiration and has evoked renewed interest in the pathogenesis of rhinosinusitis. The presence of NO in exhaled breath of humans was first established by Gustafsson et al. Later, it was shown that in physiological states almost the entire NO in the exhaled air originates from the upper airways with only a minor contribution from the lower parts. The main origin of NO measured from the nasal airway has been controversial and there are some indications that favor the mucosa of the paranasal sinuses rather than the mucosa of the nasal cavity and vice versa. NO provides a first-line defense against microorganisms by antiviral and antimicrobial activity and by up-regulation of ciliary motility.

Chronic inflammation may lead to the production of several metabolites, among them, NO, is oxidized to its more stable metabolites: nitrite (NO$_2^-$) and nitrate (NO$_3^-$). Nitrite and nitrate can then turn to cytotoxic agents such as hydroxyl and nitric dioxide. NO may have either pro- or anti-inflammatory and oxidative properties, the predominance of which will depend on such factors as the type and phase of the inflammation, the individual response, and its local concentration. Exhaled nasal NO was shown to be decreased in patients with acute rhinosinusitis and CRS. Interestingly, NO was not decreased in patients with the common cold. It was recently shown that exhaled nasal NO is increased after endoscopic sinus surgery in CRS patients and might be used as an objective evaluation of CRS therapy. The results of using NO as a biomarker for the severity of asthma in asthmatic patients and their response to treatment are promising; however, measuring NO in the gaseous form is expensive and needs an NO analyzer.

NO generation can also be indicated by the formation of more stable end products of NO nitrite and nitrate and we have recently shown that the same is true for NO and its metabolites in the sinus cavity.

This study is the first study investigating the changes of NO metabolites in nasal lavage fluid of CRS patients before and after functional endoscopic sinus surgery (FESS).

MATERIALS AND METHODS

This study was performed on 52 patients between 15 and 64 years of age, having CRS with or without nasal polyposis (NP), who did not respond to medical treatment and presented to Amir Alam Otorhinolaryngology University Hospital to undergo FESS. All patients who took part in this study gave informed consent after the nature of the experimental procedures was explained to them. This study was approved by the local ethics committee.

The patients were divided into two groups: group 1, CRS...
with NP, and group 2, CRS without NP. The medical treatment for patients contained nasal douche and local corticosteroids. Macrolides were administered for group 2 and in cases of mucosal thickening and haziness in the sinus cavity by CT scan, for group 1. Patient selection was based on history, CT scan imaging, and endonasal endoscopy. Cases with history of smoking, allergic rhinitis, asthma and chronic obstructive pulmonary disease, primary ciliary dyskinesia, and any acute inflammation in the sinonasal cavity within 1 month of surgery were excluded from the study. Patients could not have the medical treatment 2 weeks before surgery.

Nasal lavage was performed as described by Naciero et al., on the affected nasal cavity. Before nasal lavage, we ensured that the patient did not recently have intense physical activity. The sample of each subject was immediately collected into a closed, light tight test tube at 0°C and stored at −28°C.

The measurements were done according to the method of Miranda et al. After loading the plate with samples (100 μL) and adding a saturated solution of VCl3 (100 μL) to each well, Griess reagent (50 μL each) was rapidly added to the wells. Sulfanilamide and naphthyl ethylenediamine dihydrochloride were applied for the preparation of Griess reagent. The plates were incubated at 37°C for 30 minutes and absorbance was measured at 540 nm using a standard plate reader. Fresh standard solutions of nitrate were included in each experiment.

The severity of rhinosinusitis was evaluated in each patient by subjective assessment scoring, based on a visual analog scale (VAS) of 0–10 cm before and 2 months after surgery. The endoscopic sinus surgery was performed following the Messerklinger/Stammberger technique under general anesthesia. The surgeries contained anterior ethmoidectomy, sphenoethmoidectomy, and frontoethmoidectomy with or without frontal sinus drainage; however, the extent of the surgery was proportional to the extent of the disease. Nasal lavage was repeated 2 months after surgery. Seven patients could not tolerate the lavage and were replaced.

Data were presented as mean ± SEM and analyzed with SPSS for Windows Version 11.0 statistic software package. Comparisons between the results of the groups were made using paired t-test, simple t-test, and Pearsons’ test. Statistical significance was accepted for p < 0.05.

RESULTS

The NO metabolite levels in the nasal lavage fluids of subjects before and 2 months after surgery are shown in Table 1. There were no significant differences in the values of NO metabolites and VAS between CRS with and without polyposis groups (p > 0.05); therefore, we combine the data into one group and report the results before and after surgery.

NO metabolites increased significantly in patients after surgery (p < 0.01). The values of VAS also changed significantly (p < 0.01) before and after surgery, from 42.04 ± 7.3 cm to 17.56 ± 4.8 cm, respectively. The patients’ conditions improved significantly after surgery. The correlation between NO metabolite levels and the values of VAS was significant (r = −0.780) and there was a decrease in the values of VAS with increasing levels of NO metabolites.

DISCUSSION

The results of our research showed that the level of NO metabolites in the nasal lavage fluid of patients after FESS was significantly higher than that of patients before the operation. It was previously shown that gaseous NO from the nares is decreased in cases of rhinosinusitis and improvement of the disease is associated with an increase in the level of NO. We found no significant difference between the values of NO metabolites in CRS patients with and without NP. This suggests that the blockage of the osteomeatal complex prevents the NO produced in the paranasal sinuses cavities from reaching the nasal cavity. This obstruction seems to play an important role in the decreased levels of NO metabolites in nasal lavage fluid.

The findings of our current study are consistent and comparable with a study performed by Ragab et al. who showed that the levels of gaseous NO increased after CRS improvement and correlated with the radiographic and clinical scores. However, another study by the former group showed decreased exhaled NO in patients with NP that had asthma after medical and surgical treatment. Because the patients in that study had concomitant NP and asthma, the measured exhaled NO levels before treatment were probably under the influence of huge amounts of NO produced in the inflamed lower airways. With concurrent improvement of asthma and polypsis, the level of NO decreased, because of subsidence of inflammation in the lower airways. For this reason, we excluded all patients with asthma, to eliminate possible interference of lower airway NO.

In this study there was a significant correlation between the level of NO metabolites and VAS and the increase in the levels of NO metabolites was associated with patients’ improvement. Theoretically, the level of NO measured in the nasal air depends mainly on two factors: its production in the ciliated cells of the paranasal sinuses and nasal mucosa and the patency and size of the paranasal ostia. NO is produced constantly via inducible nitric oxide synthetase from the normal ciliated epithelium of sinuses. Infection and inflamma-

<table>
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<tr>
<th>Table 1</th>
<th>Nitric oxide (NO) metabolite levels in chronic rhinosinusitis patients with (CRSwNP) and without (CRSsNP) polyposis, before and after surgery</th>
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<tbody>
<tr>
<td></td>
<td>CRSwNP (n = 36)</td>
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<tr>
<td>NO metabolites before surgery (μmol/L)</td>
<td>17.66 ± 2.97</td>
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<tr>
<td>NO metabolites after surgery (μmol/L)</td>
<td>36.66 ± 4.06</td>
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Data are presented as mean ± SEM.
tion of the nose and paranasal sinuses have a definite deleterious effect on the components of the mucociliary apparatus.\textsuperscript{23} The essence of endoscopic sinus surgery is to restore normal drainage, aeration, and mucociliary clearance of the sinuses by a minimally invasive technique that maintains as much normal anatomy as possible;\textsuperscript{26} it successfully restores normal drainage and ventilation of the maxillary sinuses, which encourages regeneration of cilia and allows the outflow of the NO produced in the sinus cavity to the nasal cavity. This explains the possible reason for the increase in the level of NO metabolites found after FESS. Based on the results obtained, it can be concluded that the resolution of inflammation, accomplished by endoscopic sinus surgery,\textsuperscript{27} is associated with an increase in level of NO metabolites. The current study and our previous study that measured NO metabolites in the sinus cavity of CRS patients\textsuperscript{18} showed that they can be measured and follow a similar trend to those of NO in CRS.

Exhaled NO is measured with a chemiluminescence analyzer. Measuring NO with this device is considered to be suitable for experimental purposes, but the major disadvantages are high cost and availability. In our study, we measured nasal NO metabolites in nasal lavage by a simple, noninvasive enzymic method. Although this method is much less expensive and more available than measuring exhaled nasal NO, it can not be performed on children.

Finding higher levels of NO metabolites post-FESS in patients suffering from CRS may have clinical implications. Because NO plays an important role in the airway's defense mechanisms and is a strong stimulator for ciliary beat frequency, it may be reasonable to consider NO and its metabolite biomarkers for the well being of the sinonasal mucosa. NO and its metabolites may be used as indicators for the follow-up and improvement of CRS patients going under medical and surgical therapies. For such a purpose, additional studies on this issue are warranted.

REFERENCES