Measurement of nasal and fractional exhaled nitric oxide in children with upper airway inflammatory disease: Preliminary results

Dabo Liu *,1, Zhenyun Huang 1, Yaping Huang, Xinhua Yi, Xi Chen 1

Department of Otolaryngology, Guangzhou Women and Children’s Medical Centre, The Affiliated Hospital of Guangzhou Medical University, No. 318 Benmin Zhong Road, Guangzhou 510115, Guangdong, China

ARTICLE INFO

Article history:
Received 5 September 2015
Received in revised form 22 October 2015
Accepted 24 October 2015
Available online 31 October 2015

Keywords:
Nasal nitric oxide
Fractional exhaled nitric oxide
Sleep disordered breathing
Allergic rhinitis

ABSTRACT

Objectives: To assess the clinical significance of nasal nitric oxide (nNO) and fractional exhaled nitric oxide (FeNO) concentrations in children with upper airway inflammatory disease.

Methods: Fifteen healthy children, 30 with allergic rhinitis (AR), 10 with non-allergic rhinitis (NAR), and 30 with sleep disordered breathing (SDB) were enrolled. The FeNO and nNO concentrations were measured non-invasively using a NIOX MINO system.

Results: Both nNO and FeNO were significantly higher in children with AR than in healthy children (P = 0.000 and P = 0.000, respectively). Compared to healthy children, nNO was also significantly higher in children with NAR (P = 0.011) or SDB (P = 0.027). In contrast, FeNO did not differ from controls in children with NAR or SDB.

Conclusions: Our data suggest that nNO has potential value for diagnosing upper airway inflammation. Moreover, elevated FeNO distinguishes allergic from non-allergic rhinitis.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The concentration of exhaled nitric oxide (eNO) is correlated with airway inflammation [1]. Orally exhaled gases contain NO derived from both lower and upper airways, while the NO in nasal exhalant (nasal NO, nNO) is produced mainly in the upper respiratory tract, predominantly from the sinuses and to a lesser extent the nasal mucosa [1]. Thus, measurement of eNO and nNO concentrations may provide information on the primary location and extent of airway inflammation to help guide the management of respiratory tract diseases [1]. The 2011 American Thoracic Society (ATS) guidelines [2] provide for the use of fractional exhaled NO (FeNO) in clinical practice. Elevated FeNO is a marker of eosinophilic airway inflammation, and can be used in the diagnosis and treatment evaluation of asthma.

While a long-term (>10 year) international clinical study focused on the association between FeNO and lower airway inflammation [2], there is limited research on FeNO or nNO in upper airway inflammation. The current study was designed to compare FeNO and nNO concentrations between healthy children and children with upper airway inflammatory diseases in order to assess the clinical significance of upper airway NO release.

2. Materials and methods

2.1. Patient recruitment

Seventy-nine consecutive children (60 males and 19 females, 60–168 months of age) treated in our department between January 2014 and September 2014 were initially selected for this study. We excluded patients <60 months of age because they were too young to cooperate during the examination. Nine children were excluded because their parents would not give permission for participation. All 70 remaining patients underwent physical examination, video laryngoscopy (Olympus, Japan), polysomnography (PSG; Compumedics E-Series; USA) with continuous sleep monitoring for >7 h at night, and allergy testing using skin prick tests (SPTs) and/or serum-specific IgE screening. A mite allergen skin prick reagent kit (Recordati S.P.A., Italy) was used for skin prick testing (++ considered positive) and a special serum protein analysis kit (Beckman Coulter, USA) for serum-specific IgE screening (>3 considered positive).

Based on symptoms, physical examination, video laryngoscopy, PSG findings, and skin prick test and/or specific allergen screening (serum-specific IgE) results, the 70 patients were divided into 3 groups (Table 1), 30 with allergic rhinitis (AR), 10 with non-allergic rhinitis (NAR), and 30 with sleep disordered breathing (SDB). In the current study, the diagnostic criteria for AR and NAR met the 2008 updated Allergic Rhinitis and its Impact on Asthma (ARIA)
Table 1
Clinical condition of enrolled subjects.

<table>
<thead>
<tr>
<th></th>
<th>AR: allergic rhinitis</th>
<th>NAR: non-allergic rhinitis</th>
<th>SDB: sleep disordered breathing</th>
<th>Healthy Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Skin prick test</td>
<td>(1) and/or (2) was positive</td>
<td>Neither (1) nor (2) was positive</td>
<td>(e.g., adenoidal hypertrophy)</td>
<td>Neither (1) nor (2) was positive</td>
</tr>
<tr>
<td>(2) Serum specific IgE</td>
<td>(≥3 positive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSG parameters</td>
<td>AHI &lt; 1/h and OAI &lt; 1/h</td>
<td>AHI &lt; 1/h and OAI &lt; 1/h</td>
<td>AHI &lt; 1/h and OAI &lt; 1/h</td>
<td>AHI &lt; 1/h and OAI &lt; 1/h</td>
</tr>
</tbody>
</table>


Guidelines [3]. Non-allergic rhinitis is characterized by nasal symptoms, including anterior or posterior rhinorrhea, sneezing, nasal blockage, and/or itching of the nose, but video laryngoscopy findings have shown that there is no mucopurulent discharge from the middle meatus and/or edema/mucosal obstruction in the middle meatus. The skin prick test and/or specific allergen screening results are also negative in NAR [3]. Children with AR and NAR have normal PSG findings (Apnea-Hypopnea Index (AHI) < 1/h, and Obstructive Apnea Index (OAI) < 1/h). Sleep disordered breathing was diagnosed based on an AHI ≥ 1/h in the absence of nasal inflammatory diseases [4]. Neither the skin prick test nor the serum-specific IgE screening results are positive in children with NAR or SDB.

Fifteen healthy children matched for age and sex ratio (11 boys and 4 girls, 62–163 months of age) were recruited from the children of hospital staff during the same period as a control group. These healthy children had no clinical symptoms of disease, and the physical examination, video laryngoscopy, and skin prick test and/or specific allergen screening results were normal. PSG findings indicated an AHI < 1/h total sleep time (TST) and OAI < 1/h (Table 1). The exclusion criteria included chronic lung disease, central hypventilation syndrome, immune deficiency disease, diabetes, tuberculosis, asthma, systemic metabolic storage diseases, morbid obesity, history of upper and/or lower airway surgery, systemic infection, and use of topical or systemic drugs in the previous 15 days. None of the children smoked tobacco actively or were exposed to secondhand smoke (passive smoking).

The study was approved by the Medical Ethics Committee of Guangzhou Women and Children Medical Centre. All parents or guardians provided informed consent.

2.2. Nitric oxide measurements

The nNO and FeNO concentrations were measured non-invasively using a NIOX MINO system (Aerocrine AB, Solna, Sweden) and are expressed as parts per billion (ppb 1ppb = 10⁻⁹). All measurements were conducted between 2:00 pm and 9:00 pm in a room maintained at 20–30 °C with relative humidity of 20–60%. The environment was clean and measurements were obtained away from windows (sources of dust and pollen) and volatile gases. Interruptions from mobile phones and other strong electromagnetic signals within 2 m were avoided. Subjects were instructed not to drink or eat anything during the 2 h prior to NO testing, and not to drink liquids containing caffeine for 24 h before testing. Subjects were also instructed not to eat foods rich in nitrogen, such as sausage, organ meats, lettuce, and spinach, for 24 h before test. The children abstained from intensive physical activities on the day of the measurements.

2.2.1. Fractional exhaled NO

Exhaled NO was measured using the on-line standardized single-breath technique. In the sitting position, the children were asked to grasp the handle and mouthparts tightly and inhale deeply after a heavy exhalation. Then, the children were instructed to slowly exhale at a constant flow of 50 ml/s for 6 s. The instrument automatically determined FeNO values.

2.2.2. Nasal NO

After resting for approximately 15 min, children were seated with their mouths closed. An olivary probe was placed on the right nostril, and children continued to breathe normally. The instrument continuously pumped nasal gas into the sampling tube at 2 ml/s for 2 min and measured nNO automatically in the total sample.

2.3. Statistics

Statistical analyses were performed using SPSS software (IBM SPSS statistics 20.0). Dataset distributions were assessed by the one-sample Kolmogorov–Smirnov test. Normally distributed data are expressed as the mean ± standard deviation (SD) and non-normally distributed data by the median (25th and 75th percentiles). Continuous variables were compared by the independent samples Student’s t-test or Mann–Whitney test depending on distribution. Categorical variables were compared by the Kruskal–Wallis test. A P < 0.05 was considered statistically significant.

3. Results

3.1. Elevated nNO and/or FeNO levels in children with allergic rhinitis, non-allergic rhinitis, or sleep disordered breathing

Both FeNO and nNO concentrations were significantly higher in children with AR compared to healthy children (Table 2), while the two groups were well matched for age, sex ratio, and body mass index (all P > 0.05). Children with NAR also exhibited a significantly higher nNO concentration than healthy children (Table 3), but unlike AR patients, FeNO concentration did not differ significantly from controls. Again, NAR patients and healthy controls were well matched for age, sex ratio, and body mass index (all P > 0.05). Similar to children with NAR, children with SDB exhibited a higher nNO concentration than controls, while the FeNO concentration did not differ significantly (Table 4). As with the other intergroup comparisons, SDB patients and healthy controls were well matched for age, sex ratio, and body mass index (all P > 0.05). Thus, children with non-allergic upper airway inflammatory diseases exhibited elevated nNO, while children with AR also exhibited elevated FeNO.
Table 2
Comparison of nNO and FeNO concentrations (ppb, median (25th, 75th percentiles)) in children with AR and healthy children.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>nNO (ppb)</th>
<th>FeNO (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>30</td>
<td>187.5 (178.0,201.0)</td>
<td>46.0 (31.0,84.0)</td>
</tr>
<tr>
<td>Healthy children</td>
<td>15</td>
<td>52.0 (22.0,139.0)</td>
<td>12.0 (10.0,16.0)</td>
</tr>
<tr>
<td>Z value</td>
<td></td>
<td>-4.449</td>
<td>-5.427</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.000c</td>
<td>0.000c</td>
</tr>
</tbody>
</table>

* Kolmogorov-Smirnov test
* Mann–Whitney test

Table 3
Comparison of nNO and FeNO concentrations (ppb, median (25th, 75th percentiles)) in children with non-allergic rhinitis and healthy children.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>nNO (ppb)</th>
<th>FeNO (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-allergic rhinitis</td>
<td>10</td>
<td>167.5 (88.0,376.5)</td>
<td>10.5 (68,19.5)</td>
</tr>
<tr>
<td>Healthy children</td>
<td>15</td>
<td>52.0 (22.0,139.0)</td>
<td>12.0 (10.0,16.0)</td>
</tr>
<tr>
<td>Z value</td>
<td></td>
<td>-2.552</td>
<td>-0.946</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.011c</td>
<td>0.344c</td>
</tr>
</tbody>
</table>

* Kolmogorov-Smirnov test
* Mann–Whitney test

Table 4
Comparison of nNO and FeNO concentrations (ppb, median (25th, 75th percentiles)) in children with SDB and healthy children.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>nNO (ppb)</th>
<th>FeNO (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDB</td>
<td>30</td>
<td>111.0 (44.0,349.0)</td>
<td>12.0 (9.8,14.0)</td>
</tr>
<tr>
<td>Healthy children</td>
<td>15</td>
<td>52.0 (22.0,139.0)</td>
<td>12.0 (10.0,16.0)</td>
</tr>
<tr>
<td>Z value</td>
<td></td>
<td>-2.215</td>
<td>-0.411</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.027c</td>
<td>0.681c</td>
</tr>
</tbody>
</table>

* Kolmogorov–Smirnov test.
* Samples Student’s t-test.
* Mann–Whitney test.

4. Discussion

Airway inflammation results from the activation of mast cells and antigen–specific type 2 T-helper cells (Th2 cells), with concomitant release of cytokines including interleukin (IL)-4, IL-5, and IL-13. Release of these factors in turn causes upregulation of epithelial inducible NO synthase (iNOS) expression and higher NO generation [5]. Currently, FeNO and nNO concentrations are used in the diagnosis and treatment evaluation of lower airway inflammatory diseases [5–7] in both adults and children [8,9], but there is limited research on FeNO and nNO concentration changes during upper airway inflammation. Nasal NO concentration has been suggested as a surrogate marker of nasal inflammation and an index to evaluate the efficacy of clinical interventions for upper airway diseases [6]. However, studies comparing nNO between healthy children and those with upper airway inflammatory diseases have not yielded consistent results, possibly due to differences in subject selection criteria, age, and/or the measurement techniques and equipment used [10–13]. In the current study, subjects were recruited according to strict criteria and all groups were well matched for age, sex ratio (mostly male), and body mass index, factors that can also influence eNO. Nasal NO was markedly elevated in children with allergic rhinitis, non-allergic rhinitis, and sleep disordered breathing, while children with allergic rhinitis also exhibited elevated FeNO. Thus, nNO may be useful for the diagnosis and treatment evaluation of upper airway inflammatory disorders. In addition, FeNO may be useful for differential diagnosis of allergic versus non-allergic rhinitis.

Arnal et al. [14] reported that even in asymptomatic adult AR patients, nNO levels were higher than in healthy controls. Similarly, Proud [15] reported that symptoms of acute rhinitis were not apparent until nNO levels had increased. Thus, nNO concentration, which reflects persistent inflammation of the nasal and sinus mucosa, may be a better diagnostic index than symptoms for AR. Alexanderson et al. [16] found that nNO concentrations were similar in allergic rhinitis and perennial rhinitis and both higher than controls. In the current study, we also found that nNO levels were significantly higher in children with rhinitis than in healthy children. Children with AR exhibited higher FeNO concentrations than healthy children despite the absence of inflammatory symptoms involving the lower respiratory tract. This finding suggests that NO release from the lower airway is detectable at levels of inflammation below the threshold for clinical symptoms. Thus, FeNO may be an early sign of more severe lower respiratory tract inflammation and possible progression to a chronic condition. Indeed, asymptomatic elevation of eNO is associated with a higher risk of asthma in both children and infants [15,16]. These results underscore the utility of FeNO measurements for predicting the risk of AR evolving into asthma in children.

Nasal, tonsillar, and oropharyngeal tissues are potential sites of inflammation in patients with obstructive sleep apnea (OSA) [17]. Torretta et al. [8] reported higher nNO values, albeit with large inter-individual variability, in children with adenoid hypertrophy and obstructive symptoms but without allergic symptoms. Previously, we found higher nNO levels in children with SDB and associated tonsillar and/or adenoidal hypertrophy compared to healthy children, consistent with the findings of Torretta et al. [8]. Torretta et al. [8] suggested that the NO pathway may play a role in the pathogenesis of chronic nasopharyngeal inflammation via the stimulation of iNOS by pro-inflammatory mediators and bacterial lipopolysaccharides. We speculate that in children with SDB, mechanical trauma and hypoxemia cause local oropharyngeal inflammation, and repetitive closing and opening during apneic episodes leads to increased production of inflammatory cytokines, resulting in airway iNOS upregulation and greater NO production. A study by Vgontzas et al. [18] corroborates this view.

In contrast to nNO, studies that have evaluated FeNO levels in patients with OSA have yielded inconsistent results [7,19,20]. Culla et al. [7] and Chua et al. [21] reported that FeNO levels in OSA patients were significantly higher than in healthy subjects, while in the current study, FeNO levels were similar to those in healthy children. This discrepancy may stem from the age difference between subjects, as those in the studies by Culla et al. [7] and Chua et al. [21] were adults with longer disease histories and thus longer histories of repetitive airway closing and opening during apneic episodes; such conditions would naturally lead to increased production of inflammatory cytokines, resulting in mucosal damage. Furthermore, long-term hypoxia also leads to lower airway inflammation [7]. On the other hand, the disease histories of the children with SDB in our study are far shorter and the symptoms milder than in adults; hence, the lower airway may not yet show significant inflammation.

This study has several limitations. We did not re-measure FeNO and nNO levels in patients following successful treatment to test for possible normalization. Thus, further study is necessary to assess the clinical significance of FeNO and nNO levels for evaluating treatment of upper airway inflammatory disease. In addition, both the sample of children with NAR and the control sample of healthy children were relatively small, although they were well matched for sex ratio, age range, and levels of obesity.

In short, measurements of FeNO and nNO provide a simple, rapid, objective, reliable, and non-invasive method for monitoring airway inflammation. Moreover, nNO has potential value for diagnosing upper airway inflammation, while elevated FeNO
distinguishes allergic from non-allergic rhinitis. Children with AR who also suffer from a skin disease or urticaria, for example, may have false positive SPT. In addition, children with AR whose parents reject invasive methods (such as SPT and/or S-IgE test) could be diagnosed by FeNO and nNO tests. However, the nNO levels measured were lower than international standard values for diagnosis, so further research is necessary to determine whether FeNO and nNO can be used as screening tools for the differential diagnosis of upper airway diseases.

**Conflict of interest statement**

There is no conflict of interest and source of funding to be declared.

**Acknowledgement**

None.

**References**


