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ORIGINAL ARTICLE

Nasal nitric oxide in sleep-disordered breathing in children

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Abstract
Background Inflammation plays a role in the pathogenesis and consequences of sleep-disordered breathing (SDB). The nasal mucosa and paranasal sinuses produce high levels of nitric oxide (NO). In asthma, exhaled NO is a marker of airway inflammation. There is only limited information whether nasal NO (nNO) accompanies also chronic upper airway obstruction, specifically, SDB. The objective of this study was to investigate nNO levels in children with SDB in comparison to healthy non-snoring children.
Methods Nasal NO was measured in children who underwent overnight polysomnographic studies due to habitual snoring and suspected SDB and in healthy non-snoring controls.
Results One hundred and eleven children participated in the study: 28 with obstructive sleep apnea (OSA), 60 with primary snoring (PS), and 23 controls. Nasal NO levels were significantly higher in children with OSA and PS compared to controls (867.4±371.5, 902.0±330.9, 644.1±166.5 ppb, respectively, p=0.047). No difference was observed between children with OSA and PS. No correlations were found between nNO levels and any of the PSG variables, nor with age, BMI percentile or tonsils size.
Conclusions Compared to healthy controls, nNO is increased in children with SDB, but it is not correlated with disease severity. This is probably due to the local mechanical processes and snoring.

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Keywords Sleep-disordered breathing · Obstructive sleep apnea · Primary snoring · Nasal nitric oxide · Inflammation

Abbreviations

ADD	Attention deficit disorder
AHI	Apnea/hypopnea index
AT	Adenotonsillectomy
BMI	Body mass index
ENO	Exhaled nitric oxide
nNO	Nasal nitric oxide
NO	Nitric oxide
OSA	Obstructive sleep apnea
PPB	Parts per billion
PS	Primary snoring
PSG	Polysomnography
SDB	Sleep-disordered breathing
SpO2	Blood oxygen saturation
TST	Total sleep time

Introduction

Sleep-disordered breathing (SDB) is a spectrum of conditions that includes primary snoring (PS), upper airway resistance syndrome, and obstructive sleep apnea (OSA) [1]. In children, OSA is usually associated with adenotonsillar hypertrophy; hence, the first line of treatment is adenotonsillectomy resulting with a high rate of cure.

The role of inflammation in the pathogenesis and consequences of SDB in both children and adults has been extensively investigated with the majority of studies focusing on the role of systemic inflammation in OSA-related morbidities [2–6].

Recent studies indicate that adults and children with SDB presents with local (i.e., upper airway apparatus) inflammation and systemic correlates [4]. Studies in adults using different methodologies have shown increased upper airway inflammation in OSA [7–12]. In children, markers of local inflammation were also found [13–17]. Several studies have shown that cysteinyl leukotriene receptors are expressed in tonsillar tissue being more abundant in children with OSA and that leukotriene pathways mediate intrinsic proliferative and inflammatory signaling pathways in adenotonsillar tissues from children with OSA [13, 18, 19]. Treatment with a cysteinyl leukotriene receptor antagonist improved breathing during sleep in children with mild OSA [20, 21]. It has been shown that the mechanical forces associated with snoring can lead to upper airway inflammation [3, 22].

Nitric oxide (NO) is produced enzymatically from l-arginine by NO synthase (NOS). The inducible isoform is upregulated during inflammation. Exhaled NO (eNO) is a well-known marker of inflammation of the lower respiratory tract and is elevated in asthma [23]. The main source of nasal NO (nNO) is the mucosa of the paranasal sinuses [24]. Nasal NO concentrations are over 100-fold higher relative to those measured in the lower airways [25].

Disease state affects nNO levels. Data from adults and children shows alterations in nNO levels during allergic diseases [26–30]. Higher levels of eNO and nNO have been found in adult patients with OSA [31]. In this study, patients with upper airway disease and nasal allergies were excluded implying that the increased nNO levels did not result from an allergic state, and that additional mechanisms contributed to the rise in nNO in OSA. A possible explanation is a local inflammatory reaction. Yet, data on nNO, possibly as a marker of local inflammation, in children with SDB is scarce.

The objective of the present study was to investigate the relationship between nNO levels and the severity of SDB in children and to compare it to nNO levels in healthy non-snoring children.

Materials and methods

Study population

Consecutive children referred to full overnight polysomnographic study (PSG) at the Dana Children's Hospital, Tel Aviv Medical Center, due to snoring and suspected OSA, were included. Only cooperative children who were able to perform the single breath nNO measurement, i.e., children aged 6–18 years were recruited. Patients with craniofacial abnormalities, neurological or genetic disorders, infectious disease during recruitment, chronic inflammatory disease, and history of atopy, allergic rhinitis, or any underlying chronic medical condition were excluded. In addition, normal healthy children with no history of snoring, symptoms of OSA, or atopy were recruited from the community and served as normal controls.

The study was approved by the institutional review boards of the Tel Aviv Medical Center and the Ministry of Health (Helsinki Committee). Informed consent was obtained from the parents of all participants.

Study design

Demographics and medical history, including the presence of signs and symptoms of SDB and history of adenotonsillectomy, were collected. Physical examination including tonsil size, graded using the 1 to 4 grade scale [32], was performed by a sleep specialist during the first visit to the sleep clinic prior to PSG. Nasal NO levels were measured at the same visit. A PSG was then performed.

Polysomnographic studies

A standard full overnight multichannel polysomnographic evaluation was performed in the sleep laboratory using the EMBLA N7000 system (MedCare diagnostics, Netherlands). The PSG studies were performed and analyzed according to the accepted guidelines [33].

The obstructive apnea/hypopnea index (AHI) was defined as the number of apnea and hypopnea per hour of total sleep time (TST). OSA was defined as an AHI greater than 1 per hour of TST, while subjects with AHI less than 1 per hour of TST were defined as PS [33–35].

Nasal NO measurements

Nasal levels of NO were measured by the chemiluminescence technique using a NO analyzer (ECO MEDICS AG, Switzerland). Nasal NO levels were expressed as parts per billion (ppb). We used the single breath method against a resistor producing 10 cm H_2O pressure ensuring closure of the velum according to the 2005 ATS/ERS guidelines [36]. The child was assisted by a biofeedback to achieve the correct exhalation flow. During this maneuver, air was aspirated at constant flow rate via an olive placed inside one nostril. Measurements were performed during the morning hours, while the patient was awake and seated. Ambient NO levels during the tests were less than 10 ppb. High nNO levels were defined as levels above the 95th percentile of nNO of the control group.

Data analysis

Nasal NO levels were compared between three groups: children with OSA, PS, and normal controls. A correlation of nNO levels to patient parameters (including tonsil size) and SDB severity was performed. Comparisons

of variables according to group assignment were performed with independent t tests or analysis of variance (ANOVA) followed by post-hoc comparisons, with p-values adjusted for unequal variances when appropriate (Levene's test for equality of variances), or chi-square [χ^2] analyses with Fisher's exact test (dichotomous outcomes).

Correlations were performed using linear regression, followed by calculation of Pearson correlation coefficients. All p values reported are two-tailed with statistical significance set at <0.05. All analyses were conducted using SPSS software (version 21; SPPS Inc., Chicago, IL).

Results

One hundred and eleven children (59.8 % males) participated in the study. Of those, 88 were recruited from the sleep clinic and underwent PSG evaluation, and 23 were normal controls recruited from the community and did not undergo PSG. The mean age of the entire cohort (including controls) was 11.9± 2.9 years (range: 4–17.5), the mean BMI percentile was 60.7± 32.1 (range: 1–99). Of the 88 children who underwent PSG evaluation for suspected OSA, 28 (32 %) were diagnosed with OSA, and 60 (68 %) had PS.

Anthropometric, clinical and polysomnographic indices are presented in Table 1. Compared to healthy controls, both children with OSA and with PS had higher BMI percentiles. Children with OSA had higher percentage of enlarged tonsils compared to PS. No significant differences were observed between the two SDB groups for BMI percentile, history of adenotonsillectomy, presence of atopy, or the occurrence of attention deficit disorder (Table 1.).

Nasal NO levels were significantly different between the groups (Fig. 1). This difference was totally due to the significantly higher levels of nNO in both the OSA and the PS groups versus controls (867.4±371.5 and 902.0±330.9 vs. 644.1±166.5 ppb). No difference was observed between children with OSA and children with PS. The percentage of patients with nNO levels higher than the 95th percentile of the control group (>933 ppb) was 36 % in the OSA group, 48 % in the PS group, and 4 % in the control group. The differences between the two SDB groups compared to controls were statistically significant, but not between the OSA and PS groups (Fig. 2).

Fifteen children (17 % of the study group) had a history of adenotonsillectomy prior to enrollment and PSG: 8 had OSA (28.6 % of the OSA group) and 7 were diagnosed with PS (11.9 %). The differences in nNO levels between the study groups and the control group remained significant also after excluding these children (p=0.01). Nasal NO levels in children with a history of adenotonsillectomy were not different from children who did not underwent surgery (815.1±354.9 vs. 910.2±341.2 ppb, p=0.33).

No significant correlations were found between nNO levels and any of the PSG variables (AHI, SpO2 nadir, mean SpO2, arousal index, sleep efficiency) nor with age or BMI percentile. No correlation was observed between tonsil size and nNO levels. Comparison of nNO levels in children with the largest tonsils (3–4+) to those with no tonsillar enlargement (grade= 0) did not show a difference. Linear regression analysis with nNO as a dependent variable and age, gender, BMI percentile, height, atopy, and group assignment (OSA/PS/controls) as

Table 1 Patients characteristics and PSG results

	OSA	Primary snoring	Controls	p
N	28	60	23	
Age (years)	11.3±2.7	11.7±2.8	12.9±3.2	0.28
Gender (male %)	70	68	52	0.32
BMI percentile	75.0±27.9	60.4±30.0	31.1±25.6	<0.0001
Tonsil size (3–4+) (%)	50	10	–	0.009
Past/previous AT (%)	29	11.9	–	0.054
Atopy (%)	11.0	3.0		0.18
ADD (%)	22.0	22.0		0.11
TST (minutes)	402.8±75.0	406.5±56.5	–	0.82
Sleep efficiency (%)	87.4±9.9	89.6±9.0	–	0.31
AHI	6.6±4.7	0.4±0.38	–	<0.0001
Arousal index	9.9±5.2	8.0±3.7	–	0.053
SpO2 nadir	87.4±4.0	91.3±1.8	–	0.003
Mean SpO2	97.2±0.7	97.8±0.7	–	<0.0001

Data are presented as mean±SD. *AT* adenotonsillectomy, *ADD* attention deficit disorder, *TST* total sleep time, *AHI* apnea/hypopnea index

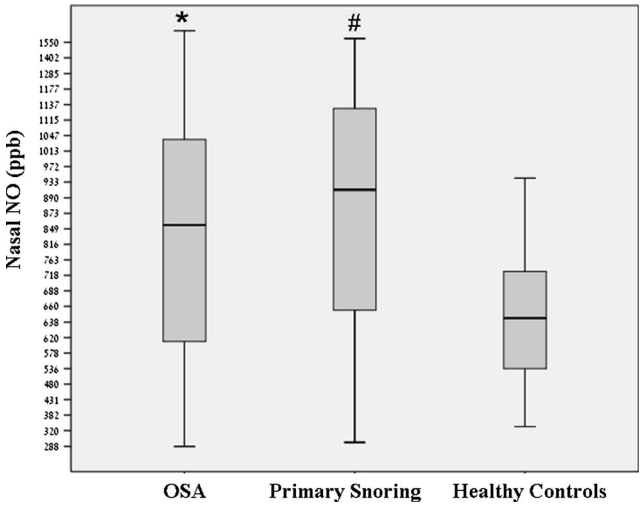

Fig. 1 Box plot of nasal NO levels in children with OSA, primary snoring, and controls. *p=0.047, OSA vs. controls; #p=0.005, PS vs. controls

covariates revealed that group assignment was the only significant variable (p<0.0001).

Discussion

This study showed that nNO levels in children with SDB (OSA and PS) are increased compared to healthy non-snoring controls. These high nNO levels were not correlated with the severity of the disease defined by the PSG results and indeed were similar also in snoring children who did not suffer from sleep apnea. Our results are in agreement with previous studies in adult patients that showed increased nNO levels, eNO, and even oral NO in OSA [31, 37, 38].

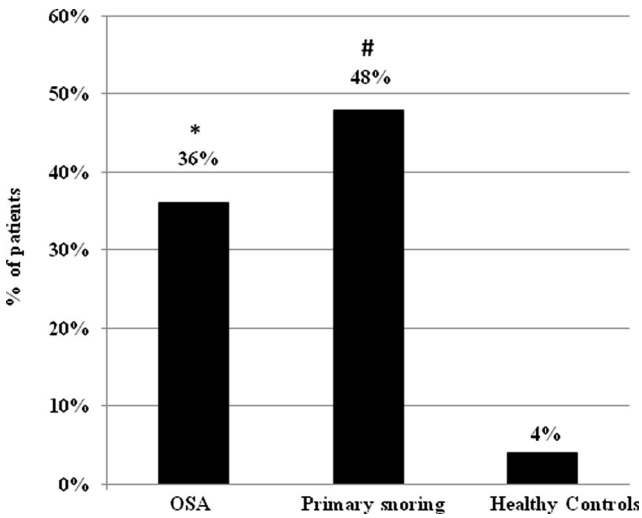

Fig. 2 Percentage of children with high nasal NO levels in the OSA, primary snoring, and control groups. *p=0.007, OSA vs. controls; #p<0.0001, PS vs. controls

NO is a marker of inflammation, especially allergic inflammation, and its production is increased during inflammation. Increased levels suggest an increased inflammatory process [23]. Since nNO levels are hundreds of times higher than lower airway NO levels and result from in situ iNOS activity and NO production, it represents a local process [24]. Hence, increased nNO levels are a marker of increased inflammation in the upper airways. This is supported by the finding that nNO is increased in allergic rhinitis [27, 28] and the findings of other inflammatory markers in the airways of patients with OSA, such as sputum neutrophils, eNO, markers from exhaled breath condensate, exhaled pentane, and many more [7–17]. The mechanism by which iNOS is upregulated and nNO is increased in snoring children is unknown. Local inflammation associated with adenotonsillar hypertrophy is appealing [3, 13, 18–21]. However, our finding of increased nNO also in snoring children post adenotonsillectomy suggests that this is not the only mechanism, focusing the process towards local mechanical influences of snoring. The fact that there was no correlation between any of the PSG variables and nNO levels and that nNO levels are increased also in snoring children who do not have OSA (PS) and in children after adenotonsillectomy supports this notion, i.e., that snoring, most probably by its mechanical effect, regardless of other components of SDB, is responsible for the local inflammation in the upper airways. The lack of correlation between tonsils size and nNO levels in children with either PS or OSA, as found in our study, is in line with this argument. Indeed, although adenotonsillar hypertrophy is the major component leading to OSA and PS in children, the relation of tonsil size to disease severity is controversial. In regards to inflammation, the tonsils and adenoids are, probably, not the only site of the inflammatory process. This may explain why nNO cannot serve as an inflammatory surrogate marker for disease severity. Separating the effect of vibration from other pathologic pathways in SDB may not be feasible in vivo; however, Almendros et al. found in an animal model that snoring-like vibration produced overexpression of tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-2 mRNA in the upper airway of rats [22]. An in vitro study showed that snoring-like vibrations alone were responsible for increased interleukin-8 released from human bronchial epithelial cells [39].

This study suffers from several limitations. These include a small sample size and the fact that we did not evaluate other factors that might influence nNO in SDB such as local eosinophil count. Another limitation is the lack of PSG for the control group. However, since the

inclusion criteria for the control group was the absence of snoring and other symptoms of SDB and since snoring occurs practically in all children with SDB [40], it may be assumed that none of the children in the control group suffered from SDB.

Conclusion

Compared to healthy controls, nasal NO is increased in children with SDB but is not correlated with disease severity. This is probably due to local mechanical processes. Future studies looking into mechanisms of local inflammation and investigating the mechanical effect of snoring are warranted. These studies may also clarify whether local inflammation manifested as increased nNO levels correlate with outcome.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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