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Research Article

Different Levels of Exhaled Nasal Nitric Oxide in Patients Diagnosed with Primary Dyskinesia

Abstract

Background: Primary ciliary dyskinesia (PCD) is a genetic disease characterized by abnormally beating cilia. In these patients levels of nasal nitric oxide (nNO) are lower than those observed in healthy subjects.

Objectives: We recorded the nNO levels in PCD patients in order to use those nNO measurements in the screening and identification of patients with symptoms suggestive of disease PCD disease.

Methods: We measured nasal NO in 36 PCD patients (3 uncooperative younger children and 33 cooperative adult patients) and did a nNO re-evaluation after 12 months in patients with higher levels of nNO.

Results: Twenty-seven PCD patients showed very low nNO levels (29.1 ppb) and nine cooperative patients had high nNO levels (583.3 ppb, $p<0.001$) (T0); the PCD patients with high nNO levels were re-evaluated after 12 months (T1).

The median T0 and T1 nNO values of the seven PCD patients were 360 ppb and 324 ppb ($p=0.0180$), respectively; in 6 patients with high levels of nNO the diagnosis of PCD was confirmed by electron microscopy, and in one subject the diagnosis was confirmed for secondary ciliary dyskinesia.

Conclusions: Low levels of nNO remain indicative of PCD disease; high levels of nNO are supportive of PCD, but cannot be used to exclude diagnosis.

These results suggest that repeated measures are warranted when nNO is occasionally high in patients with symptoms suggestive of PCD disease, and at present electron microscopy is still the only valid evaluation tool in unclear cases of PCD.

Introduction

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder characterized by a motility defect of the cilia [1] that is commonly associated with recurrent or chronic symptoms of the upper and lower respiratory airways [2,3].

Several studies have found low levels of nasal NO (nNO) in patients with PCD as compared to healthy subjects [4-8] and low nNO has also been reported in children and infants with PCD [9-11]. Therefore, an evaluation of nNO has been proposed as a screening and algorithm follow-up tool in patients for whom PCD is clinically suspected [7]. Normal nNO values are available for adults, school-aged children [5], and recently some data have been obtained also for younger children [6].

The aim of this study was to identify the nNO levels in patients diagnosed with PCD and who have Ultra structural defects confirmed by transmission electron microscopy and ciliary motion analysis.

Patients and Methods

Subjects

A total of 36 patients, adults and children, diagnosed with PCD participated in the study (16 males and 20 females, range 2-42 years, mean 15 years). The PCD cases diagnosed by electron microscopy [2,12] served as positive controls. Three subjects were uncooperative children aged between 2 and 3 years, and thirty-three were school-aged children and adults (cooperative patients) able to perform the nNO test procedures according to guidelines [13]. The subjects were selected among patients who had never used inhaled corticosteroids or nasal decongestant drugs and had not had an adeno- or tonsillectomy.

None of the PCD patients had a sputum positive culture for infection of the airways at the time of nNO evaluation. The nNO measurements were part of routine clinical evaluation. The patients' characteristics (airway comorbidity, ultrastructural ciliary defects and bronchiectasis severity score) [14] and lung function in cooperative patients were evaluated.

Nasal NO measurements

Exhaled nasal nitric oxide (nNO) levels were measured by inserting a nasal NO-inert olive in one nostril that completely occluded it to avoid ambient air sampling [15,16]. The controlateral nostril was left open. The olive was connected to the analyzer via a Teflon® tube. The nasal air of the three uncooperative children was sampled continuously with a constant trans-nasal aspiration flow of 300 mL.min-1 [12,13] for 30 sec during tidal breathing [8,12,16]. The nNO was measured "online" with a NIOX chemiluminescence analyzer (Aerocrine, Stockolm, Sweden) which was calibrated at least

every 14 days using certified calibration gas (NO, 1460 ppb). The nNO signal was sent to a computer data acquisition program (NIOX, nasal mode; Aerocrine) that displayed real-time measurements [13].

Cooperative patients were asked to take a deep breath and hold it for 10 sec while the average nNO concentration was calculated at the plateau between 7-10 sec after breath-hold according to ERS and ATS guidelines [13]. Moreover, cooperative children were asked to perform a non-cooperative test, mimicking the condition of infants; for instance, the nasal sampling was performed continuously for 30 sec during tidal breathing [8,16]. For all subjects the maneuver was performed in triplicate and the trans-nasal air flow was recorded and checked at each measurement for each subject. Measurements of ambient NO concentrations were recorded every day and it was always less than 10 ppb in the days of the study [17].

Lung function measurements

Lung function was measured in the cooperative PDC patients by an electronic spirometer (Jaeger, Master Screen IOS, Germany calibrated before the arrival of each subject with a 3 L syringe (Cardinal Health, Germany 234 GmbH). The forced vital capacity (FVC) maneuvers were carried out with the child standing and wearing a nose clip. The best value of three maneuvers was accepted and expressed as a percentage of the predicted normal values, according to ATS Guidelines [18].

Statistical analysis

Data were expressed as median values and interquartile ranges (25th to 75th quantiles). Differences between T0 and T1 nNo values were tested using the Wilcoxon matched-pairs signed-ranks test. A p value below 0.05 was considered significant.

Results

Study population

Nasal NO measurements were recorded for all 36 PCD patients (from January 2008, to December 2010), young children and adults; no adverse events were observed. Two adult patients were not compliant and did not complete the study. An analysis of nNO data showed normal distribution in our study population.

nNO levels

The distribution of nNO values at T0 in the 36 PCD patients is depicted on the left side of Figure 1. The median value was 37.8 ppb and the interquartile range was 17.8 to 233 ppb.

Twenty-seven PCD patients showed very low (below 200 ppb) nNO levels (29.1 ppb, 15.0 to 43.0 ppb) and nine (25.0%) cooperative patients under the same conditions had moderately higher nNO levels (583.3 ppb, 356.7 to 678.7 ppb, p<0.001).

Of the 36 PCD patients, 20 were females (55.6%). The median nNO was 20.7 ppb (13.2 to 29.9 ppb) for this group; it was 64.2 ppb (37.8 to 346.7 ppb) for the male group. The difference between the two groups was statistically significant (p=0.0038). The mean age was 17.0 (s.d. = 11.6) and in T0 the Spearman correlation between age and nNO was r = -0.05 (p=0.7827).

Seven of the PCD patients with high nNO levels (5 females, 71.4%) were re-evaluated after 12 months (T1), under the same conditions. Two patients were not compliant and did not complete nNO re-evaluation. The median T0 and T1 nNO values of the seven PCD patients were 360 ppb (333.3 to 610 ppb) and 324 ppb (200.0 to 350.0 ppb), respectively (Figure 1, right side). The nNO values showed a statistically significant reduction (p=0.0180). No statistically significant differences were found in nNO values between males and females. The T0-T1 decrease in nNO was significantly correlated to age (r=0.82, p=0.0234) - the older patients showed higher reductions of nNO values from T0 to T1.

Mucosa brushing and diagnosis by electron microscopy were repeated in the 7 re-evaluated patients. For 6 of these the diagnosis of PCD was confirmed but one (14.3%) subject was diagnosed with secondary ciliary dyskinesia (SCD) [19,20]. In this patient the nNO levels at first evaluation were 333.3 ppb, and 324.0 ppb at the second nNO concentration test.

No correlations were observed between nNO levels, patient characteristics (airway comorbidity like snoring and rhinitis, and bronchiectasis severity score) and lung function. There was no significant correlation between nNO concentrations and different types of ultrastructural ciliary defects.

Discussion

Primary ciliary dyskinesia (PCD) is a rare (1 in 15,000 live births, in Caucasian populations) and under-diagnosed genetic autosomal recessive disease characterized by abnormally beating cilia [1] that results in impaired mucociliary clearance of the respiratory airways [2]. In half of the patients (1 in 30,000 live births) it is associated with situs viscerum inversus (Kartagener's syndrome) [1].

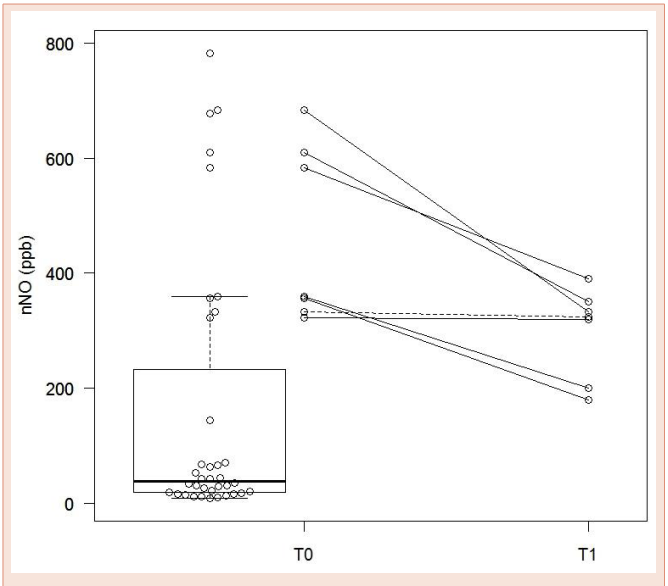

Figure 1: Distribution of nNO levels in the 36 PCD patients at T0 (left); T0 and T1 nNO levels in the 7 patients with high levels of nNO (right). The dashed line represents the nNO values of the patient with secondary ciliary dyskinesia disease.

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The diagnosis of PCD is based on the confirmation of typical Ultra structural defects by transmission electron microscopy and abnormal ciliary motion analysis and is identified in ~90% of patients; it also involves the outer dynein arms, inner dynein arms, or both [2,3]. The majority of these patients have an early onset of symptoms [1,2], and neonatal respiratory distress is common [11,12]; but there are patients with PCD that have minimal or absence of transmission electron microscopy found defects (i.e., atypical PCD or SCD), and a wide spectrum of disease variability [1,19-22]. The impaired mucociliary clearance is commonly associated with recurrent or chronic respiratory tract infections leading to sinusitis, serous otitis media, rhinitis, bronchitis and pneumonia.

Gustafsoon [23] first described nitric oxide in human exhaled air, and later Lundberg et al. [5] demonstrated that most of the exhaled air of healthy subjects originates from the upper respiratory tract, with only a minor contribution from the lower airways. The NO is synthesized by epithelial ciliated cells of the nasal cavity and paranasal sinuses where, because of its high concentration of several hundred parts per million [24], it can exert antibacterial and antiviral effects. This could be one of the main mechanisms contributing to ensure sinus sterility in healthy subjects.

It has been suggested that measurements of nNO levels may serve as a diagnostic tool because patients with PCD have very low levels of exhaled nNO compared to healthy people or those with cystic fibrosis, bronchiectasis, or asthma [3-5,7]. Several studies have shown low levels of nNO in children with PCD compared to healthy age-matched subjects [4-8]. Low levels of nNO have also been reported in infants with PCD [9-11] and in atypical PCD patients with persistent abnormal ciliary motility but who lack the classical ultrastructural defects [20].

Recommendations for standardized procedures for measuring nNO have been published [13] and normal nNO value data are available for adults, school-aged children [5] and also now for younger children [6].

A large study by Struben and coworkers [16] few years ago, has provided normal values for nasal NO in children aged 6 through 17 years. Recently our group has been demonstrated that the mean value for in healthy children nNO was 449 ppb and that it was correlated to the age of the subjects aged less than 12 years and depended on ambient nitric oxide levels [25].

Recent reports reinforce the suggestion that low levels of nNO can be used as a screening tool for the early identification of PCD patients [20,26] and that they are indicative of disease also in uncooperative children when they remain persistently low and when other diseases have been excluded [27]. Our present data confirms that nNO levels in PCD patients are very low, which is in agreement with previous studies [2,4,7].

However, in one of their previous studies Wodehouse et al. [7] observed a 100% sensitive and specific nNO in screening of PCD; in contrast, we observed that 25% of our patients with PCD had moderately high exhaled nNO concentrations (>200ppb). We repeated nNO sampling in these patients after 12 months and we observed a significant reduction of nNO levels ($p = 0.018$), however, the single levels of nNO measured are the limits with possible PCD diagnostic screening values (>150<200ppb) [20].

These observations concord with report by Narang et al. [28] that nasal NO levels <250ppb showed a sensitivity of 97% and a specificity of 90% for the diagnosis of PCD. Our data extend the observations in the study by Karadag et al. [4], where they found low levels of nNO in twenty PCD patients and reported one case of PCD with very high nNO exaled levels. The mechanism by which low nNO occurs in PCD is not clear, but low nNO is present regardless of the type of ciliary ultrastructural defect. Because NO up-regulates ciliary beat frequency, low NO in PCD may be related to the lack of motility of the cilia [29].

In the present study, in accordance with previous observations, we found no correlation between nNO levels and Ultra structural ciliary defects [27,29].

In a recent study of ours we confirmed that NO gas is implicated in the modulation of ciliary function in PCD patients [26]. This study could reflect the correlation between NO and ciliary function, which may explain some of the nNO levels and clinical variability of the disease.

The recent task force on PCD of the European Respiratory Society showed that 77% of centers have access to analyze by electron microscopy, 57% to ciliary function tests and only 36% of centers use the nasal nitric oxide test for PCD screening [30]. This task force found substantial heterogeneity in management of PCD within and between countries. This demonstrates how important it is to standardize PCD management besides the urgent need for research to simplify PCD diagnosis.

Our results suggest that in patients with symptoms suggestive of PCD low levels of nNO remain indicative of the disease; therefore high levels of nNO cannot be used to exclude diagnosis [31]. At present electron microscopy is still the only tool to use in unclear cases of disease.

The limitation of this study was the sample size of PCD patients, however the ciliary dyskinesia is rare disease.

In conclusion, further studies are necessary on large cohorts of adult and children patients in order to establish the specificity and sensitivity of nNO levels for application in the PCD diagnosis.

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