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Original Article

Decreased Levels of Nasal Nitric Oxide in Children With Midline Neuroanatomical Anomalies: A Possible Connection Between Ciliary Dysfunction and Isolated Nervous System Defects

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ABSTRACT

OBJECTIVES: Given the involvement of cilia in midline neurodevelopment, we set to determine whether children with midline neuroanatomical defects have increased prevalence of ciliary dysfunction, using nasal nitric oxide measurement, a screening test for primary ciliary dyskinesia. **STUDY DESIGN:** We measured the nasal nitric oxide levels of 26 children ages 6-17, with congenital midline central nervous system defects, who are otherwise healthy. We evaluated the effect of variables including: age, gender, and anomaly (brain, spinal cord, or combined) on our measurements. We compared our results with the previously established normal range (153.6-509.9 nL/min) and to the cutoff for children with primary ciliary dyskinesia (77 nL/min). **RESULTS:** The overall range for nasal nitric oxide in our cohort was 56.5-334.7 nL/min, with age, gender, and anomaly not having a significant effect. The overall mean, 217.7 nL/min, was significantly lower than the preestablished mean in normal children, 314.51 nL/min ($P < 0.01$). Four patients (15.4%) had nitric oxide levels below the lower end of normal, with two (7.7%) having values below the cutoff for primary ciliary dyskinesia. **CONCLUSIONS:** This is the first study to report a possible association between ciliary dysfunction and isolated congenital midline neuroanatomical defects, not in the context of any known syndrome. We suggest that genes known to cause isolated central nervous system defects may also be involved in the function of cilia. Longitudinal studies are required to investigate whether, in children with abnormal measurements, nasal nitric oxide levels normalize over time, and whether these children suffer from any respiratory sequelae.

Keywords: nasal nitric oxide, cilia, midline, neuroanatomical defects, primary ciliary dyskinesia

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Introduction

Cilia are microtubule-based structures projecting from the apical surfaces of most eukaryotic cell types and are divided into two groups: motile, and primary, or immotile cilia.[1,2] Although the former have long been known for their role in mucous clearance and sperm motility,[1] the function of the latter has been slower to emerge. For decades, primary cilia were considered a mere sensory organelle, a notion that has recently changed dramatically as studies

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began revealing their crucial role in cell division, axis determination, and migration.[2-4]

The role of cilia in human pathology has received wide recognition over the past decade, as mutations in their structure and function were found to give rise to a broad, yet overlapping, spectrum of disorders.[4] These disorders, collectively referred to as "ciliopathies," are a heterogeneous class characterized by a wide array of developmental and degenerative phenotypes affecting multiple systems.[1,2,5] Notable examples include Meckel-Gruber syndrome, Bardet-Biedl syndrome, Joubert syndrome, polycystic kidney disease, and primary ciliary dyskinesia (PCD).[5] Nervous system defects are strongly associated with a number of ciliopathies, with common findings including midline defects such as hydrocephalus secondary to aqueductal stenosis, neural tube defects, and a plethora of cortical and cerebellar abnormalities.[6-8]

Primary ciliary dyskinesia, previously known as Kartagener syndrome, was the first disorder linked to ciliary dysfunction.[9,10] Its manifestations may include chronic sinusitis/bronchitis, recurrent otitis media, situs inversus, infertility, and, in some cases, hydrocephalus.[9] One hallmark of PCD is markedly low levels of nasal nitric oxide (nNO) when compared with a normal population.[11,12] Nasal nitric oxide is the measurement of nitric oxide in the air exhaled from the nares. In healthy individuals, nNO is produced by the paranasal sinuses via the action of the enzyme nitric oxide synthase (NOS).[13] In the respiratory epithelium, the action of NOS is coupled with ciliary movement. It is suspected that low nNO in patients with PCD is secondary to impaired NOS activity, which correlates with decreased ciliary beat frequency.[13,14] Testing of nNO is highly sensitive and has been recommended as a quick and noninvasive screening tool to assess ciliary dysfunction not only in PCD,[11,12] but also in congenital heart diseases,[15] as well as in nonmotile ciliopathies such as inherited retinopathies[16] and autosomal recessive polycystic kidney disease.[17]

To date, neuroanatomical defects have been linked to ciliary dysfunction only in the context of multisystem syndromes. However, there is lack of evidence relating isolated central nervous system defects to ciliary malfunction. We hypothesized that should ciliary defects be present in children with such isolated defects, then given the ubiquity of cilia, these children may also have reduced levels of nNO. In this pilot study, we measured the nNO levels of children with congenital brain and/or spinal cord midline anomalies and compared them with the previously established values for normal population as well as to the published cutoff for patients with PCD.

Patient and Methods

Patients

Nasal nitric oxide levels were measured in children ages 6-17 years of age who were previously diagnosed with a congenital midline central nervous system anomaly. Patients were recruited from the pediatric rehabilitation, neurology, and neurosurgery outpatient clinics at the University of Alberta. All patients who met the inclusion criteria were approached by a coordinating nurse, with the intention of forming a convenience sample of 25-30 children. Only patients and families who expressed interest in participating were further contacted by the study team. Patients were excluded if they had a preexisting diagnosis of any condition that could result in a decreased nNO level. Those included children with a known ciliopathy, sinopulmonary disease, cystic fibrosis, allergic rhinitis, or any cardiac anomaly. All children with a respiratory tract infection or any other concurrent infection were also excluded for the same reason. Finally, to ensure we enrolled only those with isolated central nervous system defects, all children with any known genetic syndromes were excluded. At the time of assessment, the age, gender, and diagnosis of all patients were recorded.

Nasal nitric oxide measurement

Nasal nitric oxide assessment was carried out through placement of an inert nitric oxide sampling line with a disposable foam olive (DirectMed Inc, Glen Cove, NY, USA) into one nostril while the contralateral nostril was left open. Air was then sampled from the nostril at a constant rate of 0.33 L/min by a chemiluminescent analyzer (CLD 88 SP, ECO PHYSICS AG, Duerten, Switzerland), which was calibrated according to the manufacturer's specifications. All nNO measurements were performed with the subjects comfortably seated, following a 5-minute acclimatization period. Subjects were asked to take a deep breath and hold it for at least 10 seconds. Three nNO measurements were performed for each participant and their mean value was calculated. All maneuvers were performed according to American Thoracic Society, European Respiratory Society guidelines.[12] Measurements were reported by the analyzer as parts per billion, and converted to nanoliters per minute by multiplying the results (parts per billion) by the flow rate.

Reference values

We compared the mean measured nNO level of our patients with the normal reference range for children ages 6-17 years, as published by Struben et al., who used the same methods as ours.[18] To ease the comparison, given that Struben et al. used a device with a different flow rate than ours, their results (in parts per billion) were converted to nanoliters per minute, using the calculation as described previously. Struben et al. also suggested prediction equations for nNO, taking into account age, ambient nitric oxide, and history of adenoidectomy.[18] Therefore, we compared the results of actual nNO measurements in our study with the predicted values based on the equations suggested by Struben et al. The proposed equation for children younger than age 12 years predicts that:

$$\text{nNO (ppb)} = 314.6 + 11.5*\text{age} - 57.5*\text{history of adenoidectomy} + 0.5*\text{ambient NO}$$

The equation for children older than age 12 years, as suggested by Struben et al., predicts:

$$\text{nNO (ppb)} = 452.6 - 2.9*(\text{age} - 12) - 16.0*\text{history of adenoidectomy} + 0.5*\text{ambient NO}$$

Based on the flow rate used by Struben et al. to generate these equations, we converted them into nanoliters per minute to maintain consistency in our reported results. Our mean measurements were further compared with the nNO cutoff for children with PCD, as established by Leigh et al.[19]

Statistical analysis

Data were analyzed using PASW Statistics Version 19 (SPSS Inc, 2010). To assess the internal consistency of the three nNO measurements for each participant, we conducted the Cronbach's alpha test. For the purpose of statistical analysis, variables assessed included: (1) age ($12 \geq$ or < 12 years); (2) gender (male or female); and (3) anatomical location of defect (spinal cord, brain, or combined brain and spinal cord). The age cutoff was determined based on the findings of Struben et al., suggesting that correlation between age and nNO levels varies between these two age groups.[18]

To determine whether the anatomical location of the defect had a significant influence on mean nNO levels, we conducted a one-way analysis of variance, using the three levels of anomaly factors specified