Significance of fractional exhaled nitric oxide measurements in detecting primary ciliary dyskinesia in Saudi children

Muslim M. Al Saadi, MD, FCCP, Syed S. Habib, MD, FCPS, Badar A. Al Muqhem, MD, Abdulamajeed AlDrees, MD, PhD, Jawad F. Al Zamil, MD, Hammad A. AlSadoon, MD, ABP.

ABSTRACT

الأهداف: اختبار مدى فائدة عمل قياسات جزء أوكسيد النتريك المزفور في التنبؤ بوجود خلل في الحركة الهدبية الأولية للجهاز التنفسي عند الأطفال.

الطريقة: أجريت هذه الدراسة الوصفية في قسم طب أمراض الأطفال، وقسم علم وظائف الأعضاء بكلية الطب ومستشفى الملك خالد الجامعي التابعين لجامعة الملك سعود، الرياض، المملكة العربية السعودية، خلال الفترة من يناير 2011م حتى ديسمبر 2011م. شملت عينة الدراسة 22 طفلاً يعانون من أعراض توحي بأنهم يشتكون من وجود خلل في الحركة الهدبية الابتدائية للجهاز التنفسي وتأكد التشخيص بعد أخذ خزعة من الأهداب التنفسية. وقد تم مراعاة المبادئ المرشدة المعمول بها من قبل الجمعية الأمريكية لأمراض الصدر لدى تنفيذ قياسات جزء أوكسيد النتريك المزفور في 22 حالة ثبت عن طريق أخذ عينات من الأهداب وجود خلل في الحركة الهدبية الأولية للجهاز التنفسي عندها، كما تمت مراعاة هذه المبادئ لدى تنفيذ قياسات جزء أوكسيد النتريك المزفور في 11 طفلاً حالتهم الصحية سليمة.

النتائج: لم تظهر اختلافاتٌ تذكر على أساس العمر، أو اختبارات شهيق، وزفير الهواء بين مجموعة الأطفال الذين يعانون من خلل في الحركة الهدبية الأولية للجهاز التنفسي وبين مجموعة الأطفال الأصحاء. لكن اختلافات مهمة ظهرت على أساس قياس جزء أوكسيد النتريك المزفور إذ كان أقل عند الأطفال الذين يعانون من خلل في الحركة الهدبية الإبتدائية للجهاز التنفسي حيث سجل (6.19±1.43) بينما سجل في مجموعة المقارنة (17.00±6.30)، و(CI: -14.854 to -5.927؛ ومتوسط احتمال الإصابة أقل من $p<0.0001$. لوحظ وجود سيلان الأنف عند 7 أطفال (31.8%)، والتهاب الأذن الوسطى الحاد المتكرر عند 16 (72.7%)، والتهاب الأذن الوسطى المزمن عند 5 (22.7%) والتهاب الجيوب الأنفية المتكرر عند 5 (22.7%)، والسعال المزمن المصحوب بالقشع عند 8 (36.4%)، وتشنج القصبات الهوائية عند 11(50%)، ووجود القلب في الناحية اليمنى للصدر عند 3 (13.6%). لاتوجد علاقة تربط بين العمر في أي سنة والمؤشرات الدالة على أوكسيد النتريك المزفور ووظيفة التنفس.

خاتمة: يبدو قياسُ جزء أوكسيد النتريك المزفور مع هواء الزفير أداة مفيدة في إخضاع جميع الأطفال للفحص وفرز من منهم مصاب أو يمكن أن يصاب بمرض خلل الحركة الهدبية الأولية في الجهاز التنفسي، ويمكن اعتبار القياس جزء متمماً لفحوصات أخرى مثل أخذ خزعة من الأنف وعمل دراسة بواسطة الفحص المجهري الإلكتروني.

Objectives: To examine the usefulness of fractional exhaled nitric oxide (FENO) measurements in detecting primary ciliary dyskinesia (PCD) in children.

Methods: This observational study was conducted at the Department of Pediatrics and Physiology, College of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia from January 2011 to December 2011. The study population consisted of 22 children with symptoms suggestive of PCD and the diagnosis was confirmed by ciliary biopsy. Using the American Thoracic Society guidelines, measurements of FENO were performed in 22 subjects with proven PCD biopsies and in 11 healthy age-matched subjects.

Results: No significant differences were found on the basis of age or ventilatory function tests between the PCD patients and control groups. Fractional exhaled nitric oxide values were significantly lower in children with PCD (6.19±1.43) compared to control group (17.00±6.30) (CI: -14.854 to -5.927, $p<0.0001$). Rhinorrhea was seen in 7 (31.8%), recurrent acute otitis media in 16 (72.7%), chronic otitis media in 5 (22.7%), recurrent sinusitis in 5 (22.7%), chronic productive cough in 8 (36.4%), bronchospasm in 11 (50%), and dextrocardia in 3 (13.6%) subjects. There was no correlation between age, FENO, and ventilatory function parameters.

Conclusion: The measurement of FENO appears to be a useful tool for screening children for PCD. It can complement other tests such as nasal biopsy and electron microscopy studies.

Saudi Med J 2013; Vol. 34 (1): 24-28

From the Pulmonary Division, Department of Pediatrics (Al Saadi, Al Muqhem, Al Zamil), Department of Physiology (Habib, AlDrees), College of Medicine and King Khalid University Hospital, King Saud University, and the King Abdulaziz Medical City (AlSadoon), National Guard Health Affairs, Riyadh, Kingdom of Saudi Arabia.

Received 26th June 2012. Accepted 11th November 2012.

Address correspondence and reprint request to: Dr. Muslim M. Al Saadi, Department of Pediatrics (39), College of Medicine & King Khalid University Hospital, King Saud University, PO Box 2925, Riyadh 11461, Kingdom of Saudi Arabia. Tel. +966 (1) 4670878. Fax. +966 (1) 4672650. E-mail: alsaadi@ksu.edu.sa

Primary ciliary dyskinesia (PCD) is a genetic autosomal recessive disorder, characterized by dysmotile cilia and sperms. Clinically patients present with respiratory distress and infections from early life, with sinusitis and bronchiectasis and subfertility in later life.[1] The lack of easy and reliable screening tests may cause it difficult to diagnose PCD in children, particularly those who present with benign respiratory problems. The saccharin test, a traditional measurement of ciliary beat frequency, has been shown to miss a proportion of patients with PCD. Biopsies of the respiratory mucosa have been used for definitive diagnosis of PCD showing ultrastructural ciliary defects and abnormal ciliary motility. Moreover, measurement of ciliary beat frequency, high speed analysis of ciliary beat pattern, detailed electron microscopy of ciliary ultrastructure, and in cases where there are diagnostic uncertainties, cell culture from biopsies has been found useful.[2,3] These tests need specialized centers with adequate expertise and equipment, because secondary ciliary dyskinesia following epithelial injury from viral respiratory tract infections or exposure to pollutants is not always easy to exclude. Hence, it is not surprising that the diagnosis of PCD may be missed for a long time. Recently, genetic studies have enabled early and/or confirmation of PCD diagnosis. Data from our studies showed that analogous with cystic fibrosis CFTR p.Phe508del, screening for RSPH9 p. Lys268del (which lacks sentinel dextrocardia) in those at risk would help in the early diagnosis of PCD. This will help in tailoring clinical management, genetic counseling, and primary prevention of PCD.[4] Fraction of exhaled nitric oxide (FENO) is an emerging marker of inflammation in respiratory diseases, and it can help physicians in diagnosing and monitoring the treatment of different respiratory diseases.[5] Nitric oxide (NO) is produced from the conversion of L-arginine to NO and citrulline by nitric oxide synthases (NOSs),[6] which has 3 distinct isoforms; neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Constitutive expression of eNOS produces low levels of NO in healthy lungs. Inducible NO synthase is thought to be responsible for the increased levels of NO produced in the inflammatory states in the lung, and is markedly upregulated by cytokines, and down-regulated by corticosteroids. In response to the cytokines released by macrophages during inflammatory process, the enzyme iNOS generates extraordinarily high concentrations of NO which participates in host defense against specific organisms.[7] An unexplained feature of PCD is the very low level of NO particularly nasal NO; hence, nasal NO has been proposed as a screening test for this condition.[8] By contrast, exhaled NO levels show considerable overlap between normal individuals and PCD. There are likely at least 250 candidate genes for PCD, which are known to be on many different chromosomes,[9] so linkage disequilibration with nitric oxide synthase (NOS) is not a likely explanation for this finding. Nitric oxide is a product of L-arginine metabolism by one of the 3 isoforms of NOS; endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Bronchial iNOS is localized close to cilia,[10] and we have speculated that uncoupling of the contractile process of the cilia from NOS may result in failure of NO synthase, by analogy with the low NO production by muscle cells in patients with Duchenne muscle dystrophy. Although eNOS has also been localized to bronchial epithelium, it is iNOS which is the major source of exhaled NO. There is a lack of an easy and reliable screening test to diagnose PCD in children who often present with benign respiratory problems. In the present clinical practice, the diagnosis of PCD is made by identification of ultrastructural ciliary defects with abnormal ciliary motility in biopsies of the respiratory mucosa. Only specialized centers have adequate expertise and equipment to do these tests. Hence, the diagnosis of PCD is often missed for a long time in a number of cases.[2,3] This study was aimed at examining the usefulness of FENO measurements to detect PCD in children.

Methods. This study was conducted at the Departments of Pediatrics and Physiology, College of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia. A written informed consent was obtained from parents of all subjects and the project was approved by the College of Medicine Ethics Review Board.

The study population consisted of 22 children with symptoms suggestive of PCD. A control group comprising 11 healthy age matched subjects were also included. Primary ciliary dyskinesia diagnosis was confirmed by ciliary biopsy results in all patients. Primary ciliary dyskinesia diagnosis was based on clinical history, abnormal ciliary beat frequency on strips of epithelium harvested by nasal biopsy, and electron microscopy according to standard criteria. The inclusion criteria for the healthy controls were as follows: 1) no history or clinical evidence of acute or chronic respiratory diseases; 2) normal spirometry findings; and 3) no history of allergies.

Measurements of FENO. Fraction of exhaled NO measurements were performed according to the recent guidelines of American Thoracic Society[11] by using a Nox Eva 4000 chemiluminescence analyzer (Seres,

France) which has a sensitivity of one part per billion (ppb). All subjects were asked to refrain from eating, drinking, and strenuous exercise for 2 hours before FENO measurement. The history of recent meals was also recorded to avoid any alteration in results by nitrate containing foods. All tests were performed at the same time of the day between 09:00 am and 11:00 am to minimize any effect of circadian changes.

Firstly, the subjects were asked to inhale from residual volume to total lung capacity and then performed a slow expiratory maneuver with a constant standardized expiratory flow rate of 0.05 L/sec (± 10%) for approximately 15 seconds, into a Teflon cylinder connected to 3-mm Teflon tubing, without clipping the nose. To avoid nasal NO contamination, an expiratory resistance of 10-20 cm H_2O was applied, which was monitored by a special pressure sensor connected to restricted breathing configuration set up (Samba Sensors, Vastra Frolunda, Sweden). The subjects inspired NO free air and expired in restricted breath configuration set up. The expiratory flow rate was measured by data acquisition system BIOPAC MP-100 (Biopac Systems Inc, USA). Plateau levels of FENO against time were determined and expressed as parts ppb andmean concentrations were calculated between 5 and 15 seconds after start of the expiration. Three successive recordings were made at least at one min intervals and the mean was taken as the final result. To ensure standardization and reproducibility, the acceptable variation between the tests was kept less than 10%. Fraction of exhaled NO analyzer was calibrated before each test using a standard NO calibration gas. Ambient NO levels were also recorded, if >40 ppb analyzer was flushed with NO free gas. The laboratory set up and methods have been previously described.[5,12]

Spirometry. Spirometric tests were performed after FENO analysis, using a portable spirometer (Compact Vitalograph) calibrated before each set of measurements with one liter syringe. Three technically acceptable maneuvers were performed and the maneuver with the largest forced expiratory volume in one second (FEV1) was recorded.

Statistical analysis. We used Statisctical Package for Social Science Version 17.0 (SPSS Inc., Chicago, IL, USA) to perform the data analysis. We used test of normality to check if our data was following the normal distribution or not. By Kolmogorov-Smirnov test of normality, we found that FENO levels were not following the normal distribution. We used Wilcoxon signed-rank test to find the FENO difference between control and PCD subjects. Other parameters such as age and ventilatory function tests were analyzed by Student's t test.

Results. Table 1 shows the comparison of control and primary ciliary dyskinesia patient's clinical characteristics and ventilatory function tests. There were non-significant differences in age and ventilatory function tests between the 2 groups. Figure 1 shows

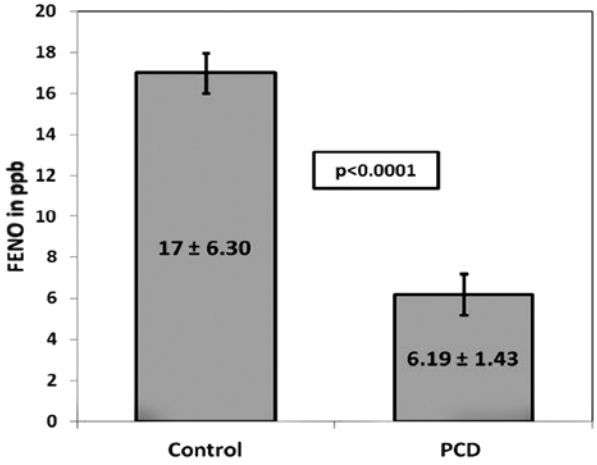

Figure 1 - Comparison of fraction of exhaled nitric oxide (FENO) levels in part per billion between control and primary ciliary dyskinesia patients. PCD - primary ciliary dyskinesia

Table 1 - Comparison of control and primary ciliary dyskinesia patient's clinical characteristics and ventilatory function tests. All values are expressed as mean±SD.

Parameters	Controls n=11	PCD n=22	95 % confidence interval of the mean (lower bound - upper bound)		*P*-value
Males/females	7/4	12/10			
Age (years)	11.00 ± 2.41	10.86 ± 3.09	-3.672	1.472	0.8949
FEV1 Liters	2.81 ± 0.96	2.28 ± 0.93	-1.180	0.838	0.2379
FVC Liters	3.00 ±0.96	2.59 ±0.94	-1.724	0.261	0.3660
FEV_1%	90.33 ± 1.83	80.07 ± 14.66	-24.694	-2.580	0.0667
FEF_{25-75}	123.03 ± 10.76	80.07 ± 33.62	-75.712	-18.037	0.0012
Inhaled corticosteroids (N)	0	15			

FVC - forced vital capacity; FEV_1 - forced expiratory volume in 1 second, FEF_{25-75} - Forced expiratory flow 25-75. PCD - primary ciliary dyskinesia

comparison of FENO levels in ppb between control and primary ciliary dyskinesia patients. Fraction of exhaled NO was significantly lower in those children with PCD (6.19±1.43) compared to healthy subjects (17.00±6.30, CI: -14.854 to -5.927, $p<0.0001$).

Rhinorrhea was present in 7 (31.8%), recurrent acute otitis media in 16 (72.7%), chronic otitis media in 5 (22.7%), recurrent sinusitis in 5 (22.7%), chronic productive cough in 8 (36.4%), bronchospasm in 11 (50.0%), and dextrocardia in 3 (13.6%) subjects. Spearman's correlation coefficients were determined between age, FENO, and ventilatory function tests. We observed that there was no significant correlation between age, FENO, and ventilatory function parameters.

Discussion. This study examined the diagnostic value of FENO in a small group of children in whom PCD was clinically suspected, and were referred for mucosal biopsy and other specific investigations. The results of our study demonstrate that FENO measurements can serve as a diagnostic tool to rule out PCD in children. We found that nasal NO levels >7 ppb excluded PCD with 82% certainty in patients with respiratory symptoms suggestive of PCD. As discussed earlier, the possibility to exclude PCD by a simple measurement of nasal NO or FENO concentrations seems clinically very useful, because the diagnosis of PCD is difficult and involves the complex assessment of ciliary structure and function by electron microscopy, which is the gold standard for the diagnosis of PCD and it can differentiate PCD from secondary ciliary dysfunction caused by acute or chronic infections. In rare cases, clear-cut abnormality of ciliary structure, is not always possible. Additionally, PCD has been described rarely without ultrastructural abnormalities, with just primary ciliary disorientation.[13,14] The reason for the low FENO concentrations in patients with PCD has not been fully elucidated. The very low NO and FENO levels appear paradoxical in an inflammatory disease such as PCD. The spirometric functions do not appear to be the reason because there was no correlation between FEV1, a measure of disease severity, and levels of FENO. The complete absence of any correlation shows that respiratory airways obstruction is not the cause of lower levels of FENO in these cases. These observations suggest that NO plays an important role in signal transduction associated with ciliary functions. The epithelial NO synthase enzyme is localized to the basal body of the microtubules of the cilia, and NO produced has been found to stimulate ciliary beat frequency. It may be the reason that the lower levels of nasal and exhaled NO in PCD are the results of reduced NO synthase activity, as levels of FENO are significantly different between children with PCD and healthy subjects.[15-17] Secondly, there is a close linkage between the gene for PCD and the gene for inducible NO synthase, which has co-inheritance of defects in both conditions. However, there are at least 200 proteins in the cilium and multiple candidate genes located on many different chromosomes, which make it difficult to undertand. However, RSPH9 inheritance may be the reason.[4] In line with our study, Narang et al reported that no patient in their study with PCD had a value of >6.1 ppb. Although in our study, 2 patients had a value of 8 ppb. However, they suggested that its usefulness is limited compared to nasal NO.[8] Similar to our results Horvath et al found that the concentration of FENO in patients with PCD and CF was significantly lower compared with healthy subjects. Their mean values were lower than our study subjects. The reason could be ethnic differences and also the methods of measurements. They performed offline FENO recordings and we did it by online methods. Additionally, they said that FENO values did not differ significantly between patients with PCD and those with CF. We excluded 2 cases of CF and in both of them the mean values were more than 10 ppb.[18]

The limitation of our study is due to the small number of study subjects and that we made only FENO measurements, and nasal NO was not measured in these cases.

In conclusion, measurements of FENO levels are helpful to screen children with clinical symptoms suggestive for PCD and to decide on the need for further and more invasive testing. The measurement of FENO appears to be a useful tool to screen children for PCD complementing the specialized tests of nasal biopsy and electron microscopy and to exclude this disease in those with high FENO levels. We suggest that if FENO is unexpectedly low in a patient with recurrent respiratory infections, the diagnosis of PCD should be actively excluded. A prospective study in a larger patient population including adults is required to establish the value of FENO and nasal NO as a valuable diagnostic tool in Saudi population.

Acknowledgment. *The authors are thankful to Mr. Timhar and Dr. Mujeeb for performing faction of exhaled nitric oxide and spirometric function studies.*

References

1. Meeks M, Bush A. Primary ciliary dyskinesia (PCD). ***Pediatr Pulmonol*** 2000; 29: 307-316.

2. Chilvers MA, O'Callaghan C. Analysis of ciliary beat pattern and beat frequency using digital high speed imaging: comparison with the photomultiplier and photodiode methods. ***Thorax*** 2000; 55: 314-317.
3. Jorissen M, Willems T, Van der Schueren B, Verbeken E, De Boeck K. Ultrastructural expression of primary ciliary dyskinesia after ciliogenesis in culture. ***Acta Otorhinolaryngol Belg*** 2000; 54: 343-346.
4. Alsaadi MM, Gaunt TR, Boustred CR, Guthrie PA, Liu X, Lenzi L, et al. From a single whole exome read to notions of clinical screening: primary ciliary dyskinesia and RSPH9 p.Lys268del in the Arabian Peninsula. ***Ann Hum Genet*** 2012; 76: 211-220.
5. Habib SS. Exhaled nitric oxide: an emerging marker of inflammation in respiratory diseases. ***Saudi Med J*** 2008; 29: 1697-1702.
6. Förstermann U, Sessa W. Nitric oxide synthases: regulation and function. ***Eur Heart J*** 2012; 33: 829-837, 837a-837d.
7. Suresh V, Mih JD, George SC. Measurement of IL-13-induced iNOS-derived gas phase nitric oxide in human bronchial epithelial cells. ***Am J Respir Cell Mol Biol*** 2007; 37: 97-104.
8. Narang I, Ersu R, Wilson NM, Bush A. Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiological significance. ***Thorax*** 2002; 57: 586-589.
9. Dutcher KS. Flagellar assembly in two hundred and fifty easy-to-follow steps. ***Trends Genet*** 1995; 11: 398-404.
10. Jiao J, Han D, Meng N, Jin S, Zhang L. Regulation of tracheal ciliary beat frequency by nitric oxide synthase substrate L-arginine. ***ORL J Otorhinolaryngol Relat Spec*** 2010; 72: 6-11.
11. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. ***Am J Respir Crit Care Med*** 2011; 184: 602-615.
12. Habib SS, Ahmed SM, Al Hadlaq A, Marzouk A. Effect of drinking Arabian Qahwa on fractional exhaled nitric oxide levels in healthy nonsmoking Saudi adults. ***Ann Thorac Med*** 2012; 7: 153-156.
13. Jorissen M, Willems T. The secondary nature of ciliary (dis) orientation in secondary and primary ciliary dyskinesia. ***Acta Otolaryngol*** 2004; 124: 527-531.
14. Biggart E, Pritchard K, Wilson R, Bush A. Primary ciliary dyskinesia syndrome associated with abnormal ciliary orientation in infants. ***Eur Respir J*** 2001; 17: 444-448.
15. Csoma Z, Bush A, Wilson NM, Donnelly L, Balint B, Barnes PJ, et al. Nitric oxide metabolites are not reduced in exhaled breath condensate of patients with primary ciliary dyskinesia. ***Chest*** 2003; 124: 633-638.
16. Xue C, Botkin SJ, Johns RA. Localization of endothelial NOS at the basal microtubule membrane in ciliated epithelium of rat lung. ***J Histochem Cytochem*** 1996; 44: 463-471.
17. Doran SA, Tran CH, Eskicioglu C, Stachniak T, Ahn KC, Goldberg JI. Constitutive and permissive roles of nitric oxide activity in embryonic ciliary cells. ***Am J Physiol Regul Integr Comp Physiol*** 2003; 285: R348–R355.
18. Horváth I, Loukides S, Wodehouse T, Csiszér E, Cole PJ, Kharitonov SA, et al. Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia. ***Thorax*** 2003; 58: 68-72.

Related Articles

Habib SS, Abba AA, Al-Zoghaibi MA, Subhan MM. Reference range values of fractional exhaled nitric oxide in healthy Arab adult males. ***Saudi Med J*** 2009; 30: 1395-1400.

Habib SS. Exhaled nitric oxide: an emerging marker of inflammation in respiratory diseases. ***Saudi Med J*** 2008; 29: 1697-1702.

Salman N, Dal D, Saridemir B, Aypar U. Spinal anesthesia in Kartageners syndrome. ***Saudi Med J*** 2006; 27: 885-887.