Nasal nitric oxide levels in primary ciliary dyskinesia, cystic fibrosis and healthy children

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Primary ciliary dyskinesia (PCD) is a rare, inherited disorder characterized by recurrent respiratory tract infections. The measurement of nasal nitric oxide (nNO) is an important test for the diagnosis of PCD. In this study, we aim to evaluate NIOX-MINO®, which is an easily applicable method for measuring nNO, in the diagnosis of patients with PCD and define diagnostic cut-off levels. Furthermore, determining the normal limits of nNO in healthy children and investigating nNO levels of children with cystic fibrosis (CF) are the other aims of this study. The children included in this study were 5 to 18.5 years old, 46 of them had PCD, 44 had CF and 200 were healthy children. To our knowledge, this work contains the widest population compared to previous studies. Subjects receiving steroids or antibiotics or those with any acute respiratory tract infection, asthma or allergic rhinitis were not included in the study. Mean nNO levels were found as 10.4, 22.8 and 21.0 ppb in PCD, CF and healthy children, respectively. The nNO levels for PCD patients were found significantly lower than children with CF and the control groups (p<0.05). In this study, the diagnostic nNO cut-off level between PCD and the other two groups was determined to be <11.5 ppb with %83.6 specificity and %67.4 sensitivity. The screening of nNO with NIOX-MINO method provides early diagnose before mucosal biopsy of patients who are suspected to have PCD and therefore, prevents co-morbidities and prolongs survival with early treatment.

Key words: primary ciliary dyskinesia, cystic fibrosis, nitric oxide, early diagnosis, case control studies.

Primary ciliary dyskinesia (PCD) is a rare (1 in 20.000-60.000 live births), typically autosomal recessive inherited disease associated with defective ciliary structure and/or function that results in abnormal mucociliary clearance.[1,2] In the 1930s, triad of situs inversus totalis, bronchiectasis and sinusitis was defined as Kartagener's Syndrome.[3] Afzelius[4] showed that ciliary abnormalities of the bronchial cilia and sperm flagella bring about chronic sinopulmonary infections and male infertility. Later, all cilia ultrastructural abnormalities similar to the ones defined above were literally termed as immotile cilia syndrome. Impaired function of cilia affects the heart and abdominal organs laterality during embryonic development. Situs inversus totalis constitutes approximately half of the cases of PCD.[5] The symptoms of PCD such as rhinitis, secretory otitis media, cough and recurrent bronchitis are common to many other diseases which often yield late diagnosis of PCD.[6] The major complication of PCD is bronchiectasis which can be prevented by early diagnosis and treatment.

Nitric oxide (NO) is a cellular signaling

molecule, functioned as modulator of ciliary function, vasodilatation, bronchodilatation, platelet aggregation, neurotransmission and immune function.[7,8] Firstly, Gustafsson et al.[9] showed NO presence in exhaled breath of humans in 1991. Nasal nitric oxide (nNO) is synthesized in respiratory airways, particularly in the paranasal sinuses.[10] The nNO level is low in individuals with PCD; thereby it can be helpful in the diagnosis before biopsy. Previously, chemiluminiscence NO analyzer was used for assessing nNO levels, however, this instrument is expensive, large and immobile. Recent studies about NIOX-MINO ® (Aerocrine AB, Sweden), which is a hand-held, non-invasive device, showed that it is useful, reliable, rapid and has a correlation with chemiluminescence NO analysis.[11,12]

Our objectives were measuring and comparing nNO levels between children with PCD, cystic fibrosis (CF) and healthy individuals via NIOX-MINO® NO analyzer, assessing healthy children normal levels, and cut-off nNO levels for PCD.

Material and Methods

Study Population

In this study, children diagnosed with PCD (including Kartagener's syndrome) and CF were recruited from Hacettepe University İhsan Doğramacı Children Hospital Respiratory Clinic. During the study period, PCD patients who accepted participation in the study were diagnosed by compatible history and symptoms, radiological findings, ciliary ultrastructure abnormalities by using electron microscopy and/or analysis of ciliary beat pattern and frequency abnormalities by using high speed video microscopy. CF was diagnosed by suggestive history, increased sweat test and/or genotyping. In this study out of 290 individuals, 46 children had PCD, 44 had CF and 200 were healthy subjects. The age of participants were distributed through 5-18.5 years. All participants were examined for nasal anatomic defects and polyps. If any of them existed in one part of the nose, the other part of the nose was preferred. Furthermore, the subjects included in this study were not receiving steroids or antibiotic and had no signs of an acute respiratory tract infection, asthma or allergic rhinitis.

Nasal NO measurements In our study, nNO was measured during quiet exhalation with NIOX-MINO®. During the tests, flow rate was 5 mL/s and a nasal olive was put into one nostril and the other nostril was closed. After the nasal olive was removed from the nostril, the time bar was filled approximately in 45 s for 5 mL/s flow rate and thus nNO measurements resulted in a few minutes. Since 5mL/s flow rate yields faster results over 2 mL/s, we preferred the former one. This method was easily performed by children; therefore, it was more practical. nNO was measured by well-trained department technicians while participants were in sitting position. NO level interval was 5-1700 ppb by NIOX-MINO® device and NO level <5 ppb couldn't be measured as a unique number. In such circumstances, NO level <5 ppb were accepted as 2.5 ppb according to the mean value between 0 and 5 with the suggestion from the statistics department.

In order to reduce the effect of air pollution, nNO was measured in May-September 2012, in which the heating systems were not used. Many environmental factors affect nNO levels, therefore, for obtaining standardization, mobile phones were closed or moved away from the NIOX-MINO® device and an isolated and ventilated room was preferred when making nNO measurement.

Statistics

The data was evaluated by IBM SPSS version 21. Data was presented with descriptive statistics such as mean, median, standard deviation, minimum, maximum numbers and percentages. Kruskal-Wallis and Mann-Whitney U tests were used for numerical values. Sensitivity, specificity and receiver operating characteristic (ROC) analysis were utilized to find best cut-off value between groups. $P<0.05$ was statistical significance level.

The study was approved by Hacettepe University Research Ethics Committee on 11.05.2012 with report number HEK 12/71. Before the measurement of nasal NO, all subjects and parents gave written informed consent to participate in this study.

Results

Table I. Nasal NO Levels of PCD, CF and Control Groups.

Groups	n	Mean (ppb)	SD (ppb)	Median (ppb)	Minimum (ppb)	Maximum (ppb)
PCD	46	10.4	8.3	9.0	2.5	42.0
CF	44	22.8	18.7	17.0	2.5	81.0
Control	200	21.0	8.9	20.0	7.0	48.0

NO: nitric oxide, PCD: primary ciliary diskinesia, CF: cystic fibrosis, ppb: parts per billion

Participants

In total, 290 subjects were included in this study. One hundred and thirty-four (46.2%) of them were girls and 156 of them (53.8%) were boys. In the PCD group, we had 46 individuals, 23 (50%) participants were girls and 23 (50%) were boys. In the CF group we had 44 individuals, 22 (50%) participants were girls and 22 (50%) were boys. In the control group we had 200 individuals, 89 (44.5%) of them were girls and 111 (55.5%) of them were boys. There was no statistically significant difference between PCD, CF and control group concerning gender (p= 0.685).

Population's age range was 5-18.5 years and the mean values for PCD, CF, healthy controls were 11.9, 10.2 and 11.1 years, respectively. There was no statistically significant difference between groups concerning ages (p=0.053). Mean diagnostic ages of PCD patients were 7.6 years and none of them were diagnosed in the newborn period. Mean diagnostic ages of CF patients were 1.8 years, furthermore 4 of them were diagnosed in the newborn period and 1 of them had a prenatal diagnose because of family's CF history.

Pulmonary function tests were successfully performed by 38 PCD and 33 CF patients. The mean and standard deviation (SD) of PCD patients for Forced expiratory volume in 1 second (FEV1), Forced vital capacity (FVC), FEV1/FVC, expiratory flow between 25% and 75% of vital capacity (FEF25-75) were 78.4 ± 19.1, 84.8 ± 17.5, 87.4 ± 11.1, 58.3 ± 21.7, respectively. The mean and SD of CF patients for FEV1, FVC, FEV1/FVC, FEF25-75 were 83.7 ± 15.9, 90.9 ± 15.8, 92.0 ± 8.5, 73.0 ± 25.7, respectively. There was no statistically significant difference between PCD and CF groups concerning pulmonary function test results except FEF25-75 parameter.

Nasal NO levels Mean, SD, median, minimum and maximum values of nNO are shown in Table I for all groups. Mean nNO levels for children with PCD, CF and healthy controls were respectively 10.4 ± 8.3, 22.8 ± 18.7 and 21 ± 8.9 ppb. nNO was statistically significantly lower in PCD when compared to CF and healthy controls (p<0.05) and there was no statistically significant difference between CF and the control group.

In our study, the nNO cut-off level was <11.5 ppb (sensitivity: %67.4, specificity: %83.6) when we compared PCD versus both control and CF groups. The nNO cut-off level was found <11.5 ppb (sensitivity: %67.4, specificity: %87.5) between PCD and control group. The nNO cut-off level was <10.5 ppb (sensitivity: %72.7, specificity: %63.0) between PCD and the CF groups. The cut-off, sensitivity and specificity values are also summarized in Table II.

ROC curves denote sensitivity and 1- specificity values for nNO levels. The more the area

Table II. Nasal NO Cut-off Values of PCD, CF and Control Groups.

	Cut-off (ppb)	Specificity (%)	Sensitivity (%)
PCD vs CF and Control	11.5	83.6	67.4
PCD vs Control	11.5	87.5	67.4
PCD vs CF	10.5	63.0	72.7

NO: nitric oxide, PCD: primary ciliary diskinesia, CF: cystic fibrosis, ppb: parts per billion
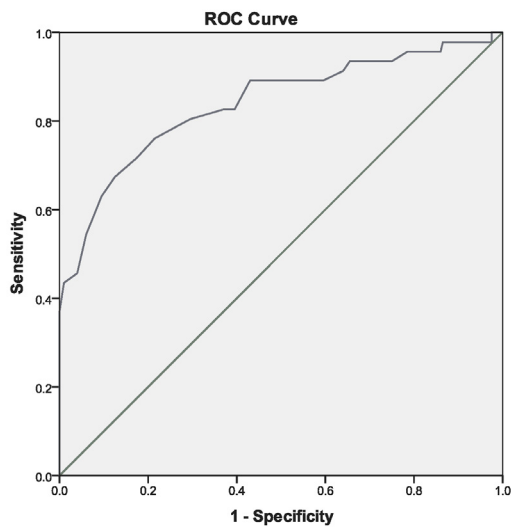
Fig. 1. ROC Curve for nNO Levels of PCD and Control Groups: Area under the ROC curve: 0.84, p: 0.00, asymptotic 95% confidence interval; lower bound: 0.76, upper bound: 0.91
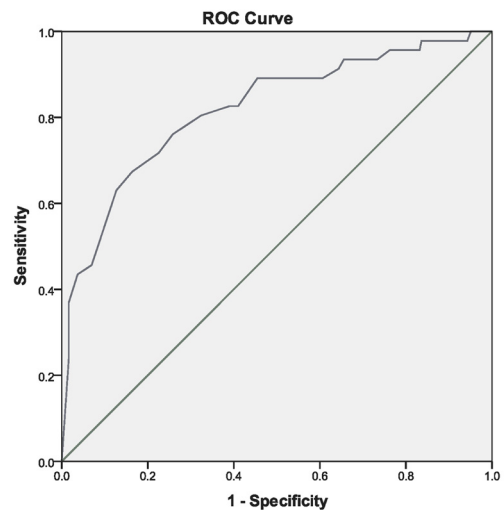

Fig. 3. ROC Curve for nNO Levels of PCD versus both the Control and CF Groups: Area under the ROC curve: 0.82, p: 0.00, asymptotic 95% confidence interval; lower bound: 0.74, upper bound: 0.89

under the ROC curve, the more significant the result is. The ROC curves obtained from the comparison of nNO levels of PCD versus healthy and CF are shown in Figure 1 and Figure 2, respectively. Moreover, Figure 3 presents the ROC curve obtained from the comparison of PCD versus both the healthy and CF. Note that, the results are found statistically significant since all of the ROC curves have p<0.05.
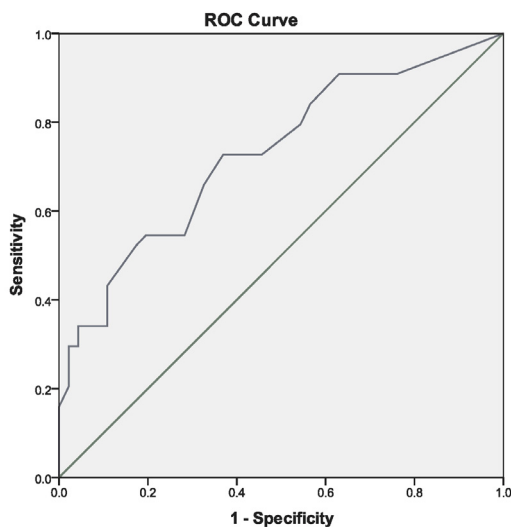

Fig. 2. ROC Curve for nNO Levels of PCD and CF Groups: Area under the ROC curve: 0.73, p: 0.00, asymptotic 95% confidence interval; lower bound: 0.62, upper bound: 0.83

Discussion

Diagnosis of PCD is often delayed because of symptoms being common to many other diseases. Clinical symptoms, family history, sputum culture results, radiographic findings, nNO levels, analysis of ciliary ultrastructure with electron microscopy, analysis of ciliary beat pattern and frequency with high speed video microscopy and genetic tests are utilized to diagnose PCD. Some studies have shown that nNO levels are very low in PCD.[13,14]

Since 2009, nNO measurement have been used for screening PCD patients in Europe.[15,16] In the literature, there exist many studies that measured nNO with chemiluminescence method and NIOX-MINO® for PCD, CF and healthy subjects and moreover they have determined correlation between these methods.[17-21] Montella et al.[17] used nasal quiet exhalation method and 0,05 L/s flow rate with NIOX-MINO® for measurement of nNO for 14 PCD, 11 CF and 13 healthy control group subjects. nNO cut-off value was determined as <23 ppb with 100% sensitivity, %85 specificity between PCD and control groups, <26 ppb with 100% sensitivity, 91% specificity between PCD and CF groups and <57 ppb with 82% sensitivity, 31% specificity between CF and control groups. In the same study, when 0.05 L/s flow rate and humming nasal exhalation method was used, PCD and

control groups were compared and nNO cut-off was demonstrated as <50 ppb with 100% sensitivity, 100% specificity. In addition, PCD and CF groups were compared and cut-off value was <21 ppb with 93% sensitivity, 91% specificity. This study showed that nNO values were influenced by the measurement technique, thus nNO cut-off values may vary for each method.

In our study, nasal quiet exhalation method was chosen because of its easy use, and also nNO values were found to be lower than previous studies. nNO cut-off values were determined as <11.5 ppb with 67.4% sensitivity, 83.6% specificity when we compared PCD versus control and CF groups, <11.5 ppb with 67.4% sensitivity, 87.5% specificity between PCD and control groups and <10.5 ppb with 72.7% sensitivity, 63.0% specificity between PCD and CF groups. In our study, we found our cut-off nNO levels using same measurement technique for a lot of healthy subjects, PCD and CF patients. As far as we know, this work has the largest study group of healthy and PCD children for measuring nNO levels with NIOX MINO® method. In our study, nNO levels were lower in PCD compared to other groups which is consistent with the previous studies [17,21,22]. However, our sensitivity and specificity values were lower than the other studies. Previous studies found that nasal quiet exhalation method with chemiluminescence analyzer had the lowest nNO values compared to the other nNO measurement methods.[23] Lower respiratory tract contamination is much more effective for this method than other methods. Note that, nNO levels in lower respiratory tract are lower than upper respiratory tract and paranasal sinuses. Therefore, the large study population and the method we used might have a role for the low values of nNO measurements.

We had some limitations such as some of the PCD and CF patients had positive sputum culture and/or colonization. In spite of that, none of the participants in the whole population had any signs of an acute infection in the 4 weeks period before the nNO measurements. In addition, NIOX-MINO® device cannot measure nNO levels <5 ppb. Therefore, we accepted 2.5 ppb for each nNO level <5 ppb according to the recommendation of the department of statistics at our university.

The common symptoms frequently cause the diagnosis of PCD to be delayed. Effective and intensive care enable the respiratory function to be stabilized and hence preventing complications for many years. History, physical examination, radiological and microscopic findings guide diagnosing PCD. Despite the fact that nNO levels obtained in this study do not show high sensitivity, this study supports the diagnosis of PCD by measuring nNO levels before the use of mucosal brush or biopsy.

Acknowledgement

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