Exhaled Nitric Oxide in Acute Respiratory Syncytial Virus Bronchiolitis

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Objective: To investigate fractional exhaled nitric oxide (FeNO) levels in infants during acute respiratory syncytial virus (RSV) bronchiolitis and during convalescence.

Design: Prospective cohort study. Comparison of FeNO levels between infants with laboratory-confirmed acute RSV bronchiolitis and 2 control groups: healthy infants and infants with recurrent wheezing.

Setting: The Department of Pediatric Emergency Medicine and the Pediatric Pulmonary Clinic of the Tel Aviv Medical Center from November 2008 to July 2009. The FeNO levels were measured at referral and at 2 visits over 4 months after convalescence. The FeNO level was measured using the multiple-breath exhalation technique.

Participants: Forty-four infants with acute RSV bronchiolitis (mean [SD] age, 6.8 [7.3] months), 21 infants with recurrent wheezing (mean [SD] age, 10.8 [7.59] months), and 32 age-matched healthy controls (mean [SD] age, 6.8 [9.1] months). Follow-up data were available for 22 children (55%) for the first follow-up visit and for 11 children (25%) for the second follow-up visit.

Exposure: Acute RSV bronchiolitis.

Main Outcome Measures: The FeNO levels during acute RSV bronchiolitis vs controls and FeNO levels during follow-up vs acute-stage disease.

Results: Mean FeNO levels for RSV-positive infants were significantly lower compared with healthy controls and infants with recurrent wheezing: mean (SD), 1.89 (1.76) parts per billion (ppb), 7.28 (4.96) ppb, and 4.86 (7.49) ppb, respectively (P<.001). The FeNO levels at the 2- and 4-month follow-up visits increased to 7.74 (5.13) ppb and 11.37 (6.29) ppb, respectively (P=.001).

Conclusions: The FeNO levels are temporarily reduced during acute RSV bronchiolitis and increase during convalescence to normal levels and higher. The mechanisms for this suppression and its relation to future wheezing and asthma need to be studied.

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Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection in children younger then 1 year, and most children have been infected by 2 years of age. Acute RSV bronchiolitis shares common clinical manifestations with asthma, including tachypnea, dyspnea, cough, and wheeze. The RSV manifests extremely restricted tropism for the respiratory epithelium, stimulating an inflammatory response. Studies using lines and cultures of respiratory epithelial cells to investigate the mechanisms of RSV-induced inflammation have shown that RSV increases inducible nitric oxide synthase (iNOS) messenger RNA and upregulates iNOS and its nitrite products. In response to RSV infection, human type 2 alveolar epithelial cells demonstrate a significant proinflammatory reaction, involving cytokines, chemokines, and other important cell-signaling molecules, including nitric oxide (NO). In a cell model, increased iNOS gene and IRF-1 gene expression were observed 4 hours after infection with RSV and lasted several hours. Although other proinflammatory cytokine (interferon β, interleukin 1β [IL-1β], and tumor necrosis factor α) gene expressions were also enhanced following RSV infection, the increased expression of iNOS occurred earlier and was independent of the stimulation of these viral-induced cytokines, which, nevertheless, further amplified iNOS production.

In RSV-infected mice, increased iNOS enzymatic activity was detected in the lung tissue 4 days after the infection, and high levels of nitrates were measured in bronchoalveolar lavage fluid. Inducible NO synthase was the main NO synthase isof orm that was increased after the RSV infection.

Respiratory syncytial virus bronchiolitis in infants poses an increased risk for...
subsequent wheezing and asthma in prepubertal children. The mechanisms underlying this increased risk and higher incidence are unclear. Evidence suggests that NO is a key mediator of eosinophilic inflammation in the airways and a well-established marker of airway inflammation in asthma. Clinical studies have shown that NO concentration in expired gas (fractional exhaled NO [FeNO]) correlates with the degree of eosinophilic airway inflammation. The FeNO level has been shown to reliably discriminate between asthmatic and nonasthmatic children and to correlate with the clinical asthmatic status and response to anti-inflammatory therapy.

Despite in vitro findings of NO involvement in acute RSV bronchiolitis, the increased risk for asthma post-RSV bronchiolitis, and the role of NO in asthma, to our knowledge, no data exist for exhaled NO levels in acute RSV bronchiolitis. Nevertheless, while childhood asthma manifests eosinophilic lower airway inflammation, this is not the characteristic finding in RSV bronchiolitis. Combined with the different profile of inflammatory and profibrotic mediators, the production of NO in the airways in RSV bronchiolitis may differ from that of asthma.

We hypothesized that high FeNO levels occur in infants with RSV bronchiolitis during the acute phase of the disease. The aim of this study was to measure FeNO levels in infants with acute RSV bronchiolitis in comparison with healthy controls and infants with recurrent wheezing and to study FeNO levels after convalescence.

**METHODS**

The FeNO level was measured in 1 study and 2 control groups.

**STUDY GROUP**

Infants referred to the Department of Emergency Medicine of the Dana Children’s Hospital at the Tel Aviv Medical Center for suspected acute RSV bronchiolitis were recruited. Inclusion criteria were healthy infants with acute RSV bronchiolitis confirmed by positive findings of antigenic fluorescent analysis of nasopharyngeal washings (LIGHT DIAGNOSTICS Respiratory Viral Screen DFA Kit; Chemicon USA, Temecula, California). Children with underlying chronic diseases and children treated with either corticosteroids by any route or bronchodilators were excluded. The FeNO level was measured during the acute stage (within 24 hours of referral). All infants were invited for future visits in the outpatient pulmonary clinic and FeNO measurements were repeated during the first and second visits after the acute RSV bronchiolitis event.

**HEALTHY CONTROL GROUP**

Healthy age-matched infants who had no significant respiratory symptoms since birth and did not have any chronic disease were recruited from the families of the hospital employees.

**RECURRENT WHEEZING GROUP**

Infants followed up in the outpatient pediatric pulmonology clinic for recurrent wheezing composed the recurrent wheezing control group. They were diagnosed by a certified pediatric pulmonologist as having the wheezy baby syndrome, defined as 3 or more reported wheezing episodes or at least 1 period of persistent wheezing longer than 2 months.

These infants were recruited during routine scheduled visits to the clinic during which FeNO level was measured. Exclusion criteria were (1) corticosteroid or leukotriene receptor antagonist treatment; (2) acute viral infection (rhinorrhea, fever, or cough); and (3) other chronic underlying diseases.

The FeNO level was measured by a rapid-response chemiluminescence analyzer (Eco Physics CLD88 NO chemiluminescence analyzer; EcoMedics AG, Duerten, Switzerland) using the multiple-breath exhalation technique according to European Respiratory Society/American Thoracic Society guidelines. Children were seated on their parent’s lap and breathed through a face mask that was gently placed on their face. Exhaled air was collected with a face mask placed over the infants’ nose and mouth during relaxed tidal breathing. A 5-cm water resistor was added to the mask so that the infants exhaled against an increased oral pressure. No sedation was used. During the test, infants were breathing NO-free air through the Denox 88 NO-free supplier module (EcoMedics AG) with online recording and FeNO levels were expressed as parts per billion.

Each infant performed at least 3 measurements until 3 acceptable test results were obtained. The FeNO measurements that were more than 10% off were discarded. Parents of all children were instructed to avoid the following prior to the test: eating or drinking for 1 hour, exposure to cigarette smoke for 24 hours, and taking any medications.

**ETHICS**

The study was approved by the Hospital and Ministry of Health ethics (Helsinki) committees.

**DATA ANALYSIS**

The FeNO levels were compared between the 3 groups. In addition, convalescent FeNO levels in the RSV-positive group were compared with the FeNO levels during acute-stage disease. Descriptive statistics are expressed as mean (SD). All analyses were performed using SPSS for Windows (release 12.0; SPSS Inc, Chicago, Illinois) by a certified biostatistician (Tel Aviv University Laboratory of Statistics). Analysis of variance was used to compare results between the 3 study groups and between the 3 consecutive measurements in the acute RSV bronchiolitis group. A P value <.05 was considered statistically significant. The Tukey post hoc test was used for homogeneous subsets.

**RESULTS**

Forty-four infants with acute RSV bronchiolitis (study group), 21 infants with wheezing (recurrent wheezing group), and 32 healthy controls were recruited. Patients’ characteristics are described in the Table. Age, height, and sex were not different between groups. Mean body weight was higher in the recurrent wheezing group compared with the other 2 groups.

No differences in FeNO levels between male and female infants were found for all 3 study groups. Seventeen infants (39%) with RSV bronchiolitis were admitted to the hospital. We did not find any difference in FeNO levels between admitted and nonadmitted infants. Mean (SD) FeNO level in the acute RSV bronchiolitis group was significantly lower compared with the healthy con-
control and recurrent wheezing groups ($P < .001$): RSV group, 1.89 (1.76) parts per billion (ppb) (95% confidence interval [CI], 1.35-2.42 ppb); healthy control group, 7.28 (4.96) ppb (95% CI, 5.49-9.06 ppb); and recurrent wheezing group, 4.86 (7.49) ppb (95% CI, 1.35-8.36 ppb). The FeNO levels did not differ between the healthy control group and the recurrent wheezing group ($P = .44$).

Twenty-four of the 44 infants (55%) in the acute RSV bronchiolitis group completed the first follow-up FeNO measurement at a mean of 2 months after the first FeNO measurement during acute-phase disease and 11 children (25%) completed a third FeNO measurement 1 to 2 months after the second evaluation and measurement. The FeNO levels increased significantly during the follow-up visits to mean (SD) 7.74 (5.13) ppb at the first follow-up visit and 11.37 (6.29) ppb at the second follow-up visit (Figure) (analysis of variance, $P < .001$). The FeNO level at the first follow-up visit was very close to the healthy control level (mean [SD], 7.74 [5.13]; 95% CI, 5.58-9.90 ppb vs 7.28 [4.96] ppb, respectively; $P = .74$). The FeNO level at the second follow-up visit was significantly higher than the healthy control level (mean [SD], 11.37 [6.29]; 95% CI, 7.14-15.6 ppb; $P = .03$).

This study shows that FeNO levels during acute RSV bronchiolitis are significantly decreased and that the levels increase back to normal levels and beyond during convalescence. These results are relevant because almost all children experience acute RSV infection and clinically significant RSV bronchiolitis disease is associated with recurrent wheezing episodes and allergic asthma in later childhood. The nature of this association is not completely understood. It is unclear whether clinical bronchiolitis due to RSV infection plays a direct causative role in subsequent allergic asthma or simply identifies infants at risk for subsequent wheezing resulting from an asthmatic genetic predisposition. Based on the role of NO in pediatric asthma and the increased FeNO levels in asthmatic children compared with healthy controls, our hypothesis was that high FeNO levels during acute RSV bronchiolitis will identify infants at increased risk for wheezing and asthma. We found significantly decreased FeNO levels in acute-stage RSV bronchiolitis, coinciding with the idea that the process of acute bronchiolitis in infants does not involve eosinophilic inflammation. The fact that FeNO levels rise after the acute phase suggests that the low levels represent active suppression of NO production in the airways. For technical and ethical reasons, we did not measure eosinophil count in the airways or alveolar fluid in our patients, but since elevated FeNO level correlates with eosinophilic inflammation, it can be assumed that this type of inflammation is absent during acute RSV bronchiolitis even though acute RSV bronchiolitis predisposes to later asthma characterized mainly by eosinophilic inflammation. Indeed, it has been shown that lower airway disease with RSV stimulates neutrophilic inflammation during the acute phase.

The finding of low FeNO levels was surprising also in regard to in vitro and animal studies that showed increased iNOS activity and NO production during the acute infection. At present, we do not have a good explanation for this discrepancy; however, there is a major difference between infection with RSV and acute bronchiolitis, the clinical respiratory disease of the lower airway, in regard to the risk of future asthma. While virtually all infants are infected with the virus by 2 years of age, only infants with a significant clinical presentation are at increased risk. In vitro and animal studies looked at infection rather than at the clinical airway disease caused by RSV. Indeed, adult volunteers experimentally infected with RSV (but not showing clinical acute bronchiolitis) did not demonstrate a change in nasal and oral NO production compared with controls. It would be interesting to measure FeNO levels in children with acute RSV infection who do not have airway disease. We plan to follow up our study group and investigate the rela-
tionship of the FeNO levels during acute illness and convalescence with the future development of asthma.

Although a clear explanation for our finding of temporary suppression of NO production cannot be provided, one possibility may involve inhibition of NO production by IL-4. In the face of type 2 helper T cell preponderance, high levels of IL-4 are released, which in turn has been shown to inhibit NO production by respiratory epithelial cells infected with RSV. To the best of our knowledge, there are no published studies that measured FeNO levels in infants during and after acute RSV bronchiolitis. Interestingly, an abstract from the American Thoracic Society 2009 annual meeting also found low FeNO levels in acute RSV bronchiolitis. This study did not use a healthy control group and used literature data for comparison. Nevertheless, this report supports our finding and the need for further research in this area.

The FeNO suppression time was limited because FeNO levels increased after acute-stage disease to normal or even higher levels. This suggests a “rebound” phenomenon. Future studies should look into this trend and into its possible relation to future wheezing and asthma.

Measurement of FeNO level in infants is difficult. It requires a constant and stable exhaled flow rate for FeNO concentration to represent FeNO production in the airways. This requires considerable cooperation of the subject and therefore can only be done in adults and older children. Children younger than 5 years can rarely meet these demands; hence, the rebreathing technique with a face mask so that the infants exhale against an increased oral pressure. This is believed to close the velum and prevent nasal contamination. With the addition of a 2- to 5-cm water resistor, this technique has been applied to infants to differentiate between healthy infants and infants with wheezing and asthma and also to follow up the course of disease and response to treatment, providing reproducible results. In our study, a 5-cm water resistor was applied.

Normal values of FeNO using the rebreathing technique vary between reports. This probably results from technical differences, the use of inline vs offline measurements with bag collection of the exhaled gas for sample analysis, the level of NO in ambient air, the use of sedation/awake/sleep state, the rate of mixing of nasal gas, and the commercial or self-made equipment applied. Normal values of FeNO obtained from our healthy controls were similar to those reported in other studies that used the tidal breathing technique. Others have reported somewhat higher levels. Hence, at this time, studies and clinical practice should not use “normal values” reported from other studies but include healthy control groups from the same laboratory for comparison. We included also a recurrent wheezing group for comparison. Their FeNO levels were not different from the healthy group. This supports that the acute infection in the RSV study group is the cause of the low FeNO levels and not the narrowed airways or wheezing. The FeNO levels in babies with wheezing have been found to be higher compared with healthy infants using the rebreathing technique.

Several limitations may affect the results of this study. The recurrent wheezing group had higher body weight by 2.2 and 1.6 kg compared with the study and control groups, respectively. Although some studies have found a positive linear correlation between age and FeNO level, which may also be interpreted as effects of body weight and height, others have not. Our overall narrow age range compared with other studies and the fact that the 3 groups differed in their FeNO levels does not support that age or body weight significantly affected the data. Another limitation of the present study was the sample size and the smaller group of infants who participated in the follow-up measurements.

In conclusion, FeNO levels are reduced in infants during the acute phase of RSV bronchiolitis compared with healthy controls and increase significantly following the acute phase. Further studies are required to investigate the relationships of these findings with future wheezing and asthma. The inclusion of infants with acute respiratory and lower airway disease caused by other viruses as an additional control group may further elucidate whether the findings of this study are specific for RSV or may be caused by other organisms as well.

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REFERENCES


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