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Original Article

Exhaled nitric oxide in acute exacerbation of pediatric asthma

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Abstract

Background Measurement of exhaled nitric oxide (eNO) is a non-invasive and easy method to monitor airway inflammation.

Objectives To compare the levels of eNO during and after an exacerbation of asthma, to evaluate the effect of glucocorticosteroids (GCS) on the levels of eNO and to correlate eNO with other markers of inflammation such as symptom scores, FEV_1 and sputum eosinophils.

Methods The observational study was performed over 24 months at a tertiary paediatric hospital. Subjects underwent eNO measurement, spirometry and sputum induction during an asthma exacerbation and then two weeks later. A symptom score was recorded everyday for two weeks. All subjects were treated with $ß_2$-adrenergic agonists and an oral glucocorticosteroid (GCS).

Results Fifteen subjects with acute asthma exacerbation aged 5 and 16 years old participated in the study. The mean level of eNO during the acute exacerbation was significantly higher than eNO levels at the follow-up visit, 11.2 (95%CI 9.2;13.2) vs. 8.0 (95%CI 5.0;11.1) ppb, P=0.03. In the acute exacerbation, eNO correlated with sputum eosinophils (P=0.04), but no correlation could be found between eNO and the other markers of inflammation during exacerbation or follow up.

Conclusions eNO level increased during asthma exacerbation and decreased after two weeks of glucocorticosteroid therapy. Measurement of eNO is a practical monitoring method in emergency management of asthmatic children. **[Paediatr Indones 2008;48:64-70]**.

Keywords: *exhaled nitric oxide, asthma exacerbations, eosinophils, FEV_1*

Asthma is a chronic inflammatory disease characterised clinically by a variable degree of airflow obstruction, bronchial hyper-responsiveness and airway inflammation.[1] Measurement of exhaled nitric oxide (eNO) is a non-invasive method to monitor diseases which involve airway inflammation.[2,3] In stable asthma the eNO level is elevated when compared with normal subjects.[4,5] During exacerbations eNO increases, and it decreases after treatment with glucocorticosteroids (GCS),[6-9] probably from increased expression of inducible nitric oxide synthase (iNOS) due to the presence of inflammatory cytokines.[10]

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The levels of eNO in normal subjects do not change in response to GCS.[11] In asthmatic subjects whether acutely ill or stable, exhaled nitric oxide is found to be correlated with other indicators of airway inflammation, i.e., eosinophils in bronchoalveolar lavage fluid and induced sputum.[12-16] eNO may therefore be an indicator of asthma severity and treatment efficacy.[6]

This study aimed to compare the levels of eNO during and after an exacerbation of asthma, to evaluate the effect of GCS therapy on the levels of eNO and to find correlation between eNO with other markers of inflammation such as symptom scores, FEV_1 (forced expiratory volume in 1 second) and sputum eosinophils.

Methods

Study design

Children with asthma exacerbations who visited to the Emergency Department of Sydney Children's Hospital (SCH) were assessed by questionnaire, spirometry, eNO, sputum induction and a diary with symptom scores and peak flow readings. The study was conducted from August 2003 to August 2004. All subjects were treated with $ß_2$-adrenergic agonists (salbutamol, GlaxoSmithKline) and an oral GCS (prednisolone) when they presented in the ED. Oral GCS therapy was administered for three days and continued with inhaled GCS (fluticasone propionate 125mg b.i.d., GlaxoSmithKline) as a controller. The study protocol was approved by the Research Ethics Committee South Eastern Sydney Area Health Service. Informed consent was obtained from parents.

Questionnaire, symptom diary, and peak flow readings

Parents completed a questionnaire detailing asthma triggers, duration and severity of asthma symptoms, and medication during the last three months.[17] The frequency of symptoms was used as a measure of asthma severity. Subjects completed a diary card with peak expiratory flow (PEF) and diurnal asthma symptoms, scored 0 to 3. Diurnal variability of PEF was calculated as previously described.[18]

Spirometry and eNO measurement

Lung function was measured by using spirometry (Vitalograph, Buckingham, U.K.). Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were recorded. Offline measurements of eNO concentrations conformed to current guidelines.[19] Samples were analysed by using a chemiluminescence nitric oxide analyser (Model 2107 Dasibi Corporation, Glendale, California, USA) within 30 mins.

Sputum collection

Sputum was induced by a 0.9% saline aerosol using a DeVilbiss Ultraneb 2000 nebuliser (Somerset, PA, USA) 10 minutes after 200 mcg salbutamol (Ventolin inhaler, GlaxoSmithKline).[20] Sputum plugs were processed within 2 hours by adding four volumes of 0.1% dithiothreitol (DTT - Sputolysin 10%, Sigma, St. Louis, USA) as previously described.[21,22] Differential counts of non-squamous cells were performed using a Diff-Quick stain.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, USA). eNO levels were log transformed to the normal distribution and geometric mean with their confidence intervals. Paired student's *t*-test was used to compare variables within subjects. $P<0.05$ was considered statistically significant.

Results

Twenty-two patients with acute asthma exacerbations aged between 5 and 16 years old were recruited from Emergency Department at SCH, but only 15 subjects completed the 2 week study. Their characteristics are presented in **Table 1.** Only one 6 year old child, presented with a first attack of asthma.

Questionnaires

The questionnaires revealed that the majority of children had been first diagnosed with asthma under five years old (15), and during the last three months,

14 visited Emergency Department and 4 had to be hospitalised (**Table 1**).

Table 1. Characteristics of study subjects. Those taking inhaled GCS were using fluticasone or fluticasone/salmeterol combination inhaler (GSK, Australia).

Variables	Number
Total	22
Age at first diagnosis	
1-4 yrs	15
5-9 yrs	6
10-15 yrs	1
GCS (preventer)	10
No GCS	12
Children with peak flow meter	7
History of the last 3 months	
Reliever as needed	17
Wheezing	20
Asthma night	18
Asthma morning	20
Asthma attacks	11
Missed school days	12
Previous ED visit	14
Hospital admission	4

GCS = Glucocorticosteroid, ED = Emergency department

Symptom scores

There was a significant decrease in asthma symptoms comparing the week after the acute exacerbation with the week before the follow up visit. The symptoms of asthma included early morning tightness (5.6, 95%CI 3.4;7.9 vs. 2.9, CI 1.2;4.6, P=0.006, r=0.6), asthma symptoms at night (3.1, CI 1.1;5.0 vs. 1.3, 95%CI 0;2.6, P=0.002, r=0.8) and wheezing during the day (6.9, 95%CI 4.1;9.7 vs. 2.3, CI 0.7;3.9, P=0.001, r=0.5).

Peak expiratory flow

A significant decrease in diurnal variability was observed between the first week compared to the 2nd week of the study, P=0.01 (**Figure 1**). The mean nadir in PEF (%) was significantly increased in the second week when compared with the first week after the exacerbation of asthma, P=0.01 (**Figure 2**).

Spirometry

The mean level of FEV_1% 2 weeks after exacerbation was significantly higher than the mean of FEV_1% during the exacerbation of asthma, P=0.005 (**Figure 3**).

Exhaled nitric oxide

eNO levels during the acute exacerbation were significantly higher than eNO levels at the follow up visit (n=14, 11.4 ppb, 95%CI 8.2;14.5 vs. 8.0 ppb, 95%CI 5.0;11.1, P=0.03, **Figure 4**) but 2 values had to be excluded due to high ambient NO levels.[23]

Table 2. Clinical data of study participants during and after the asthma exacerbation. Paired analysis was performed using the data from those subjects who attended both during the acute exacerbation and the follow-up visit.

	During exacerbation	Follow up	
Demographic			
N = 22	22	15/22	
Age, yr (mean, 95%CI)	9.6 (8.4;10.8)	10.2 (8.6;11.6)	
Sex M/F	10/12	5/10	
Height (cm)	144.1 (134.1;147.8)	142.7 (133.7;151.7)	
Clinical			
FEV_1 % predicted	69.0 (56.3 ;81.6)	90.9 (84.1;97.7)	P=0.005
FVC %	72.2 (61.2;83.2)	94.7 (87.1;102.2)	P=0.005
eNO (ppb)	11.2 (9.2;13.2)	8.0 (5.0;11.1)	P=0.03
PEF variability (%)	10.5 (6.1;14.9)	7.9 (4.1;11.8)	P=0.01
Lowest PEF (%)	69.4 (61.0;77.9)	79.8 (75.3;84.3)	P=0.01
Sputum cell counts			
Total cells count (10^6/ml)	3.2 (1.0 ;5.3)	0.9 (0.4;1.5)	P=0.03
eosinophils (%)	30.4 (21.2;39.6)	16.0 (9.9;22.2)	P=0.002
neutrophils (%)	24.9 (18.2;31.6)	20.2 (16.1;24.3)	P=0.3
macrophages (%)	36.1 (24.8;47.4)	56.1 (49.5;62.6)	P=0.004

CI= Confidence interval, FEV_1= forced expiratory in one second, FVC= forced vital capacity, PEF= peak expiratory flow

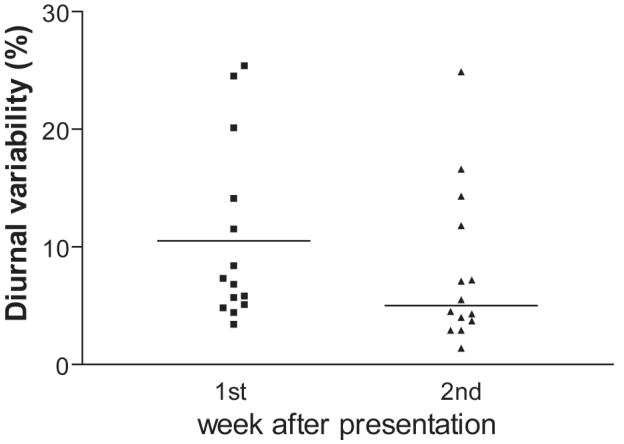

Figure 1. Levels of diurnal variability in the 14 subjects who performed PEF measurements one week after presentation at the Emergency Department and one week before follow up, P=0.01.

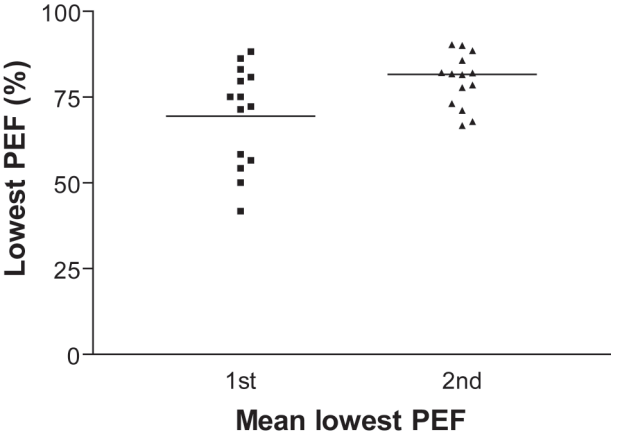

Figure 2. Levels of lowest PEF in asthma patients one week after presentation at the Emergency Department and one week before follow up, P=0.01.

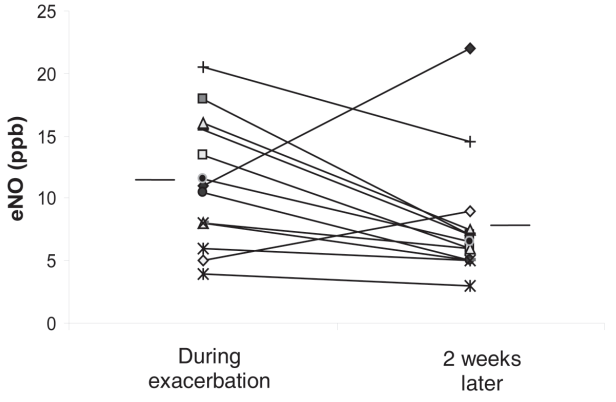

Figure 4. The individual and mean levels of eNO during the asthma exacerbation and two weeks after treated with GCS therapy, P= 0.03.

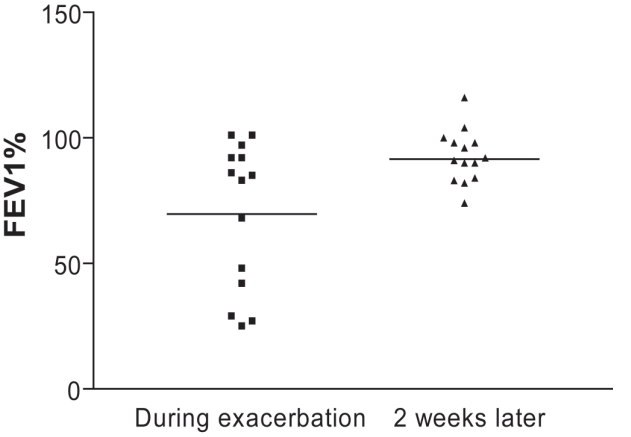

Figure 3. FEV_1% during asthma exacerbations and 2 weeks later (P=0.005). Horizontal lines represent mean values of eNO concentrations. N = 14.

Sputum analysis

The mean total cell count and percentage of eosinophils were higher during the asthma exacerbation (30.4%, 95%CI 21.1;39.6) then decreased at the follow up visit (16.0%, 95%CI 9.9;22.2), P=0.002. There was also a significant correlation between eNO concentration and percentages of sputum eosinophils during the exacerbation (P=0.04), but not two weeks later (P=0.3).

Discussion

This study shows that elevated eNO is associated with asthmatic exacerbations, and since it partially correlates with sputum eosinophilia it may measure the degree of inflammation. Measurement of eNO off-line is a practical way of assessing airway inflammation in asthma exacerbations since the patient is only required to breathe out into a gas impermeable bag. In contrast, spirometry and sputum

induction are difficult to perform in a dyspnoeic young children.

Symptom scores and PEF improved after treatment. Decreased eNO levels were accompanied by a significant improvement in the FEV_1% and PEF% (diurnal variability) when compared with the initial assessment. Symptoms and peak flow can monitor response to therapy and clinical improvement after an acute exacerbation.[24] GCS and $ß_2$-adrenergic agonists are the most important treatment and could decrease the risk of hospitalization and reduce the risk of readmission.[26-7] GCS can improve airway inflammation, reducing symptoms and airway hyper-responsiveness and also decrease eNO levels after an acute exacerbation. eNO may reflect an adequacy of GCS treatment and be an objective estimate of compliance[28] while spirometry is less sensitive.[29,30]

Sputum eosinophilia might be decreased by inhaled GCS.[31-3] It is associated with poorly controlled asthma, rather than with severity[34] and can be used to monitor the effects of anti-inflammatory treatment in a severe exacerbations.[35] Increased eNO and sputum eosinophils have been correlated with decreased lung function (FEV_1 and PEF).[36] There was a significant positive correlation between eNO and sputum eosinophils only during the exacerbation of asthma, although persisting mucosal eosinophilia after long term inhaled GCS treatment occurs in some patients.[37]

One of the obstacles in this study was recruiting asthmatic subjects with an acute exacerbation. During the course of the study, there was a marked fall in the number of hospital presentations for unclear reasons but might represent a decline in prevalence. The small sample size may influence the correlation between eNO and other markers of inflammation. Practical difficulties included obtaining sputum samples and spirometry from acutely ill children, and also the follow up.

Measurement of eNO could be useful for monitoring the degree of inflammation in children with asthma and to evaluate patient compliance in taking their asthma preventer medication. This study provides evidence that measurement of eNO is practical for emergency management of asthmatic patients by monitoring airway inflammation to avoid over-treatment with GCS. The usefulness of eNO as a method for assessing airway inflammation for controlling asthma in children could be validated with a longitudinal study that compares eNO against conventional methods,

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