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Measurement of inflammatory markers in the breath condensate of children with cystic fibrosis

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Measurement of inflammatory markers in the breath condensate of children with cystic fibrosis. S. Cunningham, J.R. McColm, L. Pei Ho, A.P. Greening, T.G. Marshall. ©ERS Journals Ltd 2000.

ABSTRACT: Identifying noninvasive markers of pulmonary inflammation would be useful in assessing new therapies in children. Breath condensate is a simple and potentially acceptable sample medium even in small children. The technique has previously been used in adults, but not children with cystic fibrosis.

The technique was assessed in 36 children with cystic fibrosis (mean age 10.4 yrs) and 17 control subjects, analysing samples for nitrite, interleukin(IL)-8 and salivary and nasal contamination. Correlations were made between levels of the inflammatory markers and forced expiratory volume in one second/forced vital capacity, chest radiograph score and use of inhaled steroids.

On samples without significant contamination (<10 u·L^{-1} amylase) nitrite was detected in 93% of samples at a median concentration of 3.0 μM compared with 50% of control samples at a median of 0.5 μM. Condensate amylase levels did not correlate with the nitrite value obtained (r=0.31). IL-8 was detected in 33% of CF samples.

Breath condensate is an acceptable method of sample collection in children. Nitrite was raised in breath condensate from patients with cystic fibrosis when compared with control subjects.

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Pulmonary inflammation in children with cystic fibrosis (CF) begins soon after birth [1], and arresting this progressive inflammation is central to CF research. It would be desirable therefore to identify a measure of pulmonary inflammation (single or combined), that is more sensitive than pulmonary function testing, noninvasive, repeatable and applicable to young children. The assessment of exhaled breath in CF has not been fruitful: nitric oxide levels are normal [2], or reduced [3] in CF when compared with control subjects, and exhaled pentane is raised in CF, but prone to gastrointestinal contamination [4].

The measurement of inflammatory mediators in breath condensate (exhaled aerosolized bronchoalveolar lining fluid), might provide a noninvasive, direct assessment of pulmonary inflammation. Condensate is capable of carrying molecules <65 kDa [5]: that could include the majority of pro-inflammatory pulmonary cytokines that are increased in CF. Nitrite [6], hydrogen peroxide [7], tumour necrosis factor-α and interleukin (IL)-6 [5] have been measured in breath condensate.

This technique, however, has not been applied to children with CF, and the aim of the current study was therefore to evaluate the reliability and acceptability of this technique, assessing whether children with CF have raised nitrite (like their adult counterparts [6]), and to assess for the first time whether IL-8, a dominant pro-inflammatory cytokine in CF [8], is found in measurable quantities in exhaled condensate.

Methods

The study was performed at the authors' regional paediatric CF centre (Edinburgh, Scotland, UK). Control samples were collected from children who were either staff relatives or attending fracture clinic with single stable fractures and no history of chronic respiratory illness. Children with CF were clinically stable at the time of assessment.

The equipment for condensate collection has previously been used successfully in adults, and consisted of a 1.5 m length of teflon tubing (internal diameter of 5 mm) immersed in ice [6]. Children were asked to make repeated blows against resistance from total lung capacity to functional residual capacity (FRC), resting if tired. They were asked to swallow any saliva. A condensate volume of 0.8–1.0 mL was obtained after 3–5 min.

Nitrite is vulnerable to rapid degeneration [6], so all samples were analysed for nitrite within 15 min of collection. Nitrite was measured on standard curves using the Griess reaction on triplicates of 200 μL condensate [9], at an absorbance wavelength of 540 nM (lower detection limit = 0.074 μM). Sample remaining was aliquotted and stored at -70°C. Measurement of IL-8 was made on batched samples by an "in house" enzyme linked immunosorbent assay (level of detection 0.5 pg·mL^{-1}).

Children with CF had routine pulmonary function tests (forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC)) performed at the time of condensate

collection. A note was made of the most recent Chrispin Norman chest radiograph score, and the use of inhaled steroids.

Two sources of contamination were possible; 1) nitric oxide is present in high concentrations in the nasal airways. Blowing against resistance raises the soft palate and obstructs nasal contamination of expired air in adults [6]. Four children who blew against resistance *via* teflon tubing as described, had no detectable helium measured in exhaled breath when 100% helium was provided to a mask placed over their nose; 2) saliva might contaminate condensate as it contains both nitrite and IL-8 [10]. Condensate amylase concentrations were therefore assessed as a marker of contamination (Synermed Europe, Burgess Hill, West Sussex, UK [11]).

Statistical analysis

Correlations between the condensate nitrite level obtained and measures of lung function and chest radiograph scores were made by Spearman Rank tests of association between nitrite level and use of inhaled steroids was by Mann Whitney U-tests. Associations between nitrite and IL-8 in CF patients and control subjects were assessed by Mann Whitney U-tests.

Consent

Informed consent was obtained from parents and children. Ethical approval was provided by the Lothian Research Ethics Committee, UK.

Results

Breath condensate samples were obtained in 36 children with CF and 17 control children. The mean age of CF children was 10.4 yrs (SD 2.7) and control children 10.2 yrs (SD 2.4). Samples were discarded from 3 children with CF and one control child because they were cloudy in appearance and therefore clearly contaminated; two CF children subsequently repeated the measurement after further instruction and clear samples were obtained. Sample analysis therefore is on 35 children with CF and 16 control children.

Assessment of contamination

Nasal. Helium was not detected in exhaled breath, indicating a lack of nasal contamination.

Saliva. Salivary contamination was assessed by measurement of α-amylase in condensate. A potentially significant level of α-amylase contamination (>10 u·L^{-1} amylase [12]) was found in 17 of 31 CF samples with sufficient volume after nitrite measurement. The median level of salivary amylase in all samples was 16 u·L^{-1} (interquartile range 3–350). In control children, amylase (>10 u·L^{-1}) was detectable in 10 of 16 samples, median 265 u·L^{-1} (interquartile range 4–1,211). Level of α-amylase contamination did not correlate with age (r=0.09), FEV1 (r=0.01) or nitrite concentration (r=0.31) in children with CF.

Table 1. – Nitrite values in breath condensate of cystic fibrosis patients and control children

Samples	NO_3- detected*	% of samples	Median value μM	Interquartile range μM
All samples				
CF	33 (35)	94	3.3	1.4–8.0
Control	9 (16)	56	1.0	0–3.7
Samples <10 u·L^{-1} amylase				
CF	13 (14)	93	3.0	0.8–6.1
Control	3 (6)	50	0.5	0–3.7

*: with number of samples in parentheses.

Nitrite measurement

Nitrite was present in 33 of 35 CF condensate samples (93% of samples, median 3.3 μM) compared with 9 of 16 control children (56% of samples, median 1.0 μM; table 1). This difference was statistically significant (p=0.018). When only condensate samples with <10 u·L^{-1} amylase were considered, 93% of CF condensate samples continued to have nitrite detected at a median of 3.0 μM (13 of 14 samples), and nitrite was detected in 50% of control samples at a median of 0.5 μM (3 of 6 samples). This difference was no longer statistically significant (p=0.34). The distribution of nitrite values in patients with CF and control children are shown in figure 1. The spread of values in patients with CF is greater than that of control children irrespective of potential contamination, and it appears that contamination had minimal effects on the results.

There was no significant correlation between condensate nitrite values (<10 u·L^{-1} amylase) and age, FEV1, FVC or chest radiograph score. At the time of study, 18 CF children were using inhaled steroids (mean age 10.7 yrs), and 17 children were not (mean age 10.2 yrs). Use of inhaled steroids by children with CF did not affect breath nitrite measurement (median 4.0 μM steroid *versus* 2.3 μM no steroids; p=0.55).

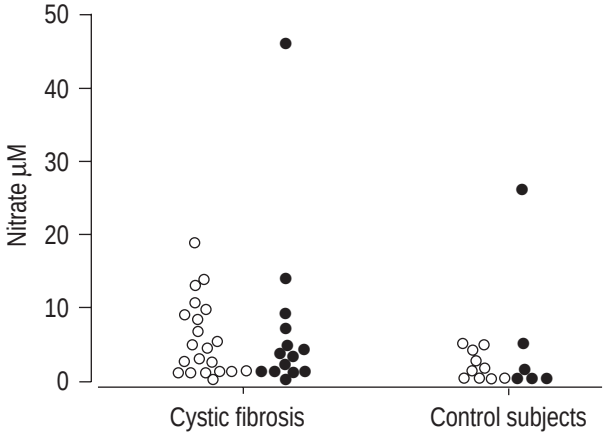

Fig. 1. – Distribution of nitrite in breath condensate of children with cystic fibrosis and control subjects. ●: uncontaminated values; ○: contaminated values.

Interleukin-8 measurement

IL-8 was present in 7 of 21 available CF condensate samples at a median of 47 $pg \cdot mL^{-1}$ (interquartile range 8–90), and 6 of 10 available control condensate samples at a median of 10 $pg \cdot mL^{-1}$ (interquartile range 5–49); this difference was not statistically different (p=0.18). IL-8 did not correlate with amylase level detected (r=-0.15). Apart from 3 CF samples, all samples contained >10 $u \cdot L^{-1}$ amylase.

Discussion

The authors have demonstrated that nitrite and IL-8 can be detected in the breath condensate of children with CF. Nitrite was raised in children with CF when compared with control children. α-Amylase was detected in many samples, though usually at extremely low levels. Figure 1 would suggest that the contribution of contamination to condensate nitrite values was minimal, with CF children continuing to have raised values compared with control children when contaminated samples were excluded. Nitrite has not previously been assessed in CF children, however, the current values are comparable with CF adults (median nitrite 1.9 μM compared with 0.3 μM in control subjects [6]). Nitric oxide is a neutrophil derived inflammatory mediator, and nitrite is presumed to result from degradation within CF mucus. Although inducible nitric oxide synthase is poorly active in CF respiratory epithelium [13], excess neutrophil presence in CF airways could account for raised nitrite values [14]. Nitrite therefore represents a potentially useful inflammatory marker in CF, that requires further investigation in longitudinal studies.

The technique used in this study has previously been used in adults without amylase contamination [6]. An earlier study found protein contamination of most samples at very low levels (using two dimensional gel electrophoresis) [5]. Other condensate studies have either not attempted to identify contamination [7, 15], or have not demonstrated contamination in healthy children [12]. This is the first study to assess potential contamination in children with respiratory disease, however there was no correlation between age or FEV_1 and amylase in condensate.

IL-8 is an important pro-inflammatory mediator in CF. Although IL-8 was measurably in condensate, disappointingly it was present in only 33% of CF samples, though values in those samples were higher than in control children. Further work is needed to correlate simultaneous sputum and condensate IL-8 values. In principle, many cytokines could be carried in condensate (*e.g.* IL-6, TNF-α, IL-10), and an investigation of these could prove useful.

The measurement of breath condensate was acceptable to children and parents, and therefore repeated measures could be made with consent.

These studies have demonstrated that breath condensate is a potentially useful technique for the assessment of inflammatory markers in breath condensate of children with cystic fibrosis. Further work should evaluate methods of concentrating samples, identifying other low molecular weight inflammatory mediators and reducing potential contamination of samples in children with respiratory disease.

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