

8-ISOPROSTANE IN NASALLY EXHALED BREATH CONDENSATE IN DIFFERENT PEDIATRIC LUNG DISEASES

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Abstract

Objective: Increased levels of 8-isoprostane were found in various human lung diseases suggesting 8-isoprostane as a marker of pulmonary oxidative stress *in vivo*. The exact role in pediatric lung diseases has not been defined yet. The goal of this study was to clarify the role of 8-isoprostane in nasally exhaled breath condensate as possible marker of oxidative stress in children with different lung diseases.

Methods: Levels of 8-isoprostane were measured in nasally exhaled breath condensate of 29 cystic fibrosis patients, 19 children with a history of wheezing episodes, 8 infants with acute respiratory tract infection and 53 healthy subjects using a specific enzyme immunoassay.

Results: Levels of 8-isoprostane did neither discriminate between different disease groups nor correlate with lung function in cystic fibrosis patients.

Conclusions: Levels of 8-isoprostane in nasally exhaled breath condensate do not reflect oxidative stress in children with different lung diseases.

Key words: Exhaled breath condensate, 8-isoprostane, cystic fibrosis, children, lung diseases

INTRODUCTION

Exhaled breath condensate (EBC) is widely studied in order to find useful biomarkers for the non-invasive evaluation of lung disease [1, 2]. 8-isoprostane is a stable peroxidative product formed by oxidative metabolism of arachidonic acid and is thought to be a reliable marker of oxidative stress [2, 3]. Increased levels of 8-isoprostane were found in a variety of inflammatory lung diseases, including patients with COPD, smokers

[4], patients with cystic fibrosis (CF) [5] and asthmatic subjects, both in adults [6] and children [7, 8]. 8-isoprostane has therefore been suggested as a marker of oxidative stress in inflammatory lung diseases [9] to assess and monitor the disease severity in chronic lung disease like cystic fibrosis or asthma. Although methodological concerns regarding the collection of breath condensate as well as the detection of 8-isoprostane have been raised [10], new studies claim the usefulness of this oxidative marker especially in pediatric lung diseases like asthma [8].

The aim of this study was to examine the role of 8-isoprostane as a disease marker in EBC of children with different lung diseases in comparison to healthy subjects. In order to be able to collect breath condensate from very young and thus non-cooperative children and even infants, EBC was collected via nasal prongs as previously described [11]. Therefore, the value of 8-isoprostane especially in these young infants could be examined.

MATERIALS AND METHODS

SUBJECTS

The population of this cross-sectional study consisted of 29 CF patients with a great variety of lung function status in order to assess a large range of disease activity, 19 children with a history of wheezing episodes during a symptom-free interval, 8 infants with acute respiratory tract infection (acute bronchitis) and 53 healthy control subjects. All subjects were non-smokers. Pulmonary function tests could be performed in 25 of the 29 CF patients. Further details of the subjects are given in Table 1. The study was approved by

Table 1. Characteristics of the subjects.

	Healthy controls	Cystic fibrosis	ARTI	wheeze
Number of subjects (male)	53 (25)	29 (15)	8 (4)	19 (10)
Age in years ¹	11.2 (0.1 – 28)*	14.2 (2 – 30)*	1.0 (0.1 – 3.2)*	2.0 (0.2 – 4.9)*
FEV1 [%-pred] ¹	n.a.	63 (20 – 117)	n.a.	n.a.

¹ given as mean and range

* there was a significant difference in age distribution between all groups, except for healthy controls and cystic fibrosis patients as well as patients with acute respiratory tract infection and history of wheezing episodes.

the Institutional review board.

COLLECTION OF BREATH CONDENSATE

EBC was collected by suction directly under the nostrils into tubes. Using a pump, the exhaled air was collected at a flow of 11.5 l/min through nasal prongs into a cold trap that holds two serially connected 50 ml plastic tubes. The whole system was closed, allowing air entrance from the nasal prongs only. A detailed description of the set-up of the apparatus as well as the reproducibility of the method is given elsewhere [11].

EBC was collected for 10 to 30 minutes when the subjects were breathing quietly through their nose and between 0.8 and 2.4 ml condensate were collected. On

the sampling days the room temperature was between 22°C and 26°C, the atmospheric pressure between 913 and 943 hPa and the humidity between 40 % and 51 %.

LABORATORY ANALYSIS

8-isoprostane concentrations in EBC were measured by a specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan, USA) according to the manufacturer's instructions. After collection, the samples were immediately frozen at -70°C. After thawing, samples were measured in duplicate and the standard values were obtained with triple measurements. The assay had a lower detection limit of 4 pg/ml and had been used for the detection of 8-isoprostane in EBC

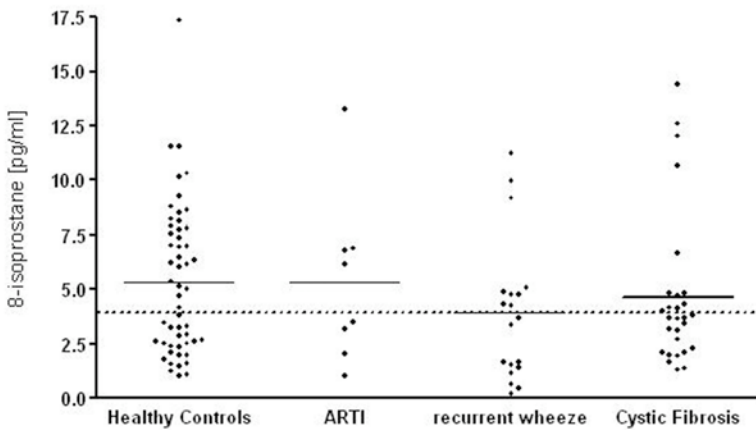


Fig. 1. Levels of 8-isoprostane in pg/ml for the different disease groups and healthy controls. The dashed line illustrates the detection limit of the assay of 4 pg/ml. The solid line indicates the mean of the respective group.

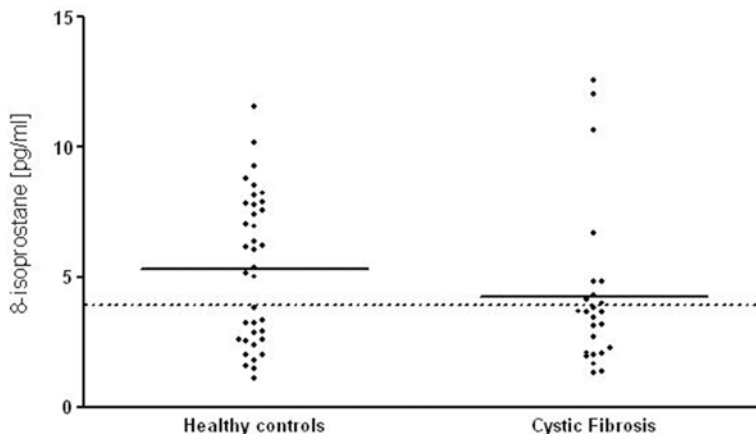
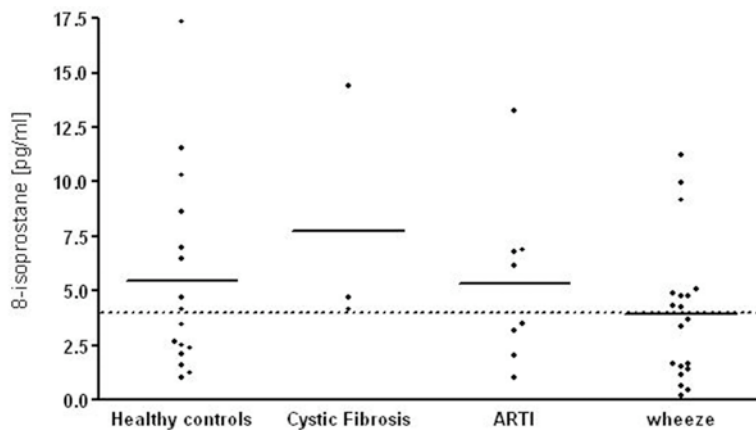


Fig. 2. Levels of 8-isoprostane in pg/ml for the different disease groups and healthy controls for subjects with age less than five years in Figure 2a) and subjects with age above five years in Figure 2b). The dashed line illustrates the detection limit of the assay of 4 pg/ml. The solid line indicates the mean of the respective group.

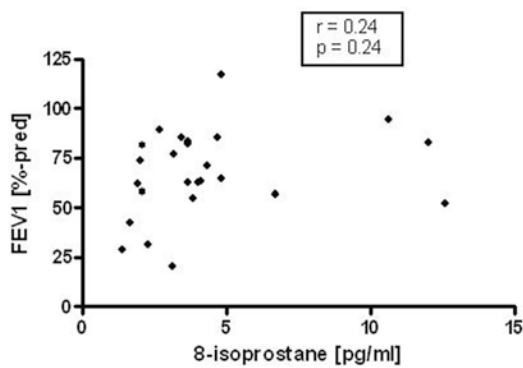


Fig. 3. Relation of lung function values as FEV1 [%-predicted] and 8-isoprostane levels [pg/ml] in cystic fibrosis patients. No correlation was found (Pearson $r = 0.24$, $p = 0.24$).

before [5].

STATISTICS

Data are given as mean \pm standard deviation if not mentioned differently. Comparisons between several groups were done with the Kruskal-Wallis Test; comparisons between two groups were performed with the Mann-Whitney-U Test. A p-value of 0.05 was regarded as significant.

RESULTS

8-isoprostane was detected in 13 of the 29 CF patients (45%), in nine of the 19 wheezing infants (47%), in four out of the eight children with acute respiratory tract infection (50%) and in 29 of the 53 healthy subjects (55%, Fig. 1).

No difference in the 8-isoprostane concentration was found between the disease groups or the controls, with 5.4 ± 3.4 pg/ml in the healthy subjects, of 4.6 ± 3.5 pg/ml in the CF group, 3.9 ± 3.2 pg/ml in the infants with a history of wheezing episodes and 5.4 ± 3.9 pg/ml in infants with acute respiratory tract infection (Fig. 1).

The detection and the concentration of 8-isoprostane were independent of the age or the gender of the subjects.

A subgroup analysis with age-matched groups did not show any different results between the groups (Fig. 2).

Despite a trend towards higher 8-isoprostane levels in children with high FEV1 values, no significant correlation was found in CF patients between their lung function values and the 8-isoprostane concentrations in exhaled breath condensate (Fig. 3). In the CF patients, no significant difference in 8-isoprostane levels was found between patients with *Ps. aeruginosa* colonization (5.0 ± 3.8 pg/ml; $n = 16$) and without *Ps. aeruginosa* colonization (4.4 ± 3.4 pg/ml; $n = 13$).

DISCUSSION

In this study we demonstrate for the first time, that 8-isoprostane is measurable in nasally collected EBC, even in infants as young as one month. We also show

that 8-isoprostane concentration in EBC lies above the limit of detection for a commercially available assay in only 55 out of 109 subjects (50%), independent of their underlying disease, their lung function or their age.

These findings are in contrast to the results of previously published studies. Montuschi et al. recently demonstrated a significant difference between adult CF patients and healthy non-smoking control subjects [5]. The measured 8-isoprostane values in their study were much higher than in our study, in the CF patients (mean 42.7 pg/ml in their study compared to 4.0 pg/ml in our study) as well as in the healthy subjects (mean 15.2 pg/ml in their study compared to 5.0 pg/ml in our study). As 8-isoprostane did not increase with age in our study and as the same group of investigators also found higher levels of 8-isoprostane in children with asthma [7], age related factors as the sole reason for the different results can be excluded.

Several methodological issues could be the reason for these different findings. The difference in the collection method could lead to different dilutions and constituents of the breath condensate [12]. The influence of the upper airways on the components of nasally collected breath condensate might be much higher in nasally collected than in orally collected breath condensate. However, this limitation applies not only to all studies that collect oral breath condensate without a nose clip (see also Table 2), but especially cannot be avoided when sampling EBC from very young and non-cooperating children. Above this, the freezing and thawing processes are crucial when examining unstable mediators in exhaled breath condensate [9] and the sensitivity of the assay at low concentrations might also contribute to above mentioned differences.

Indeed, a recently published article concluded that different collection methods as well as differences in the storage and analyzing procedures may represent the main reasons for the diverse results between the study groups [13-15]. In line with our results, van Hoydonck et al. did not detect 8-isoprostane concentrations in EBC of healthy smokers in more than half of the measurements neither [10] and van der Meer et al. were not able to obtain reproducible values in asthmatic and normal subjects [16]. They conclude, that levels of 8-isoprostane cannot be reproducibly assessed in EBC due to the low concentrations and the lack of sensitivity of the assays used [10]. The findings of our study expand this knowledge to other patient groups as well as to younger subjects. Taken together, various research groups obtained contradictory results of 8-isoprostane in oral and nasal breath condensate of patients with different lung diseases and underlying inflammatory processes (Table 2).

A recent study in cystic fibrosis patients did not show any change of 8-isoprostane levels in bronchoalveolar lavage after 14 days of inhalative anti-oxidative therapy with glutathione [17]. Nevertheless it is not clear, whether 8-isoprostane in breath condensate does really not correlate with the inflammatory status and thus is per se not a useful marker of oxidative stress or whether the difficulties in the methodological aspects are the cause of these confounding results. We

Table 2. Overview of 8-isoprostane measurements results in exhaled breath condensate in various disease groups¹.

Reference	Subject groups	EBC Sampling	Samples in which 8-isoprostane was detected	8-isoprostane in controls ²	8-isoprostane in disease groups ²
This study	29 CF patients 19 wheezing patients 8 patients with LRTI 53 control subjects	Nasal prongs	55 / 109 (50%)	5.0 (SD 3.7)	4.0 (SD 3.8) CF patients 3.9 (SD 3.2) wheezing 5.4 (SD 3.9) patients with LRTI
Shahid et al. 2005 [8]	25 asthmatics 11 control subjects	Eco Screen ^{®5} Nose-clip	24 / 36 (67%)	3.8 (SEM 0.6)	9.3* (SEM 1.7) steroid naive 6.7* (SEM 0.7) ICS ⁴ treated
Van Hoydonck et al. 2004 [10]	Eight smokers at three time points	Eco Screen ^{®5} Nose-clip	15 / 36 (42%)	n.g.	n.g.
Baraldi et al. 2003 [7]	42 asthmatics 12 control subjects	Oral sampling No nose-clip	n.g.	34.2 (SEM 4.5)	56.4* (SEM 7.7) steroid naive 47.2* (SEM 2.3) steroid treated
Zanonato et al. 2003 [20]	36 asthmatics 19 control subjects	Oral sampling No nose-clip	n.g.	3.5 (IQR 2.6 – 7.9)	16.2* (IQR 11.7 – 19.1) steroid naive 18.1* (IQR 14.8 – 20.5) ICS ⁴ treated, stable 29.7* (IQR 4.8 – 35.1) ICS ⁴ treated, unstable
Antczak et al. 2002 [21]	31 AIA patients ⁴ 26 ATA patients ⁴ 16 Controls	Oral sampling Nose clip	n.g.	21.9 (SEM 4.5)	90.0* (SEM 19.0) AIA ⁴ 79.2* (SEM 19.5) AIA ⁴
Montuschi et al. 2000 [5]	19 CF patients 10 control subjects	Oral sampling Nose clip	n.g.	15.2 (SEM 1.7)	42.7* (SEM 4.5) CF patients
Montuschi et al. 1999 [6]	44 asthmatics 10 control subjects	Oral sampling Nose clip	n.g.	15.8 (SEM 1.6)	33.7* (SEM 2.8) mild asthmatics 38.3* (SEM 3.7) moderate asthmatics 49.1* (SEM 5.0) severe asthmatics

¹ All results have been obtained using a commercially available enzyme-linked immunosorbent assay (Cayman Chemical, Ann Arbor, MI). The detection limit of the assay lies at 4 pg/ml.

² Concentrations are given in pg/ml.

³ n.g. – not given.

⁴ ICS – inhaled corticosteroids. AIA – aspirin induced asthma. ATA – aspirin tolerant asthma.

⁵ Eco Screen[®], Erich Jaeger GmbH, Hoechst, Germany

* indicates significant differences compared to control subjects.

therefore suggest first to solve methodological issues, e.g. to use mass spectrometry [18] or to improve measurement assays [14] in order to obtain reliable results. In a second step the exact role of 8-isoprostane in EBC as a marker of oxidative stress in lung disease should be assessed. Perhaps other markers in exhaled breath condensate might reflect oxidative stress in exhaled breath condensate better, as e.g. leukotriene B4 [19].

In conclusion, 8-isoprostane was detectable in about half of the subjects' nasally collected EBC samples. No difference in 8-isoprostane values was found between CF patients, children with a history of a wheezing episode, infants with acute lower respiratory tract infection and healthy control subjects. This makes 8-isoprostane in nasally collected EBC not a valuable disease marker for different pediatric lung diseases. Our findings further support the recently published opinions, that standardized and better comparable collection procedures as well as more sensitive assays are needed before biomarkers like 8-isoprostane can be used widely in research projects or even as clin-

ical application tool [2, 15].

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