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pH in nasal exhaled breath condensate in healthy adults*

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SUMMARY

This paper describes a new method to assess nasal pH in nasal exhaled breath condensate in adults.

The study included 19 healthy, non-smoking, adult volunteers without current upper respiratory disease, COPD or asthma. Expiratory breath condensate (EBC) was collected from the nose and mouth respectively. A Jaeger breath condenser was fitted with a transparent facemask and during oral sampling it was fitted with a mouthpiece. The condensates were bubbled with argon gas for 10 minutes to reduce carbon dioxide and the samples were analysed immediately using a calibrated glass electrode and a portable pH-meter.

The mean pH in the nasal EBC was 7.0 ± 0.5 and in oral EBC 6.9 ± 0.7 ($p = 0.6$). The nasal EBC-pH was well in agreement with data from previous studies, which measured pH directly on the nasal mucosa with an electrode.

In conclusion, this paper describes a method to measure pH in nasal exhaled breath condensate (EBC) in healthy adults during tidal breathing. The nasal EBC-pH related well to previous data from intranasal pH measurements suggesting that nasal EBC-pH could have a role in monitoring pH alterations in the nasal mucosa such as nasal inflammation.

Key words: nasal EBC pH, exhaled breath condensate, non-invasive, glass-electrode, nasal breathing

INTRODUCTION

Exhaled breath condensate (EBC) is a new method to indirectly assess the fluid composition of the respiratory mucosal lining by condensing the moisture in exhaled air [(1)]. This method has predominantly been applied to assess exhaled air from the lower airways by allowing airway inflammation to be monitored non-invasively. In 2000, Hunt et al. described that patients with asthma had a lower pH in EBC during exacerbations and that this measure can be used to monitor asthma patients [(2)]. Data from a study that measured pH directly on the nasal mucosa showed that the pH increases during rhinitis, which suggests that measuring nasal pH could also have a role in monitoring upper airway inflammation [(3)]. Results from measurements of nasal pH in healthy adults using intranasal pH probes varies between 5.2 and 8.1 with a mean of approximately 6-7, which rises to 7.2-8.3 in rhinitis [(3-7)] (Table 1).

Direct pH measurements on the nasal mucosa involves the use of an electrode, placed under the inferior turbinate, which is associated with a high risk of reflex mediated nasal secretion that can alter the nasal fluid pH [(8)]. This is especially important in subjects with rhinitis where nasal hyper reactivity makes the

Table 1. Shows the results from previous studies measuring intranasal pH in healthy volunteers.

Author	group	probe	mean nasal pH
Shusterman [(4)]		glass electrode	7.04-7.7
n=8	age 21-56	4 cm from nostril	no mean presented
Washington [(5)]		coupled electrodes	anterior 6.4 (5.2-8.1)
n=12	age 18-50	0-3 cm from nostril	posterior 6.3 (5.2-8.0)
Ireson [(6)]		glass electrode	
	mean age 42	4 cm from nostril	
n=24	black ethnicity		6.44
n=26	white ethnicity		6.91
Hehar [(7)]		coupled electrodes	anterior 7.1
n=4		1-4 cm from nostril	posterior 6.6

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nasal mucosa particularly sensitive. Introduction of a probe into the nasal cavity is also perceived as uncomfortable by the patients (our own experience), which further limits the utility of this method.

We have previously described how nasal EBC can be readily collected in a closed system during 10 minutes of tidal nasal breathing using a breath condenser fitted with a transparent facemask without interfering with normal nasal function and with little discomfort to the subjects [9]. The aim of the present study was to evaluate if this method can be used to assess pH in nasal EBC in healthy adults and how nasal EBC-pH relates to pH from studies using intranasal probes in healthy subjects. Exhaled air has a common origin, the lungs. We hypothesised that the different passages (nose vs mouth) could modulate the pH differently and we thus have evaluated how nasal EBC-pH relates to oral EBC-pH in the same individuals.

MATERIALS AND METHODS

Volunteers

This study included 19 healthy volunteers (11 females, 8 males, mean age 43.3 years). Exclusion criteria were current smoking, a history of perennial rhinitis or nasal hyper reactivity, previous nasal surgery, asthma, COPD or ongoing nasal medication. Subjects with a history of hay fever were not excluded since the study was performed out of the pollen season. All subjects were included after informed consent. The present study was approved by the local Ethics Committee at the Sahlgrenska Academy, Göteborg, Sweden.

Procedure

Exhaled breath condensates were collected from the nose and the mouth using an Eco Screen® breath condenser (Jaeger, Würtzberg, Germany), fitted with a two-way, non-rebreathing valve and a saliva trap (Figure 1). Depending on the measuring mode, the inlet was fitted with a mouthpiece for oral sampling or a facemask (In-Check, Clement Clarke, England) for nasal sampling. Breathing volumes were monitored with a dry spirometer (Jaeger, Würtzberg, Germany). The exhaled air was led into a Teflon coated, aluminium lamellar tube with a polyoxymethylene container embedded in the condenser at a temperature of -20°C [9].

The condensate was poured into a 1.8 ml polypropylene tube (Nunc®, Roskilde, Denmark) and argon gas (AGA, Gothenburgh, Sweden) was led through the sample for 10 minutes to minimise the CO^2 levels [2]. The pH in the condensate was measured with a glass electrode ("minitrode", Hamilton, Bonaduz, Switzerland) connected to a portable pH-meter (WTW 330, Weilheim, Germany). The glass electrode was calibrated and cleaned with distilled water before it was placed in the sample. The pH was registered after stabilisation of the reading, with the duration variying between 5 and 10 minutes.

To standardise the breathing procedure, the subjects inhaled through the nose both at nasal and oral EBC sampling. The sampling was stopped after 100 litres of expired air for each breathing mode. During the two measurements, the subjects were asked to breathe quietly and at a similar breathing rate. The total expired volume in litres and the accumulated time of the expirations in minutes were registered by the spirometer connected to the breathing valve of the condenser. The spirometer was calibrated before each measurement session using a 3-litre calibration syringe (Hans Rudolph Inc, Kansas City, USA).

The present study included human subjects. Breathing into a mouthpiece or facemask for approximately 10 minutes can be perceived as uncomfortable, however the collection of EBC was well tolerated by the subjects in this study.

Figure 1. The study set-up: An Eco Screen® breath condenser (Jaeger, Würtzberg, Germany), fitted with a two-way, non-re-breathing valve and a saliva trap. For nasal sampling the inlet was fitted with a face mask (In-Check, Clement Clarke, England) Breathing volumes were monitored with a dry spirometer (Jaeger, Würtzberg, Germany).

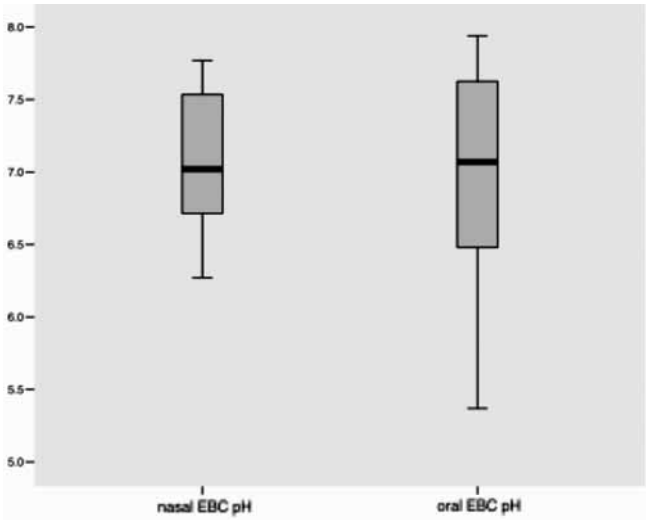

Figure 2. Box plot of the nasal (to the left) and oral (to the right) exhaled breath condensate pH for all subjects.

Table 2. Baseline data for the study population.

	Men	Women	All
n	8	11	19
Age (years)	40.4	45.5	43.3
BMI (kg/m2)	24.7	22.9	23.6
Hay fever	1	8	9

Table 3. The mean result from nasal exhaled breath condensate, EBC-pH and oral exhaled breath condensate EBC-pH. Standard deviation within brackets.

pH	Men	Women	All
Nasal EBC	7.1 (0.5)	7.0 (0.5)	7.0 (0.5)
Oral EBC	7.0 (0.8)	6.9 (0.7)	6.9 (0.7)

Difference nasal vs oral: $p = 0.6$

Statistical analyses

The comparison between nasal and oral EBC was made with paired t-test and the comparison between men and women was made with a t-test in the statistical software pack SPSS 13.0 for Windows.

RESULTS

Baseline data for the subjects are presented in Table 2. There was a predominance of women in the group, and more women than men had a history of hay fever (8 vs 1) but no subjects reported present nasal symptoms.

The mean nasal EBC-pH was 7.0 ± 0.5 and the oral EBC-pH was 6.9 ± 0.7 (Figure 2, Table 3). There was no significant difference between nasal EBC-pH and oral EBC-pH ($p = 0.6$). There was no significant difference in nasal EBC-pH or oral EBC-pH between men and women (nasal 7.1 vs 7.0, $p = 0.3$ and oral 7.0 vs 6.9, $p = 0.3$).

DISCUSSION

This is to our knowledge the first study to evaluate pH in nasal EBC collected during tidal nasal breathing in healthy adults. The nasal EBC pH was well in the range of the pH found in previous studies that measured pH directly on the nasal mucosa with a glass electrode. There was no significant difference between mean nasal EBC pH and mean oral EBC pH. Nasal EBC-pH is a non-invasive procedure that is easy to perform. The results suggest that nasal EBC-pH could have a role in monitoring upper airway inflammation.

Limited data showing that nasal pH increases during rhinitis suggest that assessment of nasal pH has a utility in monitoring upper airway inflammation [3]. There is, however, a lack of studies analysing nasal pH in large population samples. Normal nasal pH, based on intranasal pH assessments in a limited number of subjects, show a mean between 6 and 7, which is well in accordance with the mean nasal EBC-pH of 7.0 in this study (Table 1). The main advantage with nasal EBC-pH in contrast to direct measurements of nasal pH is that the procedure does not interfere with the nasal mucosa. The introduction of a catheter into the nasal cavity is often painful and can result in reflex mediated nasal discharge that could alter the intra nasal pH [8].

The EBC technique is dependent on nasal breathing which means that subjects with a severe nasal obstruction will not be able perform the test. Changes in nasal mucosa pH due to airway inflammation may also not be reflected in the nasal EBC-pH since EBC is not equal to nasal mucosal fluid. In oral EBC studies, it has for instance been found that droplets of epithelial lining fluid are diluted up to 20.000 fold with condensed water vapour [10]. Nevertheless, oral EBC-pH has proven to be useful in the assessment of lower airway inflammation [2].

When measuring pH in exhaled air collected at the nose, the relative contribution to pH from the nose and the lungs is an issue. In the standardised procedure used to collect EBC from the lungs (inhaling and exhaling through the mouth), a nose clip is applied to avoid "contamination" of air from the nose, and thus the nose is by-passed [10]. In the present study, where healthy subjects inhaled through the nose, we found no significant difference between nasal and oral EBC-pH. This means that in the absence of pulmonary disease changes in nasal EBC-pH measured by this novel method is likely to be related solely to pH changes in the nose. By adding a measurement of oral EBC-pH with a nose clip it should also be possible to determine any contribution to pH from the lower airways.

Techniques have been reported how to assess nasal EBC in children such as pumping air from nasal prongs during tidal nasal breathing into a cold-trap or using a facemask connected to a condenser similar to our method [11,12]. In order to minimize the need for co-operation when measuring children some concerns could, however, be raised regarding systems open to ambient air, long duration of EBC sampling (>10 minutes) or the subjects compliance to strict nasal breathing, all factors that can affect nasal EBC-pH. Along with age this may also explain why nasal-EBC pH in children was recently reported to be significantly lower in subjects with allergic rhinitis than in healthy controls, opposite to the findings in rhinitic adults [13].

In the sampling of oral EBC-pH it has been suggested that oral ammonia contribution to EBC could alter oral exhaled EBC-pH, however in a recently published study, oral ammonia was shown not be an important determinant of oral exhaled EBC-pH [14]. The contamination of droplets of saliva containing ammonia could also change the pH. To control for this, a saliva trap was used in the condenser in this study [10].

It has previously been described that CO_2 in the condensate lowers the pH in EBC. For that reason the condensate samples were immediately bubbled with Argon gas for 10 minutes as described by Hunt et al. to reduce CO_2 [2]. It has also been shown by Niimi et al. that previously frozen EBC samples show significantly higher pH values than corresponding fresh samples and thus samples were analysed directly on collection in this study [15].

The results from this study indicate that assessment of nasal EBC-pH, being non-invasive, fast and easy to perform should be evaluated further for its possible use in monitoring pH in the upper airways during nasal inflammation.

In summary, this paper describes a new non-invasive method to measure pH in nasal EBC in healthy adults during tidal breathing. The nasal EBC-pH related well to previous data from intranasal pH measurements suggesting that nasal EBC-pH could have a role in monitoring pH alterations in the nasal mucosa such as nasal inflammation.

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