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ORIGINAL ARTICLE

ASSESSMENT OF EXHALED BREATH CONDENSATE PH IN ASTHMATIC CHILDREN

By

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Background: *Assessment of the Exhaled breath condensate (EBC) constituents has proven to be useful and non invasive method for monitoring airway changes. On the other hand, active airway inflammation was postulated to change the pH of the airway lining fluid.*

Objective: This work was conducted to study EBC pH in children with bronchial asthma compared to other airway inflammatory markers in those children.

Methods: *Thirty asthmatic children were recruited for this study while attending the Chest Clinic of Paediatric Hospital -Ain Shams University-Cairo, Egypt. Their ages ranged between 5-16y with mean value of (8.93±4.14 years). Age and sex matched 10 normal children were selected as controls. All the children were subjected to history taking, thorough clinical examination, spirometric pulmonary function testing (for FEV1, FVC, FEF25-75%), collection of EBC was done to assess its pH and induction of sputum to determine differential cell counts.*

Results: *Our results showed that the mean values of EBC pH were significantly lower in asthmatic group when compared to controls with cut off value = 7.35 (specificity=70%, sensitivity= 89%). The mean values of EBC pH showed significant positive correlations with pulmonary function measured parameters - FEV1, FVC, and FEF25-75%- ($p<0.05$, $p<0.001$, $p<0.05$ respectively) and significant negative correlations with sputum cell counts; total leucocytic counts, oesinophilic counts and neutrophilic counts ($p<0.05$).*

Conclusion: *Exhaled Breath condensate (EBC) pH is a simple, inexpensive and non invasive promising tool to evaluate pediatric asthma.*

Keywords: *Exhaled breath condensate (EBC), pH, asthma, children.*

INTRODUCTION

Airway inflammation has an important pathophysiological role in respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD).[1] Monitoring of airway inflammation might be useful in the follow up of patients with respiratory diseases, and for guiding pharmacological therapy.[2]

Quantification of inflammation in the lungs is currently based on invasive methods including the analysis of bronchoalveolar lavage (BAL) fluid, bronchoscopy, and bronchial biopsies, semi-invasive methods such as sputum induction and the measurement of inflammatory biomarkers in plasma and urine which are likely to reflect systemic rather than lung inflammation.[3,4]

Exhaled breath consists of a gaseous phase that contains volatile compounds (e.g. nitric oxide, carbon monoxide, and hydrocarbons) and a liquid phase, termed exhaled breath condensate (EBC), that contains aerosol particles in which several nonvolatile compounds have been identified.[1,3,5]

Recently, attention has focused on EBC as a noninvasive method for studying the composition of airway lining fluid. EBC analysis of inflammatory biomarkers is a noninvasive method which has the potential to be useful for monitoring airway inflammation in patients with respiratory diseases, including children.[5] As it is completely noninvasive, EBC also is suitable for longitudinal studies and for monitoring the response to pharmacological therapy.[6]

EBC is an excellent trap for water-soluble volatile gases. These gases may be acidic or basic. Acidification of EBC, often pronounced, occurs during disease states and appears to reflect acidification of the source fluid (the airway lining fluid).[2]

The aim of the study was to assess the pH of expired breath condensate (EBC) in Egyptian children with bronchial asthma. The EBC pH was studied in comparison to other markers of airway inflammation in the studied asthmatic children.

PATIENTS AND METHOD

This study was conducted on 30 children, 15 males and 15 females. Their ages ranged between

- ***Study design:*** A cross sectional case control descriptive study performed with a sample size of 30 asthmatic children and ten healthy controls was considered appropriate (confidence level 80% of study power more than 85% - calculated using Epi Info software for windows, version 3.3.2; Atlana, CDCP; 2005). Subject details were obtained and then baseline spirometry was performed, followed by collection of exhaled breath condensate and finally sputum induction.
- ***Subjects:*** This study was conducted upon 40 Egyptian children Table 1. It included 30 asthmatic children, 16 males and 14 females attending the chest clinic of pediatric hospital, Ain Shams University .Their ages ranged from 5 -16 years with mean (8.933 ± 4.14).

Table 1. Subjects Characteristics.

	Controls	Children with asthma (30 children)			
		Mild Intermittent	Mild Persistent	Moderate	Severe
Number	10	6	10	8	6
Age (year)*	8.8±3.5	8.6±2.1	8.5±2.5	9.1±2.8	8.9±2.3
Sex M/F	5/5	3/3	6/4	5/3	4/2
FEV_1 % predicted*	93.4±1.7	91.3±3.4	86.6±3.2	70.5±4.4	51.6±2.58

The diagnosis and classification of asthma in those patients was based upon the Global Initiative for Asthma (GINA guidelines 2007.[7] All the included asthmatic children were above 5 years of age, so the child can perform the pulmonary function tests and can collect EBC. Children with any other co-morbid chronic diseases were excluded.

Healthy control subjects were recruited from siblings of children attending the Pediatric Asthma Clinic or from children of staff. A parent was interviewed to confirm the lack of past or present lower respiratory disease. This group included 10

healthy children age and sex matched. Five males and 5 females. Their ages ranged from 5-16 years with mean (8.8 ± 3.52).

Approval to conduct this study was obtained from the local ethics committee for human studies in Ain Shams University. Written informed consent was obtained from the parents of all children.

- ***Methods: Pulmonary function tests:*** was done with MIR spirobank pocket spirometer (Product Code: AW910500). Built-in software measurements were compatible with the American Thoracic Society criteria. Measured spirometric parameters were compared with predicted Mediterranean population normal values from Roca and colleagues,[8] and were reported as percent of predicted.

The data obtained from forced expiratory manoeuvre were:

- FVC: forced vital capacity.
- FEV1: forced expiratory volume in first second.
- FEV1 / FVC ratio.
- FEF25-75 %: forced expiratory flow rate over 25-75 % part of FVC.

For every parameter obtained, actual and predicted values for age, sex, height and weight were calculated and the percentages (%) from predicted values were calculated.

Expired Breath Condensate Collection: It was done by the exhaled breath condensate (EBC) tube prepared by the National Research Center for measurement of EBC pH (Fig. 2).

Collection device of EBC: The collection of breath condensate was performed in a double jacketed coiled tube of 45 cm length (an internal diameter 4 mm and external diameter 7 mm), consisting a cooling pipes with pH solution in a basket of ice. A rubber mouth piece connector – protected by a valve to trap saliva – was attached the collecting system.

Method of collection: Patient breathes in relaxed manner. It usually takes 15-20 min in children to obtain 1-3 ml of condensate. Sample collection temperature was approximately -4 to -17 c. Stable pH was achieved in all cases after de-aeration with an inert gas (Argon 350 ml /min for 10 minutes using a pulse pump). Then de-aerated EBC samples were assessed immediately using a pH meter.[9]

Sputum samples for differential cell count: Sputum induction: Sputum was obtained from asthmatics and control by nebulization with hypertonic saline, after which children were asked to cough and expectorate.[9]

Sputum processing: The collected sputum was processed immediately. Sputum plugs were selected, mixed with dithiothreitol (1:10 of 0.1% solution) and incubated at 37°C for 30 minutes. The mixture was filtered to remove the cell debris and squamous cells. Cell count (with hemocytometer) was performed on the cell suspension and corrected for dilution. The cell suspension was centrifuged, the cell pellet was resuspended with phosphate buffered saline, cytocentrifuge were prepared air dried fixed and stained with Giemsa stain for cell count.

Statistical analysis: Data analysis using SPSS version 10. Data were expressed as means ± SD. One-way analysis of variance (Kruskal–Wallis) with the Mann–Whitney test for multiple comparisons was used to compare groups. Receiver operating curve (ROC) was done to calculate the best cut off value and area under the curve (AUC) for EBC pH. The correlations were determined by nonparametric Spearman correlation analysis. Significance was defined as a value of $p < 0.05$.

Table 2. Statistical correlation between the mean values of EBC pH and the pulmonary function test parameters.

	EBC pH	Asthmatic	
		R	p
Pulmonary Functions	FEV1	0.48	0.002(sig)
	FVC	0.549	0.000(High sig)
	FEF 25-75%	0.474	0.002(sig)
Sputum Cytology	TLC×10^6/ml	-0.481	0.002 (sig.)
	%NE	-0.379	0.01 (sig.)
	%EO	-0.4	0.01 (sig.)
	%Lymph.	-0.402	0.01 (sig.)

RESULTS

- ***EBC pH study:*** The mean values of EBC pH were significantly lower in asthmatics when compared to the control (p=0.000).

  The statistical comparison between asthmatic subgroups as regard mean values of EBC pH showed significant lower values with increasing the severity of the airway inflammation. This indicates that EBC pH reflects the pH of airway lining fluid which is affected by the amount of endogenous airway inflammation.

- ***EBC pH for diagnosis of asthma:*** Receiver Operating Curve (ROC) was analyzed to detect the predectivity for EBC pH as a diagnostic tool for pediatric asthma. Results concluded that area under the curve (AUC) represented 82% which state that test was significantly predictive.

  The best cutoff value selected with best sensitivity, specificity and positive predectivity was (7.35), above which the cases were considered healthy and below which the decrease in pH reflected ongoing airway inflammation.

- ***Correlations:*** The values of EBC pH in asthmatic children showed a significant positive correlation with the measured FEV1 and FEF25-75% ($p<0.001$) and a significant negative correlation with sputum cell counts both total leucocytic counts and oesinophilic counts ($p<0.05$ and $p<0.001$ respectively).

Table 1. Comparison between cases and controls as regard age, weight (kg), height (cm), BMI (kg/m2), triceps skin fold thickness (TS) (mm), mid arm circumference (MAC) (cm), waist circumference (WC) (cm), and waist/hip ratio WHR.

Variant	Control		Cases		T	P	Sig.
	Mean	±SD	Mean	±SD			
Age	12.80	2.86	10.78	2.79	1.86	0.07	NS
Weight (kg)	42.76	8.33	67.67	20.65	3.64	0.001	**S**
Height (cm)	145.05	11.20	143.10	11.94	0.43	0.67	NS
BMI (kg/m2)	20.19	1.79	32.52	6.07	6.23	<0.0001	**HS**
Triceps skin fold thickness (mm) (TS)	18.70	4.22	29.85	5.56	5.58	<0.0001	**HS**
Mid arm circumference (cm) (MAC)	25.65	3.28	34.55	5.12	4.98	<0.0001	**HS**
Waist circumference (cm)(WC)	77.40	8.52	86.53	13.02	2.00	0.05	**S**
Waist/Hip Ratio (WHR)	0.85	0.03	1.14	0.21	4.13	<0.0001	**HS**

Fig 1. MIR Spirobank.

Fig 2. EBC collecting tube system and the pulse pump used for de-aeration of EBC samples.

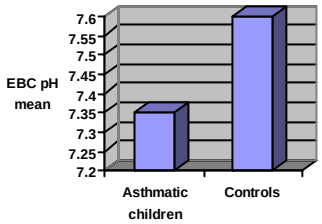

Fig 3. Bar chart for EBC pH of cases and controls.

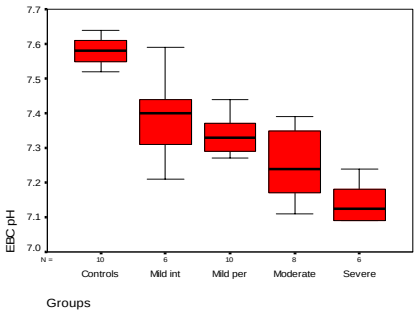

Fig 4. Box plot chart for EBC pH of asthmatic subgroups and controls.

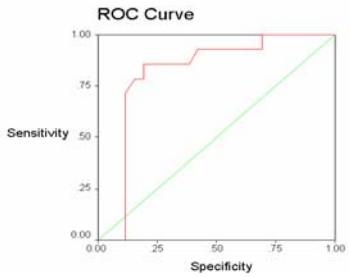

Fig 5. Receiver Operating Curve (ROC) for diagnosis of asthma using EBC pH.

Best cut off value = 7.35

Sensitivity = 89%

Specificity = 70%

Positive likelihood ratio = 5.5

DISCUSSION

Exhaled breath condensate (EBC) is a non-invasive method for studying the composition of the fluid lining the airway.[1] Researchers have reported abnormalities in EBC concentrations of at least 12 markers in individuals with inflammatory lung disorders.[2] The measurement of EBC pH is one EBC marker that is currently being investigated as a method for assessing asthma and other chronic pulmonary diseases.[7]

In this study, EBC was collected from asthmatic patients and controls using a tube prepared in National Research Center. Then EBC pH was measured using pH meter.

In our study, the mean values of EBC pH were significantly lower in asthmatics when compared to the control (p=0.000). Moreover, the statistical comparison between asthmatic subgroups as regard mean values of EBC pH showed significant lower values with increasing the severity of the airway inflammation. This indicates that EBC pH reflects the pH of airway lining fluid which is affected by the amount of endogenous airway inflammation.

Investigators have found that EBC pH values of individuals with respiratory disease (e.g., asthma and other chronic pulmonary diseases) are lower compared with those of healthy controls and that pH levels increase towards control levels after steroid treatment.[6-8]

Methodological issues: Several researchers, however, have raised concerns regarding the standardization of EBC collection and measurement methods. A recent consensus panel convened by the American Thoracic Society/European Respiratory Society Task Force on EBC[3] provided general recommendations for both EBC collection and measurement. However, some methodological aspects including the effect of ambient CO2, need for sample deaeration, and optimal time for measurement need to be addressed.

Standardization of the EBC method and validation of the analytical techniques for measuring each inflammatory biomarker are required for comparing data from different laboratories and assessing the clinical utility of EBC analysis.[9,10]

One study has shown that condensate CO2 partial pressure influences pH measurements [11]. Determination of pH at a standard CO2 level provides the most reproducible condensate pH values to date.[12]

However, another study has shown that EBC pH values in samples obtained by RTube are lower than those in samples obtained by EcoScreen condenser and that the storage for 8 weeks had a significant influence on the pH of samples analyzed without deaeration.[13]

Salivary contamination: Eicosanoids and other inflammatory mediators are present in saliva.[14] However; it was recorded that pH values in EBC are unaffected by salivary or therapeutic artifact, and identical to samples taken directly from the lower airway.[15]

pH

Measurement of pH is very reproducible and might prove clinically useful for the diagnosis and monitoring of pharmacological therapy in inflammatory airway diseases.[16]

In our study, Receiver Operating Curve (ROC) was analyzed to detect the predictivity for EBC pH for diagnosis of asthma in children. The best cutoff value of EBC pH selected with best sensitivity, specificity and positive predictivity was (7.35), above which the cases were considered healthy and below which the decrease in pH reflected ongoing airway inflammation. Statistical comparison between asthmatic subgroups around cut off value showed a significant decrease in the mean value of EBC pH with the increase in asthma severity. This confirmed the hypothesis that EBC pH is a marker of severity reflecting intensity of airway inflammation.

While the area under the curve (AUC) represented 82% which state that test was significantly predictive.

There is no comparative cut off values in literature for asthmatic children. However; screening for EBC pH in normal individuals with an extensive normal data reveals median EBC pH value is 8.0 with interquartile (25–75%) range of 7.8–8.1.[17] There were no differences based on age, sex, or race and the distribution was skewed, with 6.4% of EBC samples having a pH range <7.4.[18]

On other studies; pH of de-aerated EBC was over two log orders lower in adults with acute asthma than in healthy subjects and normalized with glucocorticoid therapy.[19]

Other authors documented that pH values in EBC in children with acute asthma are lower than those in children with stable asthma and normalize with inhaled glucocorticoid therapy.[20]

Interesting enough, there was a significantly positive correlation between EBC pH and FEV1, FVC, FEF25-75%. This means that the degree of airway acidity increases as the severity of airway obstruction increases.

Similarly, the mean values of EBC pH showed a significant negative correlation with mean values of sputum variables, total leucocytic count, eosinophils and neutrophils. This proves the robust relation between EBC pH and chronic airway inflammation and highlights the hypothesis that eosinophilic and neutrophilic inflammation contributes to endogenous acidification in asthma.

In conclusion; collection and analysis of exhaled breath condensate for pH could prove a valuable, single, noninvasive tool to evaluate and follow up children with asthma. Measurement of bio-molecules in EBC might provide insights into the patho-physiology of lung diseases. However, more studies are necessary before EBC can be recommended for clinical practice.

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