Exhaled breath condensate pH and hydrogen peroxide as non-invasive markers for asthma

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ABSTRACT

Objectives: To estimate the predictive value of exhaled breath condensate (EBC) hydrogen peroxide (H_2O_2) concentration and pH as non-invasive markers in asthma.

Methods: Fifty patients with unstable, steroid naive atopic asthma were included in the study, 25 with intermittent asthma and 25 with persistent asthma. Asthma diagnosis was according to the National Heart Lung and Blood Institute guidelines for the diagnosis and management of asthma. Forced expiratory volume in one second (FEV1) was measured by computerized spirometry. The EBC H_2O_2 assay was carried out using the colorimetric assay. The study was conducted from January to December 2005 in the Asthma and Allergy Center, Tikrit, Iraq.

Results: The EBC H_2O_2 concentration was higher in the asthmatic group (0.91 µmol) as compared with the control (0.23 µmol). There was an inverse correlation between EBC H_2O_2 concentration and FEV1 predicted percent for asthmatic patients. The mean EBC pH was lower in the asthmatic than the control group. There was a positive correlation between EBC pH and FEV1 predicted percent for asthmatic patients. There was an inverse correlation between EBC H_2O_2 concentration and pH for all asthmatic patients, intermittent, and persistent asthmatic groups.

Conclusions: Exhaled breath condensate hydrogen peroxide concentration and pH was a good non-invasive marker for asthma, whether it was with a persistent or intermittent course.

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Airway inflammation has a central role in the development and progression of asthma.[1] Chronic airway inflammation is considered responsible for symptoms and disorders of airway function associated with asthma. This process is the target of anti-inflammatory therapy. Therefore, a number of standardized, non-invasive techniques had been developed to assess it.[2] Results from studies using a wide variety of inflammatory markers have shown group differences between patients with asthma and healthy control subjects, however, evidence for the use of these markers in individual patients is scarce.[2] Exhaled breath condensate (EBC) pH and hydrogen peroxide (H_2O_2) concentration are a non invasive, simple and non expensive assay that can be performed repeatedly without adversely affecting patients.[3] The measurement of H_2O_2 and pH as markers in EBC has proven to be a useful, non-invasive technique for assessing and monitoring of airway inflammation.[4-7] Endogenous airway acidification as assessed by pH in EBC has been implicated in asthma pathophysiology.[8] The pH of the EBC was found to be substantially lower than normal during acute asthmatic exacerbations and normalized with anti-inflammatory therapy and the remission of the exacerbation.[8] Increased levels of H_2O_2 have been reported in EBC in cigarette smokers[9] and in patients with asthma[4,7,10-13] However, the relationship among exhaled H_2O_2, airway obstruction and markers of airway inflammation in asthmatic patients are not yet certain. Therefore, in this study we determined the concentration of H_2O_2 and pH in EBC in patients with steroid naive asthma of differing disease severity and compared this with forced expiratory volume in one second (FEV1). The study objective was to estimate the predictive value of EBC H_2O_2 concentration and pH as non-invasive markers in asthma.

Methods. Fifty patients with unstable, steroid naive atopic asthma recruited from the Asthma and Allergy Center, Tikrit, Iraq were included in the study, 25 had intermittent asthma and 25 had persistent asthma.

Their age ranged from 17-56 years with a median age of 35 years. Asthma diagnosis was according to National Heart Lung and Blood Institute guidelines for the diagnosis and management of asthma.[14] The atopic status was assessed by positive skin prick test to common inhalant allergens. The mean duration of asthma was 7.9±5.3 years (mean±SD). The patients were excluded if they are ex-smokers, with active allergic rhinitis, or upper respiratory tract infections during or within 4 weeks of the study, steroid dependent asthma, or receiving systemic steroids within 3 months of assessment. The study was conducted during the period from January to December 2005. The FEV1 was measured by computerized spirometry. The study was approved by the College of Medicine, Tikrit University, Iraq ethical committee. The participants were informed, and their written consent was obtained. Expired breath condensate was collected by using a glass-condensing device that was placed in a large chamber with ice. After rinsing their mouth, subjects breathe tidily with normal frequency through a mouthpiece for 20 minutes while wearing a nose clip. The H_2O_2 assay was carried out by using the colorimetric assay as described previously.[15] Briefly 100 µl of condensate was mixed with 100 µl of tetramethyl benzidine in 0.42 mol/l citrate buffer, pH, 3.8 and 10 µl of horse reddish peroxidase (52.5 U/ml). The samples were then incubated at room temperature for 20 minutes, and the reaction was stopped by addition of 10 µl 1N sulfuric acid. The reaction product was measured spectrophotometrically at 450 nm. A standard curve of H_2O_2 was performed for each assay. Exhaled breath pH was measured as previously described[8] right after the collection of the condensate by using a pH meter (HI8424).

Data concerning the comparisons among the various parameters in the study groups are given as means±standard deviation with 95% confidence intervals for the differences. Student unpaired 2-tailed t-test, and Pearson correlations (r) were used for statistical significance testing. Statistical analyses were conducted using the Microsoft Office Excel 2003.

Results. The EBC H_2O_2 concentration was higher in the asthmatic group as compared with control (**Table 1**). The above difference was statistically highly significant (p<0.0001). When asthmatic patients subdivided into the intermittent and persistent groups, the EBC H_2O_2 concentration was higher in the persistent group than the intermittent group. In addition, EBC H_2O_2 concentration was significantly higher for both groups as that for control group (p<0.0001). The mean EBC pH was 6.19±0.96 (95% CI: 5.92-6.46) for asthmatic group and this value was lower than that for control group (7.8±0.35; 95% CI: 7.16-7.99; p<0.0001). The EBC pH was lower in the persistent than the intermittent asthmatic group. Both intermittent and persistent asthmatic groups had lower EBC pH than the control, and this difference was statistically highly significant (p<0.0001) (**Table 1**). There was an inverse correlation between EBC H_2O_2 concentration and FEV1 predicted percent for all asthmatic patients. A lower correlation was observed for patients with intermittent asthma, while good inverse correlation was achieved for persistent asthmatic (**Table 2**). There was a positive correlation between EBC pH and FEV1 predicted percent for all asthmatic patients. Furthermore, there was a positive correlation between EBC pH and FEV1 predicted percent in intermittent and persistent asthma groups (**Table 2**). There was an inverse correlation between EBC H_2O_2 concentration and pH for all asthmatic patients, intermittent, and persistent asthmatic groups (**Table 2**).

Discussion. The mean concentration of expired H_2O_2 was elevated in untreated asthmatic patients in comparison with levels in normal subjects. This may

Table1 - Hydrogen peroxide (H_2O_2) breath condensate concentration (uM) and pH in patients with asthma.

Index	Asthma			Control
	Intermittent 25	Persistent 25	All 50	15
H_2O_2				
Mean	0.66	1.14	0.91	0.23
SD	0.05	0.36	0.35	0.03
95% CI	0.64-0.68	0.99-1.29	0.81-1.00	0.21-0.25
pH				
Mean	6.70	5.68	6.19	7.8
SD	0.61	0.98	0.96	0.35
95% CI	6.44-6.96	5.27-6.09	5.92-6.46	7.61-7.99
FEV1				
Mean	80	69	75	101
SD	9.07	9.87	10.9	2.25
95% CI	76-84	65-73	72-78	100-102

FEV1 - forced expiratory volume in one second, SD - standard deviation, CI - confidence interval

Table 2 - Correlation among FEV1 predicted percent, expired breath hydrogen peroxide (H_2O_2) concentration, and pH in asthma.

Group	Number	FEV1 & pH		FEV1 & H_2O_2		pH & H_2O_2	
		r	*P*-value	r	*P*-value	r	*P*-value
Asthma all	50	0.85	0.0001	- 0.79	0.0001	- 0.83	0.0001
Intermittent	25	0.80	0.001	- 0.47	0.02	- 0.51	0.01
Persistent	25	0.81	0.0001	- 0.89	0.0001	- 0.84	0.0001

r - Pearson correlation, FEV1 - forced expiratory volume in one second

indicate an enhanced production of oxidants and/or decreased antioxidant capacity of asthmatic airways.[12] Exhaled H_2O_2 levels have reported previously to be related to the eosinophil counts in induced sputum and activity of peripheral neutrophils in asthmatic patients.[16,17] Therefore an elevated concentration of H_2O_2 may result from an enhanced number and activity of inflammatory cells in the airways.[12] This may explain why patients with persistent asthma had high levels of H_2O_2 in expired breath condensate as this study indicated. Furthermore, patients with intermittent asthma had lower EBC H_2O_2 than the persistent asthma patients. However, the EBC H_2O_2 levels in intermittent asthma were higher than the control subjects, indicating that airway inflammation may present even in patients with mild asthma.

Oxidative stress is implicated in asthma and other respiratory diseases.[4,18] Hydrogen peroxide is one of the most stable of the reaction oxygen species metabolites. Due to lack of charge, it may easily penetrate cellular membranes and may generate hydroxyl radicals in presence of iron cation.[12] Hydrogen peroxide and hydroxyl radicals are able to react with membrane and lipid components of the bronchial lining fluid and cause their peroxidation.[19] The concentration of H_2O_2 in EBC is raised in patients with the inflammatory diseases of the airways.[5,6,20,21] Elevated H_2O_2 levels are associated with concentration of thiobarbituric acid reactive product in the EBC, nitric oxide in exhaled air, airway obstruction, and airway hyperresponsiveness to methacholine in asthmatic patients.[4,7,22,23] In the present study, there was an inverse correlation between EBC H_2O_2 and FEV1 predicted percent. The correlation was higher in persistent than in intermittent asthmatic patients, indicating an ongoing inflammation in persistent asthma.

The pH of EBC derived from asthmatic patients was lower than that from controls. This was in accordance with that reported for other geographical areas.[8,24] Furthermore, EBC pH was related to asthma severity depending on the finding that EBC pH was lower in the persistent asthmatic as compared to that with intermittent asthma. These observations suggest that regulation of airway pH may have a role in the pathophysiology of acute asthma or exacerbation. In addition, there was a positive correlation between EBC pH and FEV1 in asthmatic patients. Subjects with intermittent asthma had higher pH values compared to those with persistent asthma, but with lower values as compared to control. This indicates that EBC pH values differ between patients with different clinical severity. Thus, a sudden drop in airway pH during asthma exacerbation would cause extensive eosinophilic necrosis with an acute release of inflammatory, or bronchoconstricting products. However, an administration of corticosteroids control the inflammatory process of the disease,[24] and restores the pH. This means that pH is probably a consequence of the eosinophilic inflammation and not its cause.[23] The granule matrix of eosinophils contains eosinophil peroxidase, an enzyme that, in the presence of H_2O_2, can oxidize halides to form highly reactive hypohalous acid.[25] This may explain that our results showed an inverse correlation between EBC pH and H_2O_2 concentrations. This might also partly explain the lower pH that we observed in persistent asthmatic patients than those with intermittent asthma. Furthermore, in the study by Hunt et al[8] an acute fall of the pH values in exacerbated patients with asthma was reported. This might be explained by the predominance of neutrophils in the sputum of patients with asthma during the exacerbation.[26] This leads to the hypothesis that neutrophilic inflammation contributes to endogenous acidification in asthma exacerbations.[24] The present study, reported that the abnormalities in breath condensate chemistry reflects intrinsic abnormalities of the airway lining fluid.[8,24]

In conclusion, the EBC as a tool to measure airway inflammation was a non-invasive approach. Estimation of EBC pH and H_2O_2 concentration had a predictive value to differentiate between asthmatic and non-asthmatic, between different patterns and asthma severity, and may be used for monitoring of response to treatment and follow up of asthmatic patients. However, the limitation for using the EBC H_2O_2 concentration as a marker, is the high variation between different studies.[27] Thus, these study findings warrant the evaluation of the predictive value of EBC H2O2 and pH for monitoring of response to asthma treatment, prognosis, and follow-up.

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