

Effect of Steroids and Black Seed Oil on Hydrogen Peroxide Concentration and pH of Expired Breath Condensate in Asthmatic Patients

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Abstract

Asthma is a chronic inflammatory disease of airway, with increased worldwide prevalence. This study was conducted to evaluate the effect of different treatment regimens on expired breath condensate hydrogen peroxide concentration and pH. Patients and methods: Thirty two patients (17 male, 15 female), with asthma were examined during exacerbation. Asthma was diagnosed according to American Thoracic Society Criteria. The pH level was increased significantly following treatment with prednisolone. In addition, expired breath condensate hydrogen peroxide in patients with acute attack reduced significantly ($P < 0.0001$) following treatment with prednisolone. Black seed oil treatment reduced expired breath condensate H_2O_2 concentration from $1.29 \mu M$ (95% CI, 1.08-1.50) as baseline values to $0.56 \mu M$ (95% CI, 0.45-0.67) following treatment. Furthermore, pH of expired breath condensate increased from 5.54 (95% CI, 4.97-6.11) at baseline to 6.8 (95% CI, 6.28-7.32) following treatment course of black seed oil. Thus the treatment course of black seed oil significantly reduced expired breath condensate H_2O_2 concentration ($P < 0.0001$) and increased exhaled pH ($P < 0.01$). For the groups that were treated with oral prednisolone or black seed oil, there were a highly significant inverse correlation among expired breath condensate H_2O_2 with pH, Exhaled H_2O_2 with FEV1 predicted percent and positive correlation for exhaled pH with FEV1 predicted percent. Steroids and black seed are with activity to reduce expired breath condensate hydrogen peroxide concentration and pH. This may indicate that they may be used for asthma treatment.

تأثير زيت الحبة السوداء والستيرويدات على تركيز بيروكسيد الهيدروجين وعامل الحموضة في مكثف الزفير

التنفس عند مرضى الربو

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المستخلص

إن الربو القصبي مرض التهابي وتزايد نسبة حدوثه على مستوى العالم. الدراسة تهدف لتقييم تأثير العلاج على تركيز بيروكسيد الهيدروجين وعامل الحموضة في مكثف الزفير التنفسي عند مرضى الربو. شملت الدراسة اثنان وثلاثون مريضاً (١٧ ذكور و ١٥ إناث) مصاب بالربو حيث تم فحصهم عند حدوث الأزمة. تم تشخيص المرض استناداً إلى تعليمات الجمعية الأمريكية للأمراض الصدرية. إن مستوى عامل الحموضة ازداد بصورة ذات قيمة معنوية البردنزلون... يضاف لذلك فإن تركيز بيروكسيد الهيدروجين في مكثف الزفير العلاج. أن العلاج بزيت الحبة السوداء خفض بيروكسيد الهيدروجين من ١,٢٩ مايكرومول عند بدأ العلاج إلى ٥,٥٦ مايكرومول بعد العلاج. أيضاً عامل الحموضة ازداد من ٥,٥٤ قبل العلاج إلى ٦,٨ بعد العلاج بزيت الحبة السوداء. أيضاً في مجاميع العلاج كانت هناك علاقة عكسية بين تركيز بيروكسيد الهيدروجين وعامل الحموضة وكذلك بين كمية هواء التنفس و تركيز بيروكسيد الهيدروجين. أيضاً توجد علاقة طردية بين مقدار عامل الحموضة وكمية هواء التنفس لدى المجاميع التي تم معالجتها. أن العلاج بعقار البردنزلون وزيت الحبة السوداء يخفض تركيز بيروكسيد الهيدروجين ويزيد عامل الحموضة لدى مرضى الربو الذين استعملوا العلاج.

Introduction

Asthma is a chronic inflammatory disease of airway [1], with increased worldwide prevalence [2]. Inflammatory cells release superoxide anion, which undergoes spontaneous or enzymatic catalyzed dismutation to form H_2O_2 [3]. Exhaled H_2O_2 concentration predicts the severity and disease patterns [4, 5]. Increased levels of H_2O_2 and reduced pH have been reported in expired breath condensate in asthma [6-12]. However, the relationship among exhaled H_2O_2 , pH, airway obstruction, airway hyperresponsiveness and markers in asthmatic patients were reported [4,5,10,11], still there was a need for evaluation the effect of different treatment regimens on expired breath condensate hydrogen peroxide concentration and pH. Thus this study was conducted for the above objectives.

Materials and Methods

Patients

Thirty two patients (17 male, 15 female), with asthma were examined during exacerbation. Asthma was diagnosed according to American Thoracic Society Criteria [12]. All patients were non smoker. Ex smokers and patients with active allergic rhinitis or upper respiratory tract infections during or within 4 weeks of the study were excluded. Patient received the rescue medication and then allocated randomly to one of the two treatment regimens. Group A received oral prednisolone 30 mg / daily for two weeks with tapering schedules in addition to short acting, inhaled B2 agonist (salbutamol). Group B received black seed oil capsule three times daily for 4 weeks in addition to short acting B2 agonist. The study included asthmatic patients with moderate persistent asthma. There was no

significant differences at baseline between both groups in regards to age, sex and disease severity. A written Informed consent was taken from patients included in the study.

Lung Function Test

FEV1 was measured by computerized spirometry.

EBC Collection and H_2O_2 Measurement

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 twenty minutes while wearing a nose clip [5]. H_2O_2 assay was carried out by using colorimetric assay as described previously [13]. Briefly 100 μ l of condensate was mixed with 100 μ l of tetramethyl benzidine in 0.42 mol / l citrate buffer, pH 3.8 and 10 μ l 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 μ l 1 N sulfuric acid. The reaction product was measured spectrophotometrically at 450 nm. A standard curve of H_2O_2 was performed for each assay.

pH measurement

pH was measured as previously described [14] right after the collection of the condensate by using a pH meter (HI8424).

Statistical Analysis

Data concerning the comparisons among the various parameters in the study groups are given as means (SD) with 95% confidence intervals for the differences. Student unpaired two tailed t test and Pearson correlation (r) were used for statistical significance testing.

Results

The mean expired breath condensate pH value of sample from acute asthmatic was 5.49 ± 1.08 (95% CI, 4.94 – 6.04). This pH level was increased to 7.38 ± 0.63 (95% CI, 7.06-7.70) following treatment with prednisolone (Table.1). The above difference in expired breath condensate pH between pre and post treatment was statistically significant ($P < 0.001$). The expired breath condensate hydrogen peroxide in patients with acute attack was $1.17 \pm 0.46 \mu\text{M}$ (95% CI , 0.93-1.40) and this value reduced significantly ($P < 0.0001$) following treatment with prednisolone ($0.42 \pm 0.06 \mu\text{M}$, 95% CI, 0.39-0.45). Black seed oil treatment reduced expired breath condensate H_2O_2 concentration from $1.29 \pm 0.41 \mu\text{M}$ (95% CI, 1.08-1.50) as baseline values to $0.56 \pm 0.22 \mu\text{M}$ (95% CI, 0.45-0.67) following treatment. Furthermore, pH of expired breath condensate increased from 5.54 ± 1.12 (95% CI, 4.97-6.11) at baseline to 6.8 ± 1.02 (95% CI, 6.28-7.32) following treatment course of black seed oil. Thus the treatment course of black seed oil significantly reduced expired breath condensate H_2O_2 concentration ($P < 0.0001$) and

increased exhaled pH ($P < 0.01$). There was no significant difference between post treatment mean values for expired breath condensate pH ($P > 0.05$) of prednisolone and black seed groups. However, there was a marginal significant difference ($P < 0.02$) concerning exhaled H_2O_2 between the two groups. association among expired breath condensate H_2O_2 concentration, pH and FEV1 predicted percent in patients treated with either oral prednisolone or black seed are shown in Table.2. and Figs. 1-6. For the group that was treated with oral prednisolone, there was a highly significant inverse correlation among expired breath condensate H_2O_2 with pH ($r = -0.97$, $P < 0.0001$), Exhaled H_2O_2 with FEV1 predicted percent ($r = -0.95$, $P < 0.0001$) and positive correlation for exhaled pH with FEV1 predicted percent ($r = 0.84$, $P < 0.001$). The asthmatic group that received black seed oil treatment show a highly significant inverse correlation among exhaled H_2O_2 with pH ($r = -0.92$, $P < 0.0001$), Exhaled H_2O_2 with FEV1 predicted percent ($r = -0.91$, $P < 0.0001$) and positive correlation for exhaled pH with FEV1 predicted percent ($r = 0.94$, $P < 0.0001$).

Table(1):- Effect of oral prednisolone and black seed oil on pH and H_2O_2 concentration (μM) in expired breath condensate of asthmatics

Index	Prednisolone			Black seed		
	Baseline	Post treatment	P value	Baseline	Post treatment	P value
H_2O_2						
Mean	1.17	0.42	<0.0001	1.29	0.56	<0.0001
SD	0.46	0.06		0.41	0.22	
95% CI	0.935, 1.40	0.39, 0.45		1.08, 1.50	0.45, 0.67	
pH						
Mean	5.49	7.38	<0.0001	5.54	6.8	<0.001
SD	1.08	0.62		1.12	1.02	
95% CI	4.94, 6.04	7.06, 7.70		4.97, 6.11	6.28, 7.32	

FEV1						
Mean	70	94	<0.0001	70	89	<0.001
SD	12	10		12	10	
95% CI	64, 76	89, 99		64, 76	84, 94	

Table(2):- Association among H₂O₂ concentration, pH of expired breath condensate and FEV1 predicted percent in asthmatic patients treated with different regimens.

Treatment Type	H ₂ O ₂ & pH		H ₂ O ₂ & FEV1		FEV1 & pH	
	R	P	R	P	R	P
Prednisolone	-0.97	<0.0001	-0.95	<0.0001	0.83	<0.0001
Black seed	-0.92	<0.0001	-0.91	<0.0001	0.94	<0.0001

Fig.1. Correlation among PH of expired breath condensate and FEV1 percent predicted in asthmatic patients treated with prednisolone

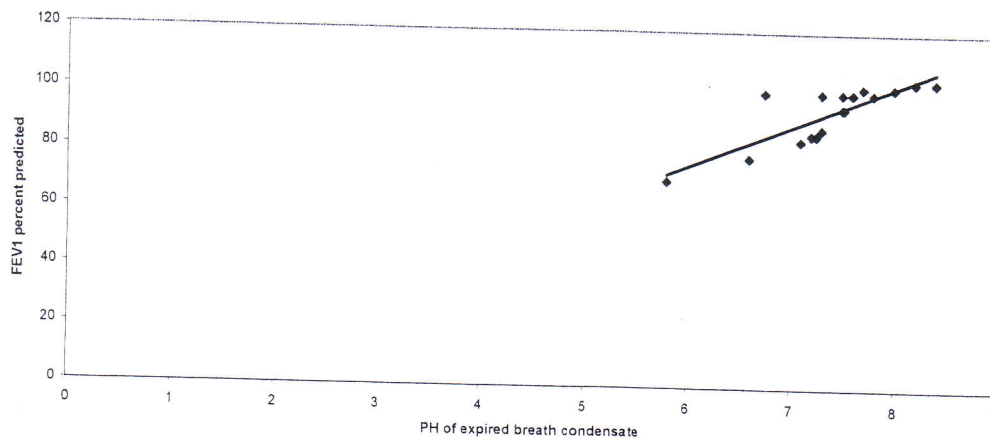


Fig.2. Correlation among H₂O₂ concentration and PH of expired breath condensate in asthmatic patients treated with prednisolone.

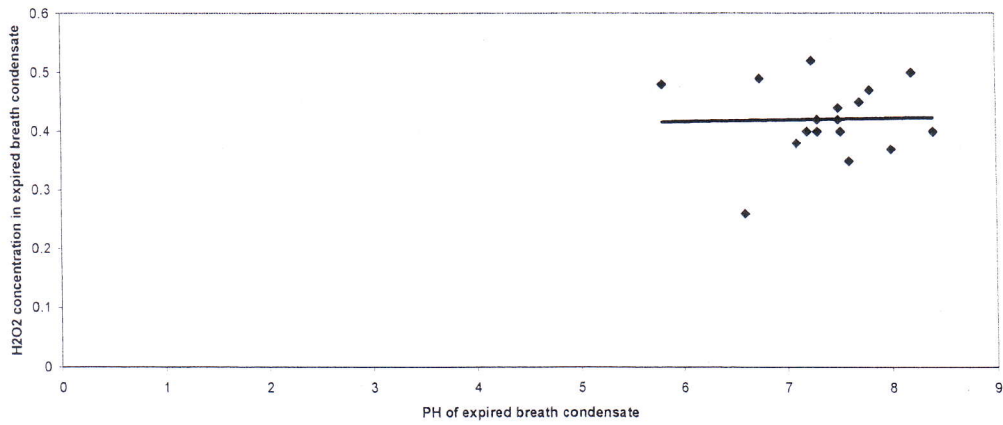


Fig.3. Correlation among H₂O₂ concentration in expired breath condensate and FEV₁ percent predicted in asthmatic patients treated with prednisolone

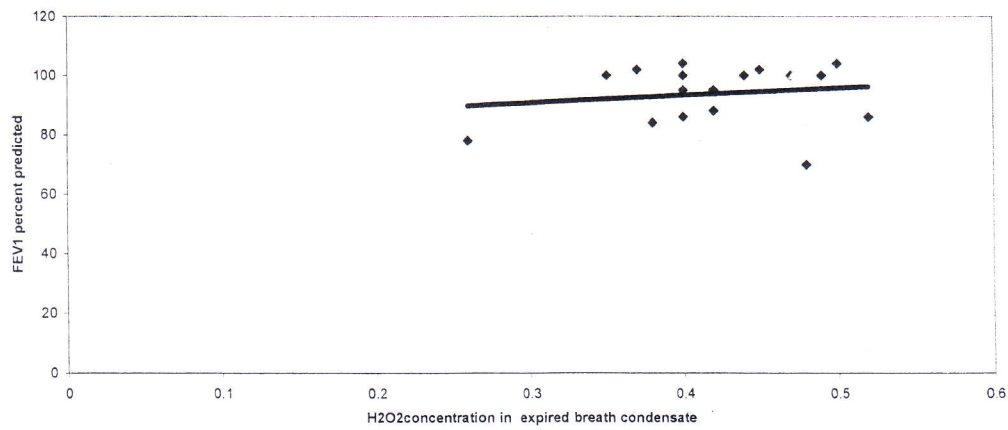


Fig.4. Correlation among PH of expired breath condensate and FEV₁ percent predicted in asthmatic patients treated with black seed oil

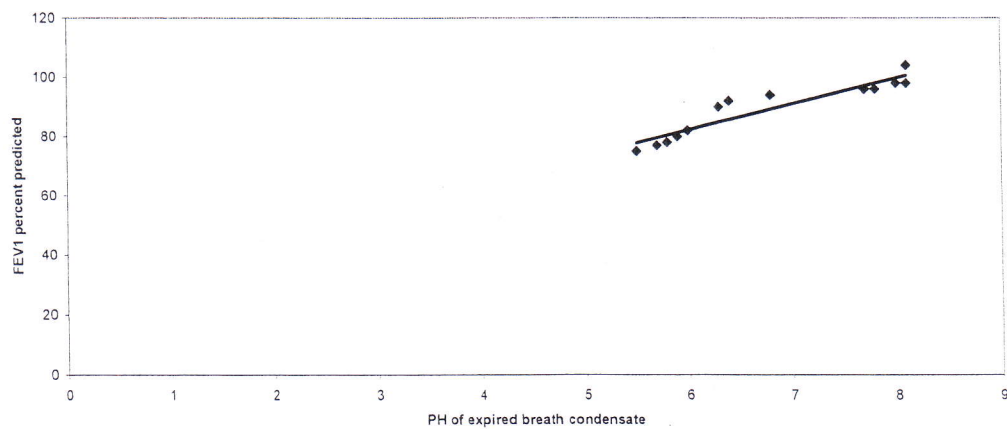


Fig.5. Correlation among H₂O₂ concentration and PH of expired breath condensate of asthmatic treated with black seed oil

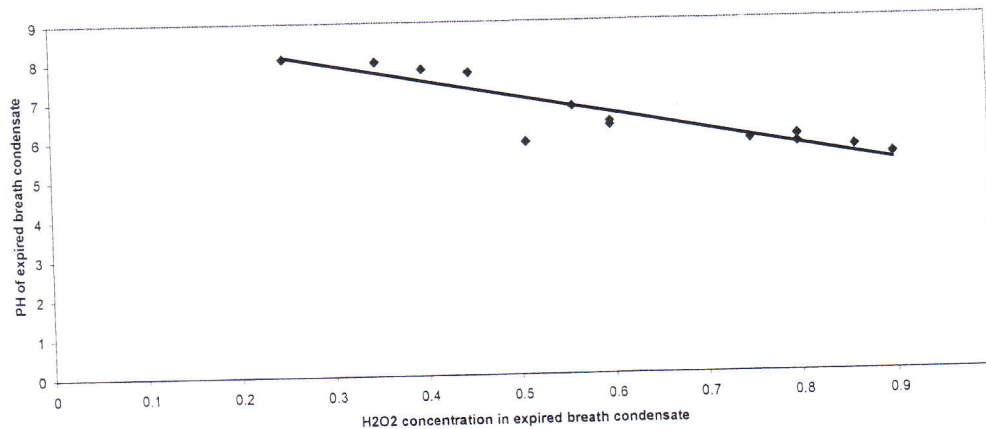
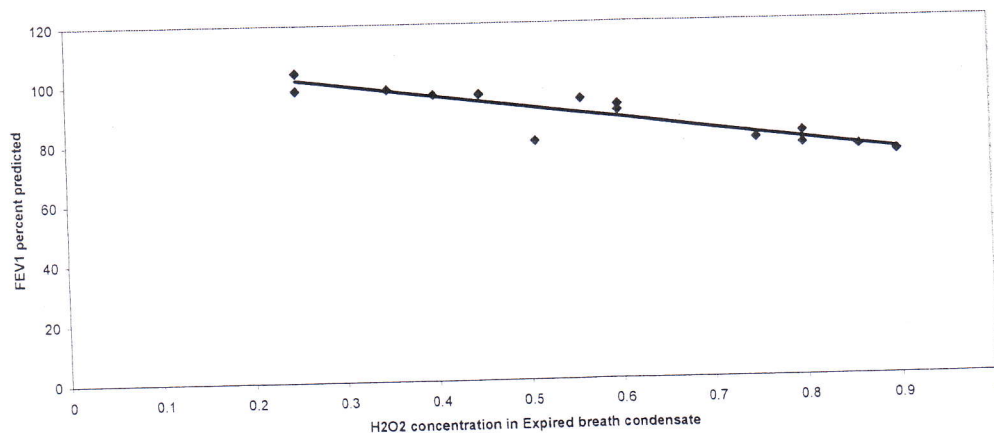


Fig.6. Correlation among H₂O₂ concentration in expired breath condensate and FEV1 percent predicted in asthmatic patients treated with balck seed oil



Discussion

The mean concentration of expired hydrogen peroxide was elevated in untreated asthmatic patients during exacerbation. This value was higher to that in normal non-asthmatic subjects [13]. All of the asthmatic patients had increased levels of H₂O₂ during acute attack. This may indicate an enhanced production of oxidative and / or decreased antioxidant capacity of asthmatic airways. Exhaled H₂O₂ levels have previously been related to the eosinophil differential counts in induced sputum and activity of

peripheral neutrophils in asthmatic patients [15, 16]. Therefore, elevated concentration of H₂O₂ may result from an enhanced number and activity of inflammatory cells in the airway [17]. This increase in H₂O₂ levels in asthmatic is affected by the use of prednisolone as this study indicated. Oral prednisolone (30 mg/day) significantly reduced exhaled H₂O₂ concentration. This reduction in expired H₂O₂ concentration was significantly inversely correlated with FEV1 predicted percent indicating the clinical improvement of asthmatic patients following suppression of the

Fig.2. Correlation among H₂O₂ concentration and PH of expired breath condensate in asthmatic patients treated with prednisolone.

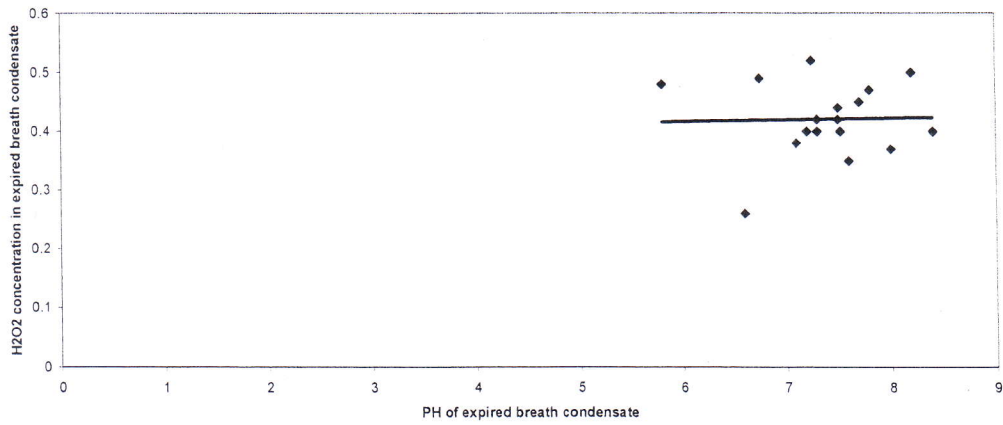


Fig.3. Correlation among H₂O₂ concentration in expired breath condensate and FEV₁ percent predicted in asthmatic patients treated with prednisolone

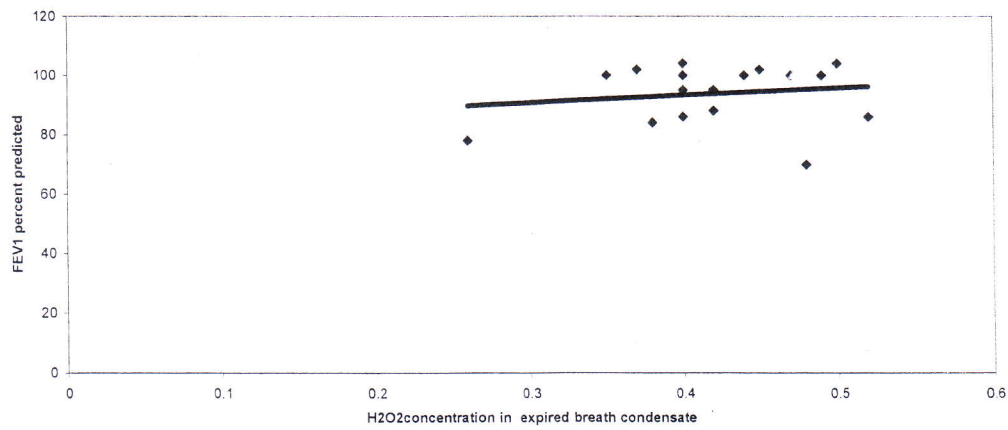
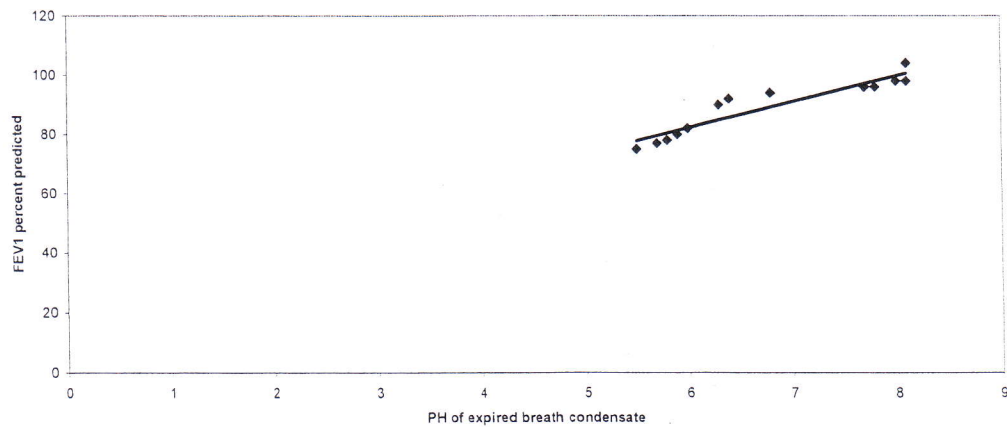


Fig.4. Correlation among PH of expired breath condensate and FEV₁ percent predicted in asthmatic patients treated with black seed oil



oxidative stress that was achieved by oral prednisolone. Increased oxidative stress is implicated in asthma [18, 19]. Hydrogen peroxide is one of the most stable of the reactive oxygen metabolites, and consequently may lead to airway inflammation, airway hyperresponsiveness and subsequent bronchoconstriction [17]. In the present study, the significant negative correlation among exhaled H_2O_2 and FEV1 indicated the association between clinical benefit with corticosteroids treatment and airway inflammation. The possibility that expired breath condensate pH may serve as a non invasive indicator of airway inflammation is supported by the tight correlations of expired breath condensate pH with inflammatory cell populations measured in induced sputum [20]. This study indicated that expired breath condensate pH was lower in asthmatic patients during the acute attack than that reported for normal subjects [5]. However, expired breath condensate pH normalized following prednisolone treatment. These observations suggest that regulation of airway pH may have a role in the pathophysiology of acute asthma [21]. Our results showed a strong correlation between amounts of pH and H_2O_2 concentration as oxidative stress marker and with FEV1 predicted percent as marker of clinical response. Hunt and coworkers [22] have reported that concentrations and bioavailability of many inflammatory markers are critically pH dependent, suggesting that low airway pH could contribute substantially to airway inflammation [19]. This may explain the positive association between airway restoring by prednisolone and FEV1 predicted percent as indicator of clinical states of the patients. The present study indicated a significant inverse correlation between expired breath condensate pH and H_2O_2

concentration. The significant correlation observed in this study might be the result of the initial cellular process, which leads through different ways to oxidative stress, to NO production, and finally to endogenous acidification [19]. This theory is partially supported by previous observations that showed a significant relationship between oxidative stress, exhaled NO and the respective inflammatory and structural cells in airways [23, 24]. Black seed oil treatment for 4 weeks duration lead to significant reduction in expired breath condensate H_2O_2 and restoring of pH. Furthermore, there was a significant improvement in lung function as measured by FEV1 predicted percent. This was the first report for effect of black seed oil on exhaled breath condensate H_2O_2 and pH. Fortunately, this effect was comparable to that induced by prednisolone. In previous reports, we demonstrate the effective treatment of allergic rhinitis and asthma with black seed oil [25, 26]. The reduction effect of expired breath condensate H_2O_2 following treatment with black seed oil was inversely consistent to the increase in exhaled pH following treatment. Furthermore, the improvement in FEV1 predicted percent was inversely correlated to expired breath condensate H_2O_2 concentrations. However, exhaled pH was correlated significantly to FEV1 predicted percent. This association indicated that black seed acts as antioxidant and anti-inflammatory drugs, which leads subsequently to improvement in lung function. Expired breath condensate is now being used by centers throughout the world in an effort to gain insight into the airway environment in multiple lung diseases [12]. It is a safe and simple procedure even in small children [27]. In deed, altering the pH of the airway environment known to affect multiple

aspects of airway function [12]. Multiple additional effects of airway acidification are expected. Mild acidification (below pH 6.5) increases mucous viscosity [28], converting it from sol to gel [29] which is perhaps relevant to mucous plugging [12]. Low environmental pH enhances inducible nitric oxide synthase 2 expression and activity in rat peritoneal macrophages through the action of tumor necrosis factor – alfa and nuclear factor –kB [30]. An increased concentration of exhaled H_2O_2 may represent an increased production of oxidants and / or a reduced free radicals scavenging capacity in the airway of asthmatic subjects [31]. In addition to the increased production of ROS in patients with asthma, there may be a deficiency of antioxidant defenses [23]. Interestingly, there is no abnormal antioxidants level in patients with mild asthma, and specifically in those whose disease is being controlled with inhaled corticosteroids. Our results showed that oxidative stress was increased during acute attack of asthma; however, it was restored after and during treatment with oral prednisolone and black seed oil. Many inflammatory and structural cells that are activated in the airway of asthma patients result in the marked production of ROS, including H_2O_2 [18]. However, the precise role of ROS in the pathophysiology of the inflammatory process remains unclear due to the difficulty in the identification of which cells produce ROS in the lungs and bronchi of asthmatic subjects as well as to the difficulty in specifying the differences in these cells among the various states of the disease [31]. It was concluded from the studies conducted so far is that eosinophilic is a characteristic feature of intermittent and mild asthma, whereas the association of eosinophilia and neutrophilia

characterizes more severe forms of the disease [32]. In the present study, there was a significant correlation among expired breath condensate H_2O_2 reduction, pH increase and lung function improvement following therapy with prednisolone and black seed oil. Furthermore, analysis of reported studies [4-11, 31] showed that there were a correlation among eosinophil counts in sputum, disease severity, lung function improvement, H_2O_2 concentration and pH of expired breath condensate. However, the present evidence suggests that the number of sputum eosinophils may possibly be the most useful parameter that directly reflects the intensity of the inflammation measured in bronchial biopsy specimens, lung function impairment and disease severity [33, 34]. Taking all these points together, and based on the above mentioned data from the literature, eosinophils are the predominant cells that generate ROS in patients with all forms of asthma, while neutrophils might be responsible for the highest levels that are observed in the more severe forms of the disease [31],35-38]. Corticosteroids suppress the eosinophilic inflammation [15], and may be neutrophil [15, 16, and 31]. Thus treatment of asthmatic patients with oral prednisolone or black seed oil may results in reduction in expired breath condensate H_2O_2 concentration and increase in pH may be via suppressing eosinophilic and neutrophilic inflammation. In a prospective controlled study [16], therapy with ICS reduced the levels of H_2O_2 in expired breath condensate, indicating an anti-inflammatory effect on oxidative stress. Data from a previous study [15] also have suggested that the differences in exhaled H_2O_2 levels between steroid treated and steroid naïve patients imply differences in oxidative stress. In conclusion, endogenous airway

acidification reflects the underlying inflammatory process in asthmatic patients. Furthermore, expired breath condensate H_2O_2 concentration related to degree of airway inflammation. This observation suggests that it might be possible to consider expired breath condensate pH and H_2O_2 concentration as markers for monitoring response to treatment in asthmatic patients. Because both pH and H_2O_2 measurement is not expensive, easy to perform and highly reproducible [12,38], prospective controlled studies are needed to confirm the effect of anti-inflammatory drugs and oxygen scavengers on both airway inflammation indexes and ROS, in order to apply the technique of expired breath condensate in the evaluation of inflammatory process in asthma. In addition, its correlations with serum and sputum eosinophil cationic protein [39] need to be evaluated.

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