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Correlation of exhaled carbon monoxide and nitric oxide with airflow obstruction in asthma and chronic obstructive pulmonary disease patients

Asrar Ahmad[1, 2], Mohammad Shameem[2], Qayyum Husain[*, 1]

[1]*Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, India*
[2]*Department of T.B. and Respiratory Diseases, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India*

ABSTRACT

The aim of this study was to quantify lung oxidative stress in patients with asthma and chronic obstructive pulmonary disease by measuring levels of exhaled carbon monoxide and nitric oxide. Levels of exhaled carbon monoxide, nitric oxide were evaluated in exhaled air of asthma and COPD patients. Moreover, correlation of CO and NO concentrations with degree of airflow obstruction (FEV1% predicted) were also measured. The mean exhaled CO level was significantly much higher among COPD (6.47 ± 0.44 ppm, p<0.01) and asthma patients (6.13 ± 0.42 ppm, p<0.05) as compared to controls (4.62 ± 0.41 ppm). There was no significant difference found in the levels of CO between asthma and COPD (p>0.05). %COHb levels were remarkably higher in COPD (p<0.01) and asthma patients (p<0.05). It was also found that the levels of exhaled NO remarkably increased in asthma (41.56 ± 3.22 ppb, p<0.001) and COPD patients (29.22 ± 2.43 ppb, p<0.01) as compared to control (17.42 ± 1.01 ppb). There was a significant negative correlation found between exhaled CO and NO with FEV1% predicted in asthma and COPD. Moreover, we have also found a remarkable positive relation between exhaled CO and NO. The present study demonstrated that the levels of CO, NO and %COHb in exhaled air might have played a significant role in lung oxidative stress and inflammation. Moreover, these biomarkers in exhaled air may provide a simple, non-invasive and sensitive approach with which to monitor airway inflammation and to assess the response to drug treatment.

Key words: Asthma, chronic obstructive pulmonary disease, carbon monoxide, nitric oxide, oxidative stress.

INTRODUCTION

Chronic inflammation is a critical feature of asthma and chronic obstructive pulmonary disease (COPD). This inflammation associated with increased production of reactive oxygen species (ROS) and causes oxidative stress in the lungs. It has played a key role in the pathogenesis of asthma and COPD [1-3]. ROS include the superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) [3-5] and cause oxidation of nucleic acids, proteins and membrane lipids [6]. To counter the oxidant-mediated toxicity in the form of increased oxidative burden generated from airways leucocytes in the blood or air spaces are scavenged by several antioxidants and antioxidant enzymes [7].

It was well documented that breath analysis has a great potential in the diagnosis and treatment of respiratory problems including asthma and COPD. Therefore, we studied exhaled carbon monoxide (CO), carboxyhaemoglobin (COHb) and exhaled nitric oxide (NO) as markers of inflammation in COPD as well as in asthma [8, 9]. Clinical research has been demonstrated that there is a useful relationship between CO and COHb obtained by a short period of breath holding by the person [10]. CO concentration demonstrates the levels of poisonous inhaled CO while the COHb shows the percentage of vital oxygen that has been replaced in the bloodstream. Some workers have also

reported that heme oxygenase is present in the pulmonary vascular endothelium [11] and alveolar macrophages [12]. Upregulation of heme oxygenase-1 (HO-1) by oxidative stress [11, 13] and inflammatory cytokines [14, 15] in airways and lung inflammation has been reported, the cause of the increased levels of exhaled CO in patients with inflammatory lung diseases such as asthma, acute pneumonia, silicosis, bronchiectasis, upper respiratory tract infections (URTIs) and allergic rhinitis [16-23]. Level of arterial blood COHb correlate to exhaled CO concentrations [24] and have also been reported as a marker in inflammatory pulmonary disease including bronchial asthma, acute pneumonia and silicosis [21-23]. It is assumed that the major site of airflow limitation in asthma and COPD is the peripheral airways. These findings entail a role of endogenous CO in airway inflammatory diseases.

Several earlier workers have found that exhaled CO may reflect inflammation in the asthma and COPD. Exhaled CO levels were elevated in untreated asthmatic patients than in non-smoking healthy controls and together with sputum eosinophils counts it decreased considerably after four weeks of treatment with inhaled corticosteroid [16, 17]. It has been also shown that exhaled CO levels increases during an asthma and COPD exacerbation [25, 26]. Therefore, measurement of exhaled CO may be a simple method of detecting and assessing airway inflammation in asthma and COPD.

Several evidence suggests that endogenous NO plays a key role in the physiologic regulation of airways as well as in pathophysiology of airway diseases [27, 28]. Thus, exhaled NO has been suggested as a marker of airway inflammation as well as oxidative stress and can be easily measured in the airways. Although, exhaled NO was magnificiently increased in asthma [29] and significantly correlates with the degree of sputum eosinophils [30] but in COPD, it increases in less and does not correlate with inflammatory indices [31, 32]. Some earlier studies showed increased NO level where as others exhibited decreased level [33, 34]. Some previous workers did not find any difference in NO concentrations between COPD and controls [35]. Both CO and NO may be influenced by inhaled steroid treatment [16, 29, 36]. It has been shown that exhaled NO rapidly decreased after treatment with oral or inhaled steroids and it depended on dose [33].

In this study we quantify lung oxidative stress in asthma and COPD patients (with and without smoking habit) by measuring CO and NO levels in exhaled air. Moreover, it established the relationship between exhaled CO and NO concentrations with degree of airflow obstruction (FEV1 % predicted).

MATERIALS AND METHODS

Study subjects

This study was comprised of two phases. All the subjects were recruited through the Department of TB and Respiratory Diseases, Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, patients attending Out Patient Department (OPD) and In Patient Departments (IPD). For this investgation, we have enrolled 55 asthmatic and 55 COPD patients, respectively. Fifty healthy control subjects were selected without respiratory abnormalities and normal lung function. All the patients had a progressive symptom like cough, productive sputum and breathlessness. As active and passive smoking influences levels of exhaled CO as well as NO and may interfere with its endogenous production, we therefore recruited only non-smoking individuals for this study. Control and asthmatic patients were non-smokers and they had none previous smoking history where as COPD patients had previous smoking history and stopped smoking before 6 months.

The clinical severity of asthma and COPD was determined using the criteria (appropriate clinical and respiratory function test) defined in the global initiative for asthma guidelines (GINA) and global obstructive lung diseases (GOLD) guidelines [25, 26]. The diagnosis of asthma and COPD was established on the basis of reversibility of airways obstruction with greater than 12% and less than 12% improvement in FEV1 after inhalation of 200 µg of salbutamol from a nebulizer. Spirometry was used in confirming the presence of airway obstruction. None of the patients were taking any antioxidant supplements and did not show any symptoms of upper and lower respiratory tract infection. Patients with systemic, vascular, renal and hepatic diseases were in exclusion criterion. No drug was allowed on the day of testing. All groups were subjected to record their demographic profile, clinical, radiological findings, pulmonary function measurement and smoking history.

The informed consent was obtained in written from all the recruited subjects and Ethics Committee of Medical College approved this study.

Pulmonary function test

Pulmonary function test was performed by Easy on PC spirometer (ndd Medizintechnik AG, Zurich, Switzerland) and the best value from 3 manoeuvres was recorded as an absolute value (in liters) and as a percentage of the predicted value.

Anthropometric measurement

All anthropometric measurements were taken according to a standardized method. Body weight was measured with participants by using a balance beam scale. Height was measured at the same time. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Exhaled CO and %COHb measurement

Exhaled CO and %COHb was measured on a portable $piCO^+$ smokerlyzer (Breath CO monitor, Bedfont Scientific Ltd., Kent, England). In this procedure, participants were said to inhale deeply and hold their breath fully for 15 sec before exhaling into a disposable mouthpiece [37]. The subjects exhaled slowly from total lung capacity with a constant flow. This procedure was repeated three times with 1 min of normal breathing between each repetition and the mean value was used for analysis. Exhaled CO level measured by the analyzer and was reported to correlate closely with blood COHb concentration [38].

Exhaled NO measurement

NO level in exhaled air was measured by a portable NO breath (F_{ENO} Monitor, Bedfont Scientific Ltd., Kent, England) with a lower detection limit of 5 part per billion (ppb) and a resolution of ±5 ppb. In this, participants are said to inhale deeply and hold their breath before exhaling rapidly. This procedure was repeated three times and there was a rest of minimum 30 sec between each repetition [39]. The mean value of three measurements in each individual was used for analysis.

Statistical analysis of data

Data have been expressed in Mean ± SEM. Statistical analyses were performed with statistical package for the social sciences for windows (Version 16.0; SPSS Inc) and Graph Pad Prism 5.01. The study parameters were compared among patients with different groups by using one-way analysis of variance (ANOVA). The relationship between different study parameters and the degree of airways obstruction was evaluated by computing the Pearson's correlation coefficient. $p<0.05$ was considered significant.

RESULTS

Table 1 depicts spirometric and demographic characteristics of the study groups. The mean age of asthma and COPD patients was 32.96 ± 1.91 and 41.73 ± 2.09 year where as the mean age of the control was 36.44 ± 2.09 years ($p<0.05$). No significant difference was found among the study groups for gender ($p>0.05$). Patients with asthma and COPD had considerably lower FEV1 (% predicted) and other spirometry indices as well as anthropometric measurements than healthy controls.

Table: 1. Spirometric and demographic characteristics of study subjects without smoking history

Characteristics	Control N = 50	Asthmatic patients N = 55	COPD patients N = 55	*p* value
Age	36.44 ± 2.09	32.96 ± 1.91	41.73 ± 2.09	0.008
Sex (Male/Female)	36/14	31/24	40/15	0.124
BMI (kg/m^2)	23.76 ± 0.60	20.35 ± 0.50	20.43 ± 0.47	<0.001
FEV1 (% predicted)	83.38 ± 1.25	56.62 ± 2.32	48.36 ± 2.54	<0.001
FVC (% predicted)	85.10 ± 1.23	67.80 ± 2.15	62.91 ± 2.07	<0.001
FEV1/FVC (% predicted)	95.98 ± 0.94	80.02 ± 2.13	72.51 ± 2.20	<0.001
FEF 25-75%	72.26 ± 3.55	36.58 ± 2.86	25.82 ± 3.01	<0.001
FIVC%	87.66 ± 1.33	64.00 ± 2.76	62.44 ± 2.32	<0.001

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF, forced expiratory flow; FIVC, forced inspiratory vital capacity. Values are expressed as mean ± SEM and one-way ANOVA analysis for compare the variables.

Table 2 shows that the mean exhaled CO level. It was found to be remarkably higher in asthmatic patients (6.13 ± 0.42 ppm, $p<0.05$) than in controls (4.62 ± 0.41 ppm). The mean exhaled CO level was significantly much higher among COPD patients (6.47 ± 0.44 ppm, $p<0.01$) while there was no significant difference between asthma and COPD ($p>0.05$). The %COHb levels were significantly higher in COPD and asthma patients as compared to control

group. The values being 1.67 ± 0.07, 1.61 ± 0.08 and 1.37 ± 0.07, respectively (COPD vs control, $p<0.01$; asthma vs control, $p<0.05$).

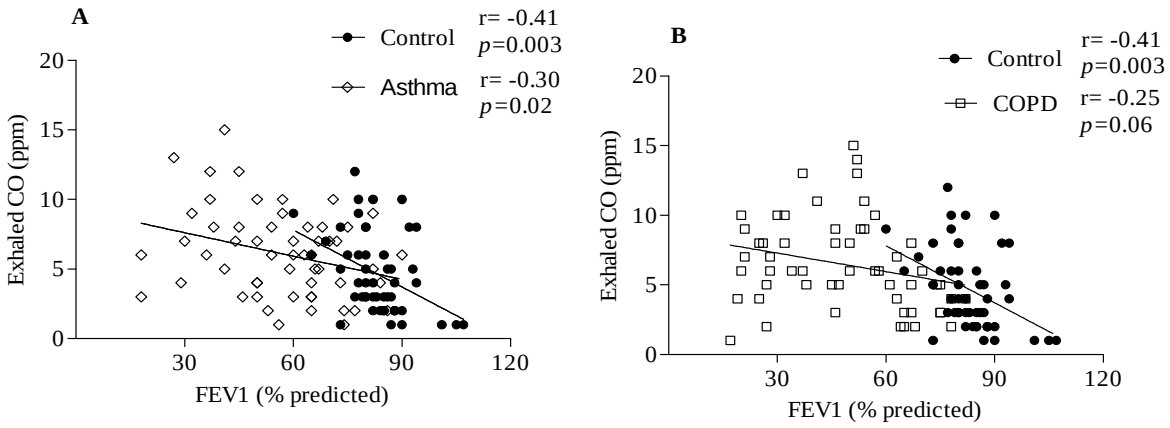

Fig 1. (A) Relationship between the level of Exhaled CO and FEV1% predicted in asthma and (B) COPD patients. Pearson correlation coefficient is denoted by 'r' and line correspond to the fitted linear regression equation.

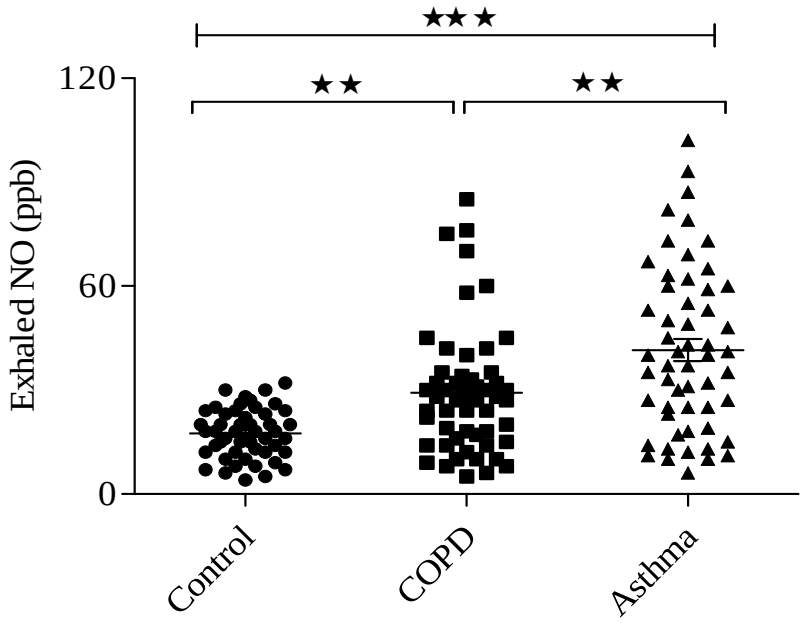

Fig 2. NO concentration in the exhaled air of healthy control, COPD and asthmatic patients $^{**}p<0.01$; $^{***}p<0.001$. Mean values are shown by horizontal bars.

Table: 2. Exhaled CO and %COHb in study subjects

Study groups	Exhaled CO level (ppm)	%COHb level
Healthy control	4.62 ± 0.41 (3.80-5.44)	1.37 ± 0.07 (1.24-1.50)
Asthmatic patients[a]	6.13 ± 0.42 (5.28-6.98)	1.61 ± 0.08 (1.47-1.74)
COPD patients[b, c]	6.47 ± 0.44 (5.58-7.36)	1.67 ± 0.07 (1.52-1.81)

Fig 1 (A and B) demonstrates the correlation between exhaled CO and FEV1 (% predicted) in asthma and COPD patients and it showed negative correlation (r=-0.30, $p<0.05$ and r=-0.25, $p>0.05$). It was also observed that the negative correlation found between exhaled CO and FEV1% predicted in healthy controls (r=-0.41, $p<0.01$).

The exhaled NO level was remarkably much higher in asthmatic patients (41.56 ± 3.22 ppb; 95% CI, 35.12 to 48.01; $p<0.001$) than in patients with COPD (29.22 ± 2.43 ppb; 95% CI, 24.34 to 34.10; $p<0.01$) and healthy controls (17.42 ± 1.01 ppb; 95% CI, 15.40 to 19.44) [Fig 2]. A negative correlation was found between exhaled NO levels and FEV1 (% predicted) in asthma (r=-0.55, $p<0.001$; Fig 3 A) as well as in COPD (r=-0.42, $p<0.01$; Fig 3 B). It was also observed that the negative correlation between exhaled NO and FEV1 (% predicted) in healthy controls (r=-0.41, $p<0.01$). Table 3 demonstrates correlation between exhaled NO levels and other demographic profiles of the study subjects.

Table: 3. Pearson's correlation coefficient between exhaled NO concentration and other demographic profiles in study population Correlation Coefficient (r)

Study parameter	Correlation Coefficient (r)		
	Control	Asthma	COPD
Age	0.35^{*}	$0.09^{\#}$	0.52^{**}
Sex	0.36^{**}	0.28^{*}	$0.25^{\#}$
BMI	$-0.18^{\#}$	-0.31^{*}	$-0.03^{\#}$
Exhaled CO	0.46^{**}	0.31^{*}	0.45^{**}
% COHb	0.47^{**}	0.31^{*}	0.45^{**}

Pearson correlation coefficient is denoted by 'r'; $^{\#}p>0.05$, Non-significant; $^{*}p<0.05$; $^{**}p<0.01$.

DISCUSSION

It has already been described by earlier workers that oxidative stress and ROS have been implicated in the pathogenesis of asthma [1] and COPD [2]. Induction of a stress response protein, HO-1 is one of the mechanisms protecting against an oxidative stress [6, 40]. Enhanced HO-1 protein expression may be due to the induction of enzyme by inflammatory cytokines and oxidants such as interleukins, tumour necrosis factor-α (TNF-α), interferon-γ, and H_2O_2 which are capable of inducing HO-1 expression in cell line and tissues [41, 42]. Induced HO-1 catalyzes the degradation of heme into bilirubin that can scavenge $HO^{\cdot}$ *in vitro* as efficiently as α-tocopherol and the by-products of HO-1 activity are free iron and CO [41]. Therefore, measurement of exhaled CO is a simple method for detecting and monitoring cytokine mediated inflammation and oxidative stress in the respiratory tract. Accurate assessment of airway inflammation and oxidative stress and its location within the lung is important for the clinical management of lung disease. Taking into consideration the complexity of inflammation and oxidative stress, it is unlikely that a single molecule measured in exhaled breath or in other biological fluids, may provide a complete profile.

In view of this, we have studied levels of CO, %COHb and NO in exhaled air of asthma and COPD patients. We observed that asthma and COPD patients have remarkable higher CO and NO values in exhaled air than control. In this study we have also demonstrated %COHb level and observed significant higher value compared to controls (Table 2). There was no significant difference found in the levels of exhaled CO and %COHb of COPD patients compared to asthmatic patients. It has also reported that treated stable asthmatics and healthy control subjects had similar exhaled CO levels [16, 17]. Yamara and co-workers [25] have been previously reported that exhaled CO increased during an asthmatic exacerbation. These results were heightened by recent research and it had supported our observations [16, 17, 25, 26].

Some workers previously reported that exhaled NO level increased in many inflammatory airway diseases including COPD and asthma [43]. Higher values of exhaled NO was found in asthmatic patients than COPD and controls because inflammatory agents triggered respiratory tract infection and allergens exhaled NO levels [44, 45]. The levels of NO in exhaled air were higher in atopic asthmatics than non-atopic asthmatics [46]. We have found a significant increased exhaled NO levels in asthma compared to COPD and controls. Despite to asthma, we have found minimally increased exhaled NO in COPD which might enhance disease progression and exacerbations [43]. Moreover, airway inflammation is concomitant with the decrease in lung function as shown by the inverse correlation between exhaled CO and NO levels with FEV1 (% predicted) in both asthma and COPD [Fig 1 (A-B) and Fig 3 (A-B)]. There was a significant correlation between exhaled CO and NO with FEV1 (% predicted) in patients with COPD and asthma [9, 47]. It was noticed that there was no remarkable correlation between CO levels and lung function; however CO reflects primarily oxidant damage and one of a key factor for the inflammatory

process [48]. The inverse correlation between exhaled CO and airway obstruction indicated that patients had more severe disease.

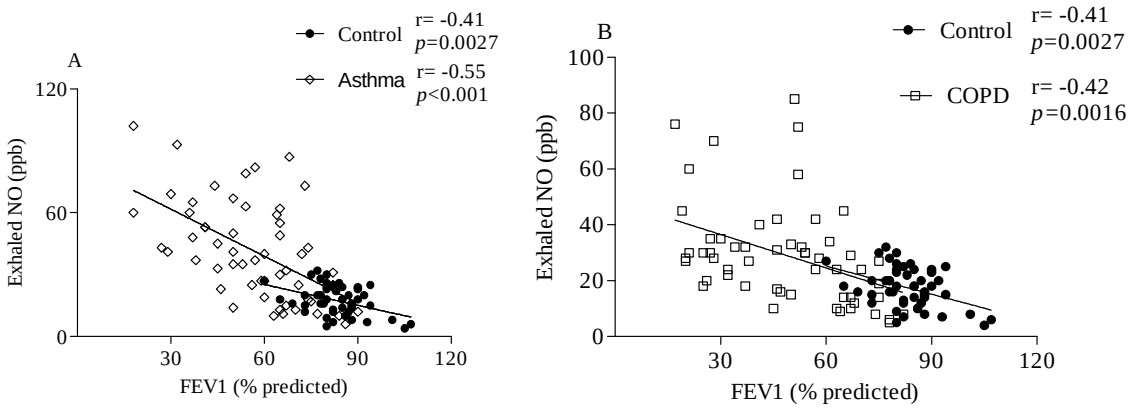

Fig 3. (A) Correlation between the level of Exhaled NO and FEV1 (% predicted) in asthma and (B) COPD patients. *r* denotes Pearson correlation coefficient and line correspond to the fitted linear regression equation.

CONCLUSION

In this study, we have shown that exhaled CO, NO and %COHb may be used to quantify lung oxidative stress and inflammation in asthma and COPD. Measurements of these biomarkers in the exhaled air may provide a simple, non-invasive, sensitive approach with which to monitor airway inflammation and to assess the response to drug treatment. Further studies, we realized the need to analyze pathophysiological mechanisms involved in lung injury related to CO poisoning.

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