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Original Article

The Levels of Exhaled Nitric Oxide in Mustard Airway Disease

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Abstract

Background: The fractional excretion of exhaled nitric oxide (FeNO) has been proposed as a noninvasive measure of airway inflammation. FeNO levels were assessed in this study to evaluate airway inflammatory characteristics in mustard airway disease (MAD).

Methods: Thirty-three MAD patients were involved in the study to determine the level of exhaled nitric oxide (NO) and its relationship to lung function; 16 MAD patients with normal symptoms and 17 MAD patients with severe symptoms were identified from this sample. To regulate their condition, severe individuals were given inhaled corticosteroids.

Results: Exhaled NO levels were greater in severe patients than in normal patients, but this was not significant. Furthermore, the findings revealed that FeNO concentrations were positively linked with carbon monoxide transfer factor in the severe group (TLCO). We were unable to find a link between pulmonary volumes and FeNO levels. We also found that 17% of patients in the severe category had FeNO levels greater than 40 ppb (cutoff point of FeNO for patients with asthma). Although, the severe group's usage of inhaled corticosteroids may lower FeNO levels.

Conclusion: Based on the FeNO results, we conclude that MAD is a diverse disorder. Exhaled NO was found to be able to detect the asthma phenotype in MAD, and FeNO was found to be a beneficial supplement to aid lung function during MAD evaluation. FeNO levels in MAD patients were similar to those in COPD patients.

Keywords: Exhaled nitric oxide, Mustard airway disease, Asthma phenotype, Mustard gas, FeNO.

Introduction

Mustard airway disease (MAD) is a pulmonary disorder of exposure to sulfur mustard (SM), which demonstrates a broad clinical heterogeneity [1]. Several phenotypes of MAD (chronic bronchitis, bronchitis obliterans, asthma, chronic obstructive pulmonary disease (COPD), and so on) have been identified amongst subjects who are suffering late pulmonary complications of SM [2]. Since these phenotypes have different therapeutic strategies, differentiating patients may help to guide the treatment decision.

Although MAD patients seem to be closer to COPD and seldom show complete reversibility after taking inhaled corticosteroids (ICS), COPD patients with high levels of fractional exhaled nitric oxide (FeNO) respond to ICS and they have an asthma-like pattern [3]. FeNO is one of the sensitive, reproducible, and noninvasive eosinophilic inflammatory markers which is elevated in patients with asthma, patients with asthma–COPD overlap syndrome (ACOS), and eosinophilic COPD [4,5]. As mentioned above, MAD has different phenotypes and these phenotypes should be diagnosed for better treatment. Accordingly, the use of FeNO for the identification and management of asthmatic phenotypes of MAD is our aim in the present study

Methods

Population

Thirty-three MAD patients who were exposed to SM 30 years ago (Iran-Iraq war during 1983-88) were studied with a mean age of 48.15 (SD: 6.55) years. All participants were collected from Sasan Hospital (Tehran, Iran). From this group, 16 patients were defined with normal PFT (FEV_1 > 80%, FEV_1/FVC

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>70% predicted) and 17 patients with severe symptoms (30% <FEV_1< 50%, FEV_1/FVC< 70% predicted). The stage of MAD patients was diagnosed according to the GOLD guidelines [6], where FEV_1 is forced expiratory volume in one second, and FVC is the forced vital capacity. All subjects were selected from male volunteers and had documented SM exposure. The participants completed a questionnaire about taking their medications, comorbidities, history of allergies, and smoking. The severe patients were receiving fluticasone 250–500 mg/salmeterol 25–50 mg per 12 h.

The inclusion criteria were diagnosis of MAD based on existing documents being a nonsmoker and nonalcoholic, and a clinically stable phase. Subjects were excluded from this study if they had concomitant illnesses (e.g. diabetes, cancer, cardiovascular disorder, renal disorders, and other pulmonary disorders such as COPD due to smoking), lung infection for four weeks before entering the study, being a smoker, and drinking alcohol. The protocols of this study were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (SBMU.REC.1392.628). Before enrolling the samples, informed consent was taken from each subject.

Measurements

2.1.1. Pulmonary function tests (PFT)

PFTs (for lung volumes) and spirometry (for lung function) were assessed for all participants according to the guidelines of the European Respiratory Society (ERS) [7] by ZAN® 530 Body Plethysmograph equipped with SB CO-Diffusion module. The transfer factor for carbon monoxide (TLCO) was done by the single-breath method.

Measurement of FeNO

Exhaled NO was measured by American Thoracic Society (ATS)/ERS guidelines using an NO analyzer (Bedfont Scientific Ltd., UK). NO-free air was inhaled to the total lung capacity, then directly exhaled into the NO analyzer at a flow rate of 50 mL/s.

Statistical analysis

Statistical analyses were performed using SPSS (version 16.0 SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to evaluate the normality of the distribution of variables. The results were expressed as mean ± standard error (SE) for normally distributed variables and mean (range) for non-normal distributed ones. The Student's t-test was used to compare continuous variables between the two groups with normally distributed data and the Mann-Whitney U test for those that were not normal. Statistical significance was considered as $p<0.05$. Bivariate correlations between different parameters and FeNO values were performed using Spearman's rank correlation.

Result

Anthropometric characteristics and pulmonary function tests

Anthropometric measurements and pulmonary function tests of the study groups were summarized in Table 1. There were no significant differences between the study groups regarding age and BMI.

As shown in Table 1, pulmonary function tests revealed that the normal MAD group had a significantly higher forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and FVC/FEV1 values compared to the severe MAD group. On the other hand, specific airway resistance (sRA), functional residual capacity (FRC), residual volume (RV), and RV/Total lung capacity (TLC) were significantly lower in normal MADs than in severe cases.

FeNO values between the normal and severe subjects

The mean FeNO value in normal spirometry MADs was 12.25 ppb, which was not significantly different from the severe group (19.12 ppb, $p>0.05$) (Table 1). Notably, the mean FeNO values were in the normal range between the two groups. The results showed that in the severe group, 17% (3 out of 17 patients) of patients had FeNO levels greater than 40 ppb (cutoff point of FeNO for asthma patients [8]). Further, 4 out of 17 severe MADs and 1 out of 16 normal spirometry MADs had FeNO above 25 ppb.

Association between FeNO values and anthropometric as well as pulmonary function tests

Spearman's rank correlation revealed that FeNO values were positively correlated with the transfer factor for carbon monoxide (TLCO) (r= 0.767, *p*= 0.016) in the severe MAD group.

However, there was no correlation between other pulmonary volumes and FeNO levels in the two study groups.

Table 1: Anthropometric and pulmonary function tests of participants

Variable	Normal MADs (n=16)	Severe MADs (n=17)	*p*-value
Age (y)	47.69±1.01	48.52±1.99	0.738
Height (cm)	173.12±2.03	171.52±1.73	0.597
Weight (kg)	81.25±1.76	74.41±4.85	0.198
BMI (kg/m^2)	26.75±0.65	24.71±1.67	0.277
FVC (% pred)	80.37±2.93	42.11±4.03	0.00
FEV$_1$ (% pred)	82.12±3.21	32.05±3.3	0.00
FVC/FEV$_1$ (%)	83.31±1.52	63.47±3.54	0.00
sRaw (% pred)	100.06±16.83	366.0±67.42	0.001
FRC (% pred)	102.56±7.25	135.94±11.22	0.019
TLC (% pred)	85.0±5.24	86.88±7.02	0.833
RV (% pred)	88.75±8.97	185.94±19.03	0.00
RV/TLC (%)	100.18±7.8	201.11±9.6	0.00
TLCO *(% pred)	110.26±7.15	107.66±18.22	0.878
KCO *(% pred)	130.26±6.16	126.0±11.85	0.728
FeNO (ppb)	12.25 (4-29)	19.12 (1-63)	0.557

Values are presented as mean±SE and mean (range). FEV$_1$: Forced expiratory volume in 1 second; FVC: Forced vital capacity; sRaw: specific airway resistance; FRC: Functional residual capacity; TLC: Total lung capacity; RV: Residual volume; TLCO: transfer factor for carbon monoxide; KCO: rate of uptake of carbon monoxide; FeNO: fractional exhaled nitric oxide; ppb: parts per billion. Comparison between two groups (normal *vs*. severe MADs) using the independent t-test (for normal distributed data), Mann-Whitney U test (for non-normal distributed data). * data is for 24 subjects.

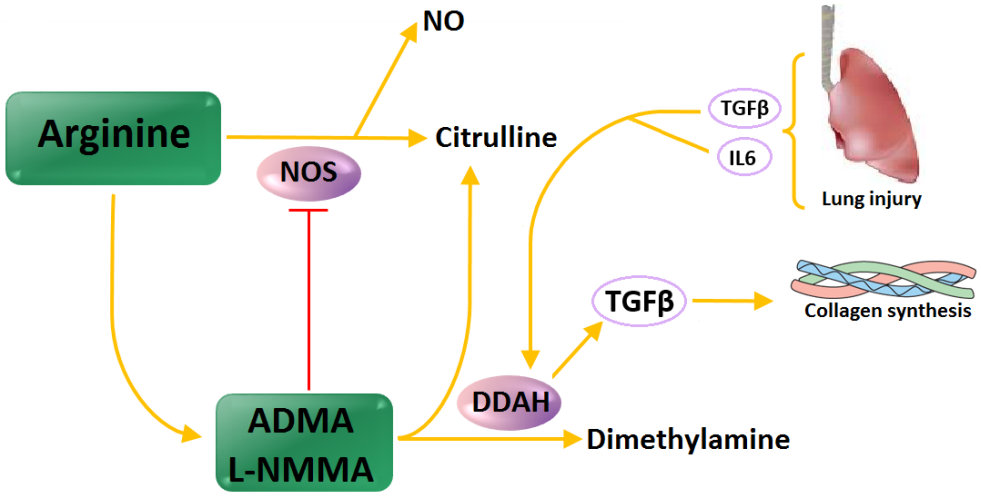

Figure 1: Increased NO levels in MAD patients. TGF-β and IL-6 can up-regulate DDAH. Then, elevated expression and activity of DDAH increases fibroblast-induced collagen production in an ADMA-independent manner.

Discussion

Airway inflammation is observed in asthmatic patients, and elevated levels of FeNO can detect eosinophilic inflammation in these patients [9]. We conducted a study to use the FeNO value to differentiate different phenotypes of MAD patients. Each MAD phenotype has a different treatment method; for example, most COPD patients with FeNO levels less than 25 ppb do not show effective responses to inhaled corticosteroids (ICS). While ICSs have a very good response to asthmatic and COPD patients with FeNO levels greater than 25 ppb [3,10]. Thus, the importance of finding a biomarker that discerns MAD phenotypes is clear. Previous investigations have measured FeNO in patients with asthma and asthma-COPD overlap syndrome (ACOS) as well as other lung diseases [11]. But there has been no study indicating the role of FeNO in MAD patients so far.

Our results demonstrated that the measurement of FeNO was helpful in the diagnosis of the asthmatic phenotype of MADs. Although other phenotypes of MADs are also inflammatory diseases, the value of FeNO was not elevated beyond 40 ppb in other subtypes. On the other hand, although in several lung diseases such as COPD or pulmonary fibrosis, FeNO production is markedly increased as compared to healthy controls, these rises are not as substantial as asthma. Notably, increased FeNO in our collected patients could suggest either more severe inflammation or inadequate treatment.

The mechanism of FeNO elevation in SM-exposed patients is unknown. The major hypothesis might be based on what happens in asthma. A previous study reported that plasma asymmetric dimethylarginine (ADMA) and monomethyl arginine (L-NMMA) diminished [12]. These metabolites were metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to form citrulline and dimethylamine [13]. Since ADMA and L-NMMA are endogenous inhibitors of nitric oxide synthase (NOS), the synthesizing enzyme for NO, reduced levels of these metabolites may cause increased levels of FeNO. Also, previous studies reported that the expression of inducible NOS (iNOS) grows in asthmatic patients [14], as it rises in MAD patients [15]. Also, the up-regulation of DDAH by transforming growth factor (TGF-β) as a pro-fibrotic cytokine has been previously reported [16]. In this regard, elevated expression and activity of DDAH increase fibroblast-induced collagen production in an ADMA-independent manner. In line with this result, Mirzamani *et al.* [17] showed that the expression of TGF-β in human airway fibroblasts from SM-exposed patients significantly increased as compared with healthy controls. Also, Adelipour *et al.* [18] reported that the mRNA expression levels of Smads, as downstream molecules of the TGF-β signaling pathway, were up-regulated in SM-exposed patients in comparison with healthy controls. Ghanei *et al.* observed that collagen depositions increased in airway walls in severe MAD patients compared to moderate cases [19]. Additionally, our prior investigations demonstrated that the serum levels of citrulline and dimethylamine rose in the severe MAD patients [20]. These findings might suggest that DDAH expression or activity may increase in severe MAD patients with high levels of FeNO and produce collagen depositions in airway walls (Fig. 1). Further studies are required to verify this hypothesis.

Another hypothesis that can be considered for our results is that SM might be a trigger or risk factor for asthma in genetically susceptible individuals. Indeed, it is possible that people had mild or intermittent asthma before SM exposure; then after exposure to this agent, their asthma was exacerbated. Alternatively, the subjects may have not had asthma but had a genetic susceptibility to asthma causing asthma after SM exposure.

The present study had some limitations that should be mentioned. First, dietary evaluations were not performed on the subjects, and the FeNO concentrations were elevated after the intake of a nitrate-rich meal [21]. Another confounding factor was the use of inhaled corticosteroids which could not be stopped for severe MAD patients, where inhaled corticosteroids reduce FeNO levels [22].

Conclusion

The FeNO concentrations were higher among asthmatic phenotypes of MAD patients. FeNO could be a non-invasive procedure for differentiating asthmatic phenotypes and deciding on the treatment strategy. Increased levels of FeNO (≥25 ppb) in patients with MAD may predict responders to ICSs, similar to patients with ACOS who might benefit from applying ICSs.

Abbreviations

MAD: Mustard airway disease, COPD: Chronic obstructive pulmonary disease, SM: Sulfur mustard, ICS: Inhaled corticosteroids, ACOS: Asthma–COPD overlap syndrome, FEV1: Forced expiratory volume in one second, FVC: Forced vital capacity, PFT: Pulmonary function tests, ERS: European Respiratory Society, ADMA: Asymmetric dimethylarginine, L-NMMA: L-NG-monomethyl arginine, DDAH: Dimethylarginine dimethylaminohydrolase, NOS: Nitric oxide synthase, TGF-β: Transforming growth factor, sRaw: Specific airway resistance, FRC: Functional residual capacity, TLC: Total lung capacity, RV: Residual volume, TLCO: transfer factor for carbon monoxide, KCO: rate of uptake of carbon monoxide.

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Authors' contributions

RA conceived the study. BFNMG performed data analysis and wrote the manuscript and both authors read and confirmed the final version of manuscript

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Disclosure statement

None declared.

Ethical Statement

The protocols of this study were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (SBMU). Prior to enrolling the samples, informed consent was taken from each subject.

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