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Exhaled nitric oxide in patients with PiZZ Phenotype-related α 1-anti-trypsin deficiency

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There is no report of exhaled NO (eNO) in subjects with different phenotypes of α 1-anti-trypsin (AAT) deficiency.

Exhaled nitric oxide was evaluated by means of single-breath chemiluminescence analysis (fractional exhaled concentration at the plateau level [pIFE_{NO}]) in 40 patients with AAT deficiency. Patients were divided according to the protease inhibitor (Pi) phenotype: PiMZ/MS, $n=25$; PiSZ, $n=6$; PiZZ, $n=9$. Nineteen healthy subjects served as controls. Levels of eNO in PiZZ patients were also compared with those of subjects, without AAT deficiency (PiMM), matched for diagnosis, sex, age, smoking habit and forced expiratory volume in 1 sec (FEV₁). In AAT deficiency subjects airway hyper-responsiveness to methacholine (PD₂₀ FEV₁) was also assessed.

pIFE_{NO} was significantly lower in the PiZZ group (4.5 ± 1.4 ppb) than in matched PiMM subjects (8.2 ± 3.8 ppb), in healthy controls (9.3 ± 2.8 ppb) and in patients of other phenotypes. Dynamic lung volumes and DL_{CO} were significantly lower in PiZZ than in other AAT-deficient patients. Bronchial hyper-responsiveness was not different among AAT phenotypes.

These results suggest that eNO may be significantly reduced in PiZZ as compared to healthy control subjects and to AAT subjects with other phenotypes, independent of the level of airway obstruction. Whether, at least potentially, eNO may be considered as an early marker of lung involvement in AAT deficiency must be confirmed with studies on larger number of subjects.

Key words: chronic airway obstruction; bronchial hyper-responsiveness; chemiluminescence.

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Introduction

α 1-anti-trypsin (AAT) deficiency is an autosomal recessive disorder caused by mutant variants of the human protease inhibitor (Pi) system locus on chromosome 14 (1). In subjects with AAT deficiency the severity of lung disease and the rate of decline in lung function vary markedly (2); the genetic profile affects susceptibility to lung destruction (3). In AAT deficiency, chronic obstructive pulmonary disease (COPD) has been found to be associated to the PiZ homozygous phenotype (PiZZ) (2–3), whereas airway hyper-responsiveness has been found to be more frequently associated to subjects with PiM heterozygous phenotypes (PiMS/MZ) (4).

Nitric oxide (NO) may regulate vascular and airway tone in the lung, thus influencing lung function (5–7). Exhaled NO (eNO) has been detected in both animals (8) and

humans (9). Increased eNO has been demonstrated in bronchial asthma (10), whereas decreased levels were found in the most severe stages of chronic diseases resulting in pulmonary hypertension such as chronic heart failure (11), COPD (12,13) and systemic sclerosis (14).

Recently, polymorphic variants of the constitutive endothelial nitric oxide synthase (cNOS) have been found to be associated with lung emphysema in subjects with AAT deficiency, suggesting a role of NO in the pathogenesis of the lung disease of these subjects (15). However, there is no report of eNO measurement in AAT deficient subjects with different phenotypes and related lung function derangements (if any). In this study eNO was compared in subjects with different phenotypic expression of AAT deficiency. PiZZ were also compared with matched subjects without AAT deficiency.

Materials and methods

Patients gave their informed consent to participate into the study, which was approved by the Ethical Committees of the Salvatore Maugeri Foundation IRCCS and the Uni-

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versity of Brescia, and was conducted according to the Declaration of Helsinki.

SUBJECTS

Forty subjects with AAT deficiency, included in the Italian National Register (16) and attending the Chest Clinic of the Institute of Internal Medicine at the University of Brescia were studied. Diagnosis of AAT deficiency was confirmed by evaluation of Pi phenotypes. According to the phenotype, subjects were divided into three groups: PiMZ/MS, PiSZ and PiZZ. Diagnosis of COPD and asthma were made according to the standard criteria (17,18). Atopy was defined by positive skin tests and/or increased level of specific serum IgE. Pulmonary emphysema was confirmed on the basis of lung function tests, DL_{CO} and computed tomography (CT) scan. Bronchiectases were assessed by ultra-thin CT scan. At the time of the study all the subjects were in a stable condition and free from acute exacerbation for at least 4 weeks. Fourteen subjects used regular treatment with inhaled bronchodilators. No patient was on systemic or inhaled steroids. One PiZZ subject suffered from chronic respiratory insufficiency and had been on long-term oxygen therapy for 1 year. The demographic, anthropometric and clinical characteristics of the patients according to the phenotypes are shown in Table 1. PiZZ subjects were compared with subjects without AAT deficiency (PiMM), matched for associated diagnosis, sex, age (± 5 years), smoking habits, post-bronchodilator forced expiratory volume in 1 sec (FEV₁) ($\pm 12\%$ predicted) and lack of use of inhaled steroids. Furthermore 19 age-related healthy volunteers (11 male, age: 45 ± 8 years) were studied as controls for eNO measurement in our laboratory.

MEASUREMENTS

Phenotypes were determined using dried blood specimens as previously described (19,20). Serum AAT was determined by radial immunodiffusion (21).

Static and dynamic volumes were measured by means of nitrogen wash-out method and pneumotachograph with volume integrator (CAD/NET System; Medical Graphic Corporation St. Paul, MN, U.S.A.), according to the European Respiratory Society (ERS) standard procedure (22). Forced vital capacity (FVC) manoeuvres were performed before and after the administration of 200 mcg of inhaled salbutamol. DL_{CO} was assessed by means of a pulmonary diagnostic system (PF/DX system, Medical Graphic Corporation) with patients in the sitting position. The predicted values according to Quanjer (23) were used.

Methacholine challenge was performed according to the guidelines (24) and the cumulative dose at which FEV₁ fell by 20% from the post-saline value (PD₂₀FEV₁) was calculated.

Measurements of eNO were performed in the Lung Function Unit of the Scientific Institute of Gussago, Salvatore Maugeri Foundation, as previously described (12,13), blind to the patient's clinical and functional status. Briefly, subjects were asked to discontinue bronchodilators

and to abstain from alcohol and caffeine at least 12 h and from food at least 4 h before the assessment. Exhaled NO was assessed on-line by means of a high-resolution (0.3 ppb) chemiluminescence analyser (LR 2000 series, Logan Research, Kent, U.K.). An internal restrictor in the breathing circuit allowed for expiration against a resistance in order to keep the soft palate closed and to prevent contamination of the exhaled air with nasal NO; a single-breath vital capacity (VC) manoeuvre at constant flow (50 ml sec⁻¹) was performed as recommended (25). The analyser was daily calibrated using a certified NO mixture (96 ppb) in nitrogen (Messer S.p.A., Collegno, Italy). Ambient air was monitored for NO concentration before starting evaluations. Measurements were only taken when ambient NO was below 40 ppb (26). Fractional exhaled NO concentration at the plateau level (pFE_{NO}) was obtained from the eNO curve as previously described (12,13). The mean value of three reproducible measurements was considered for analysis.

ANALYSIS

Data are given as frequencies and/or means \pm standard deviation (SD). Analysis of differences was performed by a Kruskal-Wallis one-way ANOVA. The Mann-Whitney *U*-test for non-parametric variables was used when appropriate. Chi-square and Cochran's Q analysis was used to test the categorized variables. Intra-patient FE_{NO} differences were analysed by ANOVA for repeated measures with Huynh-Feldt correction. As no significant within-subject difference was found, the mean FE_{NO} value of three consecutive measurements was taken into account. Spearman correlation coefficients were calculated among all the considered variables. A *P*-value less than 0.05 was considered to be statistically significant.

Results

CLINICAL STATUS

Anthropometric, demographic and clinical characteristics of the AAT subjects are shown in Table 1. Most of the subjects presented the PiM phenotypes. As expected, serum AAT levels were significantly lower in PiZZ subjects (1-3). No differences in the frequency distribution of smoking habit or atopy were found among the groups. A diagnosis of COPD and/or emphysema was found in most of the PiZZ subjects but only in one PiMZ subject. Among six COPD patients in the PiZZ group, two (33%) and four (67%) were in ATS stage I and III, respectively. Asthma was evenly represented in all groups.

LUNG FUNCTION AND AIRWAY HYPER-RESPONSIVENESS

Table 2 shows static and dynamic volumes, DL_{CO} and PD₂₀FEV₁. Mean FEV₁ and DL_{CO} were significantly lower in PiZZ than in other phenotypes. No difference in static

lung volumes was found among different phenotypes. DL_{CO} significantly correlated with FEV_1 ($r=0.74$, $P<0.0001$). Methacholine challenge was denied in three out of nine PiZZ subjects due to a baseline FEV_1 lower than 1 l. Lack of hyper-responsiveness as assessed by reaching the maximal cumulative methacholine dose of 4 mg was recorded in 18 out of 25 (72%) PiMZ/MS, five out of six (83%) PiSZ and four out of six (67%) PiZZ subjects respectively, without any significant difference among phenotypes. Mean $PD_{20}FEV_1$ did not significantly differ among these groups (Table 2).

TABLE 1. Demographic, anthropometric and clinical characteristics

	PiMZ/MS	PiSZ	PiZZ
No. of subjects (%)	25 (62)	6 (15)	9 (23)
Gender (M/F)	19/6	2/4	3/6
Age (years)	38 ± 17	45 ± 11	44 ± 11
AAT level (mg dl ⁻¹)	101 ± 20	74 ± 29	33 ± 2*
Atopy (%)	15 (60)	4 (66)	4 (44)
Smoking history			
Actual number (%)	7 (28)	1 (17)	4 (44)
Never (%)	14 (56)	2 (33)	1 (12)
Former (%)	4 (16)	3 (50)	4 (44)
Diagnosis			
COPD/emphysema (%)	1 (4)	0 (0)	6 (67)
Bronchiectasis (%)	2 (8)	0 (0)	0 (0)
Asthma (%)	3 (12)	1 (17)	1 (11)
Liver disease (%)	2 (8)	0 (0)	1 (11)
Healthy (%)	17 (68)	5 (83)	1 (11)

* $P<0.001$ vs. other groups (post-hoc test).

AAT: Serum α_1 -anti-trypsin.

TABLE 2. Lung function, diffusion capacity and airway hyper-responsiveness

	PiMZ/MS	PiSZ	PiZZ	<i>P</i> ANOVA
No.	25	6	9	
FEV_1 (%pred.)	114 ± 14	133 ± 11*	64 ± 39 ^{†‡}	0.006
VC (%pred.)	107 ± 13	125 ± 8*	94 ± 29 [†]	0.025
FEV_1/VC (%)	105 ± 7	107 ± 8	68 ± 33* [†]	0.043
RV (%pred.)	121 ± 33	115 ± 41	127 ± 40	0.940
TLC (%pred.)	112 ± 15	120 ± 11	109 ± 22	0.276
DL_{CO} (%pred.)	111 ± 20	103 ± 12	72 ± 34* [§]	0.01
$PD_{20}FEV_1$ (mg)	3.31 ± 1.14	3.18 ± 1.27	2.99 ± 1.60	0.951

* $P<0.01$ vs. PiMZ/MS (post-hoc test); [†] $P<0.05$ vs. PiMZ/MS (post-hoc test); [‡] $P<0.005$ vs. PiSZ (post-hoc test); [§] $P<0.05$ vs. PiSZ (post-hoc test).

FEV_1 : forced expiratory volume in 1 sec; VC: vital capacity; RV: residual volume, TLC: total lung capacity; DL_{CO} : lung diffusion capacity to carbon monoxide; $PD_{20}FEV_1$: cumulative dose of inhaled methacholine causing a FEV_1 20% falling from the post-saline value.

EXHALED NO

Exhaled NO was detectable in all the subjects, $plFE_{NO}$ variation coefficient (SD/mean %) of intra-patient measurements was $5 \pm 2\%$ (range, 2–10%). Figure 1 shows $plFE_{NO}$ individual and mean values in different groups. $plFE_{NO}$ was significantly lower in PiZZ than in both subjects with other phenotypes and healthy controls ($n=19$, see Methods). Table 3 shows the anthropometric, demographic, functional characteristics and $plFE_{NO}$ of individual PiZZ patients and their respective matched control PiMM subjects. eNO was significantly lower in PiZZ patients than in matched controls.

There was no significant difference in FE_{NO} values between atopic and non-atopic patients or between smokers and non-smokers. There was no significant relationship between FE_{NO} and $PD_{20}FEV_1$, post-bronchodilator FEV_1 or DL_{CO} .

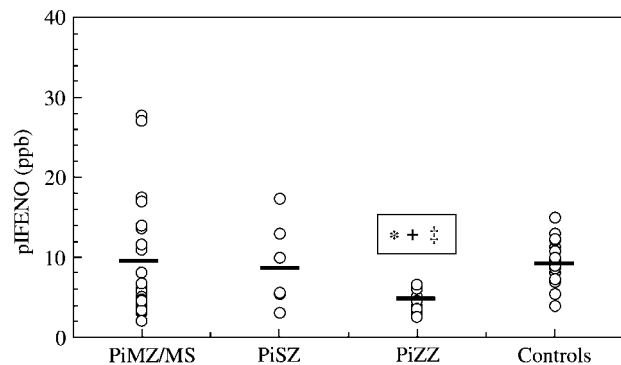


FIG. 1. Individual and mean values of $plFE_{NO}$ in the study groups (* $P=0.04$ vs. PiMZ/MS; [†] $P=0.015$ vs. PiSZ; [‡] $P=0.0002$ vs. controls).

TABLE 3. Characteristics of the PiZZ-and PiMM-matched subjects

Pair	Sex	Smoking	Inhaled steroids	Phenotype	Age	FEV ₁ (% predicted)	pFE _{NO} (ppb)
1	F	No	No	PiZZ	35	128	6.5
				PiMM	40	118	7.9
2	F	Yes	No	PiZZ	36	105	3.8
				PiMM	39	100	12.1
3	F	No	No	PiZZ	35	105	6.0
				PiMM	39	99	12.1
4	F	Yes	No	PiZZ	28	68	2.9
				PiMM	33	79	12.7
5	F	No	No	PiZZ	48	52	3.5
				PiMM	53	62	10.1
6	F	Yes	No	PiZZ	58	38	4.5
				PiMM	61	47	2.9
7	F	Yes	No	PiZZ	54	32	2.6
				PiMM	55	28	2.7
8	F	No	No	PiZZ	58	26	6.0
				PiMM	62	30	6.5
9	F	No	No	PiZZ	40	24	5.0
				PiMM	45	35	7.3
Mean \pm SD				PiZZ	43 \pm 11	64 \pm 39	4.5 \pm 1.4
				PiMM	47 \pm 10	66 \pm 34	8.2 \pm 3.8
<i>P</i>					0.426	0.894	0.014

Discussion

To our knowledge, this is the first study to report levels of eNO in subjects with different phenotypes of AAT deficiency and to relate them with lung function. When compared with AAT subjects having other phenotypes and with matched subjects without AAT with similar airway obstruction, PiZZ subjects showed significantly reduced eNO levels, without any significant correlation with lung function or airway hyper-responsiveness.

Confirming previous reports (2,27,28), our PiZZ subjects showed a greater incidence of COPD and lower mean levels of FEV₁ and DL_{CO} than the other AAT phenotypes.

PiZZ subjects of this study showed significantly reduced eNO levels in comparison with other phenotypes and with matched controls. Despite conflicting results (29), reduced FE_{NO} has been reported to be associated with the severity of airway obstruction in a stable population of COPD patients (12) and with acute induced bronchoconstriction in asthmatic subjects (30). In addition, in severe COPD patients (ATS class III) cor pulmonale has been shown to be associated with lower eNO production (13).

Reduced levels of eNO in our PiZZ patients might well be ascribed to severity of associated COPD and related clinical consequences. Indeed the mean FE_{NO} level of PiZZ patients was similar to that reported in most severe COPD patients (13). Nevertheless, some points should be addressed: (i) less than half of these subjects (three out of nine: 33%) showed a FEV₁ lower than 35% predicted; (ii)

although in this study pulmonary artery pressure was not directly assessed, none of the patients had clinical, radiological or EKG signs of cor pulmonale; (iii) in the whole population of AAT subjects, FE_{NO} did not significantly correlate with either FEV₁ or DL_{CO}. Furthermore PiZZ subjects showed lower levels of FE_{NO} when compared with associated diagnosis, age, sex, smoking habit, and FEV₁-matched PiMM subjects. A multiple logistic regression analysis would have been more appropriate, due to the number of potential confounders in eNO reading; however, the small number of PiZZ subjects makes this approach difficult to apply. Our findings only suggest that, in AAT deficiency, eNO should not be considered as a merely feature of severity of associated lung disease.

Endogenous NO has been hypothesized to have a role in airway tone modulation (31,32). With the limitation of the small number of subjects, in our study different phenotypes did not show any difference in airway hyper-responsiveness (Table 2) or in prevalence of asthma (Table 1). In the whole AAT-deficient population of our study no correlation was found between FE_{NO} and PD₂₀FEV₁, independent of the atopic status. This lack of correlation may suggest that in subjects with AAT deficiency, differently from asthmatic patients (30), NO is less involved in the pathogenesis of airway hyper-responsiveness. Conversely, when considering only five AAT deficient and asthmatic subjects (Table 1) a significant inverse correlation ($r = -0.77$, $P < 0.001$) between FE_{NO} and PD₂₀FEV₁ was found, indicating that, differently from severe asthmatic individuals (33), in AAT

subjects eNO may have no protective role in airway hyperresponsiveness.

In conclusion, exhaled NO is significantly reduced in PiZZ as compared with healthy control subjects and with subjects with other phenotypes of AAT deficiency, independent of airway obstruction. Whether eNO might be considered as an early marker of lung involvement, needing to be specifically monitored, should be evaluated in longitudinal studies on greater number of these PiZZ subjects.

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