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Elevated Plasma Levels Of Plasminogen Activator Inhibitor-1 Are Associated With Diminished Lung Function In Asthma

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Introduction

Subepithelial fibrosis is a key feature of asthma and may contribute to the irreversible component of airflow limitation in asthma.¹ Pathologic remodeling of extracellular matrix (ECM) eventually leads to altered restitution of airway structures, as exhibited in subepithelial fibrosis. The plasmin system is comprised of plasminogen that can be converted to the active enzyme, plasmin, by tissue-type plasminogen activator (t-PA) or urokinase-type PA (u-PA). Tissue-type-PA and u-PA are involved in the dissolution of fibrin and the degradation of ECM components. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of both t-PA and u-PA. PAI-1-deficient mice are protected against ECM deposition and fibrosis in the lungs after bleomycin challenge, whereas PAI-1 overexpressing mice suffer from extensive fibrotic changes.² This suggests that PAI-1 plays a key role in the deposition of ECM in the airways.

We previously reported that activated mast cells (MCs) are a major source of PAI-1 and MC-derived PAI-1 is highly expressed in patients with fatal asthma.³ We also showed that the 4G allele of the PAI-1 gene, which is associated with an elevated plasma PAI-1 level, is preferentially transmitted to asthmatics.⁴ Furthermore, we recently reported that deletion of PAI-1 prevents ECM deposition in a murine model of chronic asthma.⁵ These findings led us to hypothesize that there may be an association of elevated levels of PAI-1 and diminished lung function.

Elevation in plasma levels of PAI-1 is one of the biochemical hallmarks of obesity.⁶ This raises interesting questions since obesity has effects on both lung volume⁷ and asthma.⁸⁻¹⁰ In the present study, we sought to evaluate whether plasma PAI-1 levels were associated with worse lung function in asthmatic subjects from a community-based cohort, the Chicago Initiative to Raise Asthma Health Equity (CHIRAH).¹¹ We also evaluated whether PAI-1 levels were associated with other pro-inflammatory factors, BMI and smoking, and whether the association of these factors with lung function in this asthmatic cohort was mediated in part by PAI-1.

Materials and Methods

Study population

The methods and baseline population characteristics of the CHIRAH study have been previously described.¹¹ Briefly, CHIRAH was a community-based cohort study evaluating racial asthma health disparities conducted under the approval of the Institutional Review Boards at Northwestern University, the John H. Stroger Jr. Hospital of Cook County, Rush-Presbyterian-St. Luke's Medical Center, and Children's Memorial Hospital (Chicago). A systematic population proportionate school based sampling method was used to identify either adults aged 18-40 or children aged 8-14 years old with active asthma. For this study, we included only adult asthmatic subjects because of unavailability of enough plasma samples from the Pediatric subjects. All eligible study participants were required to have a history of physician-diagnosed persistent, symptomatic asthma, defined in this screening process as requiring at least 8 weeks of asthma medication over the previous 12 months. Five hundred and nineteen adults were verified eligible and ultimately 353 adults were enrolled and completed the baseline protocol between January 24, 2004 and July 30, 2005. Of these, 231 individuals had both PAI-1 levels measured and all the relevant clinical variables available for analysis. When we compared this subset of adult subjects to those adult subjects not analyzed, we did not find any differences in demographic characteristics, BMI, and smoking status. Once adults and caregivers were consented and enrolled, each study participant was asked to complete an in-person baseline interview. Anthropometrics (height and weight), spirometry, and blood sampling were completed on all participants.

These were conducted by trained research assistants in a community-based setting. Ethnicity, sex and smoking status were assigned by self-report for adults.

Measurement of variables

Obesity—Participants with BMI below 25 kg/m² were considered normal or underweight, those with BMI ranging from 25-29.9 were considered overweight, and those with values above 29.9 were considered obese.

Smoking status and salivary cotinine measurement—Individuals were surveyed to determine their smoking status. For the purposes of describing the smoking status of our cohort, we divided individuals into three groups; non-smoker, ex-smoker, and current smoker. Cotinine levels were measured using saliva specimens with an immunoassay (Salimetrics, Inc, State College, PA). A previous study suggested that a level of 8 ng/ml or greater is associated with active smoking for adults.¹²

Allergen sensitization—We measured specific IgE for two common indoor allergens: house dust mite (*Dermatophagoides pteronyssinus*) and cockroach (*Blattella germanica*) using the ImmunoCAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Levels of specific IgE >0.35 kU/L were considered positive.

Lung function measurement—Spirometry was performed during the baseline study visit. Trained research assistants utilized a SpiroPro® spirometer, (VIASYS Healthcare, Conshohocken, PA) following the guidelines of the American Thoracic Society/European Respiratory Society.¹³ References for spirometry values were determined utilizing age, standing height, gender and ethnicity. For subjects who self-identified as “only” white, African American (AA), or Hispanic/Mexican we used the NHANES III reference standards. For subjects of mixed or undeclared ethnic background, we used the Mexican-American standards, which fall in the middle of those for AAs and whites. This would apply to anyone with anything other than “only” white, AA, or Hispanic/Mexican (including Asians). Due to the lack of post-bronchodilator values on many subjects who had normal or near-normal baseline values, this report includes only pre-bronchodilator spirometry values.¹⁴

Use of control medications—Study participants were asked to bring their medications to the interview. For those who did not, photographs of commonly used asthma medications were available for reference. The analysis used dichotomous variables indicating use of inhaled corticosteroids (ICS) or other control medications.

PAI-1 measurement—We measured plasma PAI-1 levels using a PAI-1 ELISA kit (Diapharma, OH, USA) according to the manufacturer's instruction. The detection limit of this kit is 0.5 µg/ml.

Statistical analyses

Study population was divided into AA and non-AA because the proportion of AA in our cohort was more than 50%. Pearson's chi-square tests and Student's *t*-tests were performed to determine statistical differences of each variable between AA and non-AA. Multivariate linear regression analysis was carried out to determine the association of key determinant variables including demographic variables (age, sex, race), as well as key variables hypothesized to be associated with PAI-1 (BMI, smoking, allergen sensitization, and medications). The level of PAI-1 was also compared against BMI (normal vs overweight vs obese), and smoking status (non-smoker vs ex-smoker vs current smoker). Test statistics for PAI-1 utilized the Student's *t*-test and a 2-sided *p* value of *p*<0.05 was considered

statistically significant. We also assessed the association of PAI-1 with each of the lung function parameters (% predicted values for FEV₁, FVC, FEV₁/FVC ratio) by multiple linear regression, taking into account relevant covariates including BMI, smoking, cockroach sensitization, DM sensitization, and medications. Age, sex and race were excluded to avoid over-adjustment in this analysis because the percent predicted lung function parameters were already adjusted with age, sex and race. Finally, in a secondary analysis, we sought to evaluate the impact of PAI-1 on the association between lung function and BMI or lung function and smoking. We evaluated the association of BMI and smoking respectively with lung function, and repeated these models with the inclusion of PAI-1. To determine whether the change in association with or without the inclusion of PAI-1, the Bootstrap method was used to assess whether the models were significantly different.¹⁵ All analyses were conducted by using R version 2.7.2¹⁶ and GraphPad Prism version 4.03.

Results

Demographic characteristics

Table 1 shows demographic characteristics and general health status at baseline. The mean age was 31.7 and gender distribution was predominantly female. African Americans made up just more than half the population and the majority of the subjects were overweight or obese (23.8% and 56.7%, respectively). Twenty-five percent of the subjects had a self described history of smoking (ex-smoker or current smoker) and 31.6% of subjects had significantly elevated cotinine levels (≥ 8 ng/ml). There was a significantly higher percentage of obese individuals in AA compared with non-AA (66.6% vs 44.7%, $P < 0.05$). More AA had a history of smoking (ex-smokers and current smokers combined) and higher levels of cotinine compared with non-AA (29.4% vs 20.0%, $P < 0.05$ and 158.8 vs 78.4 ng/ml, $P < 0.05$, respectively). Interestingly, cockroach sensitization was higher in AA compared with non-AA (51.6% vs 34.3%, $P < 0.05$). 53% were using an inhaled steroid alone, 50% were using another controller alone, and 63% were using and an inhaled steroid along with another controller.

Association between PAI-1 and subject characteristics and pro-inflammatory factors

We first investigated the association of subject characteristics and other pro-inflammatory factors with PAI-1 levels (Table 2). BMI and smoking were positively associated with the plasma levels of PAI-1 ($\beta = 0.606$, $P = 0.002$ and $\beta = 7.526$, $P = 0.001$, respectively). PAI-1 levels were significantly lower in AA compared with non-AA ($\beta = -9.061$, $P = 0.01$). Age, sex, DM or cockroach sensitization, and medication use were not significantly associated with the levels of PAI-1. When dividing subjects into three groups (normal, overweight and obese) based on BMI and comparing the levels of PAI-1 in a Student's *t*-test, we found that individuals who are obese had significantly higher levels of plasma PAI-1 compared to those who have normal BMI (63.6 ± 2.2 vs 49.9 ± 3.8 ng/ml, $P < 0.01$) (Figure 1). When we divided subjects into three groups based on smoking status, individuals who were current smokers demonstrated higher levels of PAI-1 compared with non-smokers (68.4 ± 4.5 vs 56.4 ± 2.0 ng/ml, $P < 0.05$) (Fig. 2). When we further conducted multivariate analyses by adjusting the univariate Student's *t*-tests for race and smoking status as covariates, the differences in PAI-1 between individuals with normal body weight and those with obesity, and between non-smokers and current smokers remained statistical significant ($P < 0.001$ and $P < 0.01$, respectively).

PAI-1 and lung function

The primary focus of this study was to investigate the association between PAI-1 and lung function. On multivariate linear regression analysis, we found that plasma levels of PAI-1

were negatively associated with FVC (% predicted) ($\beta = -0.098$, $P = 0.011$) (Table 3), indicating an average reduction of 1% predicted FVC for each 10 ng increase of PAI-1 level. The interquartile range (IQR) for PAI-1 was 37.7 ng/ml, a range that may influence a variability of 3.8% in FVC. Since the IQR for FVC % predicted was 19.5%, it is possible that PAI-1 variability may account for about 20% of the variability in FVC. We did not find any association between PAI-1 levels and FEV₁ (% predicted) or FEV₁/FVC ratio (% predicted).

BMI, Lung function, and PAI-1

As shown in Table 2, we found that BMI and smoking were positively associated with the plasma levels of PAI-1. Given that we showed that PAI-1 was associated with reduced FVC (Table 3), we investigated whether or not BMI and smoking were also associated with diminished FVC and whether PAI-1 was a mediating factor on this association. We performed a multiple regression analysis evaluating the association of BMI and smoking with FVC, both with and without the inclusion of PAI-1 as a covariate (Table 4). The analysis without PAI-1 showed that there was a significant negative association between BMI and FVC ($\beta = -0.288$, $P = 0.009$), but not between smoking and FVC ($\beta = -0.075$, $P = 0.569$). When PAI-1 was added as another covariate, the coefficient was reduced to -0.250 ($P = 0.023$), indicating a significant impact of PAI-1 on the association between BMI and FVC (Bootstrap method, $P < 0.001$).

Discussion

In this study, we found that the plasma levels of PAI-1 are positively associated with BMI and smoking. We also showed that there was a significant association between PAI-1 and FVC. PAI-1 was also a modifying factor on the negative association between BMI and FVC. Previous animal studies by our group and others suggest that PAI-1 promotes airway fibrosis in OVA-challenged model of asthma or bleomycin-induced pulmonary fibrosis.^{2,5} We previously demonstrated that the 4G allele of the PAI-1 gene, which is associated with an elevated plasma PAI-1 level, is preferentially transmitted to asthmatics.⁴ Pampuch et al¹⁷ and others¹⁸ confirmed our findings that the frequency of the 4G allele was greater and plasma levels of PAI-1 were higher in subjects with asthma compared with normal controls. Another study showed that there were increased plasma levels of PAI-1 in young children who had repeated wheeze and the PAI-1 levels were highest in the children with frequent relapses.¹⁹

Despite multiple studies that show the association between elevated plasma levels of PAI-1 and asthma, the relationship between PAI-1 and fibrotic changes or lung function in human asthma is unclear. This study shows indirect evidence that PAI-1 may be associated with lung function, suggesting that PAI-1 may have relevance to structural changes in human asthma. Although FEV₁ is an important marker of airway obstruction and bronchoconstriction, there was no relationship between FEV₁ and PAI-1 in our population. Pampuch et al¹⁷ also showed that there was no correlation of plasma PAI-1 levels with FEV₁. In our study, elevated plasma PAI-1 was associated with decreased FVC. Although 1% reduction of FVC may not be an impressive change clinically, 20% of the variance of FVC could be attributed to plasma PAI-1 alone and therefore could be associated with a reasonable effect size. The sources of plasma PAI-1 include endothelial cells and monocytes which are quite different from local sources of PAI-1 from the lungs such as epithelial cells and mast cells. It is possible that the association of plasma PAI-1 with lung function may be less strong than that of local expression of PAI-1 with lung function.

It is possible that the reduction in FVC associated with PAI-1 levels may be related to an increase in residual volume and air trapping²⁰ which may reflect airway remodeling of small

airways as suggested by our prior murine studies. However, this remains speculation at this point and would need to be investigated by further studies using techniques such as hyperpolarized helium studies, ultrasound assisted techniques, or HRCT to correctly measure small airway changes.²¹

We showed that there is a positive association between BMI and plasma levels of PAI-1 in asthmatic subjects. The plasma levels of PAI-1 were also significantly higher in obese asthmatic subjects compared with asthmatic subjects with normal BMI. This is in keeping with the literature in non-asthmatic subjects, as an association between PAI-1 and BMI has been reported in metabolic syndrome and other cardiovascular diseases.²²

There was a positive association between smoking and the plasma levels of PAI-1 in our multiple regression analysis. Twenty five percent of subjects were smokers or ex-smokers and 31.6% of subjects had significantly elevated cotinine levels (≥ 8 ng/ml). Individuals who are current smokers also showed higher levels of PAI-1 compared with non-smokers. This is consistent with a study which found that exposure to nicotine may increase PAI-1 in subjects with chronic obstructive pulmonary disease (COPD).²³ COPD is a well known smoking-related disorder, and patients with COPD show significant tissue remodeling in small airways. Our findings suggest the need to evaluate whether elevated PAI-1 levels associated with nicotine exposure in asthma patients may facilitate airway remodeling.

Another interesting finding is that PAI-1 plays a role as a modifying factor on the association between BMI and FVC in our multiple regression analysis. Like PAI-1, BMI had a negative association with FVC, but not with FEV₁ or FEV₁/FVC ratio. When PAI-1 was added as a covariate in the regression analysis, the coefficient value was significantly reduced, suggesting that PAI-1 is potentially one of the factors on the causal pathway of the association between BMI and FVC. One can speculate that obesity may not only diminish FVC independently but also indirectly reduce FVC by elevating PAI-1. Aaron et al²⁴ demonstrated that significant weight reduction improved FEV₁ as well as FVC without significant changes in methacholine responsiveness. Our data on PAI-1 knock-out mice are consistent with this study that there was significant reduction of collagen deposition but not much difference in inflammatory cellular infiltration compared to wild type mice after chronic OVA challenge.⁵ Given these prior studies, there may be a non-inflammatory mechanism by which PAI-1 may be associated with obesity-related changes in FVC.

African Americans made up more than half the study population (55.8%) and the majority of non-African Americans were Hispanics. Our African American cohort contained a significantly higher number of obese individuals as well as higher smoking exposure, which were both associated with high PAI-1 levels. However, plasma levels of PAI-1 in African Americans were significantly lower than in non-African Americans in our study, a result that is consistent with other studies.^{25, 26} It is possible that there may be genetically determined differences in PAI-1 expression in response to these exposures or these differences may be due to other unmeasured confounders. Compared to non-Hispanic Whites or Hispanics, African Americans have significantly higher prevalence of the 5G/5G polymorphism which is correlated with low plasma levels of PAI-1.²⁵

This study suggests that subjects with elevated plasma levels of PAI-1 have diminished lung function, possibly due to tissue remodeling in the airways. However, it is still premature to say that PAI-1 can be used as a biomarker of airway remodeling in asthma due to the lack of direct evidence linking PAI-1 to remodeling. This study is based on assessment of plasma levels of PAI-1. Relating lung function changes to levels of PAI-1 in the airways measured in induced sputum or bronchoalveolar lavage fluid may provide more accurate information regarding the role of PAI-1 in the structural changes in individuals with asthma. Finally, the

population studied was a primarily minority, African American and Latino, because of the initial study design to assess inner city asthma population. Further study is needed to evaluate whether similar associations are present in whites.

In conclusion, this study demonstrates a significant association between PAI-1 and lung function in subjects with asthma. We also confirmed previous reports of an association between BMI or smoking status and PAI-1 in subjects with asthma. These results indicate that PAI-1 may play a role in the development or severity of asthma in obese individuals or smokers.

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Figure 1.

Association between PAI-1 and obesity. The plasma levels of PAI-1 were compared among normal (BMI below 25), overweight (BMI 25-29.9), and obese individuals (BMI 30 and over) using a Student's *t*-test. The levels of PAI-1 were significantly higher in obese individuals compared with normal controls (63.6 ± 2.2 vs 49.9 ± 3.8 ng/ml, $P < 0.01$).

Figure 2.

Association between PAI-1 and smoking. Asthma patients were divided into three groups by history of smoking (non-smoker, ex-smoker, and current smoker) and plasma levels of PAI-1 were compared among the three groups using a Student's *t*-test. Patients who were current smokers demonstrated higher levels of PAI-1 compared with non-smokers (68.4 ± 4.5 vs 56.4 ± 2.0 ng/ml, $P < 0.05$).

Table 1
Demographic characteristics and general health status at baseline

	Total n = 231	African Americans n = 126 (54.5%)	Non African Americans[§] n = 105 (45.5%)
Age Mean; years (SD)	31.7 (5.7)	32.2 (5.7)	31.3 (5.7)
Sex (%female)	77.5	82.5	71.4
<i>General Health and Atopy</i>			
BMI (%) [*]			
Low or Normal	19.5	18.3	21.0
Overweight	23.8	15.1	34.3
Obese	56.7	66.6^a	44.7
Smoking Status (%)			
Non-Smoker	74.9	70.6^a	80.0
Ex-Smoker	11.3	14.9^a	6.7
Current Smoker	13.8	14.5	13.3
Cotinine Level (SD)	123.8 (249.3)	158.8 (289.2) ^b	78.4 (176.5)
DM Sensitization (%)	45.0	46.0	43.8
Cockroach Sensitization (%)	43.7	51.6^a	34.3
PAI-1 Level; ng/ml (SD)	59.1 (26.0)	56.1 (25.9) ^c	62.7 (25.7)
Lung Function (SD)			
% predicted FEV ₁	86.5 (18.3)	86.8 (20.0)	86.2 (16.3)
% predicted FVC	97.7 (15.1)	97.2 (16.0)	98.3 (14.1)
%FEV ₁ /FVC ratio	75.9 (9.5)	75.4 (10.0)	76.4 (8.9)
Control medications (%) ^{**}			
Inhaled corticosteroids	52.6	50.8	54.8
Other control medications	50.2	40.5^a	61.9

AA, African American

SD, Standard Deviation

^{*} BMI, Body mass index - Participants with BMI below 25 kg/m² were considered normal or underweight, those with BMI ranging from 25-29.9 were considered overweight, and those with values equal and above 30 were considered obese.

^{**} Daily control medications were assessed within a two-week recall period. Other control medications exclude inhaled corticosteroids. Subjects with either inhaled corticosteroids or other control medications were 63.2%.

[§] NonAA- Hispanic American 29.1%, White American 14.0%, Other 2.4%

^{a,b,c} Difference between AA and Non-AA. ^a $P < 0.05$ from the chi-square test. There was a significant difference in smoking exposure by history (ex-smoker and current smoker combined) and obesity between AA and Non-AA. ^b $P < 0.05$ from the Student's *t*-test. ^c $P = 0.0528$ from the Student's *t*-test.

Table 2
Associations of Subject Characteristics with PAI-1 Levels

Covariates [§]	Coefficient (β)	Standard Error	<i>P</i> value [#]
Age (years)	0.188	0.294	0.523
Sex (Male)	4.841	4.167	0.247
Body Mass Index (kg/m²)	0.606	0.193	0.002
Smoking status *	7.526	2.319	0.001
Race (African Americans) **	-9.061	3.505	0.010
Dust mite specific IgE [†]	-0.036	0.075	0.636
Cockroach specific IgE [†]	-0.084	0.125	0.502
Inhaled corticosteroids ^{††}	1.239	4.097	0.763

[§]PAI-1 levels were used as the dependent variable and subject characteristics and other general health status as covariates in this multiple regression analysis.

[#] *P* values from a regression analysis. Statistical significant *P* values are indicated with bold.

* In the analysis, non-smokers were counted as 0, ex-smokers as 1, and current smokers as 2.

** In the analysis, non-African Americans were counted as 0 and African Americans as 1. DM or Cockroach sensitization

[†] Specific plasma IgE levels (kU/L) were used for DM or Cockroach sensitization in the analysis.

^{††} Subjects without inhaled corticosteroids (ICS) were counted as 0 and those with ICS as 1.

There was also no significant association between PAI-1 and other control medications (data not shown in the table).

Table 3
Association of Lung Function Parameters with PAI-1 Levels*

	FEV ₁ (%)	FVC	FEV ₁ /FVC ratio (%)
Serum PAI-1 (ng/ml)	-0.067 ± 0.046	-0.098 ± 0.038^a	0.016 ± 0.023
Cockroach specific IgE **	-0.196 ± 0.085 ^a	-0.084 ± 0.071	-0.131 ± 0.044 ^b
Dust mite specific IgE **	-0.063 ± 0.054	-0.026 ± 0.044	-0.036 ± 0.028
Inhaled corticosteroids	0.644 ± 2.833	-0.274 ± 2.352	0.739 ± 1.453

Results indicate coefficient β and standard error (SE)

* A multiple regression analysis was performed by using each lung function parameters as the dependent variable and PAI-1, cockroach and dust mite sensitization as covariates. Age, sex and race were excluded the lung function parameters were already adjusted with age, sex and race.

^a $P < 0.05$ and ^b $P < 0.01$ from the multiple regression analysis

[§] When BMI and smoking were included as covariates, the coefficient (β) was -0.083 with P value 0.033. When inhaled corticosteroids and other control medications were included as covariates, the coefficient did not change (-0.098), indicating that control medications were not confounders of this association.

** Specific plasma IgE levels (kU/L) for Cockroach and dust mite were used for this analysis.

Table 4
Association of FVC (% predicted) with BMI and Smoking

Model did not include plasma PAI-1 levels:			
Covariates *	Coefficient (β) **	Standard Error	P value ***
Body Mass Index	-0.288[†]	0.109	0.009
Smoking status	-0.785	1.377	0.569
Model included plasma PAI-1 levels:			
Covariates *	Coefficient (β) **	Standard Error	P value ***
Body Mass Index	-0.250[†]	0.110	0.023
Smoking status	-0.203	1.393	0.884

BMI, Body Mass Index

* Covariates used in this both regression analyses include dust mite sensitization, cockroach sensitization, BMI and smoking. Models for BMI and smoking were identical.

** β is the regression coefficient indicates the change in % predicted FVC given one unit increase in the corresponding covariate.

[†] To evaluate the statistical difference on the associations between FVC vs BMI with and without PAI-1, the Bootstrap method was used and the difference was statistically significant ($P < 0.001$).