The Effect of Secondhand Smoke Exposure on Markers of Elastin Degradation

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Background: Tobacco smoke is a major risk factor in the development of COPD. Secondhand smoke (SHS) exposure is a known risk factor in asthma, bronchitis, and coronary artery disease. Elastin is a recognized target for injury in COPD, and the amino acids desmosine and isodesmosine (D/I), which are specific for elastin degradation, are elevated in COPD. This study determined whether exposure to SHS affects elastin degradation in asymptomatic individuals.

Methods: Two cohorts of asymptomatic individuals without evidence of respiratory or circulatory disease, exposed to SHS, were studied. Both cohorts comprised normal nonsmokers, active smokers, and those exposed to SHS. D/I were measured in plasma and quantified by high-performance liquid chromatography and tandem mass spectrometry by published methods. Plasma cotinine, a metabolite of nicotine, was also measured.

Results: In each cohort, the levels of D/I in plasma were statistically significantly higher in secondhand-smoke-exposed subjects than in the normal nonexposed subjects. Smokers had the highest levels of D/I but their levels were not statistically significantly higher than those of the secondhand-smoke-exposed. Cotinine levels were elevated in secondhand-smoke-exposed subjects and active smokers but not in most nonsmoking control subjects.

Conclusions: Results indicate a tissue matrix effect of degradation of body elastin from SHS exposure and possible lung structure injury, which may result in COPD. Long-term studies of individuals exposed to SHS for the development of COPD are warranted.

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Abbreviations: D/I = desmosine and isodesmosine; LC/MS/MS = liquid chromatography tandem mass spectrometry; SHS = secondhand smoke

COPD is a growing and costly problem. According to the World Health Organization in 2007, 210 million people worldwide had suffered COPD and 3 million of those died in 2005 alone.¹ The World Health Organization estimates that, according to the current trends, COPD will become the third-leading cause of death worldwide by the year 2030. COPD is

a lengthy, chronic, progressive disease; it is debilitating and often nonreversible. Active smoking is the most important modifiable risk factor for COPD.² In comparison with previous years, overall fewer people smoke now; however, there has been a disturbing increase in active smokers in the under-30 age group.¹ Once active smoking was established as having detrimental health effects on lung disease, the focus turned to the possible adverse effects of passive smoking, the atmospheric exposure to secondhand smoke (SHS).

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SHS exposure has been implicated as a risk factor in many diseases, including asthma, bronchitis, and coronary artery disease.³ As a result, there is a worldwide campaign to eliminate passive smoking from the environment.⁴ SHS increases the risk of heart disease in adults⁵ and has been shown to increase the inflammatory state.⁶ Flouris et al³ demonstrated an increase in inflammatory cytokines in never smokers exposed to only 1 h of SHS, and these cytokines remained elevated for 3 h after the exposure ceased. In the ATTICA study, inflammatory cytokines were elevated from chronic exposure to SHS for extended periods, and the levels were similar to those of the active smokers.⁷

Given the deleterious effects of passive smoking on overall health, it is reasonable to consider its detrimental effects on lung parenchyma itself. Studies of subjects exposed heavily to SHS (ie, bar workers, flight attendants) have shown an increase in lung cancer, COPD, bronchitis, and asthma exacerbations.⁸ In the same studies, subjective health improved within 1 month of a clean air environment and objective improvement was seen within 2 months. There are studies demonstrating the effect of SHS exposure on biomarkers of inflammation in patients with cardiovascular as well as pulmonary disease.⁹⁻¹¹ However, thus far, no studies have evaluated the effect of SHS exposure on tissue matrix proteins.

Desmosine and isodesmosine (D/I) are two cross-linked pyridinoline amino acids specific to peptides, produced from elastin degradation.¹² D/I have been measured by liquid chromatography tandem mass spectrometry (LC/MS/MS) in patients with COPD and were found to be elevated compared with normal control subjects.^{12,13} For the first time to our knowledge, these peptides have been measured in subjects exposed to SHS. D/I were found to be statistically significantly elevated in patients exposed to SHS, compared with normal control subjects.

MATERIALS AND METHODS

Patients

Two cohorts of subjects, cohorts I and II, were studied. Cohort I had three subgroups of subjects who were studied for the effect of SHS exposure on D/I levels. These subjects were part of an ongoing study to determine the effect of SHS exposure on hormonal constituents in women. All subjects completed a lifestyle and nutritional questionnaire that included a description of exposure to cigarette tobacco smoke. Subjects were divided into three groups: active smokers, passive smokers, and nonexposed. Passive smokers were defined as anyone who had lived with, or had been exposed to, cigarette smoke on a daily basis, but were not smokers. Most subjects were exposed within the home.

Active smokers were persons who were smoking daily. Exclusion criteria included several medical conditions, including con-

gestive heart failure, myocardial infarction, cerebral vascular accident, asthma, bronchitis, emphysema, and any malignancy; any subjects exposed to dyes (textiles, arts and crafts) on a regular basis were also excluded. Subjects were initially screened as eligible by telephone and then came for a study visit. All patients were women and between the ages of 18 and 50 years, with the majority of them in their late 20s and 30s. They were not taking hormonal contraceptives and were not pregnant at the time of the study.

Cohort II was also subdivided into three groups: active smokers, passive smokers, and nonexposed in smaller numbers. Subjects were men and women between the ages of 22 and 69 years. They were recruited from a medical clinic in the Veterans' Administration Hospital where, after clinical evaluation, they were considered to be in normal health and were, or were not, exposed to cigarette smoke. Some subjects responded to an advertisement requesting participation in this study. Most subjects were exposed to SHS in the occupational setting (ie, bartenders, construction workers, office workers). All passive smoke exposures in cohorts I and II were current and not past.

The degree of SHS exposure in cohort I was determined from the volunteered histories given on a questionnaire. Subjects reported the number of individuals who smoked in the household, and gave estimations of the number of cigarettes each individual smoked per day and the period of time the subject lived in the household. For each smoker in the household, the following was calculated: the number of cigarettes smoked per day $\times$ 365 was divided by 20 to determine the packs that the smokers smoked per year. This number was multiplied by the number of years the exposed person lived in the household. The scores for each household smoker were added together for a final score of exposure for the exposed subject. A score $< 2,000$ was considered mild exposure, 2,000 to 10,000 moderate exposure, and $> 10,000$ severe exposure. Mild, moderate, and severe exposures were then used in the analysis.

Such detailed information on occupational and household environmental exposure was not available in cohort II. The subjects of cohort II indicated their occupation and that they were exposed to SHS and were not actively smoking themselves.

Chemicals

D/I standard (mixed 50% desmosine and 50% isodesmosine) were purchased from Elastin Products Company (Owensville, Michigan). CF1 cellulose powders were from Whatman (Clifton, New Jersey), and all other reagents were from Sigma (St. Louis, Missouri).

Preparation of Blood Samples

Plasma samples were obtained after centrifuging venous blood specimens with ethylenediaminetetraacetic acid for 2,500 revolutions/min for 15 min. Plasma samples were stored at -80°C until used. First, 0.25 mL of serum and 0.25 mL of concentrated hydrochloric acid (37%) were placed in a glass vial. After air in the sample was displaced with a stream of nitrogen, the sample was acid hydrolyzed at 110°C for 24 h. The hydrolysates were filtered and dried, and the residue was dissolved in 2 mL of the mixed solution (n-butanol/acetic acid/water, 4:1:1 by volume). The sample solution was loaded onto a 3-mL CF1 cartridge. The CF1 cartridge was prepared by introducing 3.5 mL of the slurry of 5% CF1 cellulose powder into the mixed solution between two polypropylene frits. The cartridge was washed three times with 3 mL of the mixed solution, and the D/I adsorbed in the CF1 cartridge were eluted with 3 mL of water. The eluate was evaporated to dryness under vacuum at 45°C , and the residue was dissolved in 0.1 mL of high-performance liquid chromatography mobile phase for LC/MS/MS analysis.

Samples were processed and measured in duplicate, and the results were averaged. The mean recoveries of D/I and from 0.20 ng/mL plasma were $67\% \pm 1\%$ and $72\% \pm 4\%$, respectively. Values in plasma were corrected for recovery losses.

Measurement of D/I by LC/MS/MS Analysis

The TSQ Quantum Discovery tandem LC/MS/MS system (ThermoScientific; Waltham, Massachusetts) was used for the analysis. High-performance liquid chromatography column was a 150×2 -mm (3- μ m) Atlantis dc18 (Waters, Mississippi). The mobile phase A is a solution containing 5 mM ammonium acetate and 7 mM heptafluorobutyric acid in water, and the mobile phase B is a solution containing 5 mM ammonium acetate and 7 mM heptafluorobutyric acid in acetonitrile/water (8:2 ratio). The flow rate of mobile phase is 0.2 mL/min and is programmed from mobile phase A 100% to 88% in 12 min.

Reaction ion monitoring of the transition ions, m/z 526 to m/z 481 + m/z 397, was used for the quantitative measurements of D/I. Between-run imprecision (% coefficient of variation) at D/I levels of 0.10 and 0.20 ng/mL was 4.0% and 3.9%, respectively. Institutional Review Board approval was obtained from Georgetown University Medical Center (2006-132) and from Carl T. Hayden, Veteran Administration Medical Center (Robbins 003).

Measurement of Cotinine by LC/MS/MS Analysis

To indicate exposure to cigarette tobacco smoke, plasma cotinine, a metabolite of nicotine,¹¹ was quantitated using LC/MS/MS (API-4000 [Applied Biosystems; Forest City, California] or ThermoScientific TSQ Quantum Discovery) following extraction by a solvent (ethyl acetate) extraction. Internal standard (D_3 -cotinine) solution was added to subject samples, and samples were vortexed and centrifuged. The supernatant was injected into the LC/MS/MS system. Cotinine was measured by monitoring the Q1/Q3 transition ions of cotinine at m/z 177 to 80, and D_3 -cotinine at m/z 180 to 80. Between-run imprecision (% coefficient of variation) at cotinine levels of 33, 124, and 248 ng/mL were 7.2%, 5.6%, and 3.6% respectively.

Statistical Analysis

A Student *t* test adjusted for unequal variance was used to test the null hypothesis. The level of significance was .05. The *P* values were calculated based on the summed values of D/I, using the unpaired *t* test (using GraphPad Prism 4 (2) software; La Jolla, California).

RESULTS

Results for all three groups of nonexposed, exposed, and smokers in 98 subjects (cohort I) are shown in Table 1 and Figure 1. The mean D/I level in the nonexposed group was 0.22 ± 0.07 ng/mL ($n = 30$), whereas the level in the exposed group was 0.29 ± 0.10 ng/mL ($n = 34$) and in smokers was 0.34 ± 0.12 ng/mL ($n = 34$) (Table 1). The *P* value when comparing nonexposed group D/I levels with exposed group levels was statistically significant ($P = .005$). The *P* values of nonexposed group to smokers was $< .0001$, and the value of exposed group vs smokers was near statistical significance ($P = .0533$) (Fig 1A).

Cohort II also compared D/I plasma levels and levels of cotinine in 22 subjects of nonexposed, exposed,

and smokers. The study includes men as well as women and shows the D/I levels and cotinine levels in those subjects (Fig 2, Table 2). The average D/I (ng/mL plasma) was 0.21 ± 0.03 in the nonexposed group and 0.40 ± 0.13 in the SHS-exposed group. The smoking group had a measurement of 0.55 ± 0.28 . The *P* values were .0233 for the nonexposed group vs the SHS-exposed group and .0075 for the nonexposed group vs the smoking group. The resulting *P* value when secondhand exposure was compared with active smoking was not significant ($P = .1782$) (Fig 2A).

Cotinine values for each of the cohorts are included in Tables 1 and 2, and are shown in Figures 1B and 2B. Cotinine values showed a large variation in each cohort. In cohort I, there was a significant correlation between the plasma level of D/I and the log value of plasma cotinine, as shown in Figure 3. In cohort II, with fewer subjects overall and fewer in each category of exposure, such correlations were not statistically significant. Cotinine levels in cohort I in SHS exposure exceeded those in cohort II, whereas D/I levels were higher in cohort II secondhand smokers than in cohort I.

Comparison of the mean levels of plasma D/I and cotinine in cohorts I and II showed no statistically significant difference in D/I in the nonexposed group. There were statistically significant differences in the exposed and actively smoking groups ($P = .023$ and $P = .004$, respectively for D/I but not statistically significant differences for cotinine for those groups respectively ($P = .350$ and $P = .560$). Among active smokers, the levels of D/I were higher in cohort II, consistent with their pack/year histories.

DISCUSSION

Both cohorts of subjects demonstrate a statistically significant increase in plasma levels of D/I in subjects exposed to SHS, none of whom had clinical symptoms of respiratory disease. The US Environmental Protection Agency estimates that passive smoking accounts for roughly 3,000 deaths in the United States, secondary to lung cancer annually.¹ The impact of passive smoking on overall health is clearly recognized as an independent risk factor for diseases, including the lung.⁴

D/I are crosslinking amino acids in mature elastin that exists predominantly in lung parenchyma, blood vessels, and skin. Mature elastin is a very stable compound that requires elastase activity for degradation.¹⁴ Although elastin is found in many body tissues, it is of special structural importance in the lung because elastic fibers are essential structural and functional elements in alveoli, bronchi, and pleura.¹⁴

The levels of D/I in urine and plasma in COPD have been found to be consistently elevated in many

Table 1—D/I and Cotinine Levels in 98 Plasma Samples (Cohort 1)

Nonexposed (n = 30)			Exposed (n = 34)				Smokers (n = 34)			
Age, y	D/I, ng/mL	Cotinine, ng/mL	Age, y	D/I, ng/mL	Cotinine, ng/mL	Level of Exposure	Age, y	D/I, ng/mL	Cotinine, ng/mL	Pack-y
19	0.18	...	19	0.27	93.50	Mild	42	0.28	269.50	11
43	0.25	...	25	0.21	...	Mild	24	0.23	...	1.75
41	0.16	...	34	0.18	...	NA	30	0.31	...	2.5
25	0.19	...	21	0.23	...	Moderate	24	0.25	29.10	0.8
21	0.24	...	42	0.18	...	NA	26	0.18	74.45	1.1
44	0.14	...	44	0.14	...	NA	44	0.23	...	2.55
42	0.13	0.54	44	0.38	...	Moderate	39	0.81	415.50	2.6
29	0.22	0.54	22	0.38	...	Severe	27	0.25	1.04	11
38	0.17	...	41	0.35	210.00	NA	22	0.24	...	1.5
35	0.34	...	24	0.21	...	Moderate	30	0.47	35.70	2.85
25	0.39	...	23	0.22	...	NA	44	0.21	...	26
37	0.17	...	18	0.30	...	Severe	25	0.44	...	1.75
22	0.19	...	48	0.34	...	Severe	36	0.34	...	2.4
22	0.21	...	44	0.16	17.35	NA	36	0.50	...	3.3
24	0.37	...	22	0.36	...	NA	25	0.61	68.00	0.6
26	0.13	...	35	0.32	3.12	Moderate	26	0.25	...	16
21	0.19	...	32	0.13	63.95	NA	22	0.33	...	4
37	0.17	...	41	0.26	69.50	Moderate	21	0.26	43.15	1
28	0.19	...	30	0.38	1.31	Mild	26	0.19	...	2.4
27	0.26	...	30	0.28	...	NA	41	0.31	221.00	2.3
27	0.27	...	20	0.42	722.00	NA	43	0.26	...	22
44	0.09	...	42	0.24	...	NA	23	0.26	455.00	1.5
33	0.21	...	38	0.42	24.90	Severe	36	0.38	222.00	...
28	0.23	...	22	0.29	...	Mild	39	0.51	...	8
45	0.35	...	25	0.17	...	NA	26	0.30	17.35	...
25	0.22	...	20	0.36	7.25	Moderate	30	0.24	218.00	1.3
20	0.29	...	23	0.25	12.80	NA	22	0.42	207.50	0.2
20	0.24	...	27	0.26	0.77	NA	33	0.44	127.50	0.75
20	0.22	...	22	0.24	...	NA	22	0.38	...	1.5
26	0.24	...	41	0.45	17.30	Mild	40	0.51	69.50	0.8
			42	0.57	...	NA	28	0.25	1.31	3.25
			28	0.47	20.10	Mild	23	0.32	29.80	0.9
			22	0.21	...	NA	38	0.29	1.37	15
			27	0.17	...	NA	26	0.66	54.00	1.1
30 ± 9	0.22 ± 0.07	0.04 ± 0.14	31 ± 9	0.29 ± 0.11	37.17 ± 128		31 ± 8	0.35 ± 0.15	75.32 ± 122	4.80 ± 6.50

Data in last row presented as mean ± SD. Ellipses (...) indicate below detection limit of 0.5 ng/mL. Nonexposed vs exposed, $P = .005$; nonexposed vs smokers, $P < .0001$; exposed vs smokers, $P = .053$. D/I = desmosine and isodesmosine; NA = not available.

studies by various analytic methods.^{12,13} This raises the prospect that elevated levels of D/I induced by SHS exposure may become an indicator of the risk of developing COPD in later life.

These are the first reported measurements of the effect of SHS exposure on elastin degradation in human subjects. This is a demonstration of a tissue matrix effect, which may have special significance for lung structure. The further implication of increased elastin degradation of mature elastin is increased elastase activity in tissues and possibly an upgraded inflammatory state. The patients studied in all groups were not symptomatic. Neither group had any significant medical history or evidence of lung pathology. All had equivalent and average exercise tolerance. As is the case with direct smoke exposure, in which years go by before evidence of COPD is apparent, SHS smoke exposure may produce its effects decades

later. The results also suggest that even mild passive smoke exposure could have deleterious effects on lung parenchyma, which if persistent long-term, may cause parenchymal inflammation and destruction, resulting in COPD.

The clinical significance of the findings in this study is still unclear. As mentioned earlier, patients with excessive, chronic, SHS exposure do suffer the sequelae of active smokers.³ In patients with COPD, elastin fibers have been shown to be degraded and disorganized, and the content of lung elastin is low in such patients.^{15,16} Also, once elastin breakdown has occurred, the resulting peptides may promote further parenchymal destruction because they may be antigenic and chemotactic. The peptides are chemotactic for neutrophils and macrophages, resulting in a chronic inflammatory state, which could induce further elastin degradation and possibly clinical sequelae in the long term.¹⁷

FIGURE 1. D/I and cotinine levels in plasma of 98 subjects (cohort I). The boundaries of each box indicate between the 25th and 75th percentile, the line within each box is the median, and the whiskers show the ranges of data points. A, The median D/I level in the nonexposed group ($n = 30$) was 0.22 ng/mL; in the exposed group ($n = 34$) it was 0.27 ng/mL; and in smokers ($n = 34$) it was 0.31 ng/mL (nonexposed vs exposed, $P = .0050$; exposed vs smokers, $P = .0533$; nonexposed vs smokers, $P < .0001$). B, The median cotinine level in the nonexposed group ($n = 30$) was 0 ng/mL; in the exposed group ($n = 34$) was 0 ng/mL; and in smokers ($n = 34$) was 9.36 ng/mL (nonexposed vs exposed, $P = .1161$; exposed vs smokers, $P = .2117$; nonexposed vs smokers, $P = .0012$). D/I = desmosine/isodesmosine.

The increase in levels of D/I in the plasma of subjects undergoing SHS exposure raises the question of the mechanism for the increase. Because SHS enters via the respiratory tract, the initial effect should be on

lung cells, and most notably neutrophils and macrophages, which undergo stimulation with increased synthesis and secretion of elastases. This augmented activity of elastolysis then can affect degradation of

FIGURE 2. D/I and cotinine levels in plasma of 22 subjects (cohort II). The boundaries of each box indicate between the 25th and 75th percentile, the line within each box is the median, and the whiskers show the ranges of data points. A, The median D/I level in the nonexposed group ($n = 7$) was 0.21 ng/mL; in the exposed group ($n = 6$) was 0.40 ng/mL; and in smokers ($n = 9$) was 0.45 ng/mL (nonexposed vs exposed, $P = .0223$; exposed vs smokers, $P = .1782$; nonexposed vs smokers, $P = .0075$). B, The median cotinine level in the nonexposed group ($n = 6$) was 0.84 ng/mL; in the exposed group ($n = 5$) was 0.80 ng/mL; and in smokers ($n = 9$) was 11.8 ng/mL (nonexposed vs exposed, $P = .4008$; exposed vs smokers, $P = .0740$; nonexposed vs smokers, $P = .3470$). See Figure 1 legend for expansion of abbreviations.

Table 2—D/I and Cotinine Levels in 22 Plasma Samples (Cohort II)

Nonexposed (n = 7)			Exposed (n = 6)			Smokers (n = 9)			
Subjects, Sex/Age, y	D/I, ng/mL	Cotinine, ng/mL	Subject, Sex/Age, y	D/I, ng/mL	Cotinine, ng/mL	Subjects, Sex/Age, y	D/I, ng/mL	Cotinine, ng/mL	Pack-y
M/33	0.27	1.63	M/43	0.49	5.81	M/69	0.45	344.14	30
M/46	0.21	0.5	F/49	0.39	0.8	M/69	0.92	1.08	30
F/35	0.24	0.84	F/53	0.63	0.44	M/52	0.54	1.44	30
F/20	0.16	0.78	M/25	0.28	0.43	M/57	1.05	0.59	12.5
M/19	0.19	0.97	M/34	0.24	...	M/71	0.78	0.28	84
M/47	0.21	...	F/42	0.4	27.8	M/46	0.34	550.6	20
F/45	0.21	0.29				M/42	0.31	11.08	20
						M/58	0.28	88.75	10
						M/49	0.35	515.31	35
	0.21 ± 0.03	0.84 ± 0.46		0.40 ± 0.13	7.06 ± 11.8		0.56 ± 0.28	168 ± 234	30.2 ± 21.9

Data in last row presented as mean ± SD. Ellipses (...) indicate below detection limit of 0.5 ng/mL. Nonexposed vs exposed, $P = .0223$; nonexposed vs smokers, $P = .0075$; exposed vs smokers, $P = .1782$. F = female; M = male. See Table 1 for expansion of other abbreviations.

elastin in any site in addition to the lung, such as the blood vessels and skin, which would elevate plasma concentrations of D/I.

D/I as biomarkers for COPD have been studied for some time.¹² With advances in technology and preparation of samples, they can be detected with greater sensitivity and specificity.¹³ This is significant because the levels in plasma of patients exposed to SHS may have been too low to detect previously. Plasma can be obtained easily from patients. In the past, 24-h urine has been studied as a medium for D/I measurement. Although useful, because there is an increase in free D/I in urine in active smokers and patients with COPD, it is often difficult for the patient and the practitioner to collect 24-h urine.¹³ If we are able to detect increased levels in plasma accurately and precisely, it brings us possibly one step closer to a practical marker of elastin degradation and, in effect, one step closer to understanding and quantifying lung degradation.

It would be clinically important to learn what happens to the levels of D/I over time in these individuals and whether the levels decrease if the patients are removed from passive smoke exposure, or if they are not removed, if the levels continue to increase in response to increased elastin degradation. Menzies et al⁸ illustrated previously that when the inciting factor was removed (ie, smoke in bars where they worked), the subjects rapidly returned to their previous state of health. Such findings would suggest that the levels of D/I should decrease when SHS is terminated. The clinical effects on lung function that this early degradation imposes are unclear. It is noteworthy that the levels of D/I in passive smokers were not as high as those seen in patients with COPD or α_1 antitrypsin deficiency.¹³ This finding is not surprising, given the burden of disease in patients with COPD and α_1 compared with our study population.

In this study, no significant correlations with demographics, nutritional characteristics, or life style could

be made in cohorts I or II. These confounding variables deserve consideration in a study of this type.

Certain methodologic limitations should be noted in this study. The data rely on an accurate recognition of SHS exposure by our subjects through self-reporting. This is based on an extensive questionnaire characterizing their exposure to SHS. In previous studies, a good correlation between a person's perception of SHS and the laboratory findings consistent with exposure to SHS has been shown.⁶ The questions are very specific about who smokes in the home, how much, and for how long, and how much time the subject spends within this environment. The

FIGURE 3. A statistically significant correlation is shown between the log values of plasma cotinine concentration and plasma levels of D/I in cohort 1. DES = desmosinedesmosine; IDS = isodesmosine. See Figure 1 legend for expansion of other abbreviation.

questionnaire administered to all persons participating in this study included the same questions; therefore, each study group was exposed to the same level of bias.

Of interest is the measurement of cotinine levels. Previous studies have established a positive correlation between the level of SHS exposure and inflammatory cytokine elevation.^{10,18} On average, they were significantly elevated in subjects who self-reported SHS exposure when compared with subjects without any exposure. The drawback is that cotinine remains elevated only for short periods of time post exposure, and correlations with presumed exposure may be weak.¹⁹ This could explain why certain subjects have low cotinine levels.

In the past, multiple studies illustrated that the levels of inflammatory cytokines, on average, are higher in men than in women.^{3,5,7} It is postulated that men are more sensitive to the inflammatory cytokines than are women, or that men have greater exposure to the inflammatory cytokines than do women. It is noteworthy that secondhand smokers in cohort I, which was exclusively a female population of subjects, had lower levels of D/I for the cotinine level, which was higher than that among passive smokers in cohort II. This raises the possibility that women may be less susceptible to elastin degradation by exposure to tobacco smoke, a prospect that should be explored further.

CONCLUSIONS

This study illustrates that SHS exposure may be as dangerous and harmful as active smoking on tissue matrix injury and possibly lung parenchyma. This study demonstrates that D/I can be used to detect early changes of elastin degradation, before clinically significant symptoms occur, and possibly to indicate progression of the disease. Thus, D/I can serve as biomarkers of exposure. It would be significant to determine if increased levels of D/I can be correlated with CT findings of significant parenchymal destruction, before clinical symptoms occur. Overall, having a tissue matrix component affected by SHS and detectable by chemical analysis is a useful adjunct to evaluating the clinical and physiologic consequences of environmental smoke exposure.

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Dr Lin: contributed to planning the study with collaborating investigators, analysis of the data, and editing of the final manuscript.

Dr Soldin: contributed to collecting the data on cohort I at Georgetown University and preparation of the final manuscript.

Dr Robbins: contributed to collecting the data on cohort II and preparation of the final manuscript.

Dr Turino: contributed to planning the study with collaborating investigators, analysis of the data, and editing of the final manuscript.

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