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A M E R I C A N C O L L E G E O F



P H Y S I C I A N S[®]

Regional Differences in Emphysema Scores and BAL Glutathione Levels in HIV-Infected Individuals*

Philip T. Diaz, MD; Mark D. Wewers, MD; Mark King; Joyce Wade, MD; Judy Hart; and Thomas L. Clanton, MD

Study objectives: Evidence exists that HIV-seropositive individuals may be at increased risk for the development of precocious pulmonary emphysema. HIV infection is also associated with antioxidant deficiency in both the serum and lungs, and it is therefore possible that increased oxidant stress may contribute to parenchymal lung injury occurring in the setting of HIV. We sought to determine the regional distribution of emphysema and regional distribution of glutathione (GSH) concentrations among HIV-seropositive subjects with emphysema.

Design: Cross-sectional evaluation of a prospective, longitudinal study.

Setting: University teaching hospital.

Subjects/measurements: HIV-seropositive subjects without AIDS-related pulmonary complications participating in a descriptive study of lung biology in HIV-seropositive individuals. Emphysema scoring and evaluation of emphysema lobar distribution was performed among 40 subjects with emphysema. Eleven subjects underwent BAL of the right middle lobe (RML) and right upper lobe (RUL) with measurement of epithelial lining fluid (ELF) GSH in each lobe.

Results: We found that the mean emphysema scores were much higher in the upper lobes compared to the rest of the lung. Mean GSH levels were significantly greater in the RUL compared to the RML. The regional differences were present in both smokers and nonsmokers.

Conclusions: We conclude that in the setting of HIV, emphysema is more prominent and lung GSH concentrations are higher in the upper lobes. We hypothesize that the increased GSH may represent a compensatory response to increased oxidant stress in the upper lobes.

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Key words: emphysema; glutathione; high-resolution chest CT; HIV; lung; oxidant stress

Abbreviations: ELF = epithelial lining fluid; GSH = glutathione; HRCT = high-resolution CT; RML = right middle lobe; RUL = right upper lobe

Evidence exists that HIV-seropositive individuals may be at increased risk for the development of precocious pulmonary emphysema.^{1–3} The precise pathophysiology underlying this phenomenon remains unclear. However, understanding the factors responsible for this accelerated process associated

with HIV may be relevant to emphysema pathogenesis in general. Of note, HIV infection is associated with antioxidant deficiency in both the serum and lungs,^{4,5} which may make HIV-infected individuals more susceptible to oxidant stress. It is therefore possible that increased oxidant stress may contribute to parenchymal lung injury occurring in the setting of HIV.

In a study² involving chest high-resolution CT (HRCT), we reported that HIV-seropositive individuals have higher total emphysema scores than age- and smoking-matched control subjects. In the current study, we reviewed this HRCT data to delineate the regional distribution of emphysema in the HIV-seropositive population. After finding a predilection for disease in the upper lobes, we then asked whether regional differences in oxidant stress might exist in the lungs of individuals with HIV. To address this question, we examined concentrations of gluta-

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thione (GSH), a ubiquitous antioxidant, in the epithelial lining fluid (ELF) of different lung regions.

MATERIALS AND METHODS

Subjects

All subjects were part of a cohort (n = 331) participating in a descriptive study of lung biology among HIV-seropositive individuals with no history of AIDS-related pulmonary complications. All subjects in the cohort had assessment of respiratory symptoms and pulmonary function. Some subjects, including those described in this report, participated in substudies involving more intensive testing with HRCT and/or BAL. The human subjects institutional review board approved the study, and informed consent was obtained on all individuals.

Distribution of Emphysema on HRCT

Clinical characteristics of individuals (n = 114) involved in the HRCT substudy have been previously described.² Previously, we reported the incidence of emphysema among subjects with the most substantial changes of emphysema (n = 17).² In the present study, we delineate the anatomic distribution of emphysema among individuals with any emphysema detected (n = 40). For comparison, we also evaluated the anatomic distribution of 14 HIV-seronegative subjects participating in HRCT substudy² with any emphysema detected.

Chest CT was performed with a GE9800 CT scanner (GE Medical Systems; Milwaukee, WI) or a Picker PQ 2000 CT scanner (Picker International; Solon, OH) with 1.5-mm collimation at 10-mm intervals. Scans were obtained at total lung capacity. Images were reconstructed using the high-spatial-frequency algorithm and were photographed at a lung window width of 1,500 Hounsfield units (brightness level, -700 Hounsfield units). Scans were read by two chest radiologists blinded to HIV status and clinical information. Emphysema was considered present if there was evidence of bullae, thin-walled cystic spaces, or abnormal decreases in attenuation, accompanied by vascular disruption. Emphysema severity was estimated by assigning an emphysema score (0 to 10) for each lobe according to the percentage of the lobe that was affected. For this analysis, the lingula was considered a separate lobe. The frequency of detection of emphysema in the various lobes as well as the total scores of the individual lobes were recorded.

Regional GSH Concentrations

We recruited 12 subjects for BAL of the RUL and RML. In one subject, lavage could not be analyzed because of insufficient return. This subject was excluded from analysis.

Bronchoalveolar Processing

Lavage fluid was processed as previously described.⁶ Five 20-mL aliquots of sterile saline solution were instilled into a distal segment of the RML and subsequently aspirated back into suction traps. The bronchoscope was then redirected into a distal segment of the RUL, where the procedure was repeated. Lavage fluid was immediately filtered through surgical gauze and centrifuged to separate cellular and noncellular components. Using 4 $\mu\text{mol/L}$ 5% 5-sulfosalicylic acid, the supernatant was acidified to a pH of 5.5 and immediately assayed for total GSH using the recycling assay of Sies and Akerboom.⁷ The volume of ELF was estimated by the urea dilution technique.⁸

Data Analysis

Differences in emphysema scores among the various lobes were analyzed by analysis of variance. Group mean differences in GSH levels between the upper and middle lobes were analyzed by paired *t* test. Correlations were performed using a Spearman correlation. Data represented are group means \pm SE.

RESULTS

Regional Distribution of Emphysema

Table 1 demonstrates the frequency of emphysema detection and the mean emphysema scores among the various lobes in subjects with any detectable emphysema. As is demonstrated in Table 1, both the frequency of emphysema detection as well as the mean scores are much higher in the upper lobes compared to the rest of the lung. The upper lobe predominance is evident in both the right and left lung.

BAL GSH Levels

The clinical characteristics and ELF GSH results of the study population who underwent double-lobe lavage are shown in Table 2. As is demonstrated in Table 2, in all but one case GSH levels were higher in the RUL compared to the RML. Although not shown in Table 2, there was a slightly higher lavage return from the RML compared to the RUL (51.5 ± 4.0 mL vs 41.6 ± 2.6 mL; $p = 0.022$). However, within each lobe there was no significant correlation between lavage volume and GSH concentration.

Table 1—Regional Distribution of Emphysema as Assessed by HRCT*

Variables	RUL	RML	RLL	LUL	Lingula	LLL
HIV seropositive						
Subjects	36/40	6/40	12/40	37/40	8/40	9/40
Emphysema score	2.1 ± 0.34	0.4 ± 0.17	0.6 ± 0.13	1.8 ± 0.10	0.28 ± 0.10	0.4 ± 0.12
HIV seronegative						
Subjects	13/14	2/14	3/14	12/14	2/14	3/14
Emphysema score	1.3 ± 0.34	0.1 ± 0.10	0.2 ± 0.11	0.9 ± 0.13	0.1 ± 0.10	0.2 ± 0.11

*Data are presented as No. of subjects with emphysema detected in a given lobe/total subjects, or mean \pm SE. RLL = right lower lobe; LUL = left upper lobe; LLL = left lower lobe.

Table 2—Baseline Clinical Characteristics and ELF GSH Levels Among HIV-Seropositive Subjects Undergoing Two-Lobe Lavage

Patient No.	Age yr	CD4	HAART	Smoker	Emphysema	GSH, nmol/mL ELF	
						Upper Lobe	Middle Lobe
1	26	735	Yes	Yes	No	259	53
2	28	N/A	Yes	Yes	Yes	880	320
3	32	N/A	N/A	Yes	Yes	782	407
4	44	600	Yes	Yes	Yes	793	225
5	41	399	Yes	No	No	150	34
6	44	N/A	N/A	No	No	863	373
7	37	520	No	No	N/A	396	262
8	34	323	Yes	Yes	N/A	358	255
9	31	70	Yes	No	No	414	278
10	41	270	No	Yes	No	1002	1069
11	34	378	No	Yes	No	1072	966
	35.6 ± 6.3	411.9 ± 206.2				633.6 ± 97.4	385.5 ± 100.6

Means ± SD are represented for numerical data. HAART = highly active antiretroviral therapy.

Figure 1 demonstrates the difference in mean GSH levels in lavage samples obtained from the RML compared to those in the RUL, according to smoking history. As expected, smokers had higher GSH levels compared to nonsmokers. However, in the both smokers and nonsmokers there were higher GSH levels in the upper lobes compared to the middle lobes.

DISCUSSION

Insight into mechanisms responsible for COPD development are limited by the fact that only a

minority of smokers get clinically significant disease, and by the long time course over which the disease develops. As such, examining an accelerated process as occurs in HIV may provide important insights into disease pathogenesis. Our data suggest that regional differences in oxidant stress occur in the lungs of HIV-seropositive individuals, a finding that may have relevance regarding the increased predilection for emphysema to develop in the upper lobes of both HIV- and non-HIV-seropositive smokers.

A weakness of our study involves the relatively small number of subjects recruited for double-lobe lavage and measurement of ELF GSH. Nevertheless, BAL is an invasive procedure and was done

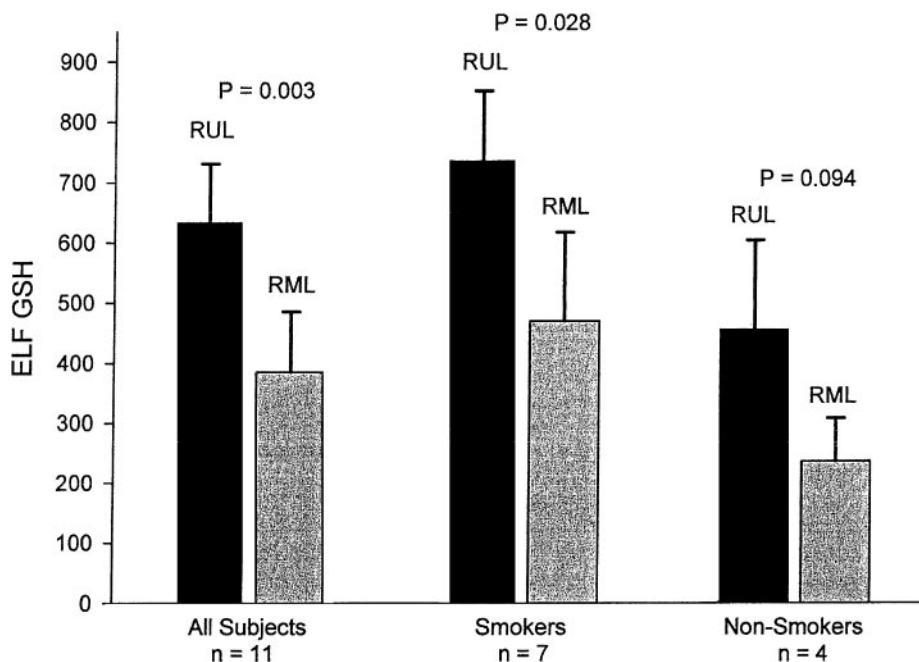


FIGURE 1. Regional ELF GSH concentrations among HIV-seropositive individuals according to smoking status. Data represented are group means ± SE. Values are nanomoles of GSH per milliliter of ELF.

entirely for research purposes. In addition, the differences between upper and middle lobes were highly significant, and similar changes were seen in both smokers and nonsmokers. Furthermore, 10 of 11 subjects had higher GSH levels in the upper lobes compared to the middle lobes. Thus, despite the relatively small numbers, we believe the regional differences in GSH concentrations are real.

We hypothesize that the increased GSH concentrations in the upper lobes represents an adaptive response to a chronic increase in oxidant stress in this region of the lung, as both *in vitro* and *in vivo* data suggest that chronic exposure to oxidant stress increases lung GSH secondary to induction of GSH synthesis.⁹ For example, *in vitro* data have demonstrated that oxidant stress produced by a variety of sources, including hyperoxia, initially results in a decrease, followed by a sustained increase in GSH levels in alveolar and bronchial epithelial cells.^{10,11} This increase appears to be secondary to up-regulation of γ -glutamylcysteine messenger RNA.⁹ Similarly, 90-day exposure to ozone in an *in vivo* model results in a 64% increase in GSH in distal bronchioles in both rats and monkeys.¹² Indeed, such an adaptive response to chronic oxidant stress has been suggested as a mechanism underlying the increase in GSH found in the ELF of long-term cigarette smokers.⁹

Our data do not address the etiology for increased GSH in the upper lung zones. However, it is interesting to note that both smokers and nonsmokers had increased GSH levels in the upper lobes compared to the middle lobes, suggesting that this difference is not merely a smoking-related phenomenon. One hypothesis may be related to higher oxygen tensions in the upper lobes resulting from higher ventilation/perfusion ratios. Alternatively, the upper zones may have greater exposure to inhaled toxins/pollutants causing relatively greater inflammatory cell trafficking with a resultant increase in oxidant stress.

The predilection for pulmonary emphysema to develop in the upper lobes in non-HIV-infected smokers is a well-recognized clinical phenomenon.^{13–15} Although the etiology is unknown, a number of hypotheses have been provided.¹³ For example, there may be less effective clearance of inhaled material from the upper lobes. Another hypothesis relates to slower transit time of leukocytes in the upper lobes compared to the lower lobes. This may allow a longer time for leukocytes to secrete inflam-

matory mediators. Each of these scenarios may be associated with increased oxidant stress in the upper lobes, and is consistent with our findings.

Whether our findings in the HIV population have relevance to the non-HIV population is not known. However, the similar tendency to develop upper lobe disease suggests that our findings among HIV-seropositive individuals may have relevance to the general population of smokers.

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