Industrial Health 2011, **49**, 746–754 **Original Article**

Oxidative Stress Markers in Exhaled Breath Condensate in Lung Fibroses Are Not Significantly Affected by Systemic Diseases

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Received August 8, 2010 and accepted September 26, 2011
Published online in J-STAGE October 20, 2011

Abstract: Exhaled breath condensate (EBC) is assumed to reflect processes in the lungs, yet it is unknown whether oxidative stress markers in EBC are affected by systemic disorders (atherosclerosis, hypertension, diabetes) or whether lung diseases increase markers in plasma and urine. 8-isoprostane, 4-hydroxy-*trans*-2-nonenale (HNE) and malondialdehyde (MDA) were measured using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS) in EBC, plasma and urine in 82 patients (45 with asbestosis and hyalinosis, and 37 with silicosis) and in 29 control subjects. 8-isoprostane and HNE in EBC, and HNE in urine were higher in both groups of patients. In addition, 8-isoprostane in plasma and urine, and MDA in urine were higher in asbestos-exposed patients and MDA in plasma in silicotics, with this marker in plasma correlated with the grade of silicosis. In all subjects, 8-isoprostane in EBC correlated with urine (r=0.38, $p<0.001$) and plasma levels (r=0.28, $p=0.003$), and HNE and MDA with urine levels (r=0.31, $p<0.001$; r=0.23, $p=0.016$, respectively). Most markers positively correlated with lung function impairment, EBC markers negatively with vitamin E supplementation. To conclude: The influence of satisfactorily controlled systemic disorders on markers in EBC in patients with pneumoconioses is not significant. In addition to oxidative stress markers in EBC, lung fibroses may increase oxidative stress markers in plasma and urine.

Key words: 8-iso-prostaglandin $F_{2\alpha}$, 4-hydroxy-*trans*-2-nonenale, Malondialdehyde, Lung fibrosis, Oxidative stress, Exhaled breath condensate, Plasma, Urine

Introduction

Lung diseases caused by asbestos and silica are incurable, incapacitating diseases and represent a worldwide problem[1)]. They exhibit unpredictable, yet progressive development; there is no treatment and there is an urgent need for simple and non-invasive tests to assess biomarkers implicated in their pathogenesis, activity and prognosis. Asbestos and silica particles induce the generation of reactive oxygen species, both directly by the Haber-Weiss reaction involving iron

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on the surface of the mineral fibers and particles, and indirectly due to activated macrophages and inflammatory cells in the lungs. Several routes of lipid oxidation can be followed. In humans, determination of the concentration of 8-iso-prostaglandin $F_{2\alpha}$ (8-isoprostane) in exhaled breath condensate (EBC) has proven to be valuable in assessing oxidative stress. The analysis of this derivative of arachidonic acid, produced in free radical-catalyzed reactions, appears to be a promising non-invasive means for assessing the activity of both silica and asbestos-induced diseases[2–4]. Moreover, no effect of sex or smoking status was observed in these studies. The quantification of oxidative stress can also be assessed by the measurement of aldehydes, such as malondialdehyde (MDA) and 4-hydroxy-*trans*-2-nonenale (HNE). Except for our pilot study[5], these markers have not yet been studied in EBC in a larger number of subjects with pneumoconioses.

However, oxidative stress products do not originate exclusively in the lungs, as they are formed *in situ* from the phospholipids of the cellular membranes and lipoproteins. After they are released to the extracellular space, they circulate in the plasma and may be detected in urine[6]. Oxidative stress is a phenomenon associated with pathogenetic mechanisms of several diseases, including atherosclerosis, cancer, diabetes mellitus and inflammatory diseases, as well as with aging processes. The levels of oxidative stress markers in plasma and urine, especially of 8-isoprostane, HNE and MDA, reportedly reflect systemic production in diseases such as diabetes[7], hypertension[8] and renal diseases[9].

On the other hand, endogenous antioxidants, e.g. uric acid and glutathione, and exogenous antioxidants from food, such as vitamin C, vitamin E and flavonoids, synthesized in plants, may decrease the level of oxidative stress. Diet and/or exercise intervention in subjects with insufficiently controlled diseases resulted in decreased lipid peroxidation, documented by lowering of oxidative stress markers in body fluids. In urine, a 35% reduction in 8-isoprostane was seen[10] after 14 days of consuming a diet with an array of fruits and vegetables. Accordingly, dietary intervention decreased plasma MDA in patients with diabetes and obesity[11], and plasma 8-isoprostane in metabolic syndrome[12].

Unfortunately, no study exists, to our knowledge, focused on a relationship among concentrations in blood, urine and EBC. The aim of our study was to evaluate the potential impact of lung fibrosis on the levels of the oxidative stress markers in blood and urine or, or the other hand, the potential interference of systemic stress with the EBC markers, primarily attributed to respiratory disorders. Therefore, in this study, also the correlation of all of the above-mentioned markers in these fluids, collected almost simultaneously within a short time interval, was investigated.

Materials and Methods

In total, 82 patients (60 men, 22 women) with occupational lung diseases were included in the study. They came for the regular follow-up and evaluation of their diseases.

Forty-five patients (24 men, 21 women, mean age 69.6 ± 2.0 yr; 15.5% smokers) had been exposed to asbestos for 23.3 ± 2.9 yr, on average, in the production of asbestos insulation and textile materials, asbestos cement roofing and pipe. Chest radiography was performed in all asbestos-exposed subjects, and high resolution computer tomography (HRCT) in 93.3% of patients with asbestos exposure. Pleural hyalinosis was found in all patients; among them, 20 subjects (44.4%) had diffuse pleural thickening. As for parenchymal changes, 28 subjects (62.2%) had radiological finding of irregular opacities grade 0/1 s/s, 1/0 s/s or 1/1 s/s; and 17 patients (37.8%) had a higher grade of opacities, corresponding to asbestosis, in the range of 1/2 s/s to 3/2 t/u[13, 14].

Thirty-seven patients, formerly exposed to silica (36 men, 1 woman, mean age 69.1 ± 2.9; 13.5% smokers), had worked as metal ore miners, tunnelers, foundry workers or stone cutters for an average of 24.8 ± 3.1 yr. According to on the chest radiographs, 17 subjects (45.9%) had the findings or rounded opacities corresponding to simple silicosis in the range 2/1 p/p to 3/3 r/r, and 20 subjects (54.1%) to complicated silicosis in the range of A–C[13].

Twenty-nine control subjects (20 men, 9 women, mean age 67.0 ± 4.6 yr; 13.8% smokers) had never been occupationally exposed to fibrogenic dusts nor diagnosed with lung fibrosis. They previously worked as office employees and safety inspectors.

Exclusion criteria in all groups were the exacerbation of chronic bronchitis and treatment with antibiotics during the past 2 months.

Subjects with systemic disorders were present in all groups examined. The percentage of subjects diagnosed with hyperlipidemia in asbestos-exposed, silica-exposed and controls was 64.4, 62.2 and 55.2; hypertension 75.6, 64.9 and 51.7; diabetes 42.2, 29.7 and 17.2; and stroke 6.7, 10.8, and 6.9, respectively.

All 3 groups of subjects were examined according to the following scheme: physical examination and a standardized questionnaire with personal and occupational history, illnesses diagnosed, including cancer, pharmaceutical treatments, daily vitamin supplements intake, dietary habits and alcohol intake. In addition, smoking,

meals and alcoholic beverages consumed during the day proceeding the day of examination and biological sample collection were recorded. Blood and a spot urine sample were taken between 8 and 12 a.m. for the blood count and following parameters: erythrocyte sedimentation (ESR), cholesterol, triacylglyceroles, glucose, urea, creatinine and liver enzymes in the blood; urinalysis; 8-isoprostane, and HNE and MDA in plasma and urine.

EBC collection was performed within the following 15–20 min with EcoScreen, Jaeger, for the analysis of 8-isoprostane, HNE and MDA. All subjects (wearing a nose-clip) breathed tidally through a mouthpiece connected to the condenser (–20°C), where vapors, aerosols and moisture condense along the walls of the tube. A constant volume of 120 l of exhaled air was maintained. Samples were immediately frozen to –80°C and stored for a period not exceeding 2 months.

Lung functions were measured using a body plethysmograph (Jaeger, Germany) in all subjects. The study was carried out according to the Helsinki Declaration and all subjects signed the informed consent.

Analysis of 8-isoprostane, MDA and HNE was performed as previously described[5, 15]. Briefly, the method consists of pre-treatment, solid-phase extraction for rapid and effective isolation of biomarkers from the biological matrices (EBC, plasma and urine) and a detection method using liquid chromatography - electrospray ionization - mass spectrometry/mass spectrometry (LC–ESI-MS/MS). Contamination of EBC with saliva eicosanoids and aldehydes was excluded by colorimetric detection of alpha-amylase (alpha-amylase-Liquid BIO-LA-TEST KIT, Pliva-Lachema, Czech Republic).

Statistical methods: Both discrete and continuous variables were included in the present study. Basic descriptive statistics (mean, mode, median, range, minimum, maximum, standard deviation, skewness and kurtosis) were calculated in the case of continuous variables. Data were verified to be free of impossible and/or unexpected values. Subsequently, the normal distribution of all variables was tested using the Kolmogorov-Smirnov test. Furthermore, the Pearson product moment correlation and Spearman rank correlation were used for normally and non-normally distributed variables, respectively. Moreover, correlations were calculated separately for selected markers in all three groups. The statistical significance of the correlations was tested on three levels: $p<0.05$, $p<0.01$ and $p<0.001$. The one-way analysis of variance (ANOVA) with the Fisher LSD post-hoc test was used to compare differences between groups. In the event of non-normally distributed variables, the Kruskal-Wallis test with respective post-hoc comparisons was used as a nonparametric alternative to the one-way ANOVA. In the case of pairs of categorical variables, data were analyzed using frequency tables and the chi-square test of homogeneity. The point-biserial correlation and Spearman correlation were used to analyze the relationships between pairs of categorical and continuous variables. This approach is an alternative to the independent samples *t*-test with the same significance levels. All analyses were conducted and verified in SPSS 17 (SPSS Inc., Chicago, IL, USA).

Results

The parameters of lung function and levels of all markers (8-isoprostane, HNE and MDA in EBC, plasma and urine, and ESR) in all groups of subjects are presented in Table 1.

8-isoprostane and HNE in EBC, and HNE in urine were higher in both groups of patients with occupational respiratory disorders due to asbestos and silica, compared to the controls.

In addition, 8-isoprostane in urine and plasma, and MDA in urine were higher in asbestos-exposed patients and MDA in plasma in silicotics, which correlated ($p=0.029$) with the grade of the disease (complicated silicosis).

In the group of all subjects, 8-isoprostane in EBC correlated with urine (r=0.38, $p<0.001$) and plasma levels (r=0.28, $p=0.003$); HNE in EBC correlated positively with urine (r=0.31, $p<0.001$), but negatively with plasma level (r=–0.22, $p=0.018$).

In the case of MDA, the correlation between EBC and urine was significant (r=0.23, $p=0.016$).

The correlations of EBC and urine 8-isoprostane in the 3 groups of subjects is shown in Fig. 1. Most oxidative stress markers in all matrices correlated with impairment of the parameters of lung function as shown in Table 2.

A part of the subjects in all groups suffered from systemic diseases; however no significant difference among the groups was seen for mean cholesterol, triacylglyceroles, glucose or liver enzymes, which indicates that the diseases were under control.

The alcohol intake in g/wk, diet regimen (quantity of fruits, vegetables, eggs, meat portions, butter/animal fat use per week) and diet on the day preceding the examination did not show a correlation with the concentration of oxidative stress markers in any biological matrix.

Among the 3 markers of oxidative stress in EBC, only MDA was not increased in the patients studied, which may be due to the fact that several correlations of MDA with biochemical markers were found, all of them negative. A negative correlation of MDA in EBC in the entire group of 111 subjects was noted for blood urea ($p=0.037$), creatinine ($p=0.001$), uric acid ($p=0.002$),

Table 1. ESR, lung functions, pH of the exhaled breath condensate (EBC) and 3 oxidative stress markers in EBC, plasma and urine in subjects with asbestos exposure, silica exposure and in the control subjects

	Arithmetic mean±confidence interval			*p*		
	Asbestos	Silica	Controls	Asbestos × Controls	Silica × Controls	Asbestos × Silica
ESR/1 h[a]	17.9 ± 4.6	19.9 ± 5.4	8.4 ± 2.8	0.002	$p<0.001$	n.s.
PEF %	92.7 ± 6.9	88.9 ± 9.6	115.07 ± 11.96	$p<0.001$	$p<0.001$	n.s.
FVC %	93.9 ± 6.7	91.8 ± 8.6	114.3 ± 5.8	$p<0.001$	$p<0.001$	n.s.
FEV1 %	88.6 ± 6.5	84.3 ± 9.1	115.9 ± 8.1	$p<0.001$	$p<0.001$	n.s.
FEV1/FVC %	75.1 ± 2.1	70.4 ± 3.6	79.7 ± 3.3	0.004	$p<0.001$	n.s.
MEF25-75 %	50.2 ± 6.1	54.5 ± 8.7	91 ± 13	$p<0.001$	$p<0.001$	n.s.
TLC %	90.9 ± 5.1	95 ± 5.4	106.8 ± 5.3	$p<0.001$	0.005	n.s.
RV/TLC %	45.1 ± 2.5	43.5 ± 3.6	38.7 ± 4.7	0.012	n.s.	n.s.
TLCO/Hb %	67.5 ± 5.7	64.3 ± 6.4	86.9 ± 6.3	$p<0.001$	$p<0.001$	n.s
pH of EBC[a]	6.26 ± 0.16	6.39 ± 0.14	6.49 ± 0.21	n.s.	n.s.	n.s.
8-iso in EBC (pg/ml)	93.5 ± 7.1	88.4 ± 7.3	62.8 ± 8.9	$p<0.001$	$p<0.001$	n.s.
8-iso in EBC (pg)	171.37 ± 17.49	159.66 ± 16.72	109.51 ± 19.87	$p<0.001$	$p<0.001$	n.s.
8-iso in plasma (pg/ml)	58.9 ± 4.0	54.9 ± 4.5	49.9 ± 6.3	0.009	n.s.	n.s.
8-iso in urine (pg/mmol creatinine)[a]	65.54 ± 16.06	42.52 ± 13.8	40.76 ± 20.61	0.011	n.s.	0.014
HNE in EBC (ng/ml)	45.7 ± 4.6	44.7 ± 4.0	32.5 ± 6.0	$p<0.001$	0.002	n.s.
HNE in EBC (ng)	83.56 ± 10.28	82.4 ± 11.26	59.77 ± 14.43	0.005	0.011	n.s.
HNE in plasma (ng/ml)[a]	49.6 ± 5.2	43.0 ± 4.7	47.5 ± 8.2	n.s.	n.s.	n.s.
HNE in urine (ng/mmol creatinine)[a]	48.2 ± 8.2	46.94 ± 11.5	34.15 ± 11.83	0.003	0.006	n.s.
MDA in EBC (ng/ml)[a]	18.6 ± 2.5	17.3 ± 2.6	17.5 ± 2.0	n.s.	n.s.	n.s.
MDA in EBC (ng)[a]	33.7 ± 5.2	31.4 ± 5.2	29.9 ± 4.8	n.s.	n.s.	n.s.
MDA in plasma (ng/ml)	49.2 ± 5.3	51.5 ± 5.3	43.2 ± 6.1	n.s.	0.048	n.s.
MDA in urine (ng/mmol creatinine)	5.0 ± 1.0	4.7 ± 1.2	4.1 ± 1.2	0.038	n.s.	n.s.

ESR-erythrocytes sedimentation, PEF-peak expiratory flow, % –percent predicted, FVC-forced vital capacity, FEV1-forced expiratory volume in 1 s, MEF 25–75 maximal expiratory flow rate at 25–75% of the vital capacity, TLC-total lung capacity, RV-residual volume, TLCO-diffuse lung capacity for carbon monoxide, EBC=exhaled breath condensate, 8-iso=8-isoprostane, HNE=4-hydroxy-trans-2-nonenale, MDA=malondialdehyde, n.s.-non significant, [a]-non-normal variable (post-hoc comparisons for the Kruskal-Walis test were used).

triglycerides (p=0.014) and gamma-glutamyl transferase (GMT) (p=0.017). A positive correlation was not found. Other EBC markers – 8-isoprostane and HNE – did not show any correlations with biochemical parameters. However, some plasma and urine markers were affected. For ESR, positive correlations were found with plasma 8-isoprostane (p=0.009); and HNE (p=0.005) and MDA (p=0.023) in urine.

As for systemic disorders and treatments in the patients with pneumoconioses, they only very rarely showed a correlation with the studied markers in plasma and urine, as shown in Table 3. The proportion of subjects in the groups with diagnosed cancer was 10.3% in the control group, 21.6 in the silicosis group and 17.8% in the asbestos-exposed group. Most frequent cancers in silicosis patients were the lung and colon cancer and

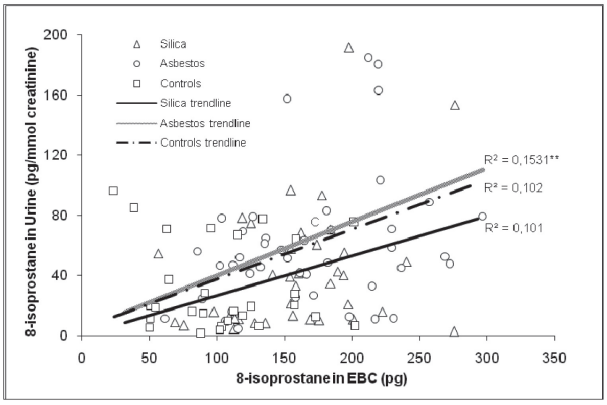

Fig. 1. Correlations of EBC and urine 8-isoprostane in the group of asbestos and silica-exposed subjects, and controls.

Table 2. Correlation of the parameters of lung functions with oxidative stress markers (8–isoprostane, MDA and HNE) in the exhaled breath condensate, plasma and urine in the group of all subjects

Marker	PEF	PEF%	FVC	FVC%	FEV1	FEV1%	FEV1/ FVC%	MMEF 75/25	MEF 25–75%	TLC%	RV/ TLC%	RV	RV%	TLCO	TLCO/ Hb	TLCO /Hb%
EBC MDA (ng/ml)	*0.028*	*0.072*	*0.019*	*0.030*	*0.013*	*0.012*	*–0.094*	*–0.014*	*0.001*	*0.010*	*–0.019*	*–0.016*	*–0.007*	*0.116*	*0.109*	*0.132*
EBC MDA (ng)	*–0.060*	*0.028*	*–0.089*	*–0.004*	*–0.096*	*–0.040*	*–0.133*	*–0.087*	*–0.055*	*0.010*	*0.039*	*–0.031*	*0.019*	*0.039*	*0.033*	*0.093*
EBC HNE (ng/ml)	–0.177	–0.105	–0.233*	–0.099	–0.292**	–0.184	–0.190*	–0.280**	–0.221*	–0.084	0.203*	0.069	0.090	–0.182	–0.183	–0.155
EBC HNE (ng)	–0.185	–0.083	–0.249**	–0.063	–0.292**	–0.133	–0.124	–0.250**	–0.172	–0.013	0.202*	0.065	0.125	–0.199*	–0.193*	–0.133
EBC 8-iso (pg/ml)	–0.194*	–0.149	–0.271**	–0.165	–0.297**	–0.208*	–0.133	–0.251**	–0.236*	–0.161	0.231*	–0.002	0.027	–0.256**	–0.281**	–0.267**
EBC 8-iso (pg)	–0.235*	–0.143	–0.342***	–0.161	–0.359***	–0.199*	–0.092	–0.295**	–0.249**	–0.089	0.262**	0.018	0.088	–0.303**	–0.318**	–0.264**
URINE_MDA (ng/mmol_creatinine)	*–0.219**	*–0.049*	*–0.314****	*–0.013*	*–0.320****	*–0.097*	*–0.194**	*–0.263***	*–0.179*	*0.010*	*0.242**	*–0.006*	*0.136*	*–0.240**	*–0.219**	*–0.092*
URINE HNE (ng/mmol_creatinine)	*–0.198**	*–0.035*	*–0.292***	*–0.061*	*–0.322****	*–0.129*	*–0.236**	*–0.267***	*–0.188*	*–0.046*	*0.159*	*–0.040*	*0.037*	*–0.269***	*–0.247**	*–0.148*
URINE 8-iso (pg/mmol creatinine)	*0.012*	*0.050*	*–0.090*	*–0.038*	*–0.076*	*–0.033*	*–0.035*	*–0.111*	*–0.097*	*–0.063*	*0.049*	*–0.065*	*–0.010*	*–0.053*	*–0.057*	*–0.018*
PLASMA MDA (ng/ml)	–0.220*	–0.233*	–0.233*	–0.277**	–0.253**	–0.282**	*–0.095*	*–0.184*	–0.164	–0.245*	0.185	0.016	–0.025	–0.224*	–0.228*	–0.263**
PLASMA HNE (ng/ml)	*0.046*	*0.046*	*–0.021*	*–0.043*	*0.054*	*0.073*	*0.251***	*0.079*	*0.092*	*–0.190**	*–0.045*	*–0.191**	*–0.251***	*–0.087*	*–0.086*	*–0.152*
PLASMA 8-iso (pg/ml)	–0.219*	–0.098	–0.262**	–0.007	–0.287**	–0.085	*–0.107*	*–0.227**	–0.168	0.048	0.183	0.015	0.099	–0.221*	–0.197*	–0.113

PEF-peak expiratory flow, %-percent predicted, FVC-forced vital capacity, FEV1-forced expiratory volume in 1 s, MEF 25–75 maximal expiratory flow rate at 25–75% of the vital capacity, TLC-total lung capacity, RV-residual volume, TLCO-diffuse lung capacity for carbon monoxide, EBC=exhaled breath condensate, 8-iso=8-isoprostane, HNE=4-hydroxy-trans-2-nonenale, MDA=malondialdehyde, *p<0.05, **p<0.01, ***p<0.001. Italic entries are Spearman rank correlations.

Table 3. Significant correlation coefficients of 8-isoprostane, HNE and malondialdehyde (MDA) levels with the diagnosed disorders and information concerning treatment (*p<0.05, **p<0.01)

Marker	Group	Source vs. Variable	Correlation
8-isoprostane	Silica	Urine vs. Nephrolithasis	*0.407**
8-isoprostane	Silica	Plasma vs. Cancer	0.343*
8-isoprostane	Controls	EBC (ng) vs. Vitamin E	–0.540**
8-isoprostane	Controls	EBC (ng/ml) vs. Statins	–0.382*
8-isoprostane	Controls	EBC (ng/ml) vs. Vitamin E	–0.493**
8-isoprostane	Controls	Plasma vs. Ischemic Heart Disease	0.455*
8-isoprostane	Controls	Plasma vs. Vitamin E	–0.403*
HNE	Silica	EBC (ng) vs. Kidney failure	–0.353*
HNE	Silica	EBC (ng) vs. Vitamin E	–0.354*
HNE	Silica	EBC (ng/ml) vs. Vitamin E	–0.346*
HNE	Silica	Urine vs. Nitrates	*0.341**
HNE	Silica	Plasma vs. Fibrates	*0.416**
HNE	Silica	Plasma vs. Beta blockers	*0.371**
HNE	Asbestos	Urine vs. Cancer	*0.303**
HNE	Asbestos	Urine vs. Vitamin C	*–0.299**
HNE	Asbestos	Urine vs. Vitamin E	*–0.299**
HNE	Asbestos	Plasma vs. Vitamin C	*0.340**
HNE	Asbestos	Plasma vs. Vitamin E	*0.340**
HNE	Controls	Urine vs. Diabetes mellitus	*0.371**
MDA	Silica	EBC (ng) vs. Vitamin E	*–0.399**
MDA	Silica	EBC (ng/ml) vs. Vitamin E	*–0.325**
MDA	Silica	Plasma vs. Vitamin E	*–0.380**
MDA	Controls	EBC (ng) vs. Ca channel blockers	*–0.418**
MDA	Controls	EBC (ng) vs. Vitamin E	*–0.382**
MDA	Controls	Plasma vs. Hypertension	0.375*

EBC=exhaled breath condensate, HNE=4-hydroxy-trans-2-nonenale, MDA=malondialdehyde, Only significant correlations are presented. Italic entries are the Spearman rank correlations.

in asbestosis patients kidney cancer, leukemia and breast cancer. As can be seen in Table 3, there was a positive correlation of 8-isoprostane in plasma with cancer in silicotics subjects; mean level was 65.77 ± 10.14 pg/ml in the patients with cancer and 52.35 ± 12.83 pg/ml without cancer. The difference was significant (p=0.014). Similarly, a positive correlation was seen in the group of asbestos-exposed and HNE concentration in urine. The level was 60.54 ± 24.75 ng/mmol creatinine in patients with cancer and 49.52 ± 27.40 ng/mmol creatinine in patients without cancer (p=0.196). Importantly, markers in EBC were not positively correlated with any other systemic diseases, and only kidney failure was negatively correlated with HNE.

The only treatment that correlated with markers in all matrices was vitamin E supplementation, which was negatively correlated with several markers in EBC in most groups of subjects; on the other hand, vitamin E was positively correlated with two plasma markers. A similar effect of vitamin C supplementation on HNE levels was noted.

Age, diet regimen, other treatments, alcohol intake and smoking status did not exhibit any correlation; accordingly, no effect was found for the composition of food, drinking of alcoholic beverages or smoking on the previous day.

Discussion

Several experimental and clinical studies have described the importance of oxidative stress in the pathophysiology of asbestos damage[16, 17], and some clinical studies found increased 8-isoprostane in EBC in pneumoconioses.

To distinguish from the potential input of systemic disorders, we compared 3 markers of oxidative stress (8-isoprostane, HNE and MDA), both in EBC and in plasma and urine, where they could potentially reflect systemic effects. This is the first study, to our knowledge, where the role of other than respiratory diseases

in EBC markers has been systematically studied.

Therefore, our controls were selected from the general population of subjects, including those treated for diabetes and hypertension. Their average biochemical parameters in blood did not significantly differ. This may explain why neither plasma nor urine oxidative stress markers showed a significant positive correlation with biochemical parameters.

In the patients with respiratory diseases, no systemic disorder positively correlated with the level of EBC marker, and only plasma and urine markers correlated with purely two diseases, nephrolithiasis and cancer.

In the controls, only plasma or urine markers were affected by ischemic heart disease, hypertension and diabetes, similarly to the studies of authors focusing on patients with uncontrolled diabetes, hypertension or hyperlipidaemia[8, 11]. As can be seen from Table 3, the correlation was positive for these diseases (nephrolithiasis, ischemic heart disease, diabetes, hypertension), where increased oxidative stress is expected, and negative for treatments with vitamin C and E, and statins, where the suppression of oxidative stress is supposed. However this simple approach cannot explain presumably complex interaction of the pathophysiological process and pharmacological treatment. The temporal relationships may also play a role, i.e. beginning of the effect of different drugs in the relationship to the time of EBC, blood and urine collection.

The groups studied did not differ in their smoking or dietary habits, including alcohol consumption in general, or on the day preceding the collection of biological samples. Among the factors studied, no correlation of all markers studied with these external factors was observed. Only vitamin E (and vitamin C to a lesser extent) might have influenced the markers studied.

A noticeable finding of this study is the fact that EBC levels of HNE, in addition to 8-isoprostane in the patients with asbestos and silica exposure, were also significantly increased. However, the most important result of this study is that EBC markers were not significantly affected by the systemic disorders of all subjects. Additionally, plasma and urine levels of the markers correlated with the impairment of lung function and plasma MDA with the severity of silicosis.

Urine levels of 8-isoprostane, HNE and MDA were more frequently correlated with EBC levels of the respective markers than their plasma levels, which suggests their potential alternative use.

Correlations of pharmacological treatment (excluding vitamin supplementation) were rare and in the patients concerned only urine and plasma HNE and treatment with fibrates, nitrates and beta-blockers. In the controls, a negative correlation of statins on 8-isoprostane in EBC was found.

As concerns the diseases, only one correlation in the patients was found, which involved urine 8-isoprostane and nephrolithiasis in the silicotics. The correlation of cancer with plasma 8-isoprostane and urinary HNE in the patients with silicosis/asbestosis might help to search for the malign complication of the pneumoconioses.

In the controls, more influence of the diseases on urine and plasma levels of the markers was registered. Plasma 8-isoprostane was positively correlated with ischemic heart disease, plasma MDA with hypertension and urine HNE with diabetes.

All these data confirm that EBC markers were not significantly affected; however, in the analysis of other than EBC markers, the interference of other factors should be considered.

Conclusion

8-isoprostane and HNE in EBC correspond to oxidative stress processes in the lungs and are not significantly affected by systemic disorders, or pharmacological or dietary factors.

In addition, lung fibroses were delineated not only in the EBC increase, but also in the elevation of several urine and plasma oxidative stress marker levels, such as HNE in urine of patients with both pneumoconioses, and 8-isoprostane and MDA in urine of patients with asbestos exposure. Additionally, plasma MDA level was higher in subjects with silicosis and correlated with its severity.

Therefore, as an option when EBC collection is not available, 8-isoprostane and HNE could potentially be measured in urine or plasma. Sufficiently controlled systemic disorders, such as ischemic heart disease, hypertension, hyperlipoproteinemia, or diabetes, and similarly with pharmacological treatment itself did not significantly influence the results concerning oxidative stress of markers in EBC, but did not even increase plasma or urine level of these markers in the patients. Only vitamin E negatively correlated with the level of HNE and MDA in EBC in silicotics; and vitamin E and statins with the level of 8-isoprostane in EBC in the controls, which may signify their antioxidant effect.

MDA in EBC in pneumoconioses was not increased; this may be caused by several negative correlations of biochemical parameters (urea, creatinine, uric acid, etc.), with a corresponding lower reliability of this marker for lung disorders.

8-isoprostane appears to be the optimal oxidative stress marker for respiratory disorders. In addition to 8-isoprostane, HNE is another marker that can be used to detect oxidative stress in pneumoconioses, as no dis-

ease or treatment has shown a positive correlation with this factor.

There might be a role of 8-isoprostane in plasma and HNE in urine to search for cancer due to their correlation with cancer in the patients with silicosis/asbestosis; however more data are needed to support this possibility.

This study supports the confidence that the non-invasive technique of EBC may contribute to a better understanding of the pathogenesis of silicosis and asbestosis and related findings, such as leukotrienes and anti-neutrophil cytoplasmic antibodies (ANCA) involvement[18–20]; and presumably to the monitoring of the activity of these still-incurable lung diseases.

Acknowledgements

The authors would like to thank project MSM 0021620807 for financial support, and Prof. Steve Diskin, PhD. for linguistic assistance.

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