Letter to the Editor

Early Changes in Serologic Markers in Workers Exposed to Indium Compounds*

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Case reports of lung disease among indium-exposed workers in Japan, the USA, and China over the past two decades have raised public concern about the toxicity of indium and indium compounds. Occupational exposure to indium compounds can result in potentially fatal indium lung disease, which is characterized by pulmonary alveolar proteinosis (PAP) and may progress to fibrosis, with or without emphysema[1]. Knowledge of the absorption, distribution, and elimination of indium in the human body is limited. However, we previously reported positive correlations between urine indium levels and the frequency of CBMN/1000 cells in a cross-sectional study of indium workers in China[2]. Therefore, the present study aimed to determine the relationships among indium exposure, serum indium, urinary indium, and markers of lung damage in indium occupational workers in China.

Epidemiological studies have demonstrated associations between biological markers of indium exposure (i.e., serum or plasma indium concentration) and various health outcomes, including changes in chest imaging and increased levels of biomarkers related to interstitial lung disease, such as KL-6 and SP-D[3-6]. However, the risks to workers exposed to indium and its compounds are largely unknown. Despite evidence of the harmful pulmonary effects of indium and its compounds, no studies have investigated the changes in biomarkers of lung disease caused by occupational exposure to indium in China. Therefore, this study measured serum biomarkers of lung damage, including lung epithelium-specific proteins (SP-A, SP-D, KL-6, and GM-CSF); cytokines and other serological parameters (monocyte chemotactic protein-1 [MCP-1], angiotensin-converting enzyme [ACE], LDH, and the cancer/fibrosis marker metalloproteinase-2,9 [MMP-2,9]); and a general marker of inflammation (TGF-β1) to assess whether there are relationships between exposure to indium compounds and the levels of these serum biomarkers in workers exposed to indium compounds in China.

The indium ingot production plant (Guangxi, China) examined in this study was selected as described previously[2]. Fifty-seven workers were mainly exposed to indium metal, but also to In_2SO_4, In_2O_3, and some $InCl_3$. Sixty-three unexposed office workers, who were matched by age, sex, smoking habits, and work experience to the exposed workers, were selected through simple random sampling. All participants were interviewed by an occupational physician using a detailed questionnaire that included demographic information, current and previous jobs, educational level, smoking history, alcohol consumption, occupational history of exposure, personal medical history, and exposure to ionizing radiation over the previous 12 months, as well as possible exposure to non-occupational sources of indium. Written informed consent was obtained from all study participants. The study was approved by the Research Ethics Committee of the North China University of Science and Technology.

The Specifications of air sampling for hazardous substances monitoring in the workplace (GBZ 159-2004) were followed when sampling for airborne indium in the breathing zones of the study participants, with continuous sampling for three days. Workers who wore personal sampling devices were asked to record the tasks they completed during working periods. Indium in the air was measured by ICP-MS (Agilent 7500a). The limit of detection (LOD) for airborne indium was estimated to be 0.045 μg/m^3, which is approximately one tenth of the acceptable exposure limit of 0.3 μg/m^3.

On the day of air sampling, blood and urine

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samples were taken from all participants at the end of their work shift. The separated serum and urine samples were stored at -80 °C until use. These biological samples were prepared for analysis by ICP-MS. A calibration curve was plotted based on the obtained data (R^2 = 0.9997, linear dynamic range: 1–100 μg/L), and the LOD for indium was 0.053 μg/L. Serum concentrations of SP-A, SP-D, KL-6, GM-CSF, MMP-2, MMP-9, TGF-β1, MCP-1, and ACE were measured using ELISA kits (R&D Systems); LDH was measured by a colorimetric assay (R&D Systems); and urinary creatinine was determined using a kit developed by Nanjing. All samples were analyzed twice, and the means are reported.

Spirometry was performed using a Vmax22 spirometer (SensorMedics Inc., USA). The parameters measured included forced vital capacity (FVC), lower forced expiratory volume measured in 1 second (FEV_1), and the FEV_1/FVC (%FEV_1/FVC) ratio. Tests of pulmonary function were performed with the participant in a sitting position *via* the closed-circuit method, measuring inhaled and exhaled air during the test cycle. The tests were repeated at least three times until appropriate and reproducible results were obtained, and then the best results were selected for analysis. The threshold levels of %FVC, %FEV_1, and the %FEV_1/FVC ratio were 80%, 80%, and 70%, respectively.

All statistical analyses were carried out using SPSS 17.0 software. Descriptive statistics, including the mean, standard deviation, range, and percentage, were used to describe data, and analytical statistics, including *t*-test, Mann-Whitney, and analysis of variance (ANOVA), were used to compare results between groups. Spearman's rank correlation test was used to evaluate the relationship between two variables. The normal distribution of the variables was assessed by the Kolmogorov-Smirnov test. *P* values less than 0.05 were considered statistically significant.

The workshop for indium production is generally divided into four sections, including workplaces for (A) indium slag leaching, extraction, and electrolytic processing; (B) indium replacement; (C) indium volatile kiln and dust collection; and (D) indium furnace post. Table 1 shows the indium concentrations in the breathing zones of the workers in the four workplaces. We collected personal breathing zone air samples from 34 indium-exposed workers and 18 office workers. The average sampling time was 375 min (range, 180–480 min). The mean airborne indium concentration of the 57 workers was 78.41 μg/m^3 (range, 1.00–1120.00 μg/m^3). The highest indium concentration was detected in samples from workers in the indium volatile kiln and dust collection section, and the lowest concentration was detected in samples from workers in the indium replacement section of the factory. None of the samples from the indium-exposed workers were below the limit of quantification (LOQ), whereas none of the samples from the controls reached a quantifiable level.

We used serum and urinary indium levels as indices for biological indium exposure. Our findings were similar to the results of other studies in Japan and Korea, which reported that workers exposed to indium compounds had significantly higher serum indium levels than non-exposed workers[3,7]. We also found significant increases in serum and urinary indium levels in the exposed groups. The highest and lowest mean urinary indium values were 13.76 and 2.23 ng/g creatinine, respectively, which were detected in the indium volatile kiln and dust collection workers and the controls. Measurements of serum indium levels showed that the highest level, 47.19 μg/L, was detected in indium volatile kiln and dust collection workers, whereas the lowest level, 4.93 μg/L, was detected in the controls. Although the mean %FEV_1 and mean %FVC were significantly lower in the indium-exposed group, the FEV_1/FVC ratio of all 57 workers was within the normal range (> 70%), and the %FVC was within the normal range (> 80%). There was no significant difference between the indium-exposed and control groups in terms of age, sex, work experience, and FEV_1/FVC ratio.

Several studies have reported SP-A, SP-D, and KL-6 as sensitive markers of pulmonary interstitial damage in humans[8]. We found that indium-exposed workers had significantly higher serum levels of SP-A, SP-D, and KL-6 as well as GM-CSF, TGF-β1, and LDH than the controls (Table 1). Thus, the observed differences between indium-exposed workers and controls may be related to the effect of indium on pulmonary cells, which is followed by stimulation of macrophages and monocytes and subsequent increased secretion. Further stratified analysis found that KL-6, GM-CSF, and LDH levels differed significantly among workers in the four workplaces and the controls, the levels of SP-A and TGF-β1 differed significantly only between indium volatile kiln or dust collection workers and the controls, and the differences in SP-D levels did not reach statistical significance.

Table 2 shows the correlations of indium exposure metrics (serum, urinary, and airborne

indium concentrations) with markers of lung function and serum biomarkers. Positive correlations were found between indium exposure and urinary indium concentrations ($P < 0.01$), serum indium concentrations ($P < 0.01$), and serum SP-A, KL-6, and LDH levels ($P < 0.01$), but no correlation was observed with serum SP-D levels. The bivariate correlations among biomonitored results were also evaluated. Serum indium was significantly correlated with urinary indium ($P < 0.001$) and serum and urinary indium were respectively significantly correlated with serum SP-A and LDH levels. In contrast, FEV_1, FVC, the FEV_1/FVC ratio, the levels of GM-CSF, MMP-2, MMP-9, TGF-β1, MCP-1, and ACE, which are indices of inflammation, were not correlated with serum or urinary indium. Our findings also showed that serum and urinary levels of indium and SP-A were significantly positively correlated with years of exposure ($P < 0.05$).

Our study showed not only associations but also dose-response relationships between indium exposure and various serum markers (Table 3). The levels of both SP-A and LDH were increased in workers with higher exposures as assessed by the gradients of serum and urinary indium concentrations, and these differences were significant ($P < 0.05$). Further analysis of the interrelationship of these two markers showed that SP-A and LDH concentrations were significantly ($r = 0.309$, $P = 0.019$) correlated with each other. These results indicate that serum and urinary indium levels may be useful early biomarkers of indium exposure when combined with other biomarkers. Nevertheless, the lack of an association for serum indium concentration with SP-D and KL-6 was unexpected, and differs from the results of a prior

Table 1. Demographic data and laboratory results of the study participants

Variable	Controls (n = 63) Mean ± SE	Exposed groups (n = 57), Mean ± SE					P_{ANOVA}	P_{trend}
		Total	A (n = 15)	B (n = 15)	C (n = 15)	D (n = 12)		
Age (yr, $\bar{x}$ ± SD)	39.32 ± 11.37	37.82 ± 9.34	39.93 ± 8.56	38.27 ± 9.42	35.20 ± 10.25	37.92 ± 11.27	0.750	0.152
Sex (male/female, %male)	46/17(73)	41/16(72)	13/2(87)	6/9(40)	12/3(80)	10/2(83)	0.030	0.895
Smoking (yes/no, %yes)	31/32(49)	21/36(37)	6/9(40)	2/13(13)	6/9(40)	7/5(58)	0.107	0.174
Drinking (yes/no, %yes)	20/43(32)	18/39(32)	5/10(33)	2/13(13)	7/8(47)	4/8(33)	0.426	0.984
%FEV_1 ($\bar{x}$ ± SD)	95.63 ± 11.96	91.46 ± 7.05	93.30 ± 8.12	92.79 ± 8.06	88.78 ± 5.80	90.83 ± 5.15	0.137	**0.020**
%FVC ($\bar{x}$ ± SD)	95.42 ± 13.34	91.03 ± 9.67	94.30 ± 0.31	94.18 ± 12.56	85.05 ± 4.61	90.50 ± 5.99	**0.036**	**0.040**
FEV_1/FVC ($\bar{x}$ ± SD)	102.49 ± 10.45	100.60 ± 10.57	102.56 ± 9.95	102.46 ± 10.79	95.84 ± 10.94	101.76 ± 9.98	0.270	0.327
Years of exposure (yr, $\bar{x}$ ± SD)	8.97 ± 8.39	8.31 ± 7.54	10.03 ± 7.76	10.27 ± 8.24	6.33 ± 6.48	6.17 ± 7.31	0.468	0.161
Airborne indium (μg/m^3, range)	–	78.41 ± 65.22, 1.00–1120.00	12.60 ± 8.80, 1.00–47.00	4.50 ± 2.03, 1.00–8.00	302.50 ± 272.69, 1.00–1120.00	10.50 ± 5.72, 1.00–24.00	–	–
Urinary indium (ng/g creatinine, range)	2.23 ± 0.25, 0.01–8.38	11.00 ± 1.67, 0.17–58.10	6.27 ± 0.92, 1.55–14.28	13.03 ± 3.69, 0.28–53.51	13.76 ± 4.40, 0.17–58.10	10.91 ± 3.08, 0.27–31.24	**< 0.001**	**< 0.001**
Serum indium (μg/L, range)	4.93 ± 0.35, 0.19–13.64	39.26 ± 3.39, 11.86–137.63	32.10 ± 2.76, 11.86–50.40	32.85 ± 3.22, 14.88–56.68	47.19 ± 9.42, 13.84–137.63	46.30 ± 9.24, 15.91–137.25	**< 0.001**	**< 0.001**
SP-A (μg/L)	117.09 ± 8.59	178.96 ± 20.11	169.01 ± 32.29	183.28 ± 34.21	189.08 ± 49.63	173.35 ± 48.03	0.078	**0.006**
SP-D (μg/L)	11.98 ± 1.18	18.49 ± 2.81	16.44 ± 4.15	16.91 ± 4.71	21.01 ± 7.22	19.90 ± 6.62	0.237	**0.036**
KL-6 (U/L)	6.56 ± 0.44	9.98 ± 1.17	9.65 ± 2.31	8.63 ± 1.48	12.02 ± 3.07	9.54 ± 2.37	**0.044**	**0.008**
GM-CSF (μg/L)	396.80 ± 19.83	623.52 ± 65.52	705.05 ± 152.29	705.02 ± 134.80	527.48 ± 95.51	539.78 ± 142.44	**0.006**	**0.002**
MMP-2 (μg/L)	591.94 ± 40.85	675.95 ± 57.66	730.64 ± 142.39	749.45 ± 113.48	640.12 ± 102.39	560.48 ± 93.07	0.477	0.230
MMP-9 (U/L)	1.53 ± 0.15	1.80 ± 0.16	2.00 ± 0.36	1.82 ± 0.31	1.55 ± 0.29	1.82 ± 0.36	0.645	0.227
TGF-β1 (U/L)	0.89 ± 0.10	1.26 ± 0.12	1.03 ± 0.18	1.31 ± 0.23	1.43 ± 0.30	1.29 ± 0.21	0.127	**0.019**
MCP-1 (μg/L)	301.31 ± 28.50	285.58 ± 30.74	278.90 ± 46.50	315.02 ± 47.98	296.48 ± 85.60	243.52 ± 61.73	0.935	0.708
ACE (μg/L)	141.32 ± 11.12	187.25 ± 20.65	203.81 ± 41.73	193.12 ± 35.32	175.80 ± 36.81	173.51 ± 57.77	0.347	0.053
LDH (μg/L)	211.28 ± 11.32	349.00 ± 16.75	353.94 ± 37.73	306.36 ± 23.75	377.20 ± 143.98	319.68 ± 120.84	**< 0.001**	**< 0.001**

study in which serum indium, KL-6, and SP-D levels declined over time after a stoppage or reduction in indium exposure[9]. It is possible that, compared with the changes in serum SP-A and LDH levels, changes in SP-D and KL-6 levels occur with higher exposure or later during exposure. The lack of any significant relationship for SP-D and KL-6 with the biomarkers of exposure assessed in our study might have been influenced by the limited number of study subjects.

In the correlation analysis of indium

Table 2. Correlations of indium exposure with serum biomarkers and markers of lung function

Variable	Serum indium (μg/L)		Urinary indium (ng/g creatinine)		Airborne indium (μg/m^3)		Years of exposure	
	r	*P*	*R*	*P*	*r*	*P*	*r*	*P*
Serum indium	–	–	0.701	**< 0.001**	0.633	**< 0.001**	0.292	**0.028**
Urinary indium	0.701	**< 0.001**	–	–	0.492	**< 0.001**	0.375	**0.004**
%FEV$_1$	−0.048	0.724	−0.160	0.233	−0.231	0.083	−0.005	0.971
%FVC	−0.091	0.502	−0.080	0.555	−0.161	0.232	0.123	0.362
FEV$_1$/FVC	0.089	0.510	0.070	0.605	−0.140	0.297	−0.118	0.381
SP-A	0.679	**< 0.001**	0.551	**< 0.001**	0.418	**0.001**	0.410	**0.002**
SP-D	−0.053	0.693	−0.076	0.575	0.178	0.186	−0.019	0.887
KL-6	0.061	0.654	0.002	0.985	0.422	**0.001**	0.125	0.354
GM-CSF	−0.147	0.271	−0.131	0.332	0.012	0.931	0.023	0.865
MMP-2	−0.072	0.596	−0.117	0.384	0.079	0.558	0.014	0.919
MMP-9	−0.064	0.638	−0.112	0.405	0.060	0.656	0.040	0.769
TGF-β1	−0.144	0.287	−0.122	0.364	−0.208	0.120	0.016	0.908
MCP-1	−0.019	0.889	−0.046	0.735	0.258	0.053	0.056	0.678
ACE	−0.174	0.195	−0.214	0.109	0.041	0.761	−0.108	0.425
LDH	0.491	**< 0.001**	0.326	**0.013**	0.539	**< 0.001**	0.164	0.222

Note. *P* values were calculated using Spearman's correlation coefficient test.

Table 3. Association of indium exposure with various serum biomarkers

Variable	Serum indium (μg/L)			Urinary indium (ng/g creatinine)		
	< 32.93, Mean ± SE (*n* = 29)	≥ 32.93, Mean ± SE (*n* = 28)	*P*	< 6.49, Mean ± SE (*n* = 29)	≥ 6.49, Mean ± SE (*n* = 28)	*P*
SP-A (μg/L)	111.43 ± 9.76	248.90 ± 35.33	**0.001**	130.03 ± 15.51	226.20 ± 34.68	**0.016**
SP-D (μg/L)	18.16 ± 3.87	18.84 ± 4.16	0.905	21.07 ± 4.37	16.00 ± 3.59	0.327
KL-6(U/L)	9.24 ± 1.52	10.76 ± 1.82	0.522	10.84 ± 1.58	9.16 ± 1.75	0.497
GM-CSF (μg/L)	644.93 ± 90.32	601.34 ± 96.58	0.743	722.10 ± 88.88	528.33 ± 94.13	0.141
MMP-2 (μg/L)	651.77 ± 76.43	700.98 ± 87.85	0.674	738.71 ± 81.33	615.35 ± 81.51	0.289
MMP-9 (U/L)	1.76 ± 0.22	1.83 ± 0.24	0.828	2.04 ± 0.23	1.56 ± 0.22	0.136
TGF-β1 (U/L)	1.30 ± 0.15	1.22 ± 0.18	0.715	1.43 ± 0.17	1.10 ± 0.15	0.166
MCP-1 (μg/L)	265.50 ± 43.14	306.39 ± 44.26	0.511	306.11 ± 46.20	265.76 ± 41.21	0.517
ACE (μg/L)	193.81 ± 30.76	180.45 ± 24.95	0.750	220.56 ± 32.00	155.08 ± 25.46	0.114
LDH (μg/L)	303.68 ± 17.66	395.94 ± 26.25	**0.005**	298.68 ± 17.14	397.59 ± 25.65	**0.002**
%FEV$_1$ ($\bar{x}$ ± s)	91.60 ± 1.25	91.31 ± 1.42	0.482	92.42 ± 1.53	90.53 ± 1.09	0.092
%FVC ($\bar{x}$ ± s)	90.93 ± 1.86	91.14 ± 1.80	0.800	91.94 ± 1.93	90.16 ± 1.71	0.821
FEV$_1$/FVC ($\bar{x}$ ± s)	99.70 ± 2.09	101.49 ± 1.87	0.320	99.61 ± 2.03	101.55 ± 1.95	0.762

concentration with early indicators of health effect, the mixed effect of the multiple metal elements encountered in the indium smelting process should be considered. One solution to this issue that was used in our approach is the simultaneous analysis of three exposure metrics (i.e., airborne, serum and urinary indium), which were important in detecting the associations. Strong interrelationships among these exposure metrics could reduce the bias in the observed associations of these indium exposure metrics with SP-A and LDH. An appropriate study population, wide variation in exposure concentrations, the consistent association of SP-A and LDH with the three exposure metrics (airborne, serum, and urinary indium), and the significant interrelationship between SP-A and LDH are the main strengths of this study. As this is the first systematic investigation of indium- and indium compound-exposed workers in China, our results are meaningful as an initial step in the identification and prevention of indium-induced lung damage.

Although this was an extensive epidemiological study aimed to determine the effects of indium exposure on serum SP-A and LDH concentrations, there were some potential limitations that warrant further discussion. First, we only assessed serum SP-A and LDH concentrations but not their activity levels, and the expression and activity levels of these proteins are not always correlated. To assess the effects of other variables in the observed associations of indium exposure with serum SP-A and LDH, adjustments were made for covariates (age, sex, and smoking and drinking habits), and the results demonstrated that indium exposure was the main contributor to the increased serum concentrations of SP-A and LDH. However, the study subjects might have been exposed to other metals that could have influenced the observed associations. Finally, as the study design was cross-sectional, a cohort-based study is needed to establish the cause-effect relationship between indium exposure and serum SP-A and LDH levels.

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