

**Research Article** 

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# Multivariate Analysis of Trace Elements Distribution in Hair of Pleural Plaques Patients and Health Group in a Rural Area from China

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### Abstract

**Background:** Little is known about trace metal distribution in hair of pleural plaques patients. Metals might promote the incidence of asbestos-related diseases. Therefore, the selected metals in hair of pleural plaques patients and health group from a rural area is investigated in this study.

**Results:** The results indicated that mean concentrations of the metals in hair usually varied between pleural plaques patients and health group. Correlation analysis showed that strong positive correlations were found between Mg-Mn, Mg-Sr, Cd-Mn, Cd-Pb in hair of pleural plaques patients, while the correlations were not obvious in the hair of health groups. Moreover, cluster analysis reveals that Mg and Sr are all strong positive correlation in both male and female.

**Conclusions:** It can be concluded that significantly different patterns of metal distribution in the hair of pleural plaques patients in comparison with health group. More evidences were needed to confirm that the metals are associated with pleural plaques.

Keywords: Pleural plaques; Metals; Hair; Cluster analysis; Natural occurring asbestos

### Introduction

In recent decades, more and more attentions have been paid to investigate concentrations of trace elements in human hair. Abundant investigations have used traces elements in human hair as an indicator of the nutritional and health status of a population [1-4]. Moreover, elements in hair have also been reported to be a reliable and convenient indicator of environmental adaption, and environmental pollution [5-7]. These studies indicated that higher levels of Cu, Pb, Zn, and many other elements in human hair were found in the areas contaminated by heavy metals compared with the other areas [8-11].

Some elements and their compounds are essential to human health, namely essential elements. They act as catalytic or structural components of larger molecules such as enzymes, vitamins and proteins. However, deficiencies or excesses of essential elements such as Ca, Zn, Se, and Cu in human body may induce diseases [12,13]. Other elements such as As, Pb, and Cd may be harmful to human health, namely toxic element. The elements have no known beneficial biological function. In human body, dynamic balance among various trace elements is responsible for many metabolic and physiological processes. Disorder of the element balance may induce many ailments [14,15]. For instance, toxic heavy metals can affect chemical synaptic transmission in the brain and the peripheral central nervous system [16]. Therefore, trace element in human hair has been considered as a tool for routine clinical screening and diagnosis of many diseases [13]. In recent years, many investigations have been conducted to determined contents of trace elements in hair of patients, such as malignant and benign breast lesions, autism, obesity and diabetes, myocardial infarction patients, benign tumor patients, hypertensive patients, gastrointestinal cancer patients, prostate cancer patients, cerebral palsy patients, respiratory system diseases patients [16-24].

However, trace elements in hair of Pleural Plaques Patients (PPPs) have not been reported at present. Pleural plaques are the most common manifestation of inhalation of asbestos fibers [25,26]. Asbestos usually contains relatively higher concentrations of trace metals such as Ni,

Cr, Mg, and Fe [27,28]. Human may also inhale abundant trace metals associated with asbestos fibers. Evidence has been presented in support of that in the induction of asbestos-related diseases; trace metals play an active role. Asbestos fibers play a passive role as a metal carrier [27]. Concentrations of trace metals in hair may vary widely among pleural plaques patients and healthy group.

Therefore, the aim of the present study is to investigate the relationships of selected trace metals in human hair between pleural plaques patients and healthy group. Multivariate analysis is also employed to investigate interrelationships of trace metals.

### Methods

### Sampling

124 subjects from Dayao County in Yunnan, NW China, were selected for hair sampling. The person who suffered from cancer, diabetes or other serious illnesses was excluded. All of the subjects were living in local area and were ethnic Han. Dietary habits were similar among the peoples. Most of foods (including rice, vegetables, meat, etc.) were produced in local. In Dayao, abundant outcrop crocidolite mineral was found in soil. Higher mortality rates of asbestos-related diseases caused by environmental exposure to asbestos have been reported by several investigations [29,30]. 59 subjects were diagnosed as pleural plaques by using imaging techniques in local hospital. These

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subjects were defined as pleural plaques patients. However, the others diagnosed without pleural plaques were defined as health group in this study. The demographic information was shown in Table 1. The ages of the subjects were range from 48 to 82 years. The average age was 62.7 years.

All of the subjects agreed to participate in this study. They also understand their hair would be used for trace metal determination. Therefore, hair sample was cut and collected from the nape of the head (close to the scalp) with scissors for each subject. Each collected hair sample was put into a paper envelope and labeled for test.

### Analysis methods

The individual hair sample was washed with detergent solution and flushed with sufficient doubly-deionized water to removed exogenous matter. Then, the clean hair samples were dried in an oven for 10 hours at 65. The dried hair sample was cut into small pieces of 0.5 to 1 cm length. Approximate 0.2 g of each hair sample was weighted for digestion.

The weighted hair sample was digested with 5 ml concentrated nitric acid at room temperature overnight. Then, the sample was further digested and heated at  $100\pm5$  until no residue in the solution. The digested sample was cooled to room temperature and diluted to 25 ml with doubly-deionized water for further analysis. The blank was prepared the same way but without the hair sample. All the reagents used were of ultrahigh purity.

The concentrations of the elements, including Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Strontium (Sr), Zinc (Zn), Nickel (Ni), Manganese (Mn), And Magnesium (Mg), were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Considering the importance of quality assurance, a parallel routine check of the accuracy of quantified results was ensured through the use of Certified Reference Material (CRM, human hair powder GBW 09101 received from the National standard Sample Study Center, China). The contents of elements estimated in the CRM were found to be consistent with the values of elements reported in the CRM.

### Data analysis

In this study, correlation analysis (Pearson correlation coefficient)

and cluster analysis were used to analyze the data. Hierarchical cluster analysis was carried out with the Ward's Method, and the distance method was square Euclidean. All statistical analyses were performed by using the Microsoft Excel 2007 and SPSS V18.0 for Windows.

### Results

# Concentrations of trace metals in total pleural plaques patients and health group

The descriptive statistical distribution parameters of the selected metals concentrations in hair of pleural plaques patients and health group are represented in Table 2. Mean concentrations of Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sr, Zn and Cd are 0.73, 9.49, 34.77, 238.58, 3.92, 0.68, 3.92, 12.39, 114.12 and  $0.08\mu g/g$  in hair of PPPs, while the values are 0.73, 10.62, 31.22, 246.61, 3.88, 0.87, 5.21, 11.11, 114.45 and  $0.13\mu g/g$  in hair of health group, respectively. Mean concentrations of Fe, Mn, and Sr in hair are slightly higher in patients than in health group, while mean concentrations of Cu, Mg, Ni, Pb, and Cd in hair of patients are lower. Cr and Zn concentrations vary little among patients and health group. It can be seen that the levels of Fe, Mg, and Zn are significantly differences between pleural plaques patients and health group. Moreover, the highest concentrations of determined metals are found in hair of PPPs are usually lower.

# Concentrations of trace metals in pleural plaques patients and health group for gender

Tables 3 and 4 listed the basic statistical results of the metals concentrations in hair of male and female, respectively. In male, mean concentrations of Cu, Fe, Mn, Ni and Sr are higher in hair of PPPS than in hair of health group, while the values of Cr, Ni and Cd vary slightly among PPPs and health group. The highest contents of Fe, Mn, Sr and Zn are also much higher in hair of PPPS than in hair of health group.

For female, mean concentrations of Fe, Sr and Zn are much higher in hair of PPPs than in hair of health group, while the values of the other metals are usually lower in hair of PPPs. The highest contents of the selected metals are mainly found in the hair of health group except for Sr. Moreover, the metal contents in hair of PPPs and health group are usually higher in female than in male, except for Cr, Cd, and Zn.

ltam	Pleural pla	aques patients	He	Tatal	
nem	Male	Female	Male	Female	I OLAI
Number	14	24	33	53	124
Mean age	63.9	65.6	60	62.8	62.7
Age range	48-80	48-81	48-77	48-82	48-82

	7				1				
Element Cr Cu Fe Mg Mn Ni Pb Sr Zn Cd		Pleural pla	aques patients	Health group					
	Mean	Median	SD	Range	Mean	Median	SD	Range	
Cr	0.73	0.73	0.11	0.52-0.94	0.73	0.74	0.14	0.37-1.1	
Cu	9.49	8.51	3.29	6.29-23.9	10.62	8.96	8.98	4.79-87.64	
Fe	34.77	21.26	34.24	9.47-166.99	31.22	21.73	27.38	7.82-191.33	
Mg	238.58	193.46	160.71	45.15-708.58	246.61	198.77	145.59	44.49-863.42	
Mn	3.92	2.01	4.9	0.13-25.17	3.88	2.32	4.96	0.28-31.38	
Ni	0.68	0.53	0.59	0.12-2.46	0.87	0.49	2.62	0.11-24.49	
Pb	3.92	3.22	3.15	0-17.22	5.21	3.34	10.86	0.28-99.02	
Sr	12.39	7.62	16.3	2.34-93.36	11.11	8.46	8.86	2.34-53.94	
Zn	114.12	101.25	36.14	40.74-188.08	114.45	114.65	39.22	42.28-257.57	
Cd	0.08	0.05	0.07	0.01-0.33	0.13	0.06	0.26	0.01-1.74	

Table 1: Characteristics of the subjects.

Table 2: Descriptive statistical data of metal concentrations in human hair (µg/g).

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### **Correlation analysis**

In order to examine the inter-relationships between metals, the correlation coefficients between determined elements in hair of PPPs and health group for both male and female are represented in Tables 5 and 6. In the hair of male with pleural plaques, strong positive

correlations are observed between Cr and Fe, Mg and Mn, Mg and Sr, Cd and Mn, Cd and Pb, while negative correlations are found between Pb and Zn, Cd and Zn (Table 5). In comparison, strong positive correlations are found for Cu and Cr, Cr and Fe, Cr and Zn, Cu and Fe, Cu and Mn, Cu and Pb, Cu and Cd, Fe and Mn, Fe and Pb, Fe and Cd,

Element		Pleural p	plaques patients		Health group					
Element	Mean	Median	SD	Range	Mean	Median	SD	Minimum		
Cr	0.74	0.71	0.13	0.58-0.94	0.75	0.74	0.14	0.37-1.10		
Cu	9	8.66	1.23	6.87-11.45	8.64	8.24	1.74	4.79-13.29		
Fe	26.02	16.15	23.98	11.27-102.07	20.44	17.68	11.67	8.48-70.00		
Mg	200.03	186.23	106.49	53.44-387.58	202.83	189.45	77.49	95.45-405.49		
Mn	3.96	1.31	6.89	0.13-25.17	1.79	1.23	2.22	0.28-12.93		
Ni	0.68	0.43	0.61	0.13-1.83	0.65	0.36	0.66	0.11-3.72		
Pb	2.92	2.81	1.97	0.22-8.04	5.42	2	16.98	0.28-99.02		
Sr	6.93	6.33	3.97	2.59-17.31	6.77	6.75	2.79	2.72-14.70		
Zn	137.42	147.9	34	80.07-188.08	141.19	143.83	18.73	104.52-176		
Cd	0.08	0.05	0.08	0.01-0.33	0.09	0.03	0.24	0.01-1.37		

Table 3: Descriptive statistical data of metal concentrations in human hair for male (µg/g).

Floment		Pleural plaqu	ues patients		Health group					
Liement	Mean	Median	SD	Range	Mean	Median	SD	Range		
Cr	0.72	0.74	0.1	0.52-0.87	0.72	0.74	0.13	0.45-1.05		
Cu	9.77	8.26	4.04	6.29-23.9	11.85	9.47	11.23	6.3-87.64		
Fe	39.87	23.64	38.56	9.47-166.99	37.93	27.58	31.97	7.82-191.33		
Mg	261.07	193.46	183.6	45.15-708.58	273.87	241.29	170.24	44.49-863.42		
Mn	3.89	3.02	3.44	0.37-11.53	5.19	3.51	5.72	0.38-31.38		
Ni	0.68	0.58	0.59	0.12-2.46	1.01	0.5	3.3	0.11-24.49		
Pb	4.51	3.39	3.57	0-17.22	5.07	3.72	3.89	0.67-20.97		
Sr	15.57	8.63	19.75	2.34-93.36	13.81	11.37	10.2	2.34-53.94		
Zn	100.52	92.84	30.4	40.74-158.95	97.81	87.25	39.53	42.28-257.57		
Cd	0.08	0.05	0.06	0.01-0.29	0.16	0.09	0.27	0.01-1.74		

Table 4: Descriptive statistical data of metal concentrations in human hair for female (µg/g).

	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn	Cd
				Pleu	ral plaques pati	ents				
Cr	1									
Cu	0.319	1								
Fe	0.578*	0.333	1							
Mg	-0.018	0.355	0.443	1						
Mn	0.048	0.046	0.457	0.713**	1					
Ni	0.008	-0.144	-0.164	-0.495	-0.122	1				
Pb	-0.237	-0.095	-0.094	0.033	0.522	0.206	1			
Sr	-0.217	0.106	0.151	0.817**	0.459	-0.306	-0.2	1		
Zn	0.406	0.352	0.139	-0.15	-0.484	-0.337	-0.577 <sup>*</sup>	-0.322	1	
Cd	-0.148	-0.119	0.203	0.34	0.826**	0.122	0.826**	0.119	-0.702**	1
					Health group					
	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn	Cd
Cr	1									
Cu	0.370 <sup>*</sup>	1								
Fe	0.348*	0.549**	1							
Mg	0.124	-0.113	-0.069	1						
Mn	0.222	0.597**	0.775**	-0.097	1					
Ni	-0.285	-0.287	0.013	0.065	0.027	1				
Pb	0.159	0.536**	0.752**	-0.126	0.913 <sup>⊷</sup>	0.045	1			
Sr	0.071	0.022	0.109	0.850**	0.152	0.228	0.076	1		
Zn	0.401 <sup>*</sup>	0.118	0.162	0.580**	-0.134	-0.208	-0.078	0.421 <sup>*</sup>	1	
Cd	0.147	0.514**	0.762**	-0.11	0.945**	0.075	0.986**	0.124	-0.095	1

\* Significant value: P < 0.05. \*\* Significant value: P < 0.01.

Table 5: Correlation coefficients between metals in hair of PPPs and health group for male.

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	0-	0	<b>F</b> -	N4-	N4	NI:	Dh	0.7	7	04
	Cr	Cu	Fe	Mg	IVIN	NI	PD	Sr	۷n	Ca
	1	1	1	Pleu	ral plaques pati	ents	1	1		
Cr	1									
Cu	0.377	1								
Fe	0.339	0.393	1							
Mg	0.448*	0.13	0.189	1						
Mn	0.487*	0.229	0.257	0.727**	1					
Ni	0.28	0.158	0.244	-0.06	-0.127	1				
Pb	0.134	0.618**	0.326	0.097	0.351	0.038	1			
Sr	0.195	0.154	0.16	0.661**	0.462*	-0.07	-0.014	1		
Zn	-0.095	-0.32	-0.488 <sup>*</sup>	0.017	-0.331	-0.163	-0.325	-0.164	1	
Cd	0.036	0.315	0.336	0.315	0.558**	-0.015	0.769**	0.206	-0.440*	1
					Health group					
	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn	Cd
Cr	1									
Cu	0.019	1								
Fe	0.004	0.236	1							
Mg	0.279 <sup>*</sup>	-0.126	0.187	1						
Mn	0.024	0.017	0.333*	0.136	1					
Ni	0.017	0.135	0.111	-0.096	-0.045	1				
Pb	0.167	0.119	0.245	-0.108	0.366**	-0.025	1			
Sr	0.164	-0.027	0.115	0.612**	0.109	-0.115	-0.174	1		
Zn	0.106	-0.117	-0.268	0.422**	-0.316*	-0.088	-0.258	0.349*	1	
Cd	0.185	-0.035	0.053	0.067	0.318*	-0.075	0.433**	0	-0.014	1

\* Significant value: P<0.05. \*\* Significant value: P<0.01.

Table 6: Correlation coefficients between metals in hair of PPPs and health group for female.

Mg and Sr, Mg and Zn, Mn and Pb, Mn and Zn, Pb and Cd, Sr and Zn in hair of health group for male.

For female, significant positive correlations are observed between Cr-Mg, Cr-Mn, Cu-Pb, Mg-Mn, Mg-Sr, Mn-Sr, Mn-Cd, Pb-Cd in hair of PPPs, while negative correlations are found for Fe-Zn, Zn-Cd. In hair of health group, significant positive correlations are found for Fe-Mn, Mg-Sr, Mg-Zn, Pb-Zn, Sr-Zn (Table 6).

## **Cluster analysis**

Cluster analysis (CA) is employed to apportion the determined metals. The results are represented in Figures 1 and 2. For male, the sample number is less than 20. Thus, cluster analysis cannot be used to analyze the relationships between the metals in hair of PPPs for male. However, based on correlation analysis, it can be found that Mg-Sr, Mn-Cd-Pb-Fe-Ni, and Cr-Cu-Zn are significant correlation in hair of PPPs for male. The clustering of the metals in hair of health group for male is represented in Fig.1. Four main clusters of the metals are found in hair of health group for male. The first cluster includes Cr-Zn-Cu-Mg-Sr-Fe. The second cluster contains Pb-Cd, while the third and the fourth cluster include Ni and Mn, respectively.

For female, three strong clusters of the determined metals are found in hair of PPPs. The first cluster includes Mg, Sr and Mn. Another strong positive cluster comprises Pb, Cd, Fe and Ni. The third cluster contains Cu and Zn. However, the cluster analysis reflects different clustering of determined metals in hair of health group. The results show that a strong positive cluster includes Cr, Mg, Zn and Sr. Another positive cluster contains Fe, Pb, Mn and Cu. The third cluster includes Ni.

### Discussions

In the study area, air and water have been significantly contaminated by asbestos fiber. Abundant trace metals are also found in asbestos and drinking water. Moreover, the asbestos contain abundant metals [31,32]. Pleural plaque is caused by exposure to asbestos. The local residents are continuous exposure to air and drinking water contaminated by asbestos fibers and metals. We have reported that Al, Fe, Zn, Mg, and Na might be accumulated in human hair derived from contamination of crocidolite asbestos fibers in the study area [33]. Therefore, metals distributions in hair may be different between pleural plaque patients and health person.

This study represents data related to the metal distribution in hair in terms of parameters such as spread around mean metal concentrations, possible correlations, and comparative evaluation of the two groups.

Tables 2 present basic distribution parameters of the determined metals in hair samples of pleural plaque patients and health person, respectively. Varied levels of the metals, particularly Fe, Mg, and Zn are found the two groups. Tables 3-4 listed the statistic data of the metals in hair of the two groups for male and female. The mean concentrations of Fe, Mn and Sr are also all higher in hair of PPPs than in hair of health group for both male and female. The highest concentration of Sr was also found in hair of PPPs. The two groups are all exposure to asbestos in air and drinking water, but concentrations of the determined metals are usually varied between then. Therefore, these results reflect that the metal concentrations of the metals are significantly different in hair among PPPs and heath group. Fe, Mn and Sr may be associated with plaque pleural.

Tables 5-6 summarize the correlation data between metals in hair of PPPs and health group for male and female. Strong positive correlations observed between Cr and Fe, Mg and Mn, Mg and Sr, Cd and Mn, Cd and Pb for male indicate that the inter-relationships between metals in hair of male are significantly different between PPPs and health group. However, Ni has no obvious relationship with other metals for both PPPs and health group, indicating its independent in hair of the population. For female significant positive correlations





observed between Cr-Mg, Cr-Mn, Cu-Pb, Mg-Mn, Mg-Sr, Mn-Sr, Mn-Cd, Pb-Cd in hair of PPPs also show that the inter-relationships between metals in hair of female are significantly different between PPPs and health group. Moreover, strong positive correlations are found between Mg and Mn, Mg and Sr, Cd and Mn, Cd and Pb in hair of PPPs for both male and female, while the relations are not obvious in the hair of health groups. This indicates that the inter-relationships of these metals may be associated with pleural plaques. Significant negative correlations are found between Pb and Zn, Cd and Zn in hair of PPPs for both male and female, indicating negative inter-relationships of the metals associated with pleural plaques.

In hair of PPPs, the results of cluster analysis are similar between male and female. Mg and Sr are all strong positive correlation in both male and female. In contrast, they are correlated with other metals in hair of health group for both male and female. This indicates that Mg and Sr may be associated with pleural plaques.

Many studies have been conducted to investigate trace metals distributions in patients and health donors [13,16,18-20,24]. Mean concentrations of Ca, Cd, Co, K, Mg, Mn, Na, Ni, Pb, Sb, Sr and Zn are significantly higher in the hair of benign tumour patients than normal donors. The results of correlation analysis, principal component

analysis and cluster analysis evidenced significantly different patterns of metal distribution in hair of benign tumour patients in comparison with normal donors [17,21]. In human serum, a significant difference in concentrations of all metals between breast cancer patients and controls are observed [34]. Therefore, the results of this study are important for investigating metals distributions in PPPs.

Metals play a vital role in diseases of unknown etiology [22]. Several researches have investigated the metals such as cadmium, lead, zinc, and copper as indicator of hypertensive status using hair as a biopsy material [35-37]. In the present study, the results may be concluded that significantly different patterns of metal distribution in the hair of pleural plaques patients in comparison with health group. Mg and Sr may be significantly associated with pleural plaques. However, the results in this paper are insufficient to indicate that the metal distributions in hair can be used as an indicator of pleural plaques.

### Conclusions

According the results of this research, the mean concentrations of the metals vary slightly in hair between PPPs and health group for both male and female, except for levels of Fe, Mg, and Zn. The results of correlation analysis shows that strong positive correlations are found between Mg and Mn, Mg and Sr, Cd and Mn, Cd and Pb in hair of PPPs for both male and female, while the relations are not obvious in the hair of health groups. Moreover, the cluster analysis evidenced significantly different apportionment of the determined metals in hair of PPPs and health group for both male and female. These results may be concluded that significantly different patterns of metal distribution in the hair of pleural plaques patients in comparison with health group. Mg and Sr may be significantly associated with pleural plaques. However, the results in this paper are insufficient to indicate that the metal distributions in hair can be used as an indicator of pleural plaques. Therefore, more evidences are needed to confirm that the metals are associated with pleural plaques.

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