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Biological monitoring of lead and cadmium in human hair and nail and their correlations with biopsy materials, age and exposure

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Hair and fingernails of exposed and unexposed subjects were analyzed for their lead (Pb) and cadmium (Cd) contents by atomic absorption spectrophotometer with graphite furnace and air-acetylene flame. Hair and nail Pb concentrations in occupationally exposed subjects ranged between 1.020-409.726 and 8.130-765.306 µg/g and in environmentally unexposed subjects 0.123-25.160 and 1.076-65.613 µg/g, respectively. Similarly, hair and nail Cd concentrations in occupationally exposed subjects ranged between 0.014-22.086 and 0.214-35.714 µg/g and in environmentally unexposed subjects 0.113-1.627 and 0.028-8.108 µg/g, respectively. A significant correlation was observed between Pb hair and nail concentrations in exposed subjects at $P<0.05$, as compared to unexposed subjects and Cd hair and nail in exposed, as well as unexposed subjects. With respect to exposure, levels of Pb in hair and nails were found to be significant in exposed subjects, compared to unexposed ones and levels of Cd were significant only in nails of exposed ones. With respect to age, no significant correlation was found between hair and nail Pb and Cd concentrations in both exposed and unexposed subjects.

Keywords: Metal body burden, trace/toxic elements, Pb and Cd in hair/nail, biomonitoring

Trace elements, even at concentration less than 0.01% of body weight play an important role in the metabolism of living organisms[1]. Urine, hair, nail, teeth and blood have been used as indicators for the estimation of metal levels. Metal body burden of trace/toxic elements is better reflected from trace element contents in hair and nail, than those in the blood, because hair and nail give record of relatively long periods, while blood shows transient levels that change with time. Furthermore, hair and nails are advantageous as they are inert and easier to sample than blood or teeth and can be stored without much technicality. Also, trace metal concentrations in these tissues are reported to be high in earlier studies[2-6]. Determining trace/toxic elements in human hair and nails has importance in biological, medical and environmental studies.

Lead is used in smelting and soldering works, painting, manufacture of batteries and motor fuels and is a potential neurotoxin. Its exposure may lead to inhibition of haeme synthesis resulting in anaemia, kidney damage, cerebral oedema, gastro-intestinal, respiratory disorders and nephropathy[7-10]. Cadmium, a highly toxic metal used as antifriction agent in batteries accumulate in the kidney and binds predominantly to metallothionein protein. Its ingestion or inhalation may cause nausea, abdominal cramps, short breath, chocking fits, renal dysfunction and inhibition of iron absorption. Catarrhal and ulcerative gastro-enteritis, congestion, pulmonary infarcts and subdural hemorrhages may be found at necropsy[11-15]. Earlier, we reported studies on Cr, Mn, Ni, Fe, Zn, Ca and Mg[3,4,7,10,16]. In view of the potential of hair and nail for assessing occupational exposure to metals, we determined Pb and Cd concentrations in these tissues in male subjects (18-60 years of age) of roadways workshop, locomotive workshop, and Pb-Cd battery units to examine their differential levels in occupationally exposed individuals and controls. The study was also aimed at investigating whether elevated Pb and Cd concentrations in hair and nail may be used as indicators of occupational exposure to these metals. A possibility of any correlation between Pb and Cd hair and nail concentrations has also been explored.

Material and Methods

Hair samples (approx. 4-5 cm in length) were collected from the nape of scalp by cutting approximately 2 mm from scalp using a pair of sterilized scissors washed with ethanol[17], from male subjects working in roadways workshop, locomotive workshop and Pb-Cd battery units, exposed to Pb and Cd in their work environment, in particular. The age of subjects range from 18-60 years. For collection of nail samples, volunteers were asked to wash their hands thoroughly with double distilled water and medicated soap, followed by drying with a clean towel or tissue paper to remove external contamination, if any. Nails were cut from fingers with stainless

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steel scissors as used in hair. All hair and nail samples were kept in separate airtight plastic bags prior to treatment and analysis. A questionnaire was got filled up for obtaining the personal and medical history of the subjects as per the recommendations of Word Health Organisation[18].

Hair samples were cut into pieces of about 1 cm. Hair and nail samples pre-washed with non-ionic detergent, triton X-100, were soaked in deionized water for 10 min, followed by soaking in acetone and washing with deionized water. Then they were dried in an oven at 110ºC for 1 hr and kept in a desiccator for subsequent analysis[19,20]. The dried samples of both were digested with 10 ml 6:1 mixture of conc. HNO_3 and conc. $HClO_4$, kept overnight at room temperature and heated at 160-180°C until complete evaporation, to obtain a crystalline white dry deposit or a water clear solution. It was then diluted with 0.1 *N* HNO_3.

The concentration of metals was determined using Perkin-Elmer AAS model-250 with graphite furnace and air-acetylene flame. A series of standards were prepared in deionized water for instrumental calibration by diluting commercial standards containing 1000 ppm of metals. All reagents were of analytical grade (Merck, Germany). A number of blanks were also prepared. The main instrumental parameters for the estimation of Cd and Pb by atomic absorption spectrophotometer were as follows, respectively: wavelength, 228.8, 217.0 nm; bandwidth, 0.7, 1.0 nm; and lamp current, 0.25, 5 mA.

Pb and Cd levels in hair and nails were expressed as arithmetic mean in μg/g ± SD and tabulated to illustrate concentration profile over each group. The statistical significance of mean values between different groups were determined by applying student '*t*' test and level of significance was set at $P < 0.05$. Regression analysis was carried out to obtain the correlation of Pb and Cd concentrations with biopsy material (hair and nail) and age. For this, the Pb and Cd concentrations obtained from elemental analysis of hair and nail samples of individual subjects of exposed and unexposed categories were subjected to statistical analysis to obtain the regression line graph to which straight line equation was applied to obtain square of correlation coefficient. The number of exposed and unexposed subjects taken for correlation study was 37 and 31, respectively.

Results and Discussion

Lead and cadmium concentrations in hair and nails of exposed and unexposed subjects after appropriate statistical treatment are given in Table 1. Wide variations were observed in their concentrations in this study, as also reported in other studies[21,22]. Such variations are expected, since the work experience is not always a function of age, particularly in case of

Table 1—Statistical significance of Pb and Cd concentrations with respect to exposure and biopsy material

Metal	Subjects	Mean±SD (μg/g)	Range (μg/g)	*t*-test at $P< 0.05$
With respect to exposure				
Pb (H)	Exposed	52.68±96.66	1.02-409.72	Significant
	Unexposed	8.28±6.87	0.12-25.16	
Cd (H)	Exposed	1.30±3.59	0.01-22.09	Non-significant
	Unexposed	0.42±0.33	0.11-1.63	
Pb (N)	Exposed	207.20±195.31	8.13-765.30	Significant
	Unexposed	30.38±17.07	1.08-65.61	
Cd (N)	Exposed	7.71±6.74	0.21-35.71	Significant
	Unexposed	2.23±2.17	0.03-8.11	
With respect to biopsy material				
Pb (N)	Exposed	207.20±195.31	8.13-765.30	Significant
Pb (H)		52.68±96.66	1.02-409.72	
Pb (N)	Unexposed	30.39±17.08	1.08-65.61	Significant
Pb (H)		8.28±6.87	0.12-25.16	
Cd (N)	Exposed	7.72±6.75	0.21-35.71	Significant
Cd (H)		1.30±3.59	0.01-22.08	
Cd (N)	Unexposed	2.24±2.17	0.03-8.11	Significant
Cd (H)		0.42±0.33	0.11-1.63	

The number of subjects were: exposed, 37; and unexposed, 31
H and N in parenthesis represent hair and nail, respectively

Table 2—Regression analysis data for Pb and Cd levels with biopsy materials/age

Correlation between	R^2	Y
Pb (H) and Pb (N) in exposed subjects	0.1289	0.7254x+168.99
Pb (H) and Pb (N) in unexposed subjects	0.0007	-0.0634x+30.913
Cd (H) and Cd (N) in exposed subjects	0.0002	-0.0093x+7.7297
Cd (H) and Cd (N) in unexposed subjects	0.0003	0.1218x+2.187
Pb (H) exposed and age	0.0004	-0.1954x+60.29
Pb (N) exposed and age	0.0732	-5.1194x+406.45
Pb (H) unexposed and age	0.0147	-0.059x+10.337
Pb (N) unexposed and age	0.0525	-0.277x+40.029
Cd (H) exposed and age	0.0063	-0.027x+2.3781
Cd (N) exposed and age	0.003	0.0113x+8.1593
Cd (H) unexposed and age	0.0131	0.0027x+0.3291
Cd (N) unexposed and age	1×10^{-7}	-5×10^{-5}+2.2402

R^2 represent square of correlation coefficient
Y represent straight line equation
H and N in parenthesis represent hair and nail, respectively

workers employed at an early age and continuously exposed to metals for a longer period, as compared to those employed at higher age.

Pb concentrations were relatively higher in hair and nails than Cd. However, among biopsy materials Pb and Cd levels were higher in nails, compared to hair; the higher Pb levels may be attributed to place of residence near heavy traffic areas and through Pb aerosol deposition on vegetables and fruits, besides occupational exposure. The difference between hair and nail metal concentrations revealed significantly high levels in nails at $P<0.05$ in exposed and unexposed subjects (Table 1). Pb levels were significant in both hair and nails of exposed subjects, but Cd levels were significant only in nails of exposed subjects. Significant Pb and Cd concentrations in hair and nails of exposed subjects, compared to unexposed ones have also been reported earlier [23,24].

The correlation of Pb and Cd in hair and nail with age was done by regression line analysis and the results are shown in Table 2. Significant correlation was observed in hair and nail Pb levels in exposed subjects, while no correlation was observed in case of unexposed ones. Earlier, significant correlation was reported between Cd blood and Cd hair levels in exposed, as compared to unexposed subjects[25]. In the present study, no correlation was observed in hair and nail Cd levels of exposed and unexposed subjects. With respect to age, nail Pb concentration in exposed and unexposed subjects exhibited higher correlation as shown by R^2 values. Significant correlation between Pb levels of hair and blood has been reported earlier in occupationally exposed workers[26,27], and also in environmentally exposed adults[28]. Such correlations are due to constant levels of the metals in the biological fluid/tissue, during longer period of exposure with intake and excretion in a steady pattern. When these conditions are not met, then it becomes difficult to evaluate such relationships. In an earlier study, a higher content of metals viz., Pb, Ni, Co, Mn and Cd was reported during 31-80 years interval, as compared to 1-30 years[29].

It can be concluded that hair and nail Pb were correlated to each other and hair concentrations of Pb are useful biological indicator for monitoring exposure to Pb in the environment — general or occupational. However, care is needed to take into account the factors influencing the incorporation of heavy metals in hair. There are many intervening events and factors, such as hair cosmetics, air-borne reagents and nutritional status that could have an effect on this correlation between hair and nail Pb in exposed subjects. Biomonitoring of metals using hair and nail can be used for assessing the exposure to metals in work environment.

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