

A COMPARATIVE STUDY BASED ON GENDER AND AGE DEPENDENCE OF SELECTED METALS IN SCALP HAIR

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(Received 29 January 2004; accepted 16 April 2004)

Abstract. Levels of 10 metals (Ca, Mg, Fe, Zn, Cu, Mn, Cd, Co, Cr and Ni) in the scalp hair of male and female donors, with age groups between 3 and 100 years, were determined by ICP-AES. In total, 58 male and 30 female hair samples were analyzed in triplicate. The donors belonged to the rural area of district Chakwal, a well-known typical non-urban site in Pakistan. Calcium showed the highest concentration of 462 $\mu\text{g/g}$ in the hair of males and 870 $\mu\text{g/g}$ in those of females followed by Zn, at 208 and 251 $\mu\text{g/g}$ for the two sexes. For male donors, Cd showed the lowest concentration (1.15 $\mu\text{g/g}$) while for female donors Co remained at minimum level (0.92 $\mu\text{g/g}$). The order of decreasing metal concentration in the hair of male donors was: Ca > Zn > Mg > Fe > Cu > Mn > Ni > Cr > Co > Cd while that for female donors it was: Ca > Zn > Mg > Fe > Cu > Mn > Cr > Ni > Cd > Co. The female group exhibited enhanced levels of all selected metals except Fe and Co in their hair as compared with the male counterparts. A strong bivariate positive correlation was found between Fe and Zn ($r = 0.841$) for the hair samples from male category while for the female category, strong positive correlations were observed between Ca–Mg ($r = 0.617$), Ca–Zn ($r = 0.569$), Ca–Mn ($r = 0.565$), Mg–Mn ($r = 0.655$), Cr–Cu ($r = 0.655$) and Cr–Ni ($r = 0.685$). The distribution of metals in the hair of donors with respect to different age groups was also investigated for both genders. The study showed that in case of males, the concentration of all selected metals decreased with increasing age except for Cu, Co and Cr. However, for females the hair metal levels increased with age, except for Co for which the concentration decreased with age. No appreciable change in the metal concentration was observed as a function of age for the combined sexes.

Keywords: age and sex dependence, hair analysis, heavy metals in scalp hair, Pakistan

1. Introduction

Recently a great deal of interest has been developed in research aimed at exploring occupational, clinical, toxicological and environmental exposure of trace metals along with their impact on human health (Vishwanathan *et al.*, 2002; Iyengar and Rapp, 2001; Hoffmann *et al.*, 2000; MacPherson and Bacso, 2000; Seifert *et al.*, 2000; Ashraf *et al.*, 1995a). To this effect, many researchers have demonstrated the usefulness of human scalp hair as an indicator of the distribution and exposure of trace metal metabolism in human body (Dombovari and Papp, 1998; Ashraf and Jaffar, 1996). Hair is an acceptable indicator for such studies as it is readily obtained, easily collected and stored and reflects a long term exposure of individuals to the

metals, providing possible correlation with a number of factors related to genetical, nutritional and geographical origin (Hintz, 2001; Carrington and Bolger, 2000; Ashraf and Jaffar, 1997).

It is recognized that metal distribution in human hair has marked dependence on age and gender (Barbosa *et al.*, 2001; Ashraf *et al.*, 1994a), and hair related environmental studies can, therefore, be used to differentiate between the health status of various segments of the population (Ashraf *et al.*, 1994b). For such studies, the need for a comparative background level of metals representing a relatively neat and clean environment is rather imperative. Such a comparison becomes still more important in the wake of recent fast urbanization and industrialization in developing countries, like Pakistan.

The changing nutritional requirements and metabolic processes associated with the aging process of the two human genders are responsible for differences in the levels of trace metals in their hair (Rivai, 2001). Dietary habits and environmental exposure may also be responsible for variations in the hair trace metal levels for male and female donors from specific geographical locations (Wilhelm *et al.*, 2002; Ashraf *et al.*, 1995b). The present study was conducted to assess the levels of ten selected metals (Ca, Mg, Fe, Zn, Cu, Mn, Cd, Co, Cr and Ni) in the scalp hair of a segment of population of district Chakwal, Pakistan, which represents a typical rural population. The principal objective was to evolve a baseline trace metal data to act as a background feedback for detailed futuristic studies on the role of a changing urban environment and its impacts on human health in rural sites. The study also envisages determination of a possible correlation between age and gender of the donor population through viable statistical tests to examine if the trace metal distribution in the two genders is different and age dependant (Chatt and Katz, 1988, Paschal *et al.*, 1989). The ICP-AES method was used for the estimation of selected metals using the HNO_3 / HClO_4 based wet digestion method.

2. Materials and Methods

Eighty eight human scalp hair samples, both from male and female donors, with ages from 3 to 100 years, were collected from the remotely located rural area of district Chakwal, Pakistan. The samples were collected from donor residents of a village with no industrial activities around within a radius of 60 km. The hair samples, cut from the nap of the neck close to scalp, as strands 3–5 cm long, with a pair of plastic scissors, were stored in zip-mouthed polythene bags (9 cm × 15 cm) and labeled with individual sample codes. Simultaneously, the details related to the donor's name, sex, his/her food habits, social behavior, education, and health status, were recorded on a separate proforma.

In order to remove any exogenous matter, the hair samples were washed prior to analysis in laboratory with 5% detergent solution. These were soaked in the solution for about 1 hour with stirring, and then rinsed with plentiful double distilled water

thoroughly (Dombovari and Papp, 1998), dried overnight at 50°C in an electric oven and subsequently cooled to room temperature in a desiccator. An accurately weighed portion (<1.0 g) of a hair sample was placed in an Erlenmeyer flask and treated with 10.0 mL of concentrated nitric acid (Suprapure, A.A. Grade 65%). The contents of the flask were then heated at 80°C for 10 min and later cooled to room temperature, followed by an addition of 5 mL of perchloric acid (70%) and heated again to a soft boil until white dense fumes were evolved marking the completion of the digestion process (Ashraf *et al.*, 1995a). A blank, without hair sample, was also prepared the same way. An inductively coupled argon plasma atomic emission spectrometer (model IRIS Thermo Jarrell Ash Co. USA) (Rodushkin and Axelsson, 2000) was used for the determination of selected metals under optimum analytical conditions. All reagents used were of ultrahigh purity (certified >99.9%) procured from E-Merck or B.D.H.

Three sub-samples for each sample were prepared the same way for digestion and these were run separately. The results, which normally agreed within $\pm 1.5\%$, are reported as average. Parallel check on the accuracy of quantified results was made through the use of SRM (RM-60, OL) obtained from National Institute of Health, Islamabad, where inter-laboratory comparison of data was also exercised. A maximum of $\pm 1.5\%$ deviation of results from the two laboratories was routinely encountered.

3. Results

Average metal contents along with some basic statistical parameters pertaining to the distribution of ten selected metals (Ca, Mg, Fe, Zn, Cu, Mn, Cd, Co, Cr, and Ni) in scalp hair of 58 male donors from the rural population of Chakwal, Pakistan are given in Table I, while the counterpart data for 30 female donors are given in Table II. Of the macronutrients (Ca and Mg), Ca showed the maximum level at 2999 and 2039 $\mu\text{g/g}$, respectively for male and female donors. For the two categories of donors, the averaged Ca concentration, again dominated at 461.7 and 869.8 $\mu\text{g/g}$, followed by that of Zn at 207.9 and 251.4 $\mu\text{g/g}$, and Mg at 113.4 and 243.4 $\mu\text{g/g}$ for hair samples from males and females. The average Fe contents of male hair samples were found at 82.66 $\mu\text{g/g}$ and for those of females at 62.82 $\mu\text{g/g}$. The order of decrease in average concentration for the metals followed the pattern: Ca > Zn > Mg > Fe > Cu > Mn and it was same for the both genders. However, the individual dispersions for the two cases varied slightly. Out of the other metals (Cd, Co, Cr, and Ni), the maximum average levels were observed for Ni at 2.374 $\mu\text{g/g}$, followed by Cr, Co and Cd respectively at 2.085, 1.247 and 1.154 $\mu\text{g/g}$ for the male donors. In comparison, for the female donors the average levels were encountered for Cr at 3.444 $\mu\text{g/g}$, Ni at 3.179 $\mu\text{g/g}$, Cd at 1.544 $\mu\text{g/g}$, and Co at 0.916 $\mu\text{g/g}$, in that order. The overall order of decrease in average concentration found in hair of male donors was thus: Ca > Zn > Mg > Fe > Cu > Mn > Ni > Cr > Co > Cd,

TABLE I
Basic statistical parameters for metal distribution in scalp hair from male donors

	Ca	Mg	Fe	Zn	Cu	Mn	Cd	Co	Cr	Ni
Minimum ($\mu\text{g/g}$)	1.630	6.820	3.290	64.370	3.680	0.011	0.012	0.020	0.014	0.001
Maximum ($\mu\text{g/g}$)	998.760	453.860	1586.780	1545.510	37.570	14.300	7.960	3.140	5.310	4.380
Mean ($\mu\text{g/g}$)	461.672	113.428	82.661	207.919	13.170	4.015	1.154	1.247	2.085	2.374
Median ($\mu\text{g/g}$)	398.740	100.790	32.505	151.575	11.015	3.226	0.344	1.475	1.800	2.055
Standard deviation	434.177	84.961	230.730	200.269	7.363	3.549	1.720	0.773	1.573	3.293
Standard error	58.019	11.253	30.296	26.297	0.967	0.466	0.226	0.101	0.207	0.432
Kurtosis	20.996	4.576	34.384	35.797	2.537	1.131	4.815	-0.945	-0.858	35.966
Skewness	3.859	1.870	5.684	5.454	1.612	1.213	2.192	0.019	0.578	5.447
Count	56	57	58	58	58	58	58	58	58	58

TABLE II
Basic statistical parameters for metal distribution in scalp hair from female donors

	Ca	Mg	Fe	Zn	Cu	Mn	Cd	Co	Cr	Ni
Minimum ($\mu\text{g/g}$)	91.940	7.950	5.559	76.710	7.500	0.360	0.003	0.034	0.173	0.019
Maximum ($\mu\text{g/g}$)	2039.190	646.120	544.340	426.920	67.430	28.040	11.438	1.987	8.730	9.560
Mean ($\mu\text{g/g}$)	869.841	243.407	62.824	251.369	24.506	6.524	1.544	0.916	3.444	3.179
Median ($\mu\text{g/g}$)	856.800	198.060	48.640	247.010	18.035	4.761	0.350	0.650	2.338	2.810
Standard deviation	461.977	178.277	93.683	91.200	16.442	5.995	2.730	0.664	2.950	2.465
Standard error	84.345	32.549	17.104	16.651	3.002	1.095	0.498	0.121	0.539	0.450
Kurtosis	0.350	0.172	26.291	-0.799	0.611	4.573	7.426	-1.501	-1.046	0.374
Skewness	0.568	0.943	4.984	0.040	1.240	1.830	2.726	0.359	0.585	0.889
Count	30	30	30	30	30	30	30	30	30	30

while for the female donors the order was: $\text{Ca} > \text{Zn} > \text{Mg} > \text{Fe} > \text{Cu} > \text{Mn} > \text{Cr} > \text{Ni} > \text{Cd} > \text{Co}$.

A linear correlation study was conducted to find any viable relationship among various metal pairs. In case of male donors (Table III), a strong positive correlation was found to exist only between Fe–Zn at $r = 0.841$, while some significant

TABLE III
Correlation coefficient matrix for selected metals in scalp hair of male donors

	Ca	Mg	Fe	Zn	Cu	Mn	Cd	Co	Cr
Mg	0.322								
Fe	-0.018	0.001							
Zn	0.035	0.265	0.841						
Cu	-0.056	0.257	-0.102	0.062					
Mn	0.056	0.382	0.221	0.218	0.322				
Cd	0.469	-0.124	-0.058	-0.172	-0.163	-0.175			
Co	-0.071	-0.345	0.072	-0.108	-0.421	-0.269	0.156		
Cr	0.069	0.137	0.158	0.281	0.218	0.249	-0.212	0.008	
Ni	-0.009	-0.142	0.046	0.071	-0.103	-0.151	0.136	0.251	0.018

Note. Values >0.219 and/or <-0.219 are significant at $p < 0.05$.

TABLE IV
Correlation coefficient matrix for selected metals in scalp hair of female donors

	Ca	Mg	Fe	Zn	Cu	Mn	Cd	Co	Cr
Mg	0.617								
Fe	0.045	0.084							
Zn	0.569	0.487	0.102						
Cu	0.199	0.087	0.101	0.423					
Mn	0.565	0.655	0.156	0.484	0.268				
Cd	0.026	-0.385	-0.164	0.009	-0.182	-0.304			
Co	-0.045	-0.261	-0.128	-0.252	-0.365	-0.183	0.243		
Cr	0.203	0.126	0.454	0.334	0.655	0.309	-0.023	-0.438	
Ni	0.028	-0.147	0.331	0.079	0.425	0.106	0.109	-0.004	0.685

Note. Values >0.307 and/or <-0.307 are significant at $p < 0.05$.

correlations were also observed for the pairs Ca–Cd ($r = 0.469$) and Cu–Co ($r = -0.421$). For the female donors (Table IV), some strong positive correlations were found between Ca and Mg ($r = 0.617$), Ca–Zn ($r = 0.569$), Ca–Mn ($r = 0.565$), Mg–Mn ($r = 0.655$), Cu–Cr ($r = 0.655$) and Cr–Ni ($r = 0.685$). In addition, significant correlations were also observed for Mg–Zn ($r = 0.487$), Fe–Cr ($r = 0.454$), Zn–Cu ($r = 0.423$), Zn–Mn ($r = 0.484$), Cu–Ni ($r = 0.425$) and Co–Cr ($r = -0.438$) metal pairs.

A comparative study based on different age groups of the two genders was also carried out (Tables V and VI, Figures 2 and 3). The donors were divided into different age groups, spanning over a period of 10 years. For male donors (Table V, Figure 2) the maximum concentrations of Ca, Mg, Fe, Zn, Cu, and Mn were encountered for the 41–50 year age group, while the toxic trace metals were

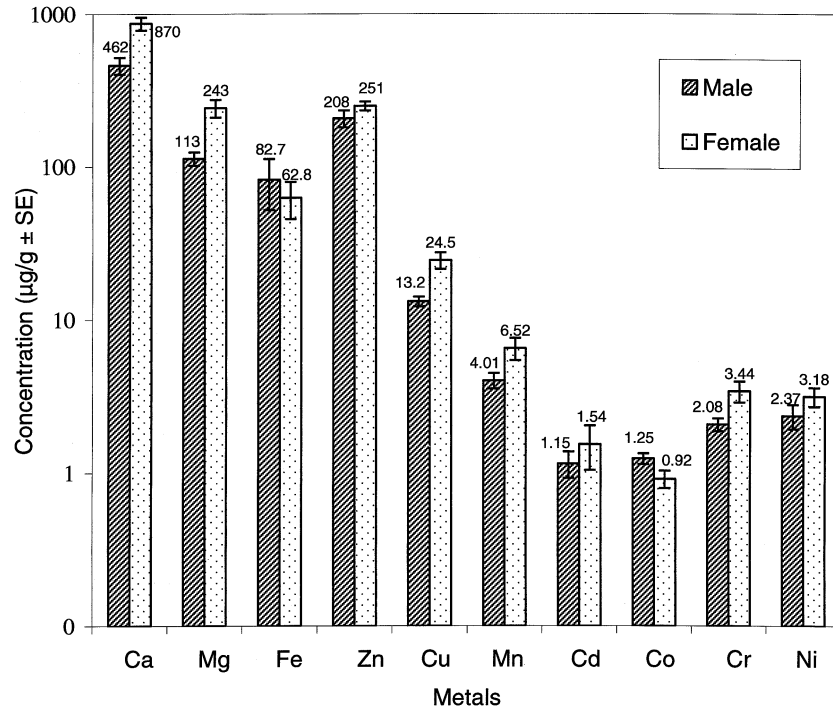


Figure 1. Average distribution of selected metals in hair of male and female donors.

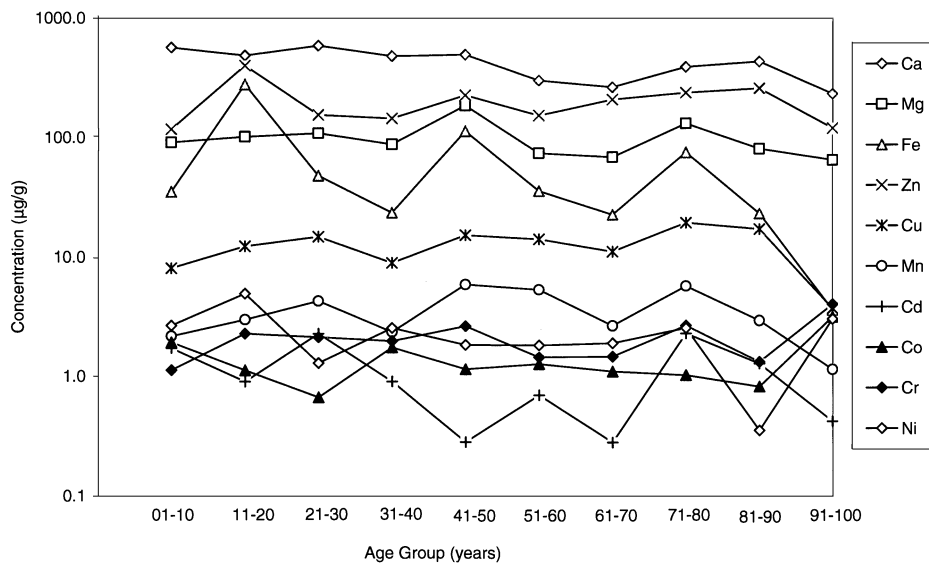


Figure 2. Dependence of selected hair metal concentration on various age groups of male donors.

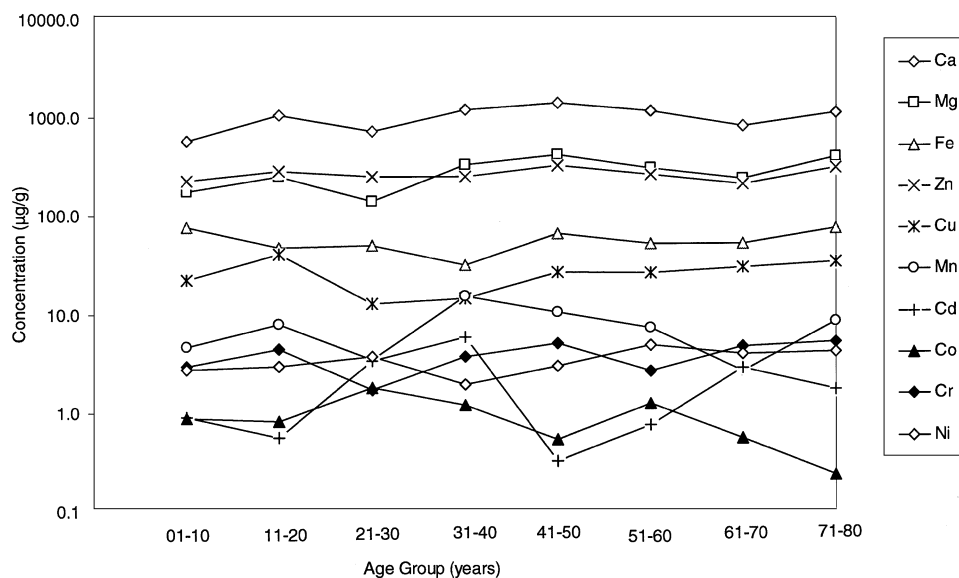


Figure 3. Dependence of selected hair metal concentration on various age groups of female donors.

found to increase with age as manifested by the maximum levels of Cd, Co, Cr, and Ni for the 70 year plus age group. The age groups of 81–90 ($n = 1$) and 91–100 ($n = 1$) did not represent a majority of donors since only one sample in each group could be arranged for analysis. These age groups therefore represented a minor segment of the sampled population and the values are given here only for an illustrative purpose. A similar trend was also observed in the case of females falling in these age groups (Table VI, Figure 3). A regression analysis was also carried out to find the trend of metal concentration in different age groups of the two genders to look into the age dependence of selected metals in a pooled segment of population (Table VII) for which no appreciable change in metal concentration was observed as a function of age.

4. Discussion

The data in Tables I and II suggested that the Fe and Cd are randomly distributed as compared with other metals in hair samples from both genders, as shown by the respective percent standard deviation. The maximum percent dispersions were shown by Fe (279%), Cd (149%), Ni (139%) and the minimum by Cu (56%) in case of male donors, while for female donors, the maximum percent dispersions were observed in the levels of Cd (177%), Fe (149%) and the minimum in the level of Zn (36%). These data are also supported by the listed computed kurtosis and skewness values which showed comparable levels. It is of interest to note

TABLE V
Average metal contents ($\mu\text{g/g} \pm \text{SE}$) of scalp hair of male donors in various age groups

Age group (y)	Count	Ca	Mg	Fe	Zn	Cu	Mn	Cd	Co	Cr	Ni
01-10	6	567.3 \pm 179.6	91.2 \pm 14.0	35.21 \pm 14.00	117.4 \pm 3.4	8.06 \pm 0.36	2.172 \pm 1.013	1.723 \pm 0.613	1.925 \pm 0.165	1.128 \pm 0.452	2.662 \pm 0.132
11-20	7	487.1 \pm 65.5	102.2 \pm 14.7	279.32 \pm 219.92	401.9 \pm 191.9	12.30 \pm 1.60	2.993 \pm 1.097	0.902 \pm 0.439	1.121 \pm 0.228	2.281 \pm 0.647	4.949 \pm 3.280
21-30	9	589.0 \pm 349.8	109.4 \pm 35.6	48.08 \pm 12.48	155.9 \pm 25.0	14.85 \pm 2.24	4.287 \pm 1.145	2.276 \pm 1.011	0.667 \pm 0.223	2.131 \pm 0.649	1.288 \pm 0.387
31-40	7	485.2 \pm 102.2	88.8 \pm 21.3	23.88 \pm 7.05	145.7 \pm 26.3	9.02 \pm 1.68	2.360 \pm 0.880	0.909 \pm 0.411	1.756 \pm 0.285	1.989 \pm 0.628	2.558 \pm 0.352
41-50	11	499.1 \pm 94.4	186.7 \pm 38.7	114.16 \pm 73.45	228.2 \pm 29.6	15.38 \pm 2.71	5.942 \pm 1.370	0.283 \pm 0.126	1.150 \pm 0.215	2.655 \pm 0.449	1.850 \pm 0.516
51-60	5	301.9 \pm 69.434	74.5 \pm 20.4	35.87 \pm 14.14	153.6 \pm 37.0	14.20 \pm 5.53	5.364 \pm 1.046	0.697 \pm 0.467	1.272 \pm 0.223	1.443 \pm 0.277	1.824 \pm 0.541
61-70	5	264.0 \pm 103.0	68.7 \pm 25.2	22.69 \pm 7.64	207.9 \pm 79.8	11.10 \pm 1.68	2.648 \pm 1.194	0.278 \pm 0.172	1.100 \pm 0.344	1.464 \pm 0.640	1.898 \pm 0.441
71-80	6	389.9 \pm 31.1	131.8 \pm 27.4	75.47 \pm 23.17	237.6 \pm 22.0	19.38 \pm 3.15	5.712 \pm 1.993	2.289 \pm 0.937	1.024 \pm 0.346	2.669 \pm 0.793	2.539 \pm 1.192
81-90	1	432.4 \pm 432.4	80.5 \pm 80.5	23.06 \pm 23.06	256.7 \pm 256.6	17.02 \pm 17.02	2.920 \pm 2.920	1.278 \pm 1.278	0.820 \pm 0.82	1.320 \pm 1.32	0.352 \pm 0.352
91-100	1	231.5 \pm 231.5	64.4 \pm 64.4	3.59 \pm 3.59	119.3 \pm 119.3	3.68 \pm 3.68	1.140 \pm 1.140	0.420 \pm 0.420	3.140 \pm 3.140	4.000 \pm 4.000	3.020 \pm 3.020

TABLE VI
Average metal contents ($\mu\text{g/g} \pm \text{SE}$) of scalp hair of female donors in various age groups

Age group (y)	Count	Ca	Mg	Fe	Zn	Cu	Mn	Cd	Co	Cr	Ni
01-10	12	577.4 \pm 69.6	175.1 \pm 37.0	77.40 \pm 42.80	225.1 \pm 29.2	22.12 \pm 4.66	4.651 \pm 0.915	0.909 \pm 0.426	0.881 \pm 0.196	2.952 \pm 1.003	2.739 \pm 0.787
11-20	3	1057.6 \pm 73.2	250.0 \pm 95.1	47.76 \pm 2.88	287.6 \pm 27.0	40.86 \pm 10.13	7.949 \pm 2.313	0.561 \pm 0.411	0.818 \pm 0.265	4.367 \pm 0.416	2.913 \pm 0.583
21-30	3	728.6 \pm 320.7	139.2 \pm 72.3	50.41 \pm 24.85	248.0 \pm 85.9	12.67 \pm 1.99	3.454 \pm 2.915	3.329 \pm 3.155	1.771 \pm 0.078	1.696 \pm 0.361	3.653 \pm 0.753
31-40	2	1208.8 \pm 328.2	327.0 \pm 319.0	31.30 \pm 16.44	248.6 \pm 62.4	14.49 \pm 4.59	15.385 \pm 12.655	5.799 \pm 5.639	1.180 \pm 0.540	3.682 \pm 1.362	1.900 \pm 0.580
41-50	3	1399.0 \pm 333.5	418.2 \pm 124.2	66.48 \pm 4.51	318.8 \pm 59.1	26.18 \pm 8.69	10.521 \pm 4.875	0.318 \pm 0.150	0.534 \pm 0.235	5.027 \pm 1.714	2.943 \pm 1.451
51-60	3	1142.4 \pm 358.8	296.1 \pm 75.8	51.25 \pm 7.62	256.7 \pm 47.9	25.61 \pm 13.64	7.169 \pm 2.440	0.732 \pm 0.714	1.218 \pm 0.549	2.571 \pm 1.827	4.713 \pm 0.945
61-70	2	810.1 \pm 527.6	231.7 \pm 27.5	50.79 \pm 2.60	203.9 \pm 13.6	29.02 \pm 14.08	2.571 \pm 1.679	2.813 \pm 0.253	0.537 \pm 0.307	4.652 \pm 3.165	3.906 \pm 3.275
71-80	2	1072.8 \pm 239.8	386.6 \pm 199.8	72.05 \pm 26.45	300.6 \pm 30.0	33.42 \pm 17.57	8.366 \pm 3.445	1.683 \pm 0.795	0.231 \pm 0.081	5.120 \pm 3.406	4.113 \pm 4.027

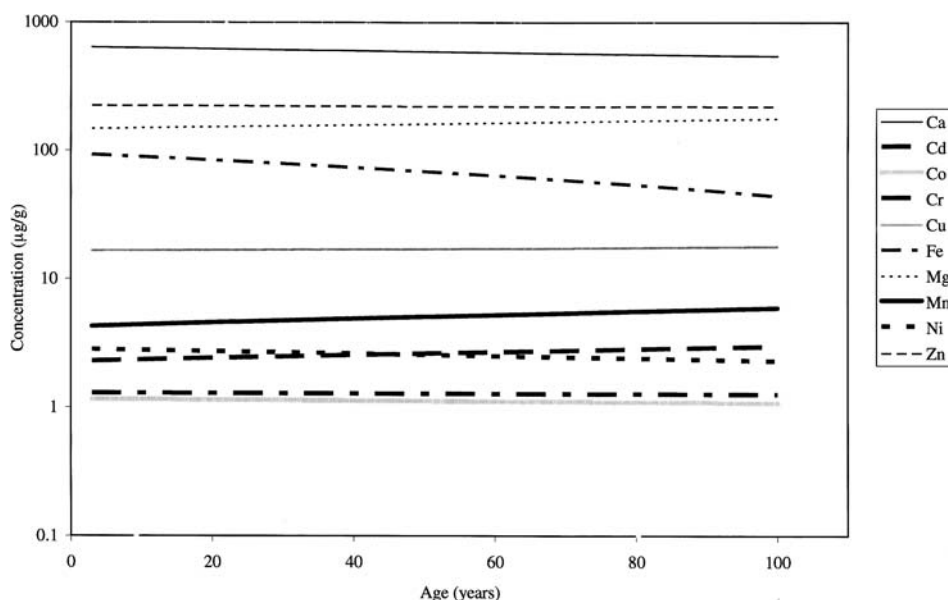


Figure 4. Overall age dependence of selected hair metal concentration.

that the average levels of selected metals were found to be higher in the hair of female donors compared with those of male donors, with the exception of Fe and Co (Figure 1). This could be explained on the basis of differences in metabolism and physiological role of the metals for two genders.

The levels of Ca, Fe, Zn, and Mg were observed to decrease considerably with age of the male donors, while Mn, Cd and Ni concentrations showed a marginal decreasing trend with age. The levels of Cu, Co and Cr were found to accumulate in the hair of elderly donors. Elevated levels of Ca, Mg, Zn, and Cu were found for the female donors, which indicated a probable metabolic retention of the specific metals related to the aging process. An insignificant accumulating behavior of metals with the age of females was noted for all selected metals, except Co which showed a slight negative dependence on age. A regression analysis of collective samples as a function of age, showed a decrease in the concentration of Ca and Fe with age while the concentration of all other selected metals showed no appreciable change with age (Figure 4). The observed behavior of Ca in the present case is different as compared with the one in counterpart data from U.S.A. (Paschal *et al.*, 1989). This could be explained as to arise on the basis of different socio-economic and health status of the two societies.

In comparison with earlier work reported for other rural areas of Pakistan (Ashraf *et al.*, 1995a), the Cu and Cd levels were found higher in the previous results for Jamrood, a rural site in North West Frontier Province. The Ca, Mg and Fe levels were comparatively found lower in rural area of district Chakwal in the present study. This could be attributed, firstly to different food habits of the two

TABLE VII

Regression equations as related to average metal concentration dependence on age of male/female/collective donors

Metals	Male	Female	Collective
Ca	$[y] = -31.886x_i + 600.09$ $R^2 = 0.5995$	$[y] = 43.597x_i + 803.4$ $R^2 = 0.1535$	$[y] = -0.9463x + 638.94$ $R^2 = 0.0023$
Mg	$[y] = -2.7522x_i + 114.96$ $R^2 = 0.0516$	$[y] = 23.218x_i + 173.5$ $R^2 = 0.3381$	$[y] = 0.3081x + 146.77$ $R^2 = 0.003$
Fe	$[y] = -12.262x_i + 133.58$ $R^2 = 0.2086$	$[y] = 0.1834x_i + 55.103$ $R^2 = 0.0009$	$[y] = -0.5033x + 94.576$ $R^2 = 0.004$
Zn	$[y] = -2.9054x_i + 218.39$ $R^2 = 0.0105$	$[y] = 2.4505x_i + 250.14$ $R^2 = 0.024$	$[y] = -0.0323x + 223.93$ $R^2 = 2E-05$
Cu	$[y] = 0.1293x_i + 11.787$ $R^2 = 0.007$	$[y] = 0.8379x_i + 21.774$ $R^2 = 0.0484$	$[y] = 0.0136x + 16.53$ $R^2 = 0.0007$
Mn	$[y] = -0.0145x_i + 3.6334$ $R^2 = 0.0007$	$[y] = 0.0642x_i + 7.2192$ $R^2 = 0.0014$	$[y] = 0.0173x + 4.2286$ $R^2 = 0.0082$
Cd	$[y] = -0.0637x_i + 1.4558$ $R^2 = 0.0636$	$[y] = 0.0405x_i + 1.8355$ $R^2 = 0.0028$	$[y] = -0.0003x + 1.2971$ $R^2 = 1E-05$
Co	$[y] = 0.0531x_i + 1.1053$ $R^2 = 0.0496$	$[y] = -0.0983x_i + 1.3388$ $R^2 = 0.2449$	$[y] = -0.0008x + 1.1629$ $R^2 = 0.0006$
Cr	$[y] = 0.1153x_i + 1.4739$ $R^2 = 0.1643$	$[y] = 0.2449x_i + 2.6564$ $R^2 = 0.23$	$[y] = 0.0072x + 2.2826$ $R^2 = 0.0062$
Ni	$[y] = -0.1497x_i + 3.1176$ $R^2 = 0.1402$	$[y] = 0.2239x_i + 2.3525$ $R^2 = 0.3711$	$[y] = -0.0054x + 2.8495$ $R^2 = 0.0019$

Note.

$[y]$ = concentration of a given metal ($\mu\text{g/g}$).

x_i = Age group ranging from $i = 1$ to $i = 10$ for various donor ages.

x = Age ranging from 1 to 100.

communities and secondly to the geological and geographical differences in the two locations.

5. Conclusions

The present study showed a distinct metal concentration dependence on sex and age. The observed variations of metal concentrations in two sexes with different age groups reflect the impact of multivariable role of metals regulating and controlling the metabolism in human body. Data from the present study are in agreement with those reported earlier from other parts of the world (Takagi *et al.*, 1990). Any differences observed in the levels of selected metals in the present study were indicative of the individual variability, sex specificity, age dependence, individual

metabolic activity, occupational exposure, geological location and food habits of the donors. In general, the local habitants of Chakwal were not critically exposed to the adverse effects of environmental trace metal pollution, as has been observed for mega cities of the world. The present data could act as baseline information for relevant futuristic studies.

Acknowledgements

Financial support by Higher Education Commission of Pakistan to undertake this project is gratefully acknowledged. We are also thankful to the Chief, Nutrition Division, National Institute of Health (NIH), Islamabad, Pakistan for allowing inter-laboratory comparison of data and providing standard reference material.

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