IS SCALP HAIR VALID INDICATOR FOR THE ASSESSMENT OF CHEMICAL ELEMENT CONTENTS IN HUMAN BODY?

S. Zaichick[1], V. Zaichick[2]

[1] *Northwestern University, Chicago, IL, 60611, USA*

[2]*Medical Radiological Research Centre of RAMS,*
Koroleva str., 4, Obninsk, 249020, Russia,
e-mail: vezai@obninsk.com

Introduction

In the last decades there is a growing fear of environmental diseases in the population of many countries and an increasing awareness of health and wellness. Chemical elementosis, a member of environmental diseases, is caused by low (deficiency) or high (excess) intake of chemical elements. Therefore there is an increasing interest in monitoring chemical element contests in humans, not only in cases of known high special burden, but also for individuals of the so-called "normal population". Blood, urine and hair are the favored indicator materials, because they can be easily obtained from a living person. If we regard the human body as a "multi-compartment model in flow equilibrium" (as frequently assumed in toxico-kinetics) there is, of course. a strong and calculable correlation between the different compartments, and therefore the same correlation between the organs and blood, urine or hair.[1] From cases with high, special burden, e.g. chronic occupational burden,[2-4] acute intoxication,[5] or mass catastrophes,[6,7] we know the benefit of such investigations. The question is: could we transfer these experiences to the normal population? Could we use blood, urine, and hair as indicators for the burden of the target organs or the whole body in a non-occupational or environmental exposed population[1]?

Hair has many advantages for assessment over the more traditional kinds of medical objects such as blood and urine because of ease of collection, transport and storage. Also, trace element contents in hair samples represent an integrated response over time compared with blood and urine levels, which can rapidly fluctuate in response to variations of nutritional and environmental conditions. The fact that contents of many chemical elements in hair are relatively high also facilitates the analysis. Commercially offered hair “mineral” analysis promises to be a simple, non-time-consuming and relatively low-priced method to determine the “body mineral status” of the donor without explanation what this term means in detail. Deriving from the “mineral status”, dietary recommendations are given and even increased risks for various adverse health effects are indicated. No wonder that hair “mineral” analysis was booming in the end of XX century not only in the USA,[8] but also in Europe[1] including Russia.

Disregarding art paper advertisements of the laboratories, which offer such analysis, there is a question of reliability of the “hair mineral test”, and the scientific validity of the “body mineral status” estimation using the “hair mineral test” results.[8-14] Therefore we performed the following study with two objectives. The first objective was to evaluate inter-

and intra-laboratory agreement concerning hair chemical element analysis. The second objective was to check correlations between chemical element contents in hair and bone tissue. Comparison to bone was made because many chemical elements have high affinity to bone tissue, thus a skeleton is a target organ for them.

Experimental

Samples of scalp hair and rib bone were obtained at postmortems from intact cadavers (38 female and 42 male, 15 to 55 year old) within 24 h of death. Each death had resulted from automobile accidents, falls, shootings, stabbing, hanging, acute alcohol poisoning, or hypothermia. All the deceased were citizens of Obninsk, a small city of non-industrial region 105 km south-west from Moscow. None of those who died a sudden death had suffered from any systematic or chronic disorders before. All studies were approved by the Forensic Medicine Department of Obninsk City Hospital and the Medical Radiological Recearch Center Ethical Committees.

A hair bunch of ≤5 cm length was taken from the proximal end of scalp hair using the titanium scalpel. The washing procedure was done according to the IAEA recommendation: washing once in acetone, twice in double deionized water and once more in acetone, for ten minutes in each case.[15] After air drying over night at room temperature, each hair sample was divided into two parts (two sub-samples) and packaged in pre-cleaned polyethylene envelopes. Identical collections of hair samples were analyzed by ICP-AES in two commercial Moscow laboratories, which offer "hair mineral" analysis today. For result quality control Laboratory 1 and Laboratory 2 use different certified reference material (CRM) - the GBW09101 (Human hair, China) and CRM-397 (trace elements in human hair) of the Community Bureau of Reference (BCR, Brussels). Good agreement of obtained results from the CRM analysis with certified data was confirmed by two laboratories. Results repeatability of the GBW09101 (Human hair, China) analysis was checked by us in Laboratory 1. For this purpose ten 50 mg sub-samples of the GBW09101 were analyzed by two operators for ten working shifts (one per shift).

Rib bone samples were analyzed in the Analytical Center of the Institute of Microelectronics, Technology and High Purity Materials (IMTHPM) by ICP-AES.[16] Standard reference materials SRM NIST 1486 Bone Meal, Tea Leaves INCT-TL-1 and Mixed Polish Herbs INCT-MPH-2 were analyzed simultaneously with rib samples to estimate the precision and accuracy of results.

Each laboratory has determined contents of a different number of elements. Ten elements (Al, Cd, Cu, Fe, Mg, Mn, Mo, P, Pb, and Zn) were determined by both "hair mineral" laboratories in hair samples and by the Analytical Center of IMTHPM in rib bone samples. In the future, for comparable reason, only the results for the common 10 elements were evaluated.

Using standard programs, the summary of statistics, arithmetic mean, relative standard deviation, relative standard error of mean, minimum and maximum values, median, percentiles with 0,025 and 0,975 levels, and correlation coefficient were calculated for chemical element contents. The reliability of difference in the results between two laboratories was evaluated by Student's *t*-test.

Results

Table 1 depicts the Laboratory 1 data of Al, Cd, Cu, Fe, Mg, Mn, Mo, P, Pb, and Zn contents in the CRM GBW09101 certified reference material (Human hair, China) and the certified values of this material.

Table 1. ICP-AES data of chemical element contents in 10 sub-samples of the certified reference material GBW09101 (Human hair, China) obtained in Laboratory 1 (mg/kg)

Element	This work results		Certificate values	
	Mean n=10	95% confidence interval	Mean	95% confidence interval
Al	10.4	9.5 – 11.7	13.3**	8.7 – 17.9
Cd	0.079	0.068 – 0.095	0.095**	0.071 – 0.119
Cu	20.3	14.8 – 32.0	23.0**	20.2 – 25.8
Fe	67.2	58.6 – 72.6	71.2**	58.0 – 84.4
Mg	94.4	86.3 – 104	105**	93 – 117
Mn	2.46	2.17 – 3.02	2.94**	2.54 – 3.34
Mo	0.51	0.42 – 0.64	0.58*	–
P	138	120 – 161	184*	–
Pb	8.17	7.58 – 9.97	7.2**	5.8 – 8.6
Zn	176	158 – 198	189**	173 – 205

** - Recommended value, * - Information value

Repeatability of ICP-AES results of chemical element contents (mg/kg) in ten 10 sub-samples of the certified reference material GBW09101 (Human hair, China) analysed by two operators for ten working shifts (one per shift) is shown in Table 2.

Table 2. Repeatability of ICP-AES results of chemical element contents (mg/kg) in the certifiedreference material GBW09101 (Human hair, China)

Element	Mean n=10	RSD %	RSEM %	Min	Max	Median	Per 0.025	Per 0.975	Max / Min
Al	10.4	7.45	2.36	9.5	11.7	10.3	9.57	11.6	1.22
Cd	0.079	12.1	3.82	0.068	0.096	0.075	0.069	0.095	1.41
Cu	20.3	29.8	9.42	14.8	34.5	20.3	14.8	32.0	2.34
Fe	67.2	7.28	2.30	58.2	73.1	68.8	58.6	72.6	1.26
Mg	94.4	6.19	1.96	85.5	105	93.5	86.3	104	1.47
Mn	2.46	11.4	3.60	2.14	3.14	2.39	2.17	3.02	1.47
Mo	0.51	14.7	4.64	0.41	0.66	0.50	0.42	0.64	1.62
P	138	10.2	3.23	119	164	138	120	161	1.38
Pb	8.17	10.6	3.35	7.54	10.4	7.94	7.58	9.97	1.39
Zn	176	8.76	2.77	158	197	174	158	198	1.25

Mean – arithmetic mean; RSD – relative standard deviation; RSEM – relative standard error of mean; min – minimal value; max – maximal value; Per 0.025 – percentile with 0.025 level; Per 0.975 – percentile with 0.975 level.

To evaluate the reproducibility within the laboratories, the results of each laboratory for two identical sub-samples of every 80 samples from hair collection were compared (Table 3). Coefficient of variation (CV) or relative standard deviation (%) was calculated as

$$CV = \sqrt{\sum \delta^2 / 2n} \times 100\%, \qquad \text{where} \qquad \delta = \frac{C_1 - C_2}{0.5(C_1 + C_2)},$$

C_1 and C_2 – are contents of chemical element in two identical sub-samples reported Laboratory 1 and Laboratory 2, respectively.

Table 3. Reproducibility of the analytical results obtained in Laboratories 1 and 2.

Element	CV or RSD %	Max (C_1/C_2)	Min (C_1/C_2)	$\frac{\text{Max } (C_1/C_2)}{\text{Min } (C_1/C_2)}$
Al	41	9.00	0.15	60
Cd	46	41.3	0.35	118
Cu	14	2.77	0.51	5.4
Fe	70	157	0.67	234
Mg	79	175	3.1	56
Mn	78	164	0.98	167
Mo	85	11.3	0.037	305
P	31	8.92	0.62	14
Pb	65	21.3	0.41	52
Zn	14	1.63	0.69	2.4

CV - coefficient of variation - $CV = \sqrt{\sum \delta^2 / 2n} \times 100\%$, where $\delta = \frac{C_1 - C_2}{0.5(C_1 + C_2)}$,

C_1 and C_2 – are contents of chemical element in two identical sub-samples reported Laboratory 1 and Laboratory 2, respectively

Tables 4 and 5 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of chemical element contents in the scalp hair of healthy humans obtained in Laboratories 1 and 2, respectively.

The comparison of results obtained in Laboratories 1 and 2 with the literature[17] and reference[18] data of chemical element contents in adult human scalp hair are given in Table 6. Table 7 shows the comparison between results of chemical element contents in the scalp hair of healthy humans obtained in Laboratories 1 and 2.

The correlation between the chemical element contents in the rib bone and the scalp hair (data Laboratory 1 and Laboratory 2) is shown in Table 8.

To estimate the usefulness of "hair mineral" results for the assessment of Pb content in human body we examined the group of deceased persons (n=14) with Pb content in the rib bone or hair above the normal region, i.e. > 90% percentile of the population (Table 9).

Table 4. Some statistical parameters of chemical element contents in the scalp hair of healthy humans obtained in Laboratory 1 (mg/kg, dry weight basis)

Element	Mean mg/kg	RSD %	RSEM %	Min mg/kg	Max mg/kg	Median mg/kg	Per0,025 mg/kg	Per 0,975 mg/kg	Max / Min
Al	33.4	214	26	2.4	454	13.3	2.7	212	189
Cd	0.21	124	15	0.035	1.76	0.14	0.039	0.772	19.8
Cu	11.0	69	8.4	6.29	66.7	9.4	7.2	22.4	10.6
Fe	120	120	15	15.3	889	67.2	19.7	499	58
Mg	156	169	20	22.9	2078	84.4	24.6	488	91
Mn	3.9	156	19	0.43	38.4	1.97	0.51	23.3	89
Mo	0.052	80	11	0.0098	0.359	0.044	0.023	0.098	37
P	425	101	12	109	2070	262	118	1862	19
Pb	3.5	115	14	0.45	21.8	2.0	0.48	15.3	48
Zn	180	23	2.8	62.1	321	179	110	286	5.2

Table 5. Some statistical parameters of chemical element contents in the scalp hair of healthy humans obtained in Laboratory 2 (mg/kg, dry weight basis)

Element	Mean mg/kg	RSD %	RSEM %	Min mg/kg	Max mg/kg	Median mg/kg	Per 0,025 mg/kg	Per 0,975 mg/kg	Max / Min
Al	22.4	85	9.8	1.51	123	18.4	4.62	65.4	81
Cd	0.11	76	8.8	0.005	0.741	0.10	0.021	0.225	148
Cu	8.4	30	3.5	3.75	14.7	8.0	4.4	13.5	3.9
Fe	78.3	86	10	17	510	67.1	22.2	207	30
Mg	79.8	75	8.6	9.99	294	59.1	12.1	268	29
Mn	1.2	154	18	0.007	12.4	0.56	0.032	5.4	1771
Mo	0.27	84	9.5	0.005	1.23	0.22	0.038	0.890	246
P	198	31	3.6	105	412	190	118	341	3.9
Pb	1.1	114	13	0.11	6.8	0.7	0.15	4.7	62
Zn	159	25	2.9	76.6	328	152	104	235	4.3

Mean – arithmetic mean; RSD – relative standard deviation; RSEM – relative standard error of mean; min – minimal value; max – maximal value; Med Per 0.025 – percentile with 0.025 level; Per 0.975 – percentile with 0.975 level.

Table 6. The comparison of results obtained in Lab. 1 and 2 with the literature and reference data of chemical element contents in adult human scalp hair (mg/kg, dry weight basis)

Element	Laboratory 1	Laboratory 2	Whole world[17]	Reference data[18]
	Mean	Mean	Min-max	Confidence range
Al	33.4	22.4	0.1-191	3 - 10
Cd	0.21	0.11	0.01-16.1	0.25 – 1.0
Cu	11.0	8.4	0.3-293	15 - 25
Fe	120	78.3	3-2400	30 - 60
Mg	156	79.8	1.5-1412	-
Mn	3.9	1.2	0.03-81.5	0.5 – 1.5
Mo	0.052	0.27	0.01-3	0.05 – 0.2?
P	425	198	18-800	-
Pb	3.5	1.1	0.004-155	2 - 20
Zn	180	159	<1-1770	150 - 250

Table 7. The comparison of results of chemical element contents in the scalp hair of healthy humans obtained in Laboratories 1 and 2 (mg/kg, dry weight basis)

Element	Laboratory 1			Laboratory 2			$\frac{M_1}{M_2}$	Student's *t*-test
	M_1 mg/kg	RSD %	Range mg/kg	M_2 mg/kg	RSD %	Range mg/kg		
Al	33.4	214	2.4 - 454	22.4	85	1.51 - 123	1.5	N.S.
Cd	0.21	124	0.035 – 1.76	0.11	76	0.005 -0.74	1.9	$p \leq 0.01$
Cu	11.0	69	6.29 – 66.7	8.4	30	3.75 – 14.7	1.3	$p \leq 0.01$
Fe	120	120	15.3 - 889	78.3	86	17 - 510	1.5	$p \leq 0.05$
Mg	156	169	22.9 - 2078	79.8	75	9.99 - 294	2.0	$p \leq 0.05$
Mn	3.9	156	0.43 – 38.4	1.2	154	0.007 – 12.4	3.3	$p \leq 0.001$
Mo	0.052	80	0.0098 – 0.359	0.27	84	0.005 – 1.23	0.19	$p \leq 0.001$
P	425	101	109 - 2070	198	31	105 - 412	2.1	$p \leq 0.001$
Pb	3.5	115	0.45 – 21.8	1.1	114	0.11 – 6.8	3.2	$p \leq 0.001$
Zn	180	23	62.1 - 321	159	25	76.6 - 328	1.1	$p \leq 0.01$

M_1 and M_2 – arithmetic mean, RSD – relative standard deviation, N.S. - not significant

Table 8. Correlation coefficient (r) of chemical element contents in scalp hair (data Laboratory 1 and Laboratory 2) and rib bone of healthy humans

Element	Laboratory 1		Laboratory 2	
	r	Confidence level	r	Confidence level
Al	0.099405	N.C.	-0.171	N.C.
Cd	-0.072936	N.C.	-0.066	N.C.
Cu	-0.023171	N.C.	0.054	N.C.
Fe	0.083923	N.C.	0.024	N.C.
Mg	0.059483	N.C.	0.047	N.C.
Mn	-0.072658	N.C.	-0.023	N.C.
Mo	-0.032984	N.C.	0.161	N.C.
P	-0.04701	N.C.	0.077	N.C.
Pb	0.295352	$p<0.01$	0.159	N.C.
Zn	-0.031486	N.C.	0.005	N.C.

N.C. – no correlation

Discussion

Detected means for the chemical element contents (mean ± SD) in the certified reference material GBW09101 (Human Hair, China) obtained in this work were within the 95% confidence intervals of all certified values, except Mn (Table 1). Mean content obtained for Mn appeared to be 16% lower than the appropriate value of the GBW09101 Human Hair certificate. Good agreement with the data of certified reference materials indicate an acceptable accuracy of the results on Al, Cd, Cu, Fe, Mg, Mn, Mo, P, Pb, and Zn contents in the scalp hair samples determined in the Laboratory 1.

Laboratory 2 used the certified reference materials CRM-397 BCR, Brussels (trace elements in human hair) for a quality control and reported only that all means of chemical element contents were within the certified region.

Table 9. Accuracy and sensitivity of the Pb excess diagnostics in the "hair mineral" Laboratory 1

Subject	Hair Percentile 0.9 7.5 mg/kg	Rib-bone Percentile 0.9 4.1 mg/kg	Coincidence
h85	0.46	**5.70**	-
h75	1.02	**5.23**	-
h96	3.53	**6.56**	-
h88	**21.8**	1.63	-
h106	1.15	**9.70**	-
h79	**17.4**	**16.48**	+
h97	0.84	**5.82**	-
h123	**14.3**	2.60	-
h119	6.4	**6.70**	-
h120	**7.8**	1.82	-
h81	**11.1**	2.43	-
h107	4.0	**4.40**	-
h116	**12.8**	2.30	-
h109	**7.9**	2.97	-
n > Percentile 0.9	n=7	n=8	Coincidence n=1

Accuracy = 1/7 (14%), Sensitivity = 1/8 (12.5%)

Repeatability of ICP-AES results in Laboratory 1 was estimated by us using data of Al, Cd, Cu, Fe, Mg, Mn, Mo, P, Pb, and Zn contents in ten sub-samples of the certified reference material GBW09101 (Human Hair, China) analyzed by two operators for ten working shifts (one per shift). Data in Table 2 show that ICP-AES analysis of single 50-100 mg sample of hair allows estimate content of Al, Fe, Mg and Zn with relative uncertainty (determined as ±2RSD) within range ±10-<20%, content of Cd, Mn, Mo, P, and Pb with relative uncertainty within range ±20-<30%, and content of Cu with relative uncertainty around ±60%. Since the CRMs are virtually ideally homogenized materials, the received estimates of the methods' possibilities may be considered at most optimistic.

Reproducibility of the analytical results was calculated by a comparison between data obtained in Laboratories 1 and 2 (Table 3). The relative uncertainty (determined as ±2RSD or ±2CV) of Cu and Zn contents in hair samples was around ±30%, Al, Cd and P - within range ±60-90%, Fe, Mg, Mn, Mo, and Pb – more than ±100%.

The means Al, Cd, Cu, Fe, Mg, Mn, Mo, P, Pb, and Zn in the scalp hair of healthy humans obtained in Laboratories 1 and 2 (Tables 4 and 5) are inside of ranges cited by other researches for the human scalp hair,[17] but, only the means Mo, Pb, and Zn agree well with reference contents in adult human hair reported by Iyengar[18] (Table 6).

The comparison between the means of chemical element contents in the scalp hair of healthy humans obtained in Laboratories 1 and 2 detected statistically significant differences for all elements except Al (Table 7).

It is well known that more than 95% of all Pb in the body of an adult is stored with a long half time in the skeleton. Statistically significant correlation between Pb content in hair (data Laboratory 1) and rib bone was found in our study (Table 8). However, the correlation is so weak that can't be used for diagnostics of Pb excess in human body. The estimation of

accuracy and sensitivity of the Pb excess diagnostics by the "hair mineral" analysis detected very low and completely unacceptable values –14% (one case from seven) and 12.5% (one case from eight), respectively (Table 9).

In conclusion, we completely agree with the statements of similar investigations[8,14] – "hair mineral" analysis from contemporary laboratories is unreliable. Moreover, "hair mineral" analysis does not reflect content of bone-seeking elements. It means that "hair mineral" analysis does not reflect content of bone-seeking elements in human body, because skeleton is the biggest pool of such elements. We must recommend to refrain from using "hair mineral" analysis to assess a "body mineral status", individual nutritional status, or suspected environmental exposure.

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