Микроэлементы в медицине 10 (1—2): 33—38

ОРИГИНАЛЬНАЯ СТАТЬЯ

A CASE REPORT ON ANALYTICAL REPRODUCIBILITY OF THE HAIR MULTIELEMENT PROFILE: A TWO YEARS FOLLOW UP

ОБ АНАЛИТИЧЕСКОЙ ВОСПРОИЗВОДИМОСТИ ЭЛЕМЕНТНОГО СОСТАВА ВОЛОС: ДВУХГОДИЧНОЕ НАБЛЮДЕНИЕ ОТДЕЛЬНОГО СЛУЧАЯ

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KEYWORDS: multielement profile, analytical reproducibility, sample storage time, hair

КЛЮЧЕВЫЕ СЛОВА: элементный профиль, аналитическая воспроизводимость, время хранения образцов, волосы

ABSTRACT: The analytical reproducibility of a multielement profile was studied in the hair sample of a 65 years old men. The hair was randomly divided into three portions, double blinded, and analyzed upon arrival, and six months and two years thereafter by the ICP MS. The coefficient of variation (%) of 41 analyzed elements differed considerably from element to element being excellent for Ge and Mo and the least reliable for Be and W (0.0 and 1.1% vs. 66.7 and 79.1%, respectively); the CV for the major bioelements (Ca, Mg, K) was less than 20%. Samples were kept at the room temperature, and over the period of two years the systemic loss was observed for I only. The observed diversity of analytical accuracy for the different elements requires further biological and chemical pondering since it appears not to be related to the sample element concentration. The observed high within the laboratory analytical reproducibility of the hair multielement profiles make such profiles a useful tool for the follow up of the changes in the nutritional and toxicological status, and for the control of unforeseen interaction is the controlled metabolic studies, respectively.

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РЕЗЮМЕ: Аналитическая воспроизводимость элементного профиля была изучена двойным слепым методом посредством повторяющегося многоэлементного анализа одного и того же образца волос (испытуемый — мужчина, 65 лет). Образец волос был случайным образом поделен на 3 порции и проанализирован методом ИСП-МС сразу, а также через 6 месяцев и 2 года после взятия. Коэффициент вариации (%) существенно различался от аналита к аналиту, из 41 определяемого элемента, будучи наилучшим для Ge и Mo и наихудшим для Be и W (0,0 и 1,1% vs. 66,7 и 79,1% соответственно); для макроэлементов (Ca, Mg, K) он был ниже 20%. Образцы хранились при комнатной температуре; за 2 года потери были обнаружены только для йода. Наблюдаемые различия в аналитической воспроизводимости для различных элементов требуют дальнейшего изучения с позиций биологии и химии, поскольку, по всей вероятности, обусловлены чем-то иным, нежели содержанием элемента в образце. Наблюдаемая высокая внутрилабораторная аналитическая воспроизводимость делает определение элементного профиля полезным инструментом для мониторинга изменений в пищевом и токсикологическом статусе, а также для контроля непредвиденных взаимодействий в контролируемых исследованиях метаболизма.

INTRODUCTION

Hair multielement profile is a powerful tool in medical research and clinical diagnosis since hair is a memory tissue not subjected to the homeostatic control like the blood (Momcilovic, 2007). The potential of hair analysis for the assessment of the nutritional status and environmental xenobiotics exposure has been realized for the decades since it is ready accessible biological matrix material easy to be transported and stored until analyzed (Passwater, Cranton, 1983; Chatt, Katz, 1988; Batzevich, 1995). However, severe criticism has been directed against hair multielement profile analysis claiming high inaccuracy of both within the laboratory and between the laboratories analytical results (Seidel et al., 2001; Klevay et al., 2002, 2004). The aim of this single subject research paper is to analyze within the laboratory reproducibility of the hair multielement profile.

MATERIAL AND METHODS

A hair sample of a 65 years old men was collected over the occipital region of the head.The sample was partitioned into three different portions of apparently equal size. Each portion was double blinded by assigning it a different name, and analyzed for the multielement profile at the time of collection and six months and two years afterwards. The samples were prepared for the analysis as described previously (Momčilović et al., 2007). A total of 41 elements was analyzed by the ICP MS at the Center for Biotic Medicine, Moscow, Russia: Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Ge, Hg, I, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pt, Rb, Sb, Se, Si, Sn, Sr, Ti, Tl, V, W, Zn, and Zr. Arithmetic mean of the sample at three different time intervals, standard deviation, and coefficient of variation was calculated for every element by the standard statistical procedures (Glantz, 2005).

RESULTS AND DISCUSSION

The results of the hair multielement profile analysis are shown in Table 1. The elements are arranged in the increasing order of their coefficient of variation (CV); means, standard deviations, and ranges for every element are also shown. The accompanied graph (Fig.1) help us to better visualize the within the subject variability of the reproduced analyses. The elements Ge and Mo showed excellent reproducibility, whereas Be and W reproducibility was the least reliable (0.0 and 1.1% vs. 66.7 and 79.1%, respectively). None of the elements in the hair seems to be affected of being kept at the room temperature except iodine. Indeed, concentrations of iodine in the aliquots of the same hair sample were, respectively (mg/kg): 2.42, 1.41, and 0.877 at the time of sample collection, and six months and two years thereafter). However, not such a conclusive

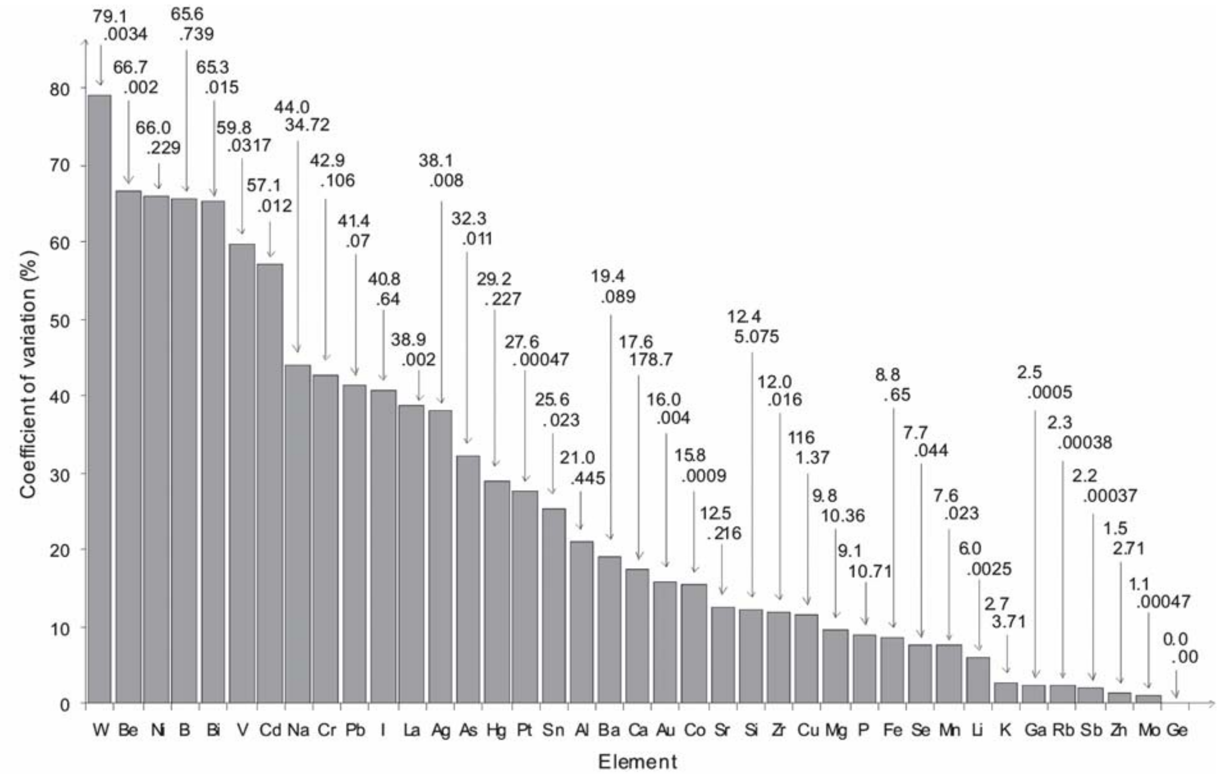

Fig. 1. *Within the subject hair multielement profile variability (upper values— coefficient of variation; lower values— standard deviation)*

Table 1. ***Reproducibility of hair multielement profile analysis (mg/kg)****

Element	Mean (n = 3)	SD	Range (Min—Max)	CV (%)
1	2	3	4	5
Ge	0.002	0.000	0.002	0.8
Mo	0.043	0.0005	0.043—0.044	1.1
Zn	181.6	2.71	177.8—183.6	1.5
Zr	0.133	0.016	0.110—0.148	2.0
Sb	0.017	0.0004	0.012—0.021	2.2
Rb	0.017	0.0004	0.013—0.022	2.3
Ga	0.030	0.0005	0.015—0.018	2.5
K	13.50	3.71	9.74—18.56	2.75
Li	0.042	0.0025	0.038—0.043	6.0
Mn	0.304	0.023	0.273—0.329	7.6
Se	0.572	0.044	0.525—0.632	7.7
Fe	7.39	0.650	6.59—8.19	8.8
P	117.9	10.71	104.1—130.2	9.1
Mg	105.5	10.36	92.71—118.1	9.8
Cu	11.76	1.37	10.17—13.51	11.6
Si	41.03	12.4	34.84—47.27	12.4
Sr	1.727	0.216	1.44—1.96	12.5
Au	0.025	0.004	0.022—0.031	16.0
Co	0.006	0.001	0.005—0.007	16.0
Ca	1014.7	178.7	822.7—1253.0	17.6
Ba	0.459	0.089	0.378—0.583	19.4
Al	2.123	0.445	1.650—2.720	21.0
Sn	0.090	0.023	0.064—0.121	25.5
Pt	0.002	0.0005	0.000—0.002	27.6
Hg	0.776	0.227	0.478—1.03	29.2
As	0.034	0.011	0.018—0.042	32.3
Ag	0.021	0.008	0.010—0.270	38.1

1	2	3	4	5
La	0.005	0.002	0.004—0.008	38.9
I**	1.569	0.640	0.877—2.42	40.8
Pb	0.169	0.070	0.106—0.267	41.4
Cr	0.247	0.106	0.097—0.332	42.9
Na	78.81	34.72	53.29—127.9	44.0
Cd	0.021	0.012	0.011—0.038	57.1
V	0.053	0.032	0.008—0.079	59.8
Bi	0.023	0.015	0.010—0.044	65.3
B	4.173	2.739	2.04—8.04	65.6
Ni	0.347	0.229	0.181—0.671	66.0
Be	0.003	0.002	0.000—0.004	66.7
W	0.004	0.003	0.001—0.009	79.1

* Elements Ti and Tl were below the detection limits.

** Iodine is the only element that systematically decreased to a great extent over the period of 0.0, 0.5, and 2.0 years.

trend of element lost was observed for the other most likely candidate, i.e., mercury (0.821, 1.03, and 0.478). Thus, to be on the safe side, hair samples aimed for both iodine and mercury analysis need to be kept refrigerated. Surprisingly enough, the similar trend of element loss was observed for Si (47.27, 40.97, and 34.84, respectively). On the other hand, Cd, Ga, P, and Pb showed a tendency towards gradual increase of the element concentration over the two years period of time, indicating subtle changes of the hair biological matrix hydration. However, Cr would first increase from the initial level to the six months level, and stay there until reanalyzed after the two years period (0.097, 0.312, and 0.330 shown in the same respective order for iodine); the inverse was true for lanthanum (0.008, 0.003, 0.0040. Apparently, the results indicate the necessity to analyze the refrigerated hair samples for such a large hair multielement profile within the period of about three months.

It is possible to make many other relevant observation on timing the multielement hair sample analysis based on the here presented within the subject data variability and what would be beyond the scope of this paper. Thus, the reproducibility of percentile data also varied from element to element (data not shown). We would only mention that the CV (%) of Ca and Mg was lower for the percentiles than for the actual element concentrations (10.0 vs. 17.6 and 7.64 vs. 9.8 for Ca and Mg, respectively). These two elements were of major concern in our recent study on Ca and Mg dietary supplementation in the postmenopausal women (Prejac et al., 2007). We expected the reproducibility results to be dependent upon the sample element concentration, but only a negligible inverse correlation was observed between the amount of the element in the sample and the corresponding coefficient of variation ($r^2 = 0.07$) (Fig. 2).

Although the collected hair was sampled from the same subject it is not biologically uniform, since every hair can be in either anagen or telogen phase, i.e., growth and rest phase, respectively (Rook, Dawber, 1982). Hence, some of the observed variability in the analytical reproducibility of the hair multielement profile may stem from the chance variability of the biological matrix itself, and what we believe to be the minor source of within the subject variability. Two other biological factors may be of the much greater importance. One is the «cross-over» inference of the different elements sharing the same mass number, since the same mass numbers may be shared by a number of elements (Momčilović et al., 2007). Indeed, selenium alone has 17 isotopes of different mass number, but there is a total of 131 isotopes sharing such numbers and coming from the Zn, Ga, Ge, As, Br, Kr, Rb, Sr, Y and Zr, and what we tentatively

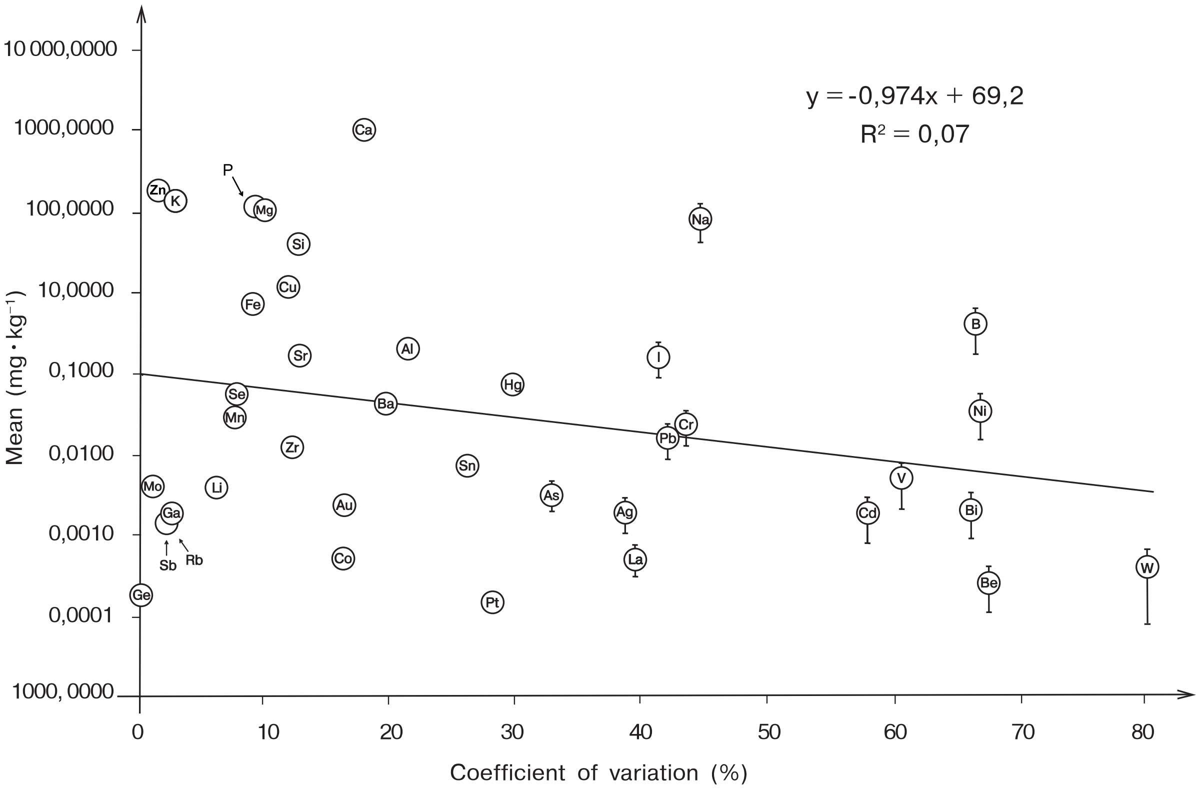

__Fig. 2.__ The relationship between the element concentration of the hair sample and the percentage coefficient of variation (CV). The bars denote standard deviation

named the Se isotope cluster. The other is the old problem of the sample preparation in general, and washing procedure in particular. Indeed, some sample preparation methods may work better for some of the elements than for the other, since it is hard to envisage the uniform procedure that would be simultaneously the best for all of the elements. Perhaps, depending upon the critical elements of the study, the washing procedure may be modified to enhance the accuracy of one group of elements at the expense of lower accuracy for the other. Nevertheless, the observed high within the laboratory analytical reproducibility of the individual hair multielement profiles make such profiles a useful tool for the follow up of the changes in the nutritional and toxicological status, respectively (Ivičić et al., 2007). Moreover, we think that any serious metabolic study on bioelements should be preceded by the hair multi-element profile analysis (or some other relevant indicator tissue biomatrix) before the commencement of such a study, and at its respective end point to cover for the unforeseen bioelement interactions.

ACKNOWLEDGEMENTS

This work was in part supported by the grants 022-0222412-2405 and 022-0222412-2403 from the Ministry of Science, Education, and Sport, Republic of Croatia. The authors would like to thank the Centre for Biotic Medicine, Moscow, Russia for analyzing the whole blood bioelements. Dr. Momcilovic would also like to thank for the generous philanthropic support of the RCSTrading Co. Ltd, Isle of Man, UK.

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