Identification of exposure to toxic metals by means of segmental hair analysis. A case report of alleged chromium intoxication

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Abstract

Hair mineral analysis has become an interesting diagnostic tool in biomonitoring of exposure to toxic elements, in the assessment of health and nutritional status. The most inconvenience of this matrix is the lack of sufficient information to define normal ranges of metal levels in a general healthy population. In this study, segmental hair analysis was used to depict a chronological scheme of exposure to arsenic, cobalt, cadmium, chromium, copper, manganese, nickel and lead in a 16-year-old girl showing signs of potential intoxication. The quantitative results obtained from consecutive segments of hair proved the exposure to chromium. In particular, segment A (0-6 cm), approximately reflecting the last 6 months of exposure, resulted in the chromium level at 5.60 µg/g. The technique of segmental analysis allowed us to establish "intra-individual" physiological variation ranges for each heavy metal hair concentration. As a consequence, these "confidence" intervals could be used as

individualized references to highlight the occurrence of atypical metal levels in any specific hair segment, possibly identifying a period of anomalous exposure and/or intoxication.

Keywords. Metals, Intoxication, Hair, Segmental analysis, Atomic Absorption Spectrophotometry

Introduction

Hair analysis currently represents a reliable and well-established means of clinical and forensic investigation [1]. The memory property of hair due to sequential accumulation of chemicals in its inner structure, together with the opportunity of conducting retrospective analysis, accounted for its success in several application contexts such as the confirmation of drug-facilitated crimes, the assessment of drug consumption history in addiction treatments, the environmental and occupational exposure to pollutants [1, 2-4]. Also workplace drug testing, driving re-licensing, withdrawal control, postmortem toxicology, prenatal exposure to drugs, and doping control extensively apply hair analysis for screening and confirmation purposes [5-13]. Upon investigating extended time-windows, as opposed to biological fluids, hair analysis has been recognized as the most powerful tool in the assessment of chronic consumption or exposure to various chemicals. The relatively constant head hair growth, with an estimated average rate of about 1.0 cm/month, allows to trace the chronological exposure profile by segmental hair analysis [14, 15]. As a matter of fact, segmental hair analysis has been repeatedly used to ascertain occasional abuse of drugs, alcohol and doping agents [1], verify the compliance of enforced abstinence [8], and outline a chronological sequence of drug exposure [16]. Lastly, hair analysis has been used to estimate the nutritional status of individuals and to assess poisoning and environmental intoxication of exposed subjects [17-19] from a variety of organic and inorganic substances, including heavy metals. From another point of view, human hair can be considered as a secondary excretion vehicle of toxic substances from the body. For example, the concentrations of heavy metals in hair are up to 10-times higher than in blood and urine [20-21]. Heavy metals, such as chromium, lead, mercury, cadmium, and arsenic – whenever biologically available - are extremely toxic to most living organisms even at very low concentrations. The presence of heavy metals in human hair usually reflects their bioavailability and therefore is generally found at ultra-trace levels. Significant excess of these elements in human

hair with respect to the expected population's average may reflect the degree of body exposure to these poisons, either from environmental pollution, workplace or food chain [17,18,22]. Hair mineral analysis has also become an interesting diagnostic tool in biomonitoring the exposure to toxic elements and the health and nutritional status assessment. Likewise organic substances and drugs, trace metal analysis on hair material presents several advantages over biological fluids, because it may provide an historical overview on the individual exposure to these elements, recognize acute vs. chronic intoxication, and monitor the nutritional status of the investigated subject over extended periods of time [23]. On the other hand, hair analysis also presents some limitations, including the lack of well-defined and generally accepted reference concentration ranges [23]. This uncertainty arises from the large differences existing in the elements' level as a function of sex, age, residence area, ethnicity, hair color, dietary habits, and individual physiological variability [24]. The present study was addressed to the evaluation of arsenic (As), cobalt (Co), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb) concentrations in the human scalp hair collected from a subject showing signs of potential intoxication, allegedly arising from exposure to toxic metals. Segmental hair analysis was performed to obtain information about the history of the patient's exposure, approximately in the preceding 3 years. Taking into account that (i) considerable interest exists in the toxicological perspectives opened by hair analysis toward the confirmation of heavy metal poisoning, and (ii) insufficient data are available in the literature about heavy metals acceptable especially for physiological hair concentrations, we decided to validate the whole analytical method and to establish an independent hair reference range for these metals in scalp hair, based on specimen collected from laboratory personnel volunteers (n=10). Furthermore, the technique of segmental analysis allowed us to establish "intra-individual" physiological variation ranges for each heavy metal hair concentration. As a consequence, these "confidence" intervals could be used as individualized references to highlight the

occurrence of atypical metal levels in any specific hair segment, possibly identifying a period of anomalous exposure and/or intoxication.

Materials and methods

Case history

A 16-year-old girl came to our laboratory to verify the possible past exposure to toxic metals by means of hair analysis. The girl had spent the previous 9 months abroad, where she lived, spent most of her time, and took her meals mostly inside a college. During this period, she presented severe symptoms, including metabolic disorders, skin irritation, nose bleeding, bronchitis and dysentery. Furthermore, recent blood tests evidenced sub-standard levels of glycemia (63 mg/dL) and increased potassium level at 5.94 mEq/L (range 3.5-5.5). The patient's parents suspected that the disorders presented by their daughter were to be attributed to a possible exposure to toxic substances arising either from the environment or the food, not only to the change in eating habits and climatic conditions.

Sample preparation

Two locks (diameter approximately 0.5 cm) of patient's hair (color: blond) were sampled in its entire length (about 40 cm) from the vertex region of the head [25], using stainless steel scissors and then stored at room temperature in a plastic box. The hair was segmented as follow: starting from the skull extremity, we considered six segments of 6 cm each and a final segment of 4 cm. The analyzed weight for each segment was in the range 400-500 mg. The hair aliquots were washed to remove potential external contaminations using the method proposed by Ohmori [26], consisting of three consecutive steps with 3 ml each of acetone, water and again

acetone. After decontamination, the samples were dried at room temperature overnight. The procedure adopted for hair digestion was based on the method commonly adopted in several literature studies [27-29]. Briefly, the dried hair aliquots were digested with a mixture of 65 % nitric acid (6 mL) and 67-72% perchloric acid (1 mL) at 70-80°C, until the hair was completely dissolved and the solution became clear (about 25 min). Lastly, each sample solution was diluted to 50 mL with demineralized Milli-Q water.

Standard solutions for calibration were prepared from a 1000 mg/L ICP Multielement solution (Merck, Milan, Italy), ranging from 0.10 to 10 µg/g. (0.50 to 50 µg/g for Cu).

Instrumentation

All analyses were performed using a Perkin-Elmer Analyst 800 atomic absorption spectrophotometer (Perkin Elmer, Norwalk, USA) equipped with an AS-800 autosampler and THGA graphite tubes with end caps (Perkin-Elmer). The instrumental parameters are described in Table 1.

Validation of analytical methods

The characteristic validation parameters for the analytical methods were determined from the analysis of blank water and standard solutions at different concentrations for each metal. These parameters, following the recommendations of ISO/IEC 17025:2005 international standard and others guidelines [25,30], included the limit of detection (LOD), limit of quantification (LOQ), linear range, precision (as CV %) and accuracy (as bias %). The linearity interval was evaluated by checking the linear regression coefficient (r^2) of the calibration curve. The linearity was considered acceptable when $r^2 > 0.995$. The linear calibration model was checked by analyzing (two replicates) blank water spiked with the working solution at five concentrations

levels in the range of 0.10-10 µg/g (0.50 to 50 µg/g for Cu). The LODs and the LOQs were extrapolated by Hubaux and Vos approach [31]. For all elements, intraday precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias %) were evaluated by spiking blank solution at low and high concentration levels at 0.10 µg/g (0.50 µg/g for Cu only) and 10 µg/g (50 µg/g for Cu only). Intraday precision was satisfactory when CV% values were below 15%. Satisfactory accuracy was achieved when the experimentally determined average concentration lied within ±15% from the expected value.

Results and discussion

Validation parameters

All the validation results are reported in Table 2. Each element showed a coefficient of determination higher than 0.995 indicating good fit and linearity for the calibration curves (0.10-10 µg/g for all elements, except for Cu, 0.50-50 µg/g). LOD values ranged from 0.012 µg/g for Pb to 0.35 µg/g for Cu, while LOQ values lied between 0.021 for Mn µg/g and 0.70 for Cu µg/g. Intraday precision and accuracy were satisfactory for most but not all analytes. In particular, at the lowest calibration level, accuracy (as bias %) for As, Cd, Cu, and Mn exceeded the accepted values, while at high concentration level (10 µg/g), only Cd exceeded the accepted values. At last, intraday precision (as CV %) exceeding the accepted interval of ±15% was observed for Co, Cr, Mn, and Pb at low concentrations, while modest deviation from 15% was observed at high concentration for As.

Analysis of real hair samples

Physiological reference values and ranges for all heavy metals in hair were determined on ten healthy volunteers, four females and six males. For women's hair, segmental hair analysis was used whenever possible, but no concentration difference was found among the segments arising from the same subject. Table 3 reports the median and reference ranges obtained thereby. Table 3 also reports the expected concentration ranges of trace metals in the hair of healthy subjects, as were determined within a systematic ICP-MS study reported in the literature (n=45) [32]. These values can be compared with those obtained within the present study from young laboratory personnel hair (n=10, six of which were browns, two blondes and two blacks) using electro-thermal atomization AAS. While the lower limits of the reference concentration ranges appear to be similar in the two studies, the same did not apply to the upper limits of Co, Cr, and Mn, that are 4-8 times higher in the present study than in the one previously published [32], despite the lower number of subjects involved in the present study. For these metals, also the median values are appreciably higher. The results obtained on the hair sample from our case in question are summarized in Table 4 and visually represented in Fig. 1. The visual examination of the data obtained from the seven segments reveals that the level of Cr in segment A (0-6 cm), approximately reflecting the last 6 months of exposure, is much higher than in the preceding segments. In fact, segments B-G have an average Cr concentration of 0.74±0.39 μg/g; a Student t-test made with the level found in segment A (5.6 μg/g) produces a residual probability $\alpha < 10^{-20}$. Analogous t-tests calculated with respect to the reference Cr levels of both the present and Goullé et al. [32] studies (see Table 3) show a highly significant difference for segment A ($\alpha < 10^{-12}$). Although no literature data are available on the Cr scalp hair concentration in patients suffering from subchronic Cr intoxication, the exceedingly high Cr concentration found in hair segment A - corresponding to the period that the patient spent abroad - is consistent with the patient's symptoms in that period and the

hypothesis of her intoxication by Cr exposure, which can produce the severe health effects actually observed (metabolic disorders, severe skin irritation, and dysentery). Indeed chromium is an essential nutrient in our diet that helps insulin to maintain normal glucose level [33]. Because $Cr^{(III)}$ is poorly absorbed by any route, the toxicity of chromium is mainly attributable to the $Cr^{(VI)}$ form. It can be absorbed by the lung and gastrointestinal tract, and even to a certain extent by intact skin. It is known that Cr toxicity is commonly associated with stomach upsets, ulcer, and kidney and liver damages [17]. Inhaling high levels of Cr can cause irritation to the lining of the nose, nose ulcers and breathing problems. Long term exposure can cause damages to the liver and kidney, and can cause circulatory and nerve disorders, as well as severe skin irritation [34]. For the remaining metals (As, Cd, Co, Cu, Ni, Pb), the concentrations found in the patient's hair segments are consistently within the expected reference ranges (see Tables 3 and 4). Only the Mn level is somehow higher than the published [32] upper reference limit, but this result is not considered significant because the Mn hair concentration was found approximately constant throughout the seven segments and lower than the upper reference limit observed in the present study (Table 3).

Conclusions

The human exposure to toxic metals is generally monitored by determining their concentrations in conventional body fluids such as blood and urine. Just recently, other non-conventional matrices, in particular scalp hair, are gaining importance in the investigation of possible excessive exposure to toxic metals. Unlike conventional matrices, human scalp hair, and particularly segmental analysis on long hair, provide historical and chronological information on trace element concentrations in the body, that portrays a unique profiling of exposure. Compared to the screening for drugs in the keratin matrix, the detection of (heavy) metals is less influenced by the redistribution along the hair shaft and less affected by washing out effects. Therefore, a segmental hair analysis is much more effective in order to obtain an over months

chronological information about poisoning/exposure with/to heavy metals. In reporting an interesting real case of alleged intoxication by Cr, the present study demonstrates that segmental hair analysis allows to compare the heavy metal exposure during a specific period of time with that during other time intervals, possibly corresponding to different external conditions (i.e., different environmental, occupational, or domiciliary exposure, or even deliberate poisoning). This unique intra-individual comparison is referred to the subject under study, and proves to be more specific than any comparison made with a generic reference population. The patient under examination presented severe symptoms possibly associated to heavy metal intoxication, and indeed the concentration of Cr in her hair segment grown during the period when she changed her domicile and living habits was found to be exceedingly high, as compared with those in the hair segments grown in preceding time periods. Moreover, the most severe symptoms that she accused (i.e. hypoglycemia, diarrhea and erythema), were consistent with the hypothesis of sub-chronic intoxication by Cr. The present study is likely to contribute to the scientific knowledge about the relationship existing between exposure to toxic metals and their expected concentration in the scalp hair grown in the corresponding period.

Conflict of Interest. The authors declare that they have no conflict of interest.

Ethical approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study

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230 **Figure caption**

231 **Figure 1** Graphical representation of metal concentrations in each segment. The
232 segmentation (cm) is reported on x-axis. The y-scale for Cu was reduced by one order
233 of magnitude

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Table 1 Experimental parameters used in electro-thermal atomization for atomic absorption spectrophotometry (AAS) analyses.

Parameters	As	Cd	Co	Cr	Cu	Mn	Ni	Pb
Wavelength (nm)	193.7	228.8	240.7	357.9	324.8	279.5	232.0	283.3
Slit-width (nm)	0.7	0.7	0.2	0.7	0.7	0.2	0.2	0.7
Pretreatment temperature (°C)	1200	700	1400	1500	700	900	1500	1900
Atomization temperature (°C)	2000	1900	2450	2300	2250	2400	2300	2450
Sample volume (µL)	20	20	20	20	20	20	20	20

As: arsenic, *Cd*: cadmium, *Co*: cobalt, *Cr*: chromium, *Cu*: copper, *Mn*: manganese, *Ni*: nichel, *Pb*: lead

Table 2 Limits of detection (LODs), limits of quantitation (LOQs), coefficient of determination (r^2), and data of accuracy and precision for determination of As, Cd, Co, Cr, Cu, Mn, Ni, and Pb by electro-thermal atomization AAS

Parameter	As	Cd	Co	Cr	Cu	Mn	Ni	Pb
LOD (µg/g)	0.10	0.050	0.10	0.12	0.35	0.021	0.050	0.012
LOQ (µg/g)	0.20	0.10	0.20	0.25	0.70	0.042	0.10	0.024
r^2	[a]0.9988	0.9962	0.9988	0.9984	0.9972	0.9999	0.9997	0.9999
Accuracy (bias %)	[b]+37.3	+21.4	+7.4	+15.7	+18.8	+18.2	+1.0	+13.3
	[c]-0.04	-39.9	+0.07	-2.8	+5.5	-1.9	+3.2	-7.9
Precision (CV%)	[b]8.9	2.0	21.2	31.1	13.4	22.3	11.0	26.3
	[c]16.5	5.4	0.8	2.2	0.6	1.3	4.4	4.2

Limits of Detection and Quantification, Squared Correlation Coefficient, Accuracy and Precision data are reported.

[a]The linearity range tested was 0.10-10 µg/g (0.50 -50 µg/g for Cu)

[b]Low concentration 0.10 µg/g (0.50 µg/g for Cu)

[c]High concentration 10 µg/g (50 µg/g for Cu)

Table 3 Comparison of heavy metal levels in hair obtained from the healthy subjects in the present study with those reported by Goullè et al. [32]

	Present study (*n*=10)		Goullé, J.P. et al. [32] (*n*=45)	
Element	**Reference range (μg/g or ppm)**	**Median (μg/g or ppm)**	**Reference range (μg/g or ppm)**	**Median (μg/g or ppm)**
As	< 0.10	-	0.03-0.08	0.05
Cd	< 0.050	-	0.04-0.17	0.011
Co	0.10-1.1	0.19	0.004-0.14	0.023
Cr	0.12-2.4	0.34	0.11-0.52	0.20
Mn	0.022-3.8	0.35	0.016-0.57	0.067
Ni	0.05-1.9	0.66	0.08-0.90	0.23
Cu	6.5-42	11	9.0-61	20
Pb	0.013-1.4	0.42	0.13-4.6	0.41

Table 4 Analytical results for the case under study. Concentration of trace elements in the scalp hair of the patient are reported as µg/g or ppm.

Segment (cm)		As	Cd	Co	Cr	Ni	Pb	Cu	Mn
A	0-6	< 0.10	< 0.10	< 0.10	5.6	0.29	0.38	16	1.1
B	6-12	< 0.10	< 0.10	< 0.10	0.26	0.15	0.32	17	1.1
C	12-18	< 0.10	0.26	< 0.10	1.1	0.26	0.31	17	1.1
D	18-24	< 0.10	0.15	0.29	0.55	0.37	0.56	19	1.8
E	24-30	< 0.10	0.25	0.22	0.39	0.94	0.77	23	1.7
F	30-36	< 0.10	0.25	< 0.10	1.1	0.57	1.6	27	1.9
G	36-40	< 0.10	0.52	< 0.10	1.1	0.52	1.8	25	2.0

Figure 1

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