ORIGINAL ARTICLE

Reliability on Intra-Laboratory and Inter-Laboratory Data of Hair Mineral Analysis Comparing with Blood Analysis

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Background: Nowadays, although its clinical value remains controversial institutions utilize hair mineral analysis. Arguments about the reliability of hair mineral analysis persist, and there have been evaluations of commercial laboratories performing hair mineral analysis. **Objective:** The objective of this study was to assess the reliability of intra-laboratory and inter-laboratory data at three commercial laboratories conducting hair mineral analysis, compared to serum mineral analysis. **Methods:** Two divided hair samples taken from near the scalp were submitted for analysis at the same time, to all laboratories, from one healthy volunteer. Each laboratory sent a report consisting of quantitative results and their interpretation of health implications. Differences among intra-laboratory and inter-laboratory data were analyzed using SPSS version 12.0 (SPSS Inc., USA). **Results:** All the laboratories used identical methods for quantitative analysis, and they generated consistent numerical results according to Friedman analysis of variance. However, the normal reference ranges of each laboratory varied. As such, each laboratory interpreted the patient's health differently. On intra-laboratory data, Wilcoxon analysis suggested they generated relatively coherent data, but laboratory B could not in one element, so its reliability was doubtful. In comparison with the blood test, laboratory C generated identical results, but not laboratory A and B. **Conclusion:** Hair mineral analysis has its limitations, considering the reliability of inter and intra laboratory analysis comparing with blood analysis. As such, clinicians should be cautious when applying hair mineral analysis as an ancillary tool. Each laboratory included in this study requires continuous refinement from now on for inducing standardized normal reference levels. **(Ann Dermatol 25(1) 67~72, 2013)**

-Keywords-
Blood minerals, Hair minerals, Minerals, Scalp hair

INTRODUCTION

Scalp hair has been widely accepted for the evaluation of toxic element exposure and has been performed by clinical laboratories since it was first used to assess systemic levels of the elements in 1929[1]. Researchers have found correlations between essential elements and diseases, metabolic disorders, environmental exposures, and nutritional status[2-7].

However, according to several studies that assessed the reliability of commercial laboratories performing hair mineral analysis, it has been suggested that using hair to measure essential element is meaningless[8-13].

The usefulness of hair mineral analysis remains controversial. Nevertheless, much technical progress has been made in the laboratory field in the last 10 years. The aim of this study was to evaluate the performance of current commercial laboratories performing hair mineral analysis in terms of intra-laboratory and inter-laboratory reliability, compared to the analysis of serum minerals.

MATERIALS AND METHODS

Enrolled laboratories

At the time of this study, there were three commercial institutions conducting hair mineral analysis in Korea. Two of them are based on Korea, and one is a branch of a large laboratory headquartered in the USA, which receives samples from Korea for analysis. All three laboratories were included in this study.

Donor selection

Hair was donated by a 23 year-old healthy, black-haired, Asian man. He had no medical or drug history. Routine blood tests and serum levels of copper, zinc, iron and magnesium were within the normal limits. Electro-cardiogram (EKG) and chest radiograph were normal. This study was approved by the Institutional Review Board of the Dankook University Hospital, and the hair donor provided informed consent prior to participation.

Methods of hair analysis

Hair was cut adjacent to the scalp from several areas of the parieto-occipital regions, using clean stainless steel scissors. Hairs measuring 3 cm from the scalp were included. Collected hairs were intermingled, weighed, and divided into 6 groups according to the amounts required by each laboratory, ranging from 60~80 mg. For each laboratory, two divided samples were submitted at the same time with different IDs.

Statistical analysis

Differences among laboratories were analyzed by Friedman analysis of variance, using the mean value of two results at each laboratory. Differences between intra-laboratory data were analyzed by the Wilcoxon signed rank sum test. In all analyses, SPSS for windows (version 12.0; SPSS Inc., Chicago, IL, USA) was used. Data were valued within a confidence interval of 95%. A p-value of less than 0.05 was considered statistically significantly different ($p < 0.05$).

RESULTS

Features of the laboratories

The features of the laboratories are summarized in Table 1. All 3 laboratories analyzed hair samples with inductively coupled plasma mass spectrometry (ICP-MS). The advantage of ICP-MS is its lower detection threshold compared to inductively coupled plasma atomic emission spectrometry (ICP-AES). The required quantity for analysis at each laboratory was 60, 60 and 80 mg, respectively. Laboratory A analyzed 30 elements, and B and C analyzed 26 and 38 elements, respectively. In total, 24 elements including aluminum, arsenic, barium, bismuth, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, molybdenum, mercury, phosphorus, potassium, selenium, sodium, sulfur, uranium, vanadium, and zinc were commonly analyzed by all 3 laboratories. Laboratories provided numerical results and interpretation, which included an evaluation of health status, prediction of possible illnessess in the future, and recommendation on dietary habit. Two laboratories (B, C) added promotions of branded supplements according to the results.

Each laboratory presented a normal reference range for each analyzed element. They were summarized in Table 2. In many elements, all 3 laboratories used different reference ranges, based on studies. As seen in Table 2, the inter-laboratory differences of the reference range for several elements were sufficiently wide in that they did not overlap.

Reliability of inter-laboratory data

The laboratories presented their results on split hair samples as illustrated in Table 3. Statistically, the Friedman test of variance indicated no significant difference between different numerical results from 6 samples ($p = 0.513$).

In the interpretation of their own results, the laboratories did not reach a general consensus although their numerical results were coherent, due to different reference ranges. As summarized in Table 4, laboratory A commented that the donor lacked iron and selenium. Laboratory B

Table 1. Characteristics of the selected laboratories

Characteristic	Laboratory A	Laboratory V	Laboratory C
Analytic instrumentation	MS	MS	MS
Hair sample weight required for analysis (mg)	60	60	80
Numbers of analyzed elements	30	26	38
Toxic elements	10	10	8
Nutrients	20	16	30

MS: mass spectrometry.

Table 2. Reference value of each laboratory

Number	Element	Laboratory A (mg%)	Laboratory B (mg%)	Laboratory C (mg%)
1	Aluminum	<16	<10	<18
2	Antimony	<0.05	NA	NA
3	Arsenic	<0.5	<1	<0.2
4	Barium	<2	<1.5	<2.6
5	Beryllium	<0.02	NA	<0.01
6	Bismuth	<0.5	<1	<0. 39
7	Boron	0.1~10	NA	0.3~9.1
8	Cadmium	<0.1	<0.15	<1.4
9	Calcium	190~738	180~760	220~970
10	Cesium	NA	<0.01	NA
11	Chromium	0.23~0.86	0.2~1.2	0.2~0.8
12	Cobalt	0~0.1	0.01~0.05	0.01~0.03
13	Copper	5~27	15~35	9~39
14	Germanium	0~0.2	NA	0.07~1.1
15	Iron	7~21	6~15	5~16
16	Lead	<2	<2	<3
17	Lithium	0~0.12	0.01~0.2	0.02~0.06
18	Magnesium	15~115	18~78	20~110
19	Manganese	0.2~0.8	0.2~0.8	0.1~1.3
20	Molybdenum	0.01~0.4	0.02~0.1	0.03~0.08
21	Mercury	<1.1	<1	<1.8
22	Nickel	NA	NA	<1
23	Phosphorus	130~220	145~250	110~200
24	Platinum	NA	NA	0~0.02
25	Potassium	10~206	5~40	20~240
26	Rubidium	NA	NA	0~0.19
27	Selenium	0.55~3.75	0.6~1.6	0.4~1.8
28	Sodium	40~480	18~85	40~360
29	Strontium	0.6~7	NA	0~5
30	Sulfur	35,000~55,000	30,000~55,000	35,460~53,360
31	Thallium	NA	<0.01	0~0.06
32	Tin	0~0.6	NA	0~0.3
33	Titanium	NA	NA	0~2
34	Tungsten	NA	NA	0.0~0.11
35	Uranium	<0.5	<1	<0.5
36	Vanadium	0.01~0.3	0.02~0.1	0.02~0.14
37	Zirconium	NA	NA	0~0.9
38	Zinc	81~175	150~250	110~210

NA: not analyzed.

identified a cobalt, copper, selenium, zinc and phosphorus deficiency. According to laboratory C, the donor was only deficient in molybdenum. As a result, these differences resulted in the laboratories recommending different nutritional supplements and treatments to the hair donor.

Reliability of intra-laboratory data

The laboratories did not reproduce identical results, but statistically they were coherent numerical results according to the Wilcoxon signed rank sum test ($p =$ 0.513).

On interpretation, laboratory B failed to produce consistent results. B detected a cobalt, copper, selenium, and zinc deficiency in one hair sample, and a deficiency in cobalt, copper, selenium, and phosphorus in another one.

Comparison of blood test and hair mineral analysis

The laboratories indicated that the donor might experience symptoms such as chronic fatigue, dizziness, insomnia, depression, and anxiety. They also claimed he was at increased risk of developing osteomalasia, skin disease, arthralgia, anemia, hypercholesterolemia, alopecia and myalagia. In actuality, the donor did not experience health problems, and his blood test, EKG, and chest radiograph were unremarkable. Specifically, his serum levels of copper, zinc, iron, and magnesium was normal, but his iron level was below the normal limit according to laboratory A. Laboratory B concluded that he was copper

Table 3. Results of generated at each laboratory

Number	Element	A-1 (mg%)	A-2 (mg%)	B-1 (mg%)	B-2 (mg%)	C-1 (mg%)	C-2 (mg%)
1	Aluminum	6	6.9	4.282	4.001	5	5
2	Antimony	0.009	0.007	NA	NA	NA	NA
3	Arsenic	0.04	0.02	0.104	0.102	0.06	0.07
4	Barium	0.91	0.89	0.531	0.599	1	0.9
5	Beryllium	0.002	0.003	NA	NA	0.01	0.01
6	Bismuth	0.01	0	0.03	0.038	0.02	0.02
7	Boron	1.91	1.85	NA	NA	0.6	1.8
8	Cadmium	0.02	0.02	0.024	0.019	0.02	0.03
9	Calcium	568	514	353.6	418	730	670
10	Cesium	NA	NA	0.001	0.001	NA	NA
11	Chromium	0.58	0.59	0.215	0.224	0.4	0.5
12	Cobalt	0.001	0	0.009*	0.009*	0.01	0. 01
13	Copper	7.9	7.8	10.58*	10.98*	9	9
14	Germanium	0.019	0.018	NA	NA	0.07	0.07
15	Iron	4.2*	4.2*	6.861	6.63	5	5
16	Lead	0.22	0.24	0.43	0.395	1	1
17	Lithium	0.06	0.056	0.012	0.012	0.01	0.01
18	Magnesium	60	55	25.33	32.98	58	50
19	Manganese	0.36	0.37	0.298	0.333	0.5	0.42
20	Molybdenum	0.017	0.014	0.023	0.023	0.02*	0.02*
21	Mercury	0.18	0.19	0.59	0.625	0.8	0.8
22	Nickel	NA	NA	NA	NA	0.1	0.1
23	Phosphorus	158	156	164.5	141.6*	150	150
24	Platinum	NA	NA	NA	NA	0.01	0.01
25	Potassium	117	140	17.04	16.13	100	120
26	Rubidium	NA	NA	NA	NA	0.104	0.124
27	Selenium	0.44*	0.37*	0.528*	0.517*	0.4	0.4
28	Sodium	216	274	35.93	31.74	210	240
29	Strontium	2.37	2.25	NA	NA	3.3	2.8
30	Sulfur	42080	41595	33262	34244	39240	40270
31	Thallium	NA	NA	0.001	0.001	0.005	0.005
32	Tin	0.096	0.081	NA	NA	0.1	0.1
33	Titanium	NA	NA	NA	NA	0.7	0.6
34	Tungsten	NA	NA	NA	NA	0.01	0.01
35	Uranium	0.01	0.01	0.015	0.006	0.005	0.005
36	Vanadium	0.076	0.072	0.033	0.037	0.04	0.05
37	Zirconium	NA	NA	NA	NA	0.1	0.1
38	Zinc	173	166	136*	155.5	190	180

NA: not analyzed. *Above or below normal reference range.

Table 4. Elements below the normal reference range at each laboratory

	A-1	A-2	B-1	B-2	C-1	C-2
Elements	Iron Selenium	Iron Selenium	Cobalt Copper Selenium Zinc	Cobalt Copper Selenium Phosphorus	Molybdenum	Molybdenum

and zinc deficient (Table 5).

DISCUSSION

Hair analysis provides good evidence in the fields of forensic and clinical toxicology, doping control, and occupational medicine, but do not demonstrate clear evidence in the fields of general health screening. While urine and blood tests show the recent and current body status, hair represents a longer time frame, which can incorporate many years[1]. Since the elements are present in the hair at higher levels, more sensitive and accurate analysis results can be expected[9]. As such, it has been suggested that hair mineral analysis can be a cost-effective

Table 5. Comparison of the result between blood test and hair mineral analysis

Element	Blood test	Laboratory A	Laboratory B	Laboratory B
Iron	Normal	Below normal*	Normal	Normal
Magnesium	Normal	Normal	Normal	Normal
Zinc	Normal	Normal	Below normal*	Normal
Copper	Normal	Normal	Below normal*	Normal
Selenium	Not done	Below normal*	Below normal*	Normal

*Below normal reference range.

means of screening an individual, or monitoring a patient during putative toxic exposure or its therapy[10].

However, the analysis of elements in hair remains controversial. There exists little or no evidence to support hair as a true biological marker. Analytical accuracy and lack of standardization have been cited as the main challenge. In the field of dermatology, trichothiodystrophy is a well known candidate disease for hair mineral analysis, but other skin diseases including hair diseases are not. In 1985, Barrett[8] asserted that commercial use of hair analysis is unscientific and economically wasteful after assessing the reliability of commercial hair mineral test results. Seidel et al.[11] concluded that commercial laboratories failed to generate consistent results in 2001. Seidel et al.[11] also called the varied normal reference ranges into question. In a similar study conducted in Germany in 2002, the accuracy of hair mineral analysis was refuted[12].

In a matter of accuracy, it displayed different results in our study compared to those previously. All 3 commercial laboratories generated coherent results on identical hair samples in repeated analyses. There were imperceptible numerical gaps, but were deemed insignificant by statistical analysis. This may partly be due to identical analysis methods, unlike those studied previously. The non-standardized reference ranges remained a major challenge. In inter-laboratory comparison, each laboratory gave different interpretations on the same patient, because they applied different normal ranges. Insufficient elements were detected and possible predicted future health problems varied, and each laboratory recommended different nutritional supplements.

In intra-laboratory comparison, laboratory A, C made the same interpretations but laboratory B did not. Laboratory B needs to improve its detection consistency.

Compared with serum copper, zinc, iron and magnesium, laboratory A gave a different interpretation in iron and B gave one in copper, and zinc. Laboratory C made an identical interpretation in all three elements. These differences represent a challenge in the evaluation of a patient's health. More research is required to evaluate for the correlation between clinical state, hair mineral analysis and blood test.

For now, it appears that we can apply hair analysis carefully as an ancillary tool to evaluate patients, in addition to history taking, physical examination, diet, lifestyle, the environment, and other laboratory data because each laboratory can generate precise numerical results[1,14]. However, hair mineral analysis cannot be a primary tool for the assessment of individual health without a well-established normal reference range, because the interpretation of results varies greatly according to the wide diverse reference range of each laboratory. It remains a challenge to establish 'normal' reference ranges for minor and trace elements in human hair due to the natural variance of hair compositions[9]. Thus, well-designed large-scale studies are needed to standardize international reference ranges for hair mineral elements.

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