

Metallomics study using hair mineral analysis and multiple logistic regression analysis: relationship between cancer and minerals

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Received: 25 December 2008 / Accepted: 14 May 2009
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Abstract

Objectives The objective of this metallomics study is to investigate comprehensively some relationships between cancer risk and minerals, including essential and toxic metals.

Methods Twenty-four minerals including essential and toxic metals in scalp hair samples from 124 solid-cancer patients and 86 control subjects were measured with inductively coupled plasma mass spectrometry (ICP-MS), and the association of cancer with minerals was statistically analyzed with multiple logistic regression analysis.

Results Multiple logistic regression analysis demonstrated that several minerals are significantly correlated to cancer, positively or inversely. The most cancer-correlated mineral was iodine (I) with the highest correlation coefficient of $r = 0.301$, followed by arsenic (As; $r = 0.267$), zinc (Zn; $r = 0.261$), and sodium (Na; $r = 0.190$), with $p < 0.01$ for each case. In contrast, selenium (Se) was

inversely correlated to cancer ($r = -0.161$, $p < 0.05$), followed by vanadium (V) ($r = -0.128$). Multiple linear regression value was highly significantly correlated with probability of cancer ($R^2 = 0.437$, $p < 0.0001$), and the area under the receiver-operating characteristic (ROC) curve was calculated to be 0.918. In addition, using contingency table analysis and the chi-square test, the precision of discrimination for cancer was estimated to be 0.871 (chi-square = 99.1, $p < 0.0001$).

Conclusions These findings suggest that some minerals such as arsenic, selenium, and probably iodine, zinc, sodium, and vanadium contribute to regulation of cancer and also that metallomics study using multiple logistic regression analysis is a useful tool for estimating cancer risk.

Keywords Cancer · Metallomics · Arsenic · Selenium · Multiple logistic regression analysis

“Metallomics,” as well as genomics, transcriptomics, proteomics, metabolomics, and so on, is one of the “omics” sciences investigating metals and metalloids comprehensively; this term was coined by Prof. Haraguchi in 2001. A new international journal named *Metallomics* has been published since 2008 by the Royal Society of Chemistry.

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Introduction

Cancer has been the first cause of death in developed countries, and worldwide there are more than 10 million new cancer cases each year [1, 2]. Therefore, a large number of experimental and epidemiological studies have been undertaken to identify potential risk factors for cancer, among which the association with some carcinogenic metals such as cadmium, nickel, beryllium or arsenic has been reported [2–5]. However, there is no metallomics study comprehensively examining the relationship between cancer risk and multiple biometals.

For the last few years, we have measured scalp hair minerals in over 60,000 subjects, ranging from infant to elderly, in order to assess exposure to toxic metals, deficiency/excess profile of essential minerals, and some

relationships between minerals and physical or mental disorders [6–8].

The purpose of this study is to examine comprehensively some roles of toxic and essential minerals in cancer risk. Twenty-four kinds of minerals in hair samples were measured for cancer and control subjects aged 20–80 years, and the association between cancer and these multiple minerals was examined with multiple logistic regression analysis.

Materials and methods

Materials

On the basis of informed consent, scalp hair samples (about 0.2 g) were collected from 124 solid-cancer patients suffering from breast ($n = 28$), stomach ($n = 22$), lung ($n = 11$), colon ($n = 10$), prostate ($n = 9$), liver ($n = 7$), pancreas ($n = 5$), uterus ($n = 5$), ovary ($n = 4$), esophagus ($n = 4$), malignant lymphoma ($n = 4$), kidney ($n = 3$), thyroid ($n = 2$) or other tumors who had been cared for and treated in Somon Hachioji Clinic (Table 1). In addition, control hair samples from healthy subjects aged 20–80 years old from the Tokyo metropolitan district were collected. The study protocol was approved by the ethical committee of this laboratory. All of the data obtained are held securely in such a form as to ensure anonymity.

Hair mineral analysis

Hair sample of 75 mg was weighed into 50 ml plastic tube, and washed twice with acetone and then with 0.01% Triton solution, in accordance with the procedures recommended by the Hair Analysis Standardization Board [9]. The washed hair sample was mixed with 10 ml 6.25% tetramethylammonium hydroxide (TMAH, Tama Chemical) and 50 μ l 0.1% gold solution (SPEX Certi Prep.), and then dissolved at 75°C with shaking for 2 h. After cooling of the solution to room temperature, internal standard (Sc, Ga, and In) solution was added and, adjusting its volume gravimetric, the obtained solution was used for mineral analysis. The mineral concentrations were measured with

inductively coupled plasma mass spectrometry (ICP-MS; Agilent-7500ce) by the internal standard method [6–8, 10], and are expressed as ng/g hair (ppb). For quality control of the mineral analysis, the human hair certified reference material supplied from the National Institute for Environmental Studies of Japan (NIES CRM no. 13) [11] was used.

Statistical analysis

The hair mineral concentrations were log-normal distributed, and geometric rather than arithmetic means were used as representative of hair mineral levels. For statistical analysis, the values of mineral levels were converted to logarithms. The relation between cancer risk and 24 minerals was investigated with multiple logistic regression analysis (JMP6; SAS Institute).

Results

Twenty-four kinds of mineral concentrations in the scalp hair of 124 solid-cancer patients and 86 control subjects were measured, and the association between cancer and each mineral was statistically examined with multiple logistic regression analysis. Some minerals were found to be significantly correlated to cancer, positively or inversely, and the multiple coefficient of determination (R^2) for estimating the probability of cancer risk was estimated to be 0.465 (Table 2). The most cancer-correlated mineral with the highest partial correlation coefficient was iodine (I; $r = 0.301$), followed by arsenic (As; $r = 0.267$), zinc (Zn; $r = 0.261$), and sodium (Na; $r = 0.190$), with $p < 0.01$ for each element. These correlation coefficients were higher than that of ageing, a well-known risk factor of cancer. Regression charts for the best three minerals exhibiting significantly high positive correlation with cancer are shown in Fig. 1a–c. In contrast, the most inverse-correlated mineral to cancer was selenium (Se; $r = -0.161$) (Fig. 1d), followed by manganese (Mn) and vanadium (V) (Table 2).

Figure 2 exhibits a significantly high correlation of cancer with multiple linear regression value obtained by the following equation ($R^2 = 0.447$, $p < 0.0001$):

$$\begin{aligned} \text{Multiple linear regression} = & \\ & \pm 0.70 (\text{Sex}) + 0.023 \times \text{Age} \\ & + 1.75 \times \text{Log I} + 3.59 \times \text{Log As} \\ & + 5.38 \times \text{Log Zn} + 6.67 \times \text{Log Fe} \\ & + 2.04 \times \text{Log Na} - 6.47 \times \text{Log Se} \\ & - 1.48 \times \text{Log K} - 1.47 \times \text{Log Mn} \\ & - 50.88 \end{aligned}$$

Thus, the area under the receiver-operating characteristic (ROC) curve for discriminating cancer was

Table 1 Tested subjects ($n = 210$)

	No.	Age (mean \pm SD), years
Male		
Cancer	52	60.9 \pm 12.2
Control	53	51.6 \pm 11.9
Female		
Cancer	72	54.9 \pm 10.6
Control	33	53.7 \pm 16.0

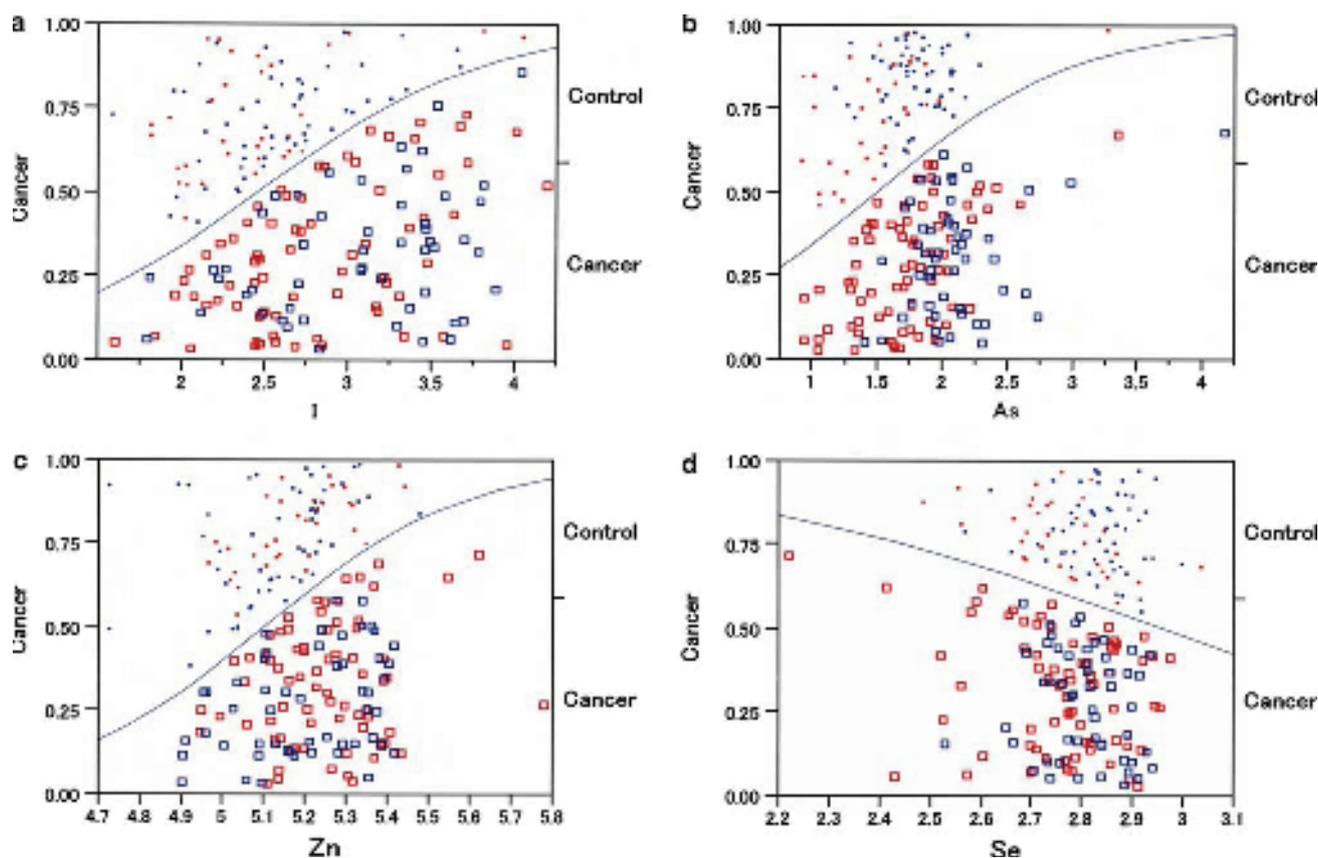


Fig. 1 Positive or inverse relationship between cancer and minerals in multiple logistic regression analysis (**a** iodine, **b** arsenic, **c** zinc, **d** selenium). The relationship between minerals and probability of cancer was obtained by logistic multiple regression analysis of data for 24 minerals from 124 cancer (*open square*) and 86 control (*dots*)

subjects. The abscissa and ordinate express the logarithmic values of hair mineral (**a** iodine, **b** arsenic, **c** zinc, **d** selenium) level and probability of cancer, respectively. The *blue* and *red* marks show male and female subjects, respectively

estimated as 0.918 for multiple linear regression, and significantly greater than 0.704 for iodine, 0.647 for arsenic, and 0.662 for zinc. The accuracy of the discrimination of cancer was also assessed using contingency table analysis and the chi-square test (Table 3). From the contingency table, the precision of discriminating cancer patients was estimated to be 0.871 ($108/124 = 87.1\%$; chi-square = 99.1, $p < 0.0001$).

The mean hair arsenic level in the patient group was significantly higher than that in the control group, in both sexes, and gender difference was observed (Table 4).

Discussion

Hair mineral analysis has been used in forensic medicine, in screening populations for toxic metal poisoning, and in monitoring environmental pollutants [6, 12–14]. Furthermore, its clinical use has been tried for diagnosis of some diseases and symptoms [7, 8], but reliability remained to be confirmed [15, 16]. Our previous studies demonstrated that

hair levels of some toxic metals such as lead, cadmium, and aluminum are extraordinarily high in infants and children [6, 17]. In addition, we reported that autistic children are suffered from global mineral deficiency of various essential trace elements and also that, in autistic children, there are some subgroups with high accumulation of mercury, cadmium, and lead, or aluminum and iron [7].

In the present metallomics study for cancer patients, the multiple logistic regression analysis exhibited a significant association between some minerals and cancer. In particular, carcinogenic arsenic and its antagonistic element, selenium, was confirmed to correlate to cancer risk positively and inversely, respectively (Fig. 1b, d, Table 2).

A significantly high close relationship between probability of cancer and multiple linear regression value was demonstrated ($R^2 = 0.447$, $p < 0.0001$) (Fig. 2), and the corresponding area under the receiver-operating characteristic (ROC) curve for diagnosis of cancer was estimated to be 0.918. Furthermore, this close relationship was confirmed by the results of contingency table analysis: the precision of discrimination for cancer patients was

Table 2 Relationship between cancer risk and minerals (multiple logistic regression analysis)

	Part. coeff.	Corr. coeff.	p value	Significance		Part. coeff.	Corr. coeff.	p value	Significance
I	0.3012	0.3484	0.0000	****	Se	-0.1614	-0.1114	0.0282	*
As	0.2668	0.2319	0.0002	***	Mn	-0.1533	0.1337	0.0372	*
Zn	0.2612	0.2754	0.0003	***	V	-0.1279	-0.1237	0.0827	
Na	0.1903	0.2172	0.0095	**	K	-0.0722	0.0747	0.3291	
Br	0.1839	0.2213	0.0122	*	Mg	-0.0661	0.0793	0.3711	
Fe	0.1275	0.2623	0.0837		Ni	-0.0456	0.0003	0.5377	
Al	0.0873	0.0189	0.2375		B	-0.0372	-0.0204	0.6149	
Cr	0.0849	0.0821	0.2504		Mo	-0.0227	-0.1123	0.7595	
Co	0.0804	0.1554	0.2767		P	-0.0140	0.0918	0.8499	
Ca	0.0766	0.0947	0.3000						
Cu	0.0617	0.1186	0.4043						
Ge	0.0551	0.2917	0.4566						
Pb	0.0072	0.0486	0.9225						
Cd	0.0051	0.1087	0.9446						
Hg	0.0016	-0.0396	0.9833						
Age	0.1491	0.1955	0.0428	*					

Determination coefficient: 0.465

The relationship between cancer and minerals was analyzed with multiple logistic regression analysis of data for 24 minerals from 124 cancer patients and 86 control subjects

Part. coeff. partial correlation coefficient by logistic multiple regression analysis, *Corr. coeff.* simple correlation coefficient by simple regression analysis

Statistically significant association is shown with asterisk marks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$

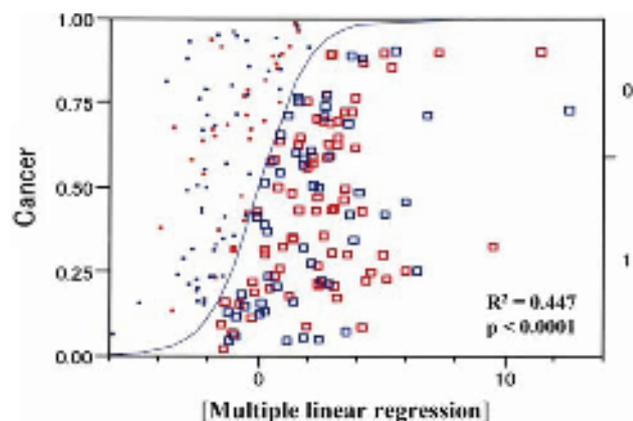


Fig. 2 Significantly high relationship between cancer and multiple linear regression value in multiple logistic regression analysis. The relationship between multiple linear regression value and probability of cancer was obtained by multiple logistic regression analysis of data for 24 minerals from 124 cancer (*open square*) and 86 control (*dots*) subjects. The abscissa and ordinate express the calculated multiple linear regression value of the tested subjects and the probability of cancer, respectively. The *blue* and *red* marks show male and female, respectively

estimated to be 0.871 ($108/124 = 87.1\%$) and the accuracy to be 0.838 [$(108 + 68)/210$ (chi-square = 99.1, $p < 0.0001$)] (Table 3).

These findings suggest that there is a significantly high close relationship between probability of cancer and trace bioelements, including toxic and essential minerals.

The multiple coefficient of determination ($= R^2$) for estimating the probability of cancer risk was calculated to be 0.465 ($p < 0.0001$). This means that the cancer risk of an individual case can be evaluated with 46.5% probability by using hair mineral analysis and multiple logistic regression analysis.

From the regression curve shown in Fig. 1b, the probability of cancer in a subject with a logarithmic arsenic value of 2.5 was estimated to be about 80%, and about 30% increment of the probability was estimated to be associated with a tenfold increase in hair arsenic level from 10 to 100 ppb (odds ratio 36.2; 95% CI 7.1–234). Arsenic has been known to be toxic and induce chromosomal aberrations and carcinogenesis via various cellular changes including alterations in cell differentiation and proliferation [4, 18, 19]. Cells exposed to arsenic have been shown to increase cellular tyrosine phosphorylation, which is related to the aberrant cell signaling and uncontrolled cell growth associated with cancer development. Some ecologic studies in areas known for high levels of arsenic in drinking water have suggested that this element is associated with increased risks of lung and bladder cancer [4, 20–23]. In

Table 3 Contingency table

	No.	Judgment	
		Negative	Positive
Cancer	124		
Count		16	108
Row %		0.129	0.871
Control	86		
Count		68	18
Row %		0.791	0.209
	210	84	126

Using contingency table analysis and the chi-square test, the accuracy and precision of discrimination of cancer patients was assessed
 chi-square = 99.1 ($p < 0.0001$)

Table 4 High arsenic concentrations in cancer patients

	No.	Hair arsenic concentration		<i>p</i> value
		Geomean	Log As (mean ± SD)	
Male				
Control	53	61.9	1.7914 ± 0.2425	
Cancer	52	123.7	2.0924 ± 0.4173	0.0000****
Female				
Control	33	31.4	1.4975 ± 0.4324	
Cancer	72	52.9	1.7231 ± 0.4058	0.0141*

The hair arsenic concentrations in the 124 cancer patients are compared with those in the 86 control subjects. Each value of arsenic concentration was converted to its logarithm, and the geometric mean was used as the average hair arsenic level. Statistical significance was determined using Welch's *t* test

Statistically significant difference is shown with asterisk marks: * $p < 0.05$ and **** $p < 0.0001$

Japan, much of the arsenic accumulated in the human body is known to derive from dietary intake of arsenic-containing foods, such as seaweeds and shellfishes, and also from environmental exposure.

The most strongly correlated mineral, iodine, possibly makes some contribution to cancer pathogenesis, although its association with cancer remains to be studied further.

Zinc has an important role in cell-mediated immune functions, and zinc deficiency was reported to be associated with increased risk of cancer [24–26]. However, in this metallomics study, zinc level in hair was found to be positively correlated with cancer risk (Table 2; Fig. 1c). This discrepancy may be explained by the hypothesis that hair zinc level behaves paradoxically when deficient in the human body. Further studies are needed for clarifying the role of zinc in cancer risk.

Selenium, a representative competitive mineral against arsenic, correlated inversely with cancer risk; the higher the level of this element, the lower the probability of cancer

risk (Fig. 1d; Table 2). This finding is consistent with the hypothesis of anticancer activity of selenium. Selenium is the key element of glutathione peroxidase and other selenium proteins, and is essential for counteracting reactive oxygen species, protecting from oxidative stress, and regulating the redox status in cells [2]. This essential mineral also appears to correlate with stimulation of metabolic detoxification and excretion of arsenic [27, 28]. Thus, selenium is expected to reduce the toxicity and cancer risk of arsenic [29, 30], although high body burden of selenium is toxic. Furthermore, selenium is known to have an ability to compete with other toxic metals such as mercury, cadmium, and lead.

The present study suggests that some minerals such as not only arsenic and selenium but also iodine, zinc, sodium, and vanadium play a pivotal role in cancer pathogenesis, indicating that metallomics analysis using multiple logistic regression analysis seems to be a good tool for estimating cancer risk. Further studies for each type/nature of solid cancer are needed for certifying the usefulness of this analysis.

Hair mineral analysis can provide global information for human metallomics study, i.e., the deficiency/excess profile of multiple trace bioelements that contribute to various physiological and/or adverse events, and therefore will be useful as a biomarker for monitoring health and risk assessment.

Conclusion

This metallomics study for cancer patients demonstrated that there is a close relationship between cancer risk and trace bioelements including toxic and essential minerals, and that many patients have some mineral imbalance in their body. These findings suggest the possibility that metallomics study using hair mineral analysis and multiple logistic regression analysis will be a useful tool for estimating cancer risk and also for cancer prevention.

Acknowledgments The authors sincerely appreciate Emeritus Prof. Toshiro Haga for his useful advice regarding the statistical analysis based on multiple logistic regression analysis in this study.

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