대한예방한의학회지 제13권 제1호(2009년 4월)
Kor. J. Oriental Preventive Medical Society 2009 ; 13(1) : 93-103

Cross-sectional Study for Blood Metal Concentration in Patients with Herbal Medicine Intake

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Abstract

Evidences from various countries suggest that toxic heavy metals in herbal medicine may constitute a serious health problem. In order to evaluate whether the toxic heavy metals caused by herbal medicine intake, blood samples collected from 222 patients taking herbal medicine were analyzed. In average levels of analyzed metals, 0.4~33.9% of total samples for 8 metals such as Cd, Co, Cu, Hg, Mn, Ni, Pb and Zn except Cr and Fe exceeded the upper limit for WHO reference value. In analysis of regression coefficients indicating the levels of metals increased or decreased after taking herbal medicine for one month, however, there were different aspects by intake types for herbal medicine. For example, the metals increased by taking decoction in blood samples were as follows; Cd and Pb whether Mn, Ni and Pb as increased metals were identified in the group taking pill and decoction(combined intake group). The odds ratio showing values higher than 1 indicating that people who take herbal medicine would have possibility higher for metal accumulation in blood than that from people who do not take herbal medicine. The metals showing the odds ratio higher than 1 were Hg and Ni in decoction group, and Cd and Hg in combined intake group. However, eight of the total, 10 metals showed the odds ratios lower than 1 by taking herbal medicine. Thus, this may explain the possible role of herbal medicine as a chelator for heavy metals in body.

Key words : Herbal Medicine Intake, Blood Metal Concentration, Cross-sectional Study

· 접수 : 2009년 4월 15일 · 수정접수 : 2009년 4월 24일 · 채택 : 2009년 4월 25일

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I. Introduction

Some herbal remedies may do more harm than good. It was reported that 20percent of herbal medicine products sampled contained dangerous levels of heavy metals.[1-3] Use of medical herbs for heavy metal pollutants in man and animals is receiving attention worldwide. Most of the herbs were collected from hills, forests, and areas least inhabited by humans. Ironically, medicinal plants today are cultivated commercially in polluted environments, where soil, water, and air contain rather high levels of heavy metals. Indeed, one obvious safety issue relates to the possibility that some herbs contain heavy metals[1), 4), 5)] as herbal medicine was found to be responsible for several cases of metal poisoning.

However, the methods for taking herbal medicine would influence greatly on uptake or accumulation of the heavy metal in human body even if heavy metals are contaminated in herbal materials. In western countries, raw plant materials are directly taken as dietary supplements which is processed or not. This direct uptake would give people more chance to be exposed by heavy metals than decoction which is defined as herbal medicine boiled and filtered in water to yield herbal extract. In korea the herbal medicine are usually taken as decoction type. This type would cause lower possibility for heavy metals to be taken and accumulated in body because of the process such as extraction filtration. In our previous study,[6)] indeed, the levels of heavy metals from 308 patients taking herbal medicine through decoction and pill were analyzed in urine and hair. some of metals in samples from people taking herbal medicine showed the decreased level the low odds ratio compared to those of the control group. This may explain the possible role of herbal medicine as a chelator for heavy metals in body.

In the previous study,[6)] concentrations of heavy metals in urine and hair were analyzed in urineand hair from patients taking herbal medicine through decoction and pill. Some of metals in samples from people taking herbal medicine in the previous study showed the decreased level the low odds ratio compared to those of the control group. In spite of the possible role of herbal medicine as a chelator, there was still numerous case reports and case series of heavy metal poisoning associated with the use of herbal medicine. In terms of serial study, concentrations of heavy metals were analyzed in blood rather than in urine and hair from patients taking herbal medicine through decoction and pill. This study would be helpful to understand one of possible roles of herbal medicine as a chelator for metals was evaluated. Furthermore, considering different methods of taking herbal medicine, such as decoction and pill type, it is believed that differences in methods would affect the physiological concentration or accumulation of metals.

II. Materilas and Methods

1. Study Population

Patients with Cerebral Vascular accident, low back pain and other various disease were selected from one of Oriental Medical Clinics located in Wonju, Korea. The total of 222 spot blood samples were collected from patients admitted between Sep, 2000 and Jan, 2001. Patients were classified into three groups as non-herbal group, herbal group and combined group based on therapy tools. The herbal group had taken herbal medicine as decoction for duration ranged from 2 to 130 days(mean ≒ 26 days). The combined group had taken both decoction, pill and powder, simultaneously for duration ranged from 1 to 100 days(mean ≒ 20 days). "Decoction" is defined as herbal medicine boiled in water to yield herbal extract. A "pill" is a mixture of raw herbs without boiling. "Powder" is a herbal medicine in powder form. Patients were asked to fill out a questionnaire relating to job history, residence, alcohol intake, smoking, etc. A comparison of general characteristics between the subjects with complete data and those with missing data was summarized in table 1.

2. Blood collection

Approximately 10ml of blood was drawn from each subject, 4ml of which poured into tube which contained K-EDTA as an anti-coagulant and mixed for 10minutes. Blood samples for elemental analysis were stored

Table1. Socio-demographic characteristics of the groups(N=222)

	Non-herbal(%)		Decoction only(%)		Combined intake(%)		p-value
Age(years)	34	13.3%	49	22.1%	139	62.6%	
Sex							0.818
Male	14	41.2%	20	40.8%	51	36.7%	
Female	20	58.8%	29	59.2%	88	63.3%	
Marriage							0.203
Yes	33	100%	46	93.9%	127	91.4%	
No			3	6.1%	12	8.6%	
Alcohol							0.918
Yes	21	74.4%	36	73.5%	105	7.5%	
No	8	27.6%	13	26.5%	34	24.5%	
Smoking							0.251
Yes	22	75.9%	33	67.3%	110	79.1%	
No	7	24.1%	16	32.7%	29	20.9%	
Residential area							0.539
Rural	8	27.6%	19	38.8%	44	31.7%	
Not rural	21	72.4%	30	61.2%	95	68.3%	
Job							0.337
Farmer & Fishermen	7	24.1%	14	28.6%	24	17.3%	
Service	17	58.6%	22	44.9%	83	59.7%	
Etc	5	17.2%	13	26.5%	32	23.0%	
Disease							0.440
Non-stroke	20	58.8%	25	51.0%	65	46.8%	
Stroke	14	41.2%	24	49.0%	74	53.2%	

at -20°C until analysis. Samples were diluted 5 times for serum with high purity deionized water.

3. Sample pretreatment and metal analysis

Sample pretreatment and analytical methods followed US EPA procedures, in part, Goulle's and Mortada's study.[7-9)]

Cd, Co, Cr, Cu, Fe, Hg Mn, Ni, Pb and Zn : Samples were treated using 65ml of cleaning solution(186ml of ethyl alcohol, 93ml of acetone and 371ml of n-hexane to 1L volumetric flask) for 12hours. Each aliquot(0.1~0.2g) from well homogenized samples was moved to a digestion vessel. The aliquots were mixed with 10ml of 1 : 1 HNO_3, and then covered with a watch glass. Treated samples were heated to 95±5 and refluxed for 10 to 15 minutes without boiling. After adding 5ml of concentrated HNO_3, the samples were allowed to be cool and were refluxed for 30 minutes. This step was repeated until no brown fume was given off by the sample, showing the complete reaction with HNO_3. Using a ribbed watch glass, the solution was evaporated to approximately 5ml at 95±5℃ for two hours. The samples were cooled again and 3ml of 30% H_2O_2 was added. In order for the peroxide reaction the vessels were covered with a watch glass and placed on the heat source for warming until effervescence subsides. Then peroxide solution was added in 1 ml aliquots. The aliquots were warmed until the effervescence was minimal. The aliquots were covered with a ribbed watch glass. The acid-peroxide digestate of the aliquot was heated until the volume was reduced to approximately 5ml. The solution was covered over the bottom of the vessel at all times. After cooling, it was diluted to 50ml with water. The particulates in digestates were then removed by filtration. The filtered samples were analyzed by ICP-MS.[7)] The ICP-MS analysis for seven elements(Cu, Cd, and Pb) was carried out using ICP-MS spectrometer Varian Ultramass 700(USA, 1998) with cross flow nebulizer. The operating conditions are given in Table 2.

Table 2. ICP-MS operating conditions

Nebulizer	Babington
Spray chamber	Scott, 2°C
Radio Frequency(RF) power	1300W
Sampling depth	6.4 mm
Plasma gas flow rate	15.0 l min^{-1}
Auxiliary gas flow rate	1.0 l min^{-1}
Nebulizer gas flow rate	1.05 l min^{-1}
Sampler	0.5 mm, Ni
Skimmer	0.5 mm, Ni
Integration time	0.3 s/channel (three channels per mass)
Repetitions	5

Hg : Samples were treated by using 65ml of cleaning solution(186ml of ethyl alcohol, 93ml of acetone and 371ml of n-hexane to 1L volumetric flask) for 12hours. Each aliquot (0.1, 0.2g) from well homogenized samples was placed in the bottom of a BOD bottle. The aliquots(0, 1, 3, 5ml) of mercury working standard containing 0~5ug/L of mercury were transfer to a series of BOD bottles. The reagent water(5ml) and concentrated sulfuric acid(5ml) were added to the aliquots of standard and sample. Also 2.5ml of concentrated Nitric acid was added to them and then they were heated for two minutes at 95±3℃. After cooling, samples and standard were added by 15ml of 5% potassium permanganate solution and mixed. They were heated for 30minutes at 95±3℃. After cooling, they were mixed with sodium chloride-hydroxylamine hydrochloride to reduce the excess permanganate. The standard and sample were diluted to 100ml with de-ionized water. The particulates in digestates were removed by filtration. The filtered samples and standards were analyze by mercury analyzer(USA, Cetac, M-6000A).

4. Reagents

All reagents were of analytical reagent grade. High purity de-ionized water(Milli-Q system, Millipore, USA) was used throughout. Analytical reagent nitric acid(Merck, 70%) was used after additional purification by sub-boiling distillation in quartz still. Plastic bottles and glassware were cleaned by soaking in 20%(v/v) HNO_3 for 24h. This material was then rinsed three times with de-ionized water.

5. Statistics

Statistical analyses were performed separately by two groups. We calculated geometric means for Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn in blood samples of the test subjects. We performed ANOVA to compare the geometric means between the experimental groups and the control group. We also calculated percents exceeding the upper limit of the reference value ranges for each metal. We adjusted for potential confounders such as age, sex, smoking, drinking, job, and residence in the ANOVA. Multiple regression values(β(SE)) for duration taking herbal medicine were calculated after adjusting for potential confounders. Dependent variables were the natural logarithm of each metal level plus one, for example, log(Al+1). Odds Ratios were taken for exceeding reference values in blood, adjusting for potential confounders. Odds ratios of two groups were compared after adjusting for potential confounders. For statistical analysis, Stata(2001) statistical package was used for mean ± standard deviation(SD), geometric mean, ANOVA, multiple regression, and odds ratios.[10)]

III. Results

Table 3 shows arithmetic mean and standard deviation(SD) of heavy metal concentrations in blood from all samples. Also, WHO reference values(trace elements in Human Nutrition and Health) for heavy metals analyzed are listed and compared to those of samples. There were 1.40 ± 3.64, 0.47 ± 1.11, 144.93 ±

Table 3. Arithmetic mean(AM) and SD of various metal concentrations in blood(N=222)

Variables (㎍/L)	Min value	Max value	AM	SD	% exceeding upper limit reference	Reference value(WHO)[11]
Cd	N.D	50.6	1.4	3.6	31.7%	0.3-1.2[a], 1-4[b]
Co	N.D	14.9	0.5	1.1	0.4%	5-10
Cr	N.D	708.9	144.9	87.8	-	-
Cu	192.8	3295.7	689.3	266.2	0.9%	800-1100[c], 1000-1400[d]
Fe	147569.6	963720.3	390789.2	94978.2	-	-
Hg	N.D	188.6	13.1	24.8	20.4%	2-20
Mn	N.D	1525.6	32.5	137.4	33.9%	8-12
Ni	N.D	175.2	11.4	25.7	31.7%	1-5
Pb	N.D	426.3	36.8	53.2	3.5%	50-150
Zn	1738.9	9743.6	3730.1	962.6	0.9%	6000-7000

a : non-smoker, b : smoker, c : males, d : females

Table 4. Adjusted geometric mean* and SD of metal concentrations in blood by groups

Variables (㎍/L)	Non-herbal (N=24)	Decoction only (N=49)	Combined intakes† (N=139)	p value
log Cd	0.61(0.64)	0.59(0.53)	0.62(0.62)	0.93
log Co	0.32(0.32)	0.28(0.25)	0.30(0.37)	0.84
log Cr	4.83(0.63)	4.85(0.47)	4.79(0.71)	0.85
log Cu	6.49(0.38)	6.52(0.27)	6.48(0.30)	0.70
log Fe	12.87(0.25)	12.85(0.21)	12.84(0.25)	0.74
log Hg	1.28(1.45)	1.39(1.54)	1.65(1.50)	0.33
log Mn	1.93(1.96)	1.81(1.82)	1.71(1.67)	0.75
log Ni	1.29(1.62)	1.44(1.61)	1.08(1.45)	0.33
log Pb	2.62(1.72)	2.28(2.10)	2.35(1.89)	0.70
log Zn	8.21(0.28)	8.19(0.22)	8.20(0.23)	0.97

* Geometric means(SD) are natural logarithm of each metal level plus one, for example, log(Cd+1).
† Combined intake : administration of both decoction, pill and powder

87.79, 689.30 ± 266.22, 390,789.20 ± 94978.15, 13.05 ± 24.88, 32.50 ± 137.44, 11.42 ± 25.74, 36.83 ± 53.23 and 3730.09 ± 962.62㎍/ℓ blood for Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn, respectively. Compared to the range of WHO reference value for each metal, 0.4~33.9% of total samples for metals except Cr and Fe exceeded the upper limit for WHO reference value.

Table 4 shows geometric means and SD of metal concentrations after adjusting potentially confounding factors such as age, sex, smoking, drinking, job, and residence. The p-value range for all metal concentrations at the level of p = 0.05 between non-herbal group and herbal groups such as decoction and combined intakes was from 0.33~0.97. Especially, the adjusted geometric mean for Zn showed a significant difference between non-herbal group and herbal groups.

Table 5 presents regression coefficients and standard error(SE) for duration of herbal intake from multiple regressions of metals in blood samples. The regression coefficients indicate the levels of metals increased or decreased in blood after taking herbal medicine for one month, compared to those of the non-herbal group. Regression coefficient for duration of decoction intake ranged from -0.21 to 0.30μg/kg blood for all metals. Regression coefficient for duration of combined intake ranged from -0.01 to 0.16μg/kg blood for all metals. However, all metals did not show any significance in values of regression coefficients. This indicates that all levels of 10 metals would not be increased in blood when patients take herbal medicine for one month.

Table 6 presents odds ratio and standard error from case-control study, using the samples which exceed the upper limit of WHO reference values. There were 0.96, 0.95, 0.96, 0.97, 0.98, 1.34, 0.90, 1.19, 0.56 and 0.99 as an odds ratio for Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn in decoction group against non-herbal group. There were 1.03, 0.98, 0.88, 0.91, 0.96, 1.79, 0.75, 0.89, 0.46 and 0.98 as an odds ratio for Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn in combined intake group against non-herbal group. There were two of the total metals such as Hg and Ni in a case of decoction group, and two metals such as Cd and Hg in a case of combined intake group showing values higher than 1 as a odds ratio for each metal. However, all metals did not show any significant odds ratio higher than 1. This indicates that people who take herbal medicine would have no possibility higher for metal accumulation in blood than that from people who do not take herbal medicine.

Table 5. Regression coefficients(SE) for duration by groups from multiple regression of metals in blood adjusted for potential confounders(N=222)

Variables (μg/L)*	Duration of decoction only(weeks) Beta(SE)	Duration of combined intake(weeks) Beta(SE)
log Cd	0.00(0.05)	0.03(0.03)
log Co	-0.01(0.04)	0.03(0.02)
log Cr	-0.11(0.04)	-0.01(0.03)
log Cu	0.03(0.02)	0.01(0.01)
log Fe	0.03(0.02)	-0.01(0.01)
log Hg	-0.21(0.20)	0.02(0.07)
log Mn	0.30(0.15)	0.16(0.07)
log Ni	0.03(0.14)	0.05(0.08)
log Pb	0.00(0.20)	0.06(0.09)
log Zn	0.02(0.01)	0.00(0.01)

* Dependent Variables are natural logarithm of each metal level plus one, for example, log(Cd+1).

Table 6. Odds ratios^ for metal concentrations in blood adjusted* for potential confounders

Variables (μg/L)†	Duration of decoction only				Duration of combined intake			
	OR	95% C.I.		p	OR	95% C.I.		p
log Cd	0.96	0.72	1.27	0.76	1.03	0.80	1.34	0.80
log Co	0.95	0.80	1.11	0.49	0.98	0.84	1.13	0.76
log Cr	0.96	0.70	1.31	0.78	0.88	0.66	1.17	0.36
log Cu	0.97	0.84	1.11	0.66	0.91	0.81	1.04	0.17
log Fe	0.98	0.87	1.10	0.69	0.96	0.86	1.07	0.42
log Hg	1.34	0.66	2.74	0.42	1.79	0.93	3.45	0.08
log Mn	0.90	0.39	2.06	0.80	0.75	0.35	1.60	0.45
log Ni	1.19	0.57	2.48	0.64	0.89	0.46	1.75	0.74
log Pb	0.56	0.23	1.36	0.20	0.46	0.20	1.04	0.06
log Zn	0.99	0.88	1.11	0.86	0.98	0.88	1.08	0.63

* Adjusted for sex, age, job, smoking, alcohol, residence

† Odds ratios are natural logarithm of each metal level plus one, for example, log(Cd+1)

Ⅳ. Discussions

Evidence from various countries suggest that toxic heavy metals in Asian herbal medicine may constitute a serious health problem.[2), 12), 13)] However, the majority of the data is anecdotal and insufficient to define prevalent figures. Our study was carried out using the samples from people taking herbal medicine for a long-term, ranging from 2 to 130days(mean ≒ 26days). This fact would reflect a relative figure of risk by heavy metals in herbal medicine. The results from this study were discused and compared to the results from the the previous study.[6)]

In this study, 0.4~33.9% of total samples for 8 metals such as Cd, Co, Cu, Hg, Mn, Ni, Pb and Zn except Cr and Fe exceeded the upper limit for WHO reference value, compared to the range of WHO reference value for each metal.[11)] In the previous study,[6)] 11.69%, 26.95%, 20.79% and 12.66% of the total samples showed concentrations exceeding the upper limit of WHO reference value for Cd, Cu, Hg and Pb of 9 metals such as As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn, respectively. However, the metal concentrations of Co, Mn, Ni and Zn not exceeding WHO reference value in the previous study showed higher than WHO reference concentrations in this study. This difference would be due to the longer duration for taking herbal medicine in this study than that in the previous study.

It is not easy to estimate the potential risk of these results obtained from the comparison with WHO reference value, since the reference values were obtained in different fashion. Thus in other to retrieve toxicological information of metal in herbal medications, our analytical approaches for this study has been diversely carried out. After adjusting confounding factors, all levels of the decoction and combined groups for metals were compared to those of the control group not taking any herbal medications. The levels for 10 metals, did not showed significantly different mean compared to those for metals in the non-herbal group. The similar result was also identified in an analysis of adjusted geometric mean. Thus, the mean levels for all metals in blood samples would be not increased by taking herbal medicine as types of decoction and combined intake.

In accordance with the result from an analysis of adjusted geometric mean, the result in an analysis of regression coefficients and standard error(SE) for duration of decoction and combined intake from multiple regressions of metals in blood samples did not show any significance. The regression coefficients indicate the levels of metals increased(+) or decreased(-) in blood after taking herbal medicine for one month, compared to those of the non-herbal group. In this study, regression coefficient for duration of decoction intake ranged from -0.21 to 0.30μg/kg blood for all metals. Regression coefficient for duration of combined intake ranged from -0.01 to 0.16μg/kg blood for all metals. The metals decreased by taking decoction in blood samples were as follows ; Co, Cr and Hg. In contrast, the metals increased by taking decoction in blood samples were as follows ; Cu, Fe, Mn, Ni and Zn. In addition, the metals increased by taking decoction in blood samples were as follows ; Cd and Pb. In a case of combined intake, there were Cr and Fe as decreased metals, Cd, Co, Cu, He, Mn, Ni and Pb as increased metals, Zn as unchanged metal in terms of concentrations in blood samples. In both decoction and combined intake, the highest increased metal in blood sample was Mn (Manganese). Manganese is an essential trace element and is necessary for good health. Manganese can be found in several food items, including grains and cereals, and is found in high amounts in other foods, such as tea. Thus, this increased level of Mn would be considered as one of beneficial effects for herbal-medicine intakes.

In blood samples, the metals showing the highest level decreased by herbal intake were Hg and Cr for decoction group and combined intake group, respectively. Both heavy metals have no function in the body and can be highly toxic. The average person's body contains about 10～15mg of mercury and chromium. Both metals are also eliminated daily through the urine and feces. Thus it is believed that herbal medicine caused the possible decrease of metals in human body. The decreased levels of metals would be due to the role of herbal components for chelating metals. In general, heavy metals to be excreted should be captured or bound to chelating agents. There are a number of natural(non-synthetic) chelators such as herbs, amino acids, and other nutritional supplements.[14] One of many herbal component, flavonoid and derivatives, dislodges heavy metal molecules from cell receptors and captures them so that they may be excreted from the body.[15), 16)]

However, the assumption for the role of herbal medicine as a chelator was not supported by the comparison of odds ratio from case-control study, using the sample exceeding the upper limit of WHO reference values. The odds ratio showing values higher than 1 indicates that people who take herbal medicine would have possibility higher for metal accumulation in blood than that from people who do not take herbal medicine. The metals showing the odds ratio higher than 1 were Hg and Ni in decoction group, and Cd and Hg in combined intake group. However, there were very low p values for the odds ratio higher than 1 in both decoction and combined intake group. This indicates that all levels of 10 metals would not be increased in blood when patients take herbal medicine even if there would be the possibility for slightly increase level of Cd and Hg in blood.

In summary, the results from this study were in accordance with the results from the previous study.[6] Similarly the levels of metals exceeding WHO reference values were observed in blood samples. In addition, some of metals showed the possibility of accumulation in body and the high odds ratio after adjusting confounding factors. However, some of metals in samples from people taking herbal medicine showed the decreased level and the low odds ratio compared to those of the control group. This may explain the possible role of herbal medicine as a chelator for heavy metals in body. Nonetheless, despite small number of experiment samples, the results obtained in the study suggest that heavy metal contamination in the herbs don't necessarily cause metal poisoning because of the possible role of herbal medicine as a chelator.

Acknowledgements

This study was sponsored by Youngdeungpo Oriental medical clinic in Seoul and by active participation from the patients. Youngdeungpo Oriental medical clinic administered variety of herbal medicines to the patients suffering from chronic skin disorders of vitiligo and psoriasis. Our deep gratitude goes to Youngdeungpo Oriental medical clinic and the patients for their participation.

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