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Hair Tissue Mineral Analysis and Metabolic Syndrome

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Abstract Deficiency of minerals causes functional abnormality of enzymes, frequently resulting in metabolic disturbance. We investigated possible relationship between minerals and metabolic syndrome by analysis of hair tissue minerals. We selected 848 subjects older than 20 years of age at Ajou University Hospital from May 2004 to February 2007. We excluded the subjects who had cancers, steroid and thyroid medication, and incomplete record from the study. Finally, 343 subjects were eligible. We performed cross-sectional analysis for the relationship between minerals and metabolic syndrome. The contents of calcium, magnesium, and copper in the metabolic syndrome group were significantly lower than those of the normal group, whereas the amounts of sodium, potassium, and mercury in the metabolic syndrome group were significantly higher than those of the normal group. By dividing the subjects into quartile with the level of calcium, magnesium, and mercury concentrations, we carried out logistic regression analysis to study the subjects and found that the subjects in the third quartile of calcium and magnesium concentrations had significantly lower odds ratio (OR) of the metabolic syndrome compared with that of the lowest quartile group [OR=0.30, confidence interval (CI)=0.10–0.89; OR=0.189, CI=0.063–0.566] and that the subjects in the highest mercury quartile had significantly higher OR of the metabolic syndrome compared with that of the lowest mercury quartile group (OR=7.35, CI=1.73–31.1). As part of the metabolic syndrome, the optimal calcium and magnesium concentrations in hair tissue may reflect decreased risk of metabolic syndrome, whereas high mercury concentration in hair tissue may indicate increased risk of metabolic syndrome.

Keywords Hair tissue analysis · Mineral · Metabolic syndrome

Introduction

The adjusted prevalence of metabolic syndrome for Koreans in 2001 was 15.1% in total, being 15.7% for men and 14.4% for women, and the prevalence increases from 40s for men

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and 50s for women [1]. According to the guideline of the National Cholesterol Education Program–Adult Treatment Panel III (NCEP-ATP III) in the USA [2], the prevalence of metabolic syndrome for adult Koreans has steadily increased and was 31.6% totally [3]. Furthermore, prospective analysis [4] revealed that the risk of cardiovascular disease in metabolic syndrome patients has increased three times more. As the mechanism involved in the conversion of nutrients to energy in human body and the effect of cardiovascular risk factors are well known, micronutrients have been considered to play critical roles [5].

Because minerals are components of many enzymes and play important roles in the activation of enzymes, deficiency of minerals causes functional abnormality of enzymes, resulting in endocrinologic and metabolic disorder [6]. For examples, insulin is secreted through calcium-dependent process and is associated with the incidence of diabetes if the disturbance of calcium channel develops [7–9]. Deficiency of magnesium in cells causes the disturbance of tyrosine kinase activation and then increases insulin resistance [10]. Zinc has an effect on insulin, such as stimulation of secretion and activation of insulin, insulin-like effects on glucose transport directly, and stabilization of DNA binding site on four-dimensional structure of peroxisome proliferator-activated receptors associated with energy balance [11–14]. An increased insulin secretion due to insulin resistance causes zinc deficiency in pancreas; the antioxidant enzyme induces metallothioneine synthesis in pancreatic islets of mice and protects against diabetes [15–18]. When copper is nutritionally deficient, decreasing activity of superoxide dismutase is observed in red blood cells [19].

Mineral content in blood cells has been measured; however, the procedure is complex. However, hair is easily available, and it is also possible to measure essential trace elements with appropriate specificity, sensitivity, speed, sampling, simplicity, and cost to satisfy the skeptical chemist [20].

The use of hair for tissue mineral analysis is very efficient and relatively accurately reflects mineral status in human body, and minerals may be risk factors as part of the metabolic syndrome. Therefore, this study was undertaken to evaluate the relationship between hair tissue and metabolic syndrome.

Materials and Methods

Population

We scrutinized 848 subjects who visited Ajou University Hospital from May 2004 to February 2007. We excluded 505 persons, and 343 subjects were finally included in the study. Exclusion criteria were cancers, steroid and thyroid medication, and incomplete survey among participants.

Methods

The sociodemographic characteristics of subjects were surveyed by questionnaire. We surveyed smoking status, alcohol drinking status, and activity status as well.

Height and weight were measured using bioelectrical impedance analysis (Inbody 3.0, Biospace, Korea, 2001) following overnight fast. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) [21]. Waist circumference was measured at middle part between the lower rib and iliac crest by a trained nurse. Blood pressure was measured electrically (TM-2650A, PMS Instruments, Tokyo, Japan) after a rest of at least 15 min.

Venous blood was drawn following an 8-h overnight fast and 24-h abstinence from vigorous activity. Standard enzymatic measurements of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and fasting glucose were made on fresh serum samples by oxidase–peroxidase enzymatic assay (TBA-200FR, Toshiba, Tokyo, Japan).

Only patients with uncolored hair and with no shampooing were accepted for the study. Hair sample was sent to the US TEI (Dallas, TX, USA) through Korea TEI (Trace Elements, Inc.). After washing with acetone, water, and extran (1% *v*/*v*) and water, aliquots of hair samples were wet-ashed according to the following procedure. A 250-mg sample was wet digested overnight with 2.5 ml of HNO_3 in a closed, graduated polypropylene tube (50 ml) at room temperature and then for 1 h at 60 to 70°C in a drying oven. After cooling to room temperature, the sample was diluted to a final volume of 25 ml with Milli Q water. This solution was analyzed by Perkin-Elmer Mass Spectrometer (Sciex Elan 6100, Perkin-Elmer corporation, Foster, CA, USA) [22]. Mineral concentrations are shown as mg% (mg/100 g of hair).

Analysis

We followed the NCEP-ATP III Asian guideline components to define metabolic syndrome, which consisted of central obesity (waist circumference ≥90 cm for men and ≥85 cm for women), blood pressure ≥130/80 mmHg, triglyceride ≥150 mg/dl, fasting glucose ≥110 mg/dl, and low high-density lipoprotein cholesterol (men<40 mg/dL and women<50 mg/dL). In that guideline, subjects who had more than three abnormal values mentioned above were defined as metabolic syndrome subjects.

We used chi-square test and Student's *t* test to calculate the mean values of general characteristics and laboratory tests. The logistic regression analysis was employed to evaluate metabolic syndrome according to calcium, magnesium, and mercury concentrations. All significant values were defined by $p<0.05$ as determined by SPSS in version 11.5.

Results

General Characteristics of the Study Subjects

Total number of subjects was 343 including 224 men and 119 women. The mean age of subjects was 46.5±9.2 years old, and the mean BMI was 23.9±3.2 kg/m^2. The number of subjects in the metabolic syndrome group was 73 while 270 in normal group, and the number of men was significantly higher than women. Alcohol drinking status and activity grade were not significantly different between two groups; however, smoking status was significantly higher in the metabolic syndrome group. The mean age of the metabolic syndrome group was 48.6±10.7 years, and the mean age of normal group was 45.8±8.7 years, so there was no significant difference between two groups. The mean BMI of the metabolic syndrome group was 26.9±2.9 kg/m^2, whereas that of the normal group was 23.0±2.7 kg/m^2, showing significant difference between two groups. The mean waist circumference of the metabolic syndrome group was 91.5±7.8 cm, whereas that of the normal group was 80.6±8.2 cm; therefore, there was significant difference between two groups. In the metabolic syndrome group, all metabolic markers were significantly different compared with normal group ($p<0.05$) (Tables 1, 2, and 3).

Table 1 General Characteristics of the Study Subjects

	Male (n=224)	Female (n=119)	p value[a]
Age (years)	46.9±8.1	45.6±10.8	0.223
BMI (kg/m^2)	24.3±3.2	22.9±3.0	0.000
WC (cm)	85.4±8.7	78.4±8.5	0.000
SBP (mmHg)	124.1±15.4	115.1±15.2	0.000
DBP (mmHg)	81.4±10.6	73.5±11.2	0.000
MS (Y/N)	63/161	10/109	

Mean±SD

BMI body mass index, *WC* waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MS* metabolic syndrome

[a] By independent t test

Mineral Concentrations and Metabolic Syndrome

The concentrations of calcium, magnesium, and copper in the metabolic syndrome group were significantly lower than those in the normal group. On the other hand, the concentrations of sodium and potassium in the metabolic syndrome group were significantly higher than those in normal group (p<0.05). Table 4 shows that the concentrations of zinc, phosphorus, iron, manganese, chromium, and selenium were not significantly different between the two groups.

The Concentrations of Toxic Elements and Metabolic Syndrome

Mercury concentration in the metabolic syndrome group was significantly higher than that in normal subjects (p=0.000), whereas the concentrations of cadmium, lead, and aluminum were not significantly different between two groups (Table 5).

The Ratios of Mineral and Toxic Elements According to Metabolic Syndrome

With the mineral concentrations, the ratio of calcium/phosphorus was significantly higher in the metabolic syndrome group (Table 6). As for the toxic elements, the ratios of calcium/

Table 2 Sociodemographic Characteristics of the Study Subjects

	Normal group (n=270)	MS group (n=73)	p value[a]
Gender (M/F)	161/109	63/10	0.000
Smoking (yes/no)	131/117	188/129	0.000
Alcohol intake(yes/no)	163/85	52/15	0.165
Activity grade			
Sedentary	173	51	0.662
Regular activity	51	10	

Mean±SD

MS metabolic syndrome, *BMI* body mass index, *WC* waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBS* fasting blood sugar, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol

[a] By chi-square test

Table 3 Metabolic Markers by Metabolic Syndrome

	Normal group (n=270)	MS group (n=73)	p value[a]
Age (years)	45.8±8.7	48.6±10.4	0.223
BMI (kg/m^2)	23.0±2.7	26.9±2.9	0.000
WC (cm)	80.6±8.2	91.5±±7.8	0.000
SBP (mmHg)	118.9±15.5	128.8±14.9	0.000
DBP (mmHg)	77.3±11.3	8.35±10.5	0.000
FBS (mg/dL)	93.8±11.3	117.2±38.8	0.000
TG (mg/dL)	107.3±65.4	233.9±114.4	0.000
HDL (mg/dL)	57.5±13.2	43.7±8.9	0.000

Mean±SD

MS metabolic syndrome, *BMI* body mass index, *WC* waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBS* fasting blood sugar, *TG* triglyceride, *HDL* high density lipoprotein-cholesterol

[a] By Student's t test

lead, selenium/mercury, zinc/cadmium, and zinc/mercury were significantly higher in the metabolic syndrome group (Table 7).

Odds Ratios of Calcium, Magnesium, and Mercury Concentrations According to Metabolic Syndrome

After dividing the subjects into quartile with the level of calcium, magnesium, and mercury concentrations, we carried out logistic regression analysis after adjustment of age, gender, smoking status, alcohol drinking status, and activity grade. Table 8 shows that the subjects with the third quartile of calcium and magnesium concentrations had significantly lower odds ratio (OR) in metabolic syndrome compared with the lowest quartile group [OR=0.30,

Table 4 Mineral Concentrations

	Normal group (n=270)	MS group (n=73)	p value[a]
Ca (mg%)	101.58±65.59	79.34±56.57	0.009
Mg (mg%)	7.38±5.52	5.76±4.15	0.021
Na (mg%)	19.20±21.58	26.18±23.80	0.017
K (mg%)	10.71±12.53	16.38±20.93	0.030
Cu (mg%)	4.29±4.74	3.36±2.86	0.036
Zn (mg%)	16.54±4.92	15.38±3.31	0.059
P (mg%)	14.35±2.15	14.23±1.94	0.669
Fe (mg%)	0.90±2.53	0.68±0.20	0.449
Mn (mg%)	0.04±0.10	0.03±0.03	0.256
Cr (mg%)	0.05±0.01	0.05±0.02	0.256
Se (mg%)	0.08±0.20	0.07±0.01	0.719

Mean±SD

MS metabolic syndrome, *Ca* calcium, *Mg* magnesium, *Na* sodium, *K* potassium, *Cu* copper, *Zn* zinc, *P* phosphorus, *Fe* iron, *Mn* manganese, *Cr* chromium, *Se* selenium

[a] By independent t test

Table 5 The Concentration of Toxic Elements

	Normal group (n=270)	MS group (n=73)	*p* value[a]
Hg (mg%)	0.17±0.12	0.29±0.18	0.000
Cd (mg%)	0.002±0.005	0.003±0.004	0.509
Pb (mg%)	0.13±0.11	0.16±0.20	0.191
Al (mg%)	0.60±0.45	0.57±0.39	0.636

Mean±SD

MS metabolic syndrome, *Hg* mercury, *Cd* cadmium, *Pb* lead, *Al* aluminum

[a] By independent *t* test

confidence interval (CI)=0.10–0.89; OR=0.189, CI=0.063–0.566] and that the subjects with the highest mercury quartile had significantly higher OR in metabolic syndrome compared with the lowest mercury quartile group (OR=7.35, CI=1.73–31.1).

Discussion

Since the acute-phase reactant has been shown to be one of the most powerful predictors of risk of cardiovascular events, mild, chronic inflammation in the endothelium has been suggested to be a further feature of metabolic syndrome: Pannacciulli et al. [23] has shown relationship between central fat accumulation, insulin resistance, and C-reactive protein (CRP) plasma levels, thus suggesting that mild, chronic inflammation may be a further component of metabolic syndrome and a mediator of the atherogenic profile of this syndrome. In metabolic syndrome, pH and magnesium concentration in cells are low, or concentration of calcium in cells tends to be high. Furthermore, the abnormality of zinc/copper ratio is considered to be the risk factor of hyperlipidemia and coronary heart disease [24]. The above multifactorial metabolic abnormalities occur in patients with metabolic syndrome; however, the relation of micronutrients to metabolic syndrome has not been well known.

In the present study, the concentrations of calcium, magnesium, and copper in the metabolic syndrome group were significantly lower than those of the normal group. Zinc

Table 6 The Ratios of Mineral Elements According to Metabolic Syndrome

	Normal group (*n*=270)	MS group (*n*=73)	*p* value[a]
Ca/P	7.26±4.96	5.63±3.89	0.003
Na/K	2.69±2.66	2.50±2.14	0.583
Ca/K	32.05±42.49	21.41±47.73	0.065
Zn/Cu	7.51±5.91	7.52±4.86	0.993
Na/Mg	4.61±8.33	8.21±14.51	0.045
Ca/Mg	15.08±4.05	14.87±3.93	0.691
Fe/Cu	0.47±2.32	0.33±0.22	0.600

Mean±SD

MS metabolic syndrome, *Ca* calcium, *P* phosphorus, *Na* sodium, *K* potassium, *Zn* zinc, *Cu* copper, *Mg* magnesium, *Fe* iron

[a] By independent *t* test

Table 7 The Ratios of Toxic Elements According to Metabolic Syndrome

	Normal group (n=270)	MS group (n=73)	p value[a]
Ca/Pb	958.9±672.2	715.6±602.3	0.005
Fe/Pb	8.4±25.4	5.8±2.3	0.390
Fe/Hg	7.5±17.6	3.5±2.8	0.051
Se/Hg	0.6±0.9	0.3±0.3	0.029
Zn/Cd	12,026.7±6,780.6	10,365.0±16,545.2	0.062
Zn/Hg	138.1±98.5	76.7±52.5	0.000

Mean±SD

MS metabolic syndrome, *Ca* calcium, *Fe* iron, *Se* selenium, *Zn* zinc, *Pb* lead, *Hg* mercury, *Cd* cadmium

[a] By independent t test

concentration in the metabolic syndrome group was lower than that of the normal group but not statistically significant. Intracellular calcium modulates the affinity of insulin receptor and insulin sensitivity [25] and affects insulin secretion [7–8]. Moreover, calcium channel in muscle cells influences glucose uptake [26, 27], and calcium in adipose cells induces weight gain and aggravates insulin resistance after a while [28]. Severe magnesium deficiency has been shown to increase in inflammatory cytokines as well as to change circulating leukocyte subpopulations [29]. It is apparent that elevated circulating levels of neutrophils and lymphocytes contribute to both the acute and chronic phases of the inflammatory responses in the Mg-deficient rats [30], and CRP was elevated in obese subjects [31]. Furthermore, it has even been proposed that there is a causal link between magnesium deficiency, oxidative stress, and the inflammatory state [32–34]. Cellular magnesium, which is competitive with calcium, shows opposite effects on insulin sensitivity contrarily. Cellular magnesium concentration in insulin-resistant patients tends to be low compared with calcium [35, 36]. Disturbance of cellular magnesium intake results in the development of insulin resistance [37–39], while sufficient magnesium intake decreases the risk of diabetes [40]. However, Bogdan et al. [41] showed that calcium and magnesium concentrations in metabolic syndrome patients were not different compared with the control group. Therefore, there are many different views concerning whether measurement of hair tissue minerals truthfully reflects mineral concentrations in human body cells [42, 43]. Yakinici et al. [44] reported that copper concentration was significantly

Table 8 Odds ratios for prevalence of metabolic syndrome

		Quartile			
		Lowest	Mid-lowest	Mid-highest	Highest
Ca	OR[a] (95%CI)	1.00	1.36 (0.60–3.10)	0.30 (0.10–0.89)	1.46 (0.54–3.96)
	p value		0.456	0.031	0.456
Mg	OR[a] (95%CI)	1.00	0.83 (0.36–1.92)	0.19 (0.06–0.57)	1.03 (0.41–2.62)
	p value		0.669	0.003	0.951
Hg	OR[a] (95%CI)	1.00	0.75 (0.17–3.21)	3.14 (0.79–12.51)	7.35 (1.73–31.1)
	p value		0.700	0.104	0.007

Ca calcium, *Mg* magnesium, *Hg* mercury, *OR* odds ratio, *CI* confidence interval

[a] Adjusted for age, sex, smoking, alcohol intake, and physical activity

higher in the obesity group than in the normal group, whereas other studies reported no difference between two groups [45, 46]. Furthermore, copper concentration in diabetes patients was found to be lower than that of normal group [47].

In most mammalian species, insulin is stored in the pancreatic β cells as Zn crystals [48]. The attenuated immunological activity of Zn^{2+}-free insulin indicates that removal of Zn^{2+} significantly perturbed the antigenic determinants of insulin [49]. Metabolic abnormality of zinc has been suggested to result in the role for the development and complication of diabetes [50]. In this study, zinc level in hair tissue of the metabolic syndrome group tended to be lower than that of the normal group; however, the difference was not significant between the two groups, suggesting that zinc could not sensitively reflect insulin resistance. Earlier studies showed that chromium is decreased in diabetes patients [51–53], and the level of chromium in Korean diabetes patients is lower than that of non-diabetes group [54]. Furthermore, there are studies to indicate that the action of insulin was improved through supplementation of chromium [55, 56]. In the present study, however, chromium level was not significantly different between the two groups. Selenium for glutathione peroxidase is regarded as a protector for lipid peroxidation and rigidity of cell membranes. Deficiency of selenium affects enzymatic antioxidant capacity of liver tissue and aggravates the damage of liver tissue due to heavy metals, such as mercury and cadmium, by acidification or chemical induction [57]. According to a recent study [58], selenium supplementation does not seem to prevent type 2 diabetes but rather increase the risk of diabetes. More researches on its metabolism are needed.

Many earlier studies have shown that mercury induces the functional abnormality of vascular endothelium [59], increases oxidative stress [60], and induces the hyperplasia of vascular smooth muscles [61]; the higher the mean mercury content, the higher the risk of high blood pressure and cardiovascular disease. In the present study, the subjects in the highest mercury quartile had significantly higher OR (OR=7.35, CI=1.73–31.1) of metabolic syndrome compared with the lowest mercury quartile group.

There are some limitations in this study. Firstly, we did not compare mineral contents of hair tissue with those of serum or plasma and did not measure the enzymes, transcription factors, hormones, or immunological status, which are dependent on minerals. Secondly, we are not certain whether the mineral contents of hair tissue truthfully reflected that of human body tissues. Thirdly, toxic minerals enter into human body via various pathways such as smoking, foods, or polluted air; nevertheless, we did not collect the data. Finally, further researches are needed to investigate what effect the imbalance of interrelated minerals has and how it is related to metabolic syndrome.

Conclusions

As part of the metabolic syndrome, the optimal calcium and magnesium concentrations in hair tissue may decrease the risk of metabolic syndrome, and high mercury concentration in hair tissue may increase the risk of metabolic syndrome. Therefore, further studies on the effect of mineral contents by mineral supplementation and follow-up studies are needed.

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