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Clinical note

# Relationship between self-reported osteoporosis and mineral concentrations in female hair

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## ABSTRACT

**Introduction:** Hair mineral analysis is a method used to predict the development of hay fever and schizophrenia, and is becoming common in Japan. Meanwhile, the medical checkup rate for osteoporosis in Japan was reported to be as low as 5.0%, and it is difficult to educate young people about the risk of bone mineral density loss. To prevent osteoporosis, it is necessary to increase the peak bone mass until the early 20s. Therefore, this study aimed to clarify the relationship between the concentration of minerals in hair and self-reported osteoporosis, for the primary prevention of osteoporosis.

**Methods:** A questionnaire survey about lifestyle habits was conducted and the concentrations of five different minerals in the hair shaft were measured in 200 female participants.

**Results:** The results indicated that self-reported osteoporosis is significantly associated with advanced age ($p<0.05$) and low concentration of phosphorus (P) in the hair ($p<0.01$). Additionally, the multivariate logistic analysis model, which examined age and phosphorus (P) and calcium (Ca) concentrations in hair, showed statistical significance ($p=0.014$). The odds ratios for age, P, and Ca were 1.11, 0.00, and 0.77, respectively. Furthermore, the predictive ability of this model was moderate. Measurement of P and Ca concentrations in hair can be used to evaluate the risk of osteoporosis because these two minerals are components of hydroxyapatite.

**Conclusion:** The results of this study suggest that measuring the concentration of minerals in hair during regular haircuts would be a useful method to predict the risk of osteoporosis among young people. The measuring of hair P and Ca concentrations may contribute to primary prevention of osteoporosis in young female.

## INTRODUCTION

Calcium (Ca), phosphorus (P), and sulfur (S) are macro-minerals, which means that their total mass in the body is >10 g and the required daily intake of these nutrients is >100 mg. Ca and P exist in bone and teeth as components of hydroxyapatite, and S is a component of amino acids such as methionine and cysteine. In contrast, zinc (Zn) and copper (Cu) are micro-minerals, the required daily intake of which is <100 mg despite the important roles they play in the body. In addition, Ca, P, Zn, and Cu are subject to the regulations described in the Dietary Reference Intakes for Japanese [1]. Thus, it is important to consume the proper amounts of these minerals and assess their physiological concentrations on a regular basis.

Minerals taken into the body are mainly excreted in urine and feces but may also be excreted through the hair via the blood capillaries. Approximately 90% of the hair on the human scalp is composed of a keratin protein with disulfide bonds, and hair tests have been used to detect exposure biomarkers such as arsenic and mercury. The concentrations of minerals in hair are generally higher [2, 3], less susceptible to daily fluctuations, and more stable as a result of temporal changes [2, 4] than those in blood or urine. Accordingly, hair minerals should be measured to better understand the risks for the development of certain health conditions. Previous studies have demonstrated an association between the risk of hay fever and low concentrations of iron, Ca, chromium, and cadmium, and high concentration of selenium in hair [5]; an association between schizophrenia and low concentrations of Zn and Ca and high concentrations of Cu and cadmium in the hair of adult men [6]; and a relationship between low concentrations of Zn in hair during childhood and autism spectrum disorder [7]. Therefore, measurement of hair mineral concentrations during regular haircuts may contribute to the early detection of diseases and improve health consciousness. As the medical expenses in Japan are increasing, hair analysis as a tool for the primary prevention of diseases is becoming more common.

The 2000 National Institutes of Health Consensus Conference defined osteoporosis as a skeletal disorder characterized by compromised bone strength, which predisposes patients to an increased risk of fracture [8]. Bone strength is determined by assessing the overall bone quality and bone mineral density (BMD). Approximately 70% of bone strength is determined by BMD, whereas the remaining 30% is determined by factors such as bone microstructure and rate of turnover [9]. Lifestyle, diabetes, chronic kidney disease, and a family history of fractures also influence the fracture risk, leading to an increase in the number of patients with osteoporosis in recent years. The number of patients aged >40 years with osteoporosis of the lumbar spine or femoral neck is estimated to be 3.0 million in male and 9.8 million in female [10, 11], and the intervention is needed to improve bone mineral density of Japanese female. Furthermore, previous studies reported that the rate of medical checkups for osteoporosis in Japan was as low as 5.0% in 2015, and care is required in areas where the checkup rate is low [12]. Hence, there is a concern about a decreased quality of life in patients with severe osteoporosis who often eventually become bedridden.

To prevent osteoporosis, it is necessary to increase the peak bone mass until the early 20s; however, efforts to educate young people about osteoporosis have been deemed insufficient. For these reasons, we aimed to primary prevent osteoporosis using the concentrations of minerals in hair by investigating the relationship between self-reported osteoporosis, hair mineral concentrations, and lifestyle diseases.

## Methods

**1. Population**

A total of 200 Japanese women between the ages of 20 and 59 years (52, 76, 50, and 22 women aged 20-29, 30-39, 40-49, and 50-59 years, respectively) participated in the questionnaire survey from May to July in 2015.

**2. Questionnaire survey and definition of self-reported osteoporosis**

The items included in the questionnaire focused on the participants' age and disease history. The diseases included in the questionnaire were diabetes, hypercholesterolemia, anemia, osteoporosis, hay fever, dermatitis, and protracted wound healing. The participants who answered they have low bone density were classified self-reported osteoporosis. We explained the requirements of this study to all participants. Once informed consent was obtained, we collected hair samples and the responses to the questionnaire.

**3. Measurement of mineral concentrations in hair**

Unless otherwise noted, the following procedures were performed at room temperature. Acetone, Triton-X100, and nitric acid were purchased from Fuji Film Wako Pure Chemical Co. (Osaka, Japan). The hair samples (0.1 g) were washed twice with 5.0 mL acetone for 30 min at 100 rpm and dried for 30 min at 50 °C. Thereafter, the hair samples were washed with 0.01% Triton-X100 (5.0 mL) for 1 h at 100 rpm and dried for 48 h at 50 °C. The washed hair samples were dissolved in concentrated nitric acid (2.5 mL) for 24 h at 100 rpm, and then purified water (15.0 mL) was added. The solutions were filtered through a 0.45-µm membrane filter (Toyo Roshi Kaisha, Ltd., Japan). The concentrations of S, Ca, Zn, P, and Cu in the hair samples were analyzed using inductively coupled plasma optical emission spectrometry (iCAP-7600; Thermo Fisher Scientific Inc., Tokyo, Japan). The mineral concentration was calculated calibration curve method, and the following conditions: the wavelengths, which were S (180.731, 182.034, and 182.624 nm), Ca (422.673, 396.847, and 393.366 nm), Zn (213.856, 202.548, and 206.200 nm), P (177.495, and 178.284nm), and Cu (324.754, 327.396, and 224.700 nm); the concentrations of calibration curve, which were S (0, 5.0, 10.0, 20.0, 50.0, and 100.0 mg/L), Ca (0, 1.0, 2.0, 5.0, 8.0, 10.0, 50.0, and 100.0 mg/L), and Zn, P, and Cu (0, 0.1, 0.2, 0.5, 0.8, 1.0, 5.0, and 10.0 mg/L).

**4. Statistical analysis**

Statistical analyses were conducted using JMP version 13.2.1 for Windows (SAS Institute Inc., Cary, NC, USA). The significance level was set at 0.05. The relationship between self-reported osteoporosis and diseases was analyzed using the chi-square test. Additionally, the relationship between self-reported osteoporosis and aging was analyzed using the Cochran-Armitage trend Test. The correlation coefficients of age and hair mineral concentrations were analyzed using the correlation analysis. The mineral concentrations were calculated for each age class (20-29, 30-39, 40-49, and 50-59 years) because the concentrations seem to change with age. Accordingly, we calculated the average, median, and standard deviation value of the concentration of each mineral among the four age classes. The relationship between mineral concentrations and self-reported osteoporosis was analyzed using the Mann-Whitney U test. Furthermore, multivariate logistic regression analysis was performed to determine the effect of age and hair mineral concentrations on self-reported osteoporosis (objective variable). Finally, we analyzed the area under the receiver operating characteristic (ROC) curve (AUC) to assess the predictive performance of this logistic model.

**5. Compliance with ethical standards**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Research Ethics Committee of Kindai University (14-064) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The participants were informed that the obtained data would be used only for this study, that their participation in the study was voluntary, and that their privacy would be guaranteed.

## RESULTS

**1. Relationships between self-reported osteoporosis and aging or diseases**

The proportions of participants in each age group (20-29, 30-39, 40-49, and 50-59 years) who were self-reported osteoporosis were 0.00% (0/52), 5.26% (4/76), 6.00% (3/50), and 13.64% (3/19), respectively. This proportion was significantly increased with aging ($p=0.019$). The relationships between self-reported osteoporosis and diseases are shown in Table 1. A significant difference was found between the questions "Do you have low bone density?" and "Do you have high blood sugar or high hemoglobin A1c (HbA1c)?,"

Table 1 Relationship between self-reported osteoporosis and diseases.

| Questionnaire | Self-reported osteoporosis (%) Yes (n=10) | Self-reported osteoporosis (%) No (n=190) | Odds ratio | 95%CI Lower | 95%CI Upper | p value |
|---|---|---|---|---|---|---|
| Do you have high blood sugar or high HbA1c? | 20.00 | 1.58 | 15.58 | 2.28 | 106.72 | 0.015 |
| Do you have high cholesterol? | 20.00 | 14.70 | 1.45 | 0.29 | 7.17 | 0.662 |
| Do you have anemia? | 20.00 | 23.16 | 0.83 | 0.17 | 4.05 | 0.814 |
| Do you have hay fever? | 60.00 | 49.47 | 1.53 | 0.42 | 5.60 | 0.515 |
| Do you have dermatitis? | 20.00 | 20.00 | 1.00 | 0.20 | 4.90 | 1.000 |
| Have you been told or felt that the healing is slow? | 30.00 | 27.90 | 1.11 | 0.28 | 4.44 | 0.886 |

CI: confidence interval, p: statistical significance obtained using the chi-squared test.

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Table 2 Correlation coefficients from single regression analysis of age and hair mineral concentrations.

| | Age | | S | | Ca | | Zn | | P | | Cu | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p | r | p | r | p |
| Age | 1.00 | | | | | | | | | | | |
| S | -0.07 | 0.307 | 1.00 | | | | | | | | | |
| Ca | -0.06 | 0.374 | 0.08 | 0.266 | 1.00 | | | | | | | |
| Zn | 0.03 | 0.628 | 0.09 | 0.194 | 0.16 | 0.028 | 1.00 | | | | | |
| P | 0.13 | 0.074 | 0.25 | <0.001 | -0.30 | <0.001 | 0.05 | 0.479 | 1.00 | | | |
| Cu | 0.01 | 0.897 | 0.08 | 0.282 | 0.25 | <0.001 | 0.03 | 0.633 | -0.18 | 0.012 | 1.00 | |

r: correlation coefficient, p: statistical significance obtained using correlation analysis.

and the odds ratio was 15.58 (p=0.015). However, no significant differences were found between the questions "Do you have low bone density?" and "Do you have high cholesterol?" (p=0.662), "Do you have anemia?" (p=0.814), "Do you have hay fever?" (p=0.515), "Do you have dermatitis?" (p=1.000), or "Have you been told or felt that the healing is slow?" (p=0.886), and the odds ratios were 1.45, 0.83, 1.53, 1.00, or 1.11, respectively.

**2. Mineral concentrations in hair**

The correlations between age and hair mineral concentrations are shown in Table 2. The concentration of Ca in the hair samples was correlated with the Zn, P, and Cu concentrations, with correlation coefficients of 0.16, -0.30, and 0.25, respectively. Similarly, the P concentration in the hair samples was correlated with the S and Cu concentrations, with correlation coefficients of 0.25 and -0.18, respectively. No significant differences between the hair mineral concentrations were found among the age groups (p=0.074-0.897). The mean and standard deviation values of hair mineral concentrations in different age groups are provided in Table 3. The median concentrations of S, Ca, Zn, P, and Cu in the hair samples were 44.33-45.26, 1.79-2.19, 0.16, 0.11-0.12, and 0.04-0.06 mg/g, respectively. The median concentrations of the hair minerals did not substantially vary among the age groups.

**3. Relationship between self-reported osteoporosis and hair mineral concentrations**

The relationships between hair mineral concentrations and self-reported osteoporosis are shown in Table 4. The P concentration in the hair samples was correlated with self-reported osteoporosis. However, the Ca concentration did not correlate with self-reported osteoporosis. The results of the multivariate logistic regression analysis using age, P, and Ca as explanatory variables, and self-reported osteoporosis as an objective variable are shown in Table 5. Age (p=0.005) and P concentrations in the hair samples (p=0.044) were found to be significantly associated with self-reported osteoporosis using this model. Additionally, the cutoff values for the P and Ca concentrations were 0.94 and 2.07 mg/g, respectively. The odds ratios for age, P, and Ca were 1.11, 0.00, and 0.77, respectively. Furthermore, we analyzed the AUC to assess the predictive performance of this logistic model. The AUC of the model was 0.79, indicating a fair predictive performance.

Table 3 Hair mineral concentrations in different age groups.

| Age | | Minerals (mg/g) | | | | |
|---|---|---|---|---|---|---|
| | | S | Ca | Zn | P | Cu |
| 20-29 (n=52) | AM | 45.88 | 2.23 | 0.24 | 0.11 | 0.07 |
| | P50 | 45.26 | 2.19 | 0.16 | 0.11 | 0.05 |
| | SD | 3.39 | 1.21 | 0.22 | 0.02 | 0.11 |
| 30-39 (n=76) | AM | 44.96 | 2.02 | 0.24 | 0.11 | 0.11 |
| | P50 | 44.33 | 1.79 | 0.16 | 0.11 | 0.04 |
| | SD | 4.23 | 1.13 | 0.18 | 0.02 | 0.23 |
| 40-49 (n=50) | AM | 44.42 | 1.96 | 0.24 | 0.11 | 0.10 |
| | P50 | 44.94 | 2.07 | 0.16 | 0.11 | 0.04 |
| | SD | 6.60 | 0.91 | 0.19 | 0.02 | 0.12 |
| 50-59 (n=22) | AM | 44.69 | 2.26 | 0.23 | 0.12 | 0.09 |
| | P50 | 45.14 | 1.96 | 0.16 | 0.12 | 0.06 |
| | SD | 3.71 | 1.16 | 0.16 | 0.02 | 0.09 |

AM: arithmetic mean, P50: 50th percentile, SD: standard deviation.

Table 4 The relationship between mineral concentrations in the hair and self-reported osteoporosis.

| | Self-reported osteoporosis | | p value |
|---|---|---|---|
| | Yes | No | |
| S (mg/g) | 43.71 ± 8.58 | 45.11 ± 9.46 | 0.339 |
| Ca (mg/g) | 1.93 ± 1.34 | 2.09 ± 2.24 | 0.864 |
| Zn (mg/g) | 0.26 ± 0.42 | 0.24 ± 0.38 | 0.993 |
| P (mg/g) | 0.10 ± 0.02 | 0.11 ± 0.04 | 0.046 |
| Cu (mg/g) | 0.10 ± 0.24 | 0.10 ± 0.34 | 0.876 |
| Ca (mol)/P (mol) | 14.92 ± 12.08 | 15.44 ± 19.14 | 0.820 |

Mean±2SD. p: statistical significance obtained using the Mann-Whitney U test.

Table 5 Logistic regression analysis of self-reported osteoporosis and age or hair mineral concentrations.

| Factor | Odds ratio | 95%CI | | p value | Cutoff value |
|---|---|---|---|---|---|
| | | Lower | Upper | | |
| Age (year) | 1.11 | 1.03 | 1.20 | 0.005 | 39 year |
| P (mg/g) | 0.00 | 0.00 | 0.52 | 0.044 | 0.94 mg/g |
| Ca (mg/g) | 0.77 | 0.39 | 1.53 | 0.433 | 2.07 mg/g |

CI: confidence interval, p: statistical significance obtained using multivariate logistic regression analysis. The p value of the model was 0.014.

## DISCUSSION

### 1. Relationships between self-reported osteoporosis and aging or diseases

The proportion of participants with self-reported osteoporosis increased with age. Yoshimura et al. [11] reported that the prevalence rate of bone fracture was 14.0% (among Japanese women aged 50-59 years), which agrees with our result. The proportion of participants with high blood glucose or HbA1c levels in addition to self-reported osteoporosis was high. Discussion about the relationships between osteoporosis and diseases such as diabetes, hypertension, hyperlipidemia, and chronic kidney disease have dominated the research field in recent years. Moreover, there is evidence that diabetes and chronic kidney disease may cause osteoporosis. Vestergaard et al. [13] reported that the risk of femoral bone fracture among patients with type 2 diabetes was approximately 1.4 times higher than that in nondiabetic patients. Additionally, Yamamoto et al. [14] reported that the presence of type 2 diabetes was a risk factor for prevalent vertebral fracture with odds ratios of 4.73 in men and 1.86 in women. Previous studies have demonstrated that the risk of bone fracture was increased in patients with diabetes. We concluded that high blood glucose or HbA1c levels were risk factors for self-reported osteoporosis with an odds ratio of 15.58. Furthermore, in previous studies, the risk of bone fracture among patients with diabetes was attributed to oxidative stress caused by high blood glucose [15] and inhibition of osteoblast differentiation by insulin resistance [16]. This risk seems to be attributable to the degradation of bone quality caused by diabetes. It is important to decrease the blood glucose levels because preventing diabetes improves the risk of osteoporosis and lifestyle diseases such as obesity. In conclusion, our questionnaire survey may be used to monitor BMD conditions.

### 2. Mineral concentrations in hair

The concentrations of Ca in hair were correlated with those of Zn, P, and Cu, whereas the concentrations of P were correlated with those of Cu and S. Qayyum et al. [17] reported that among Pakistani individuals between 37 and 74 years of age, there was a significant correlation between Cu and Zn. However, there was no significant correlation between Ca and Zn or Cu. Fedor et al. [18] reported that there were no significant correlations between age and the concentrations of Zn or Cu, or between Zn and Cu. In this study, the mineral concentrations in the hair of the participants did not significantly vary with age (p=0.074-0.897). Skalnaya et al. [19] reported that median concentrations of Cu, and Zn in the hair of Russian individuals aged 40-49 years were 14.57 and 208.5 μg/g, respectively. Hao et al. [20] reported that the median concentrations of Ca, Cu, P, and Zn in the hair of Chinese individuals aged 100 years were 1550.49, 8.09, 147.30, and 170.59 mg/kg, respectively. Previous studies have proven that the mineral concentrations in the hair of the participants were adequate; however, the concentration of Cu in this study was higher than that reported in previous studies. The effects of age, sex, and race on hair analysis have not been established; therefore, it is important to accumulate extensive evidence about the concentrations of minerals in hair.

**3. Relationship between self-reported osteoporosis and hair mineral concentrations**

Approximately 60% of the bone is composed of hydroxyapatite, a calcium hydroxide phosphate with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. In this study, the P concentration in the hair of the participants was significantly correlated with self-reported osteoporosis (p=0.046) because it is a major component of hydroxyapatite. Song et al. [21] assessed the relationships between BMD and Ca or Mg concentrations in hair using multiple linear regression analysis, and reported significant correlations between lumbar BMD loss and these minerals. However, in the present study, no significant difference was found between the Ca concentration in hair and self-reported osteoporosis (p=0.864). The average molar ratio of Ca to P in the hair of the participants was 15.42 (range 1.67-55.10) in Table 4. The concentration of Ca in the hair was higher than that of P, and there was no significant difference between the molar ratio of Ca to P and self-reported osteoporosis (p=0.820). The molar ratio of Ca to P in hydroxyapatite is 2.16, and the desired ratio for dietary intake of Ca to P ranges from 1:1 to 1:2. Excess intake of P results in a negative balance of Ca. This suggests that the Ca concentration in hair could not directly indicate the status of self-reported osteoporosis. For this reason, the Ca and P in hair needs to be measured to correctly determine the risk for BMD loss. Multivariate logistic regression analysis was performed to examine the factors associated with self-reported osteoporosis. In this model, age (p=0.005) and P concentration in hair (p=0.044) were significantly associated with self-reported osteoporosis, and the AUC of 0.79 indicated a moderate predictive ability. The value of AUC is used to evaluate the predictive ability of a model. The predictive ability of an ROC curve may be assessed as follows: 0.50-0.70, low; 0.70-0.90, moderate; and 0.90-1.0, high. This result suggests that age and hair mineral concentrations may be used to primary prevent osteoporosis. To prevent osteoporosis, it is important to increase the peak bone mass until the early 20s. Kubota et al. [22] reported that adequate intake of n-3 fatty acids, phosphate, and vitamin D may help maximize the peak bone mass at the hip among young women. Zhu et al. [23] suggested that regular exercise increases the peak bone mass. Previous studies have demonstrated that appropriate lifestyle habits during early life may prevent osteoporosis later in life. It is assumed that regular hair mineral analysis can be an effective lifestyle habit intervention. Effective methods for preventing osteoporosis include regular exercise and appropriate intake of nutrients such as Ca. It is important to provide information about the influence of appropriate lifestyle habits on the risk of osteoporosis; however, individuals must have an understanding of their health condition to conduct an effective lifestyle habit intervention. In conclusion, this study suggests that it is possible to understand the risk of diseases such as osteoporosis early in life by measuring the mineral concentrations in hair. However, this study had some limitations. First, the questionnaire was based on self-assessment and there was no detailed information about BMD loss. The prevalence rate of self-reported osteoporosis was the same as in previous studies [10, 11]; however, the degree of BMD loss may differ in terms of the subjective view of the participants. Second, there was no information related to osteoporosis such as BMI, fracture history, smoking, and alcohol. Future studies should evaluate BMD and these factors to improve the predictive ability. Finally, in the case of multivariate logistic analysis, the number of outcomes was low. In general, multivariate logistic analyses require 10 times the number of outcomes compared with the number of factors to achieve robust results. However, the model was statistically significant (p=0.014), and the factors of the model agreed with those of previous studies.

Although our findings should be interpreted with caution due to there are these limitations, this study may contribute to primary prevent of osteoporosis.

## Conflict of Interest

The authors declare no conflict of interest.

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