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Concentrations of trace element in human dentin by sex and age

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ABSTRACT

Teeth are recently drawing attention for their potential as biological modeling investigation samples due to their ability to be collected and their slow substance metabolism. There is no active metabolism of elements after the completion of dentin. Dentin is surrounded by enamel and cementum, and is not affected by the oral environment. Therefore, the amount of trace elements in dentin may change with age, and this is considered to be a reliable biological load index. The objectives in this study are to demonstrate concentrations of elements in the dentin of healthy Japanese subjects by sex and age, and to reveal the relationship between element levels and age. 121 healthy teeth samples were extracted due to periodontal disease or orthodontic treatment. Each tooth was sliced from the crown to the root apex into 0.5–1 mm thickness, then enamel, cementum, and the pulp were removed; the dentins were used as samples. The concentration of 10 trace elements (B, Mn, Co, Cu, Zn, Rb, Sr, Mo, Cd, and Pb) in the dentin was measured using inductively coupled plasma mass spectrometer (ICP-MS). The differences Co and Pb in the dentin between men and women were significant ($p < 0.01$). Significant positive correlation was observed between B, Co, Cu, Zn, Sr and Pb concentrations in the dentin and age ($p < 0.001$). The results of the present study suggest that human dentin is an appropriate substance for relativity with sex and age at further future research.

1. Introduction

Humans ingest elements, and some are accumulated in the hard tissues. Concentrations of elements in these tissues, therefore, may be the index of cumulative ingestion of elements. However, some hard tissues cannot be used as reliable indices for various reasons. For example, human bone is very difficult to be collected for measurement, and finger nails and hair are often contaminated with dust, shampoo, and nail polish, so these samples are not ideal to observe biological changes.

Teeth are recently drawing attention for their potential as biological modeling investigation samples due to their easy extraction and slow substance metabolism [1]. Concentration analysis of teeth has been conducted to investigate the relationship between sample and environment. The relationship between lack or overdose of trace elements in deciduous teeth and disease and nutritional investigation have been conducted by measuring trace elements in deciduous teeth [2,3]. In particular, protoplasmic protuberances of odontoblasts remain after the completion of dentin development, and some metabolism is mediated through these growths. There is no active metabolism of elements after the completion of dentin. Dentin is surrounded by enamel and cementum, and is not affected by the oral environment. Since the amount of trace elements in dentin may change with age, this is considered to be a reliable biological load index.

The objectives in this study are to demonstrate concentrations of elements in dentin of healthy Japanese subjects by sex and age, and to reveal the relationship between element levels and age.

2. Materials and methods

2.1. Subjects

Samples include single root canines, first and second premolars, as well as second and third molars that were extracted due to periodontal disease or orthodontic treatment. A total of 121 healthy teeth were extracted from patients between 14 and 91 of age (mean = 49.7 years of age), of which 55 were men and 66 were women. We obtained informed consent from patients to use their teeth in the study. Table 1 shows the number, age, and sex of patients who contributed to the study. Fluoride (F) concentrations of water in patients' living areas were below 0.15 ppm.

2.2. Determination of elements in dentin

Soft tissue around the teeth was removed, and each tooth was sliced buccolingually from the crown to the root apex into 0.5–1 mm thickness with a diamond disc (HORICO Hopf, Ringleb & Co. GmbH & Cie., Berlin, Germany) for the analysis. Enamel, cementum, and the pulp were removed with diamond disc under a magnifying glass and stereoscopic microscope, and dentins of about 100 mg dry weight were used for measurement. 1 mL of a high purity nitric acid (Wako Pure

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Table 1
Sex and age distribution of subjects. A total of 121 healthy teeth were extracted from patients between 14 and 91 of age (mean = 49.7 years of age), of which 55 were men and 66 were women.

Age groups (years)	Men	Women	Total
<29	10	21	31
30–49	7	12	19
50–69	31	20	51
70<	7	13	20
Total	55	66	121

Men: 14–91 years (mean: 51.8 years). Women: 18–83 years (mean: 47.0 years).

Chemical Industries, Ltd., Osaka) was added to the sample in a Teflon tube (San-ai Kagaku Co. Ltd., Nagoya, Japan), and the tube was maintained at 120 °C for 12 h using the hot block bath. After decomposition, the resultant solution was transferred to a Teflon jar (San-ai Kagaku Co. Ltd., Nagoya, Japan). The solution was maintained at 120 °C for 5 h using a hot plate. After drying, 20 mL of 5% high purity nitric acid solution was added to the jar and capped. The solution was put on a hot plate and maintained at 50 °C to dissolve the sample matter.

Concentration of 10 trace elements, boron (B), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), rubidium (Rb), strontium (Sr), molybdenum (Mo), cadmium (Cd), and lead (Pb) were determined by inductively coupled plasma mass spectrometer (ICP-MS; SCIEX, ELAN6000, Perkin Elmer, Inc., Massachusetts, USA).

Accuracy control of the measurements was conducted using the following methods. Five blank samples of only nitric acid were prepared, and trace elements in these samples were measured in the same manner as the tooth samples. The measurements of trace element concentration in these samples were deducted from the measurements from the tooth samples. Calcium (Ca) concentration in the solution sample was also monitored by ion chromatography to evaluate the influence of Ca.

2.3. Statistical analysis

Student's *t*-test was used for comparison between men and women. Regression analysis was performed and Pearson's correlation coefficients were obtained between element concentration and age. One-way analysis of variance was used to compare elements concentration among age groups. B, Mn, Cu, Sr, Cd and Pb were log-transformed before analysis since it was similar to the log-normal distribution.

3. Results

Table 2 shows the comparison of mean levels of elements concentrations in dentin by sex. B, Mn, Cu, Sr, Cd and Pb were indicated as geometrical average and standard deviation, and others were indicated as average and standard deviation. The differences in Co and Pb between men and women were significant ($p < 0.01$). Co was higher in women than men, and Pb was higher in men than women.

Table 3 shows the correlation coefficients between elements concentrations in dentin and age. Significant positive correlation was observed between B, Co, Cu, Zn, Sr, and Pb concentration and age ($p < 0.001$). Fig. 1 shows the comparison between levels of trace elements in dentin and age. Fig. 2 shows the comparison between these element levels and age groups. A significant difference was observed in Sr concentration between the group of 50–69 year olds and that of 70 years or older ($p < 0.001$). A significant difference was also observed in Co concentration between groups of the same age ($p < 0.01$). No clear increases in concentrations of other trace elements were observed in age groups of 50 years of age or older.

We compared the concentrations of trace elements between different teeth, but no significant difference was observed (data not shown).

Table 3
Correlation coefficients between elements concentrations in dentin and age. Significant positive correlation was observed between B, Co, Cu, Zn, Sr, and Pb concentration and age ($p < 0.001$).

	Elements				
	B	Mn	**Co**	**Cu**	**Zn**
Correlation coefficient	**0.686**	0.144	**0.491**	**0.494**	**0.384**
p value	**<0.001**	0.115	**<0.001**	**<0.001**	**<0.001**

	Elements					
	Rb	**Sr**	Mo	Cd	**Pb**	Ca
Correlation coefficient	0.130	**0.823**	0.098	0.141	**0.863**	−0.091
p value	0.155	**<0.001**	0.283	0.126	**<0.001**	0.320

The bold values show the element that significant correlation was observed with age.

4. Discussion

The previous studies regarding trace elements in teeth had been difficult due to the influence by high Ca concentration and detection limit of trace elements [2–9]. ICP-MS was applied to determine trace elements concentrations due to its high sensitivity and quantitative analysis.

Ichida reported that Pb in dentin is higher in men than women [9]. The results of the present study also showed higher Pb in men. A significant difference was observed in Co between men and women, but the reasons for this are still unclear. It is reported that a smoker's Pb level of blood shows a high value [10]. Further studies are required in which the consideration of various factors such as the difference in preferred foods or smoking habits are examined in order to discover the reasons for divergence.

The results of the present study showed that many elements in dentin increase with age until the age of 50 years old. This is considered to be due to the fact that dentin contains a large amount

Table 2
Comparison of mean level of elements concentrations in dentin by sex. B, Mn, Cu, Sr, Cd and Pb were indicated as geometrical average and standard deviation, and others were indicated as average and standard deviation. The differences in Co and Pb between men and women were significant ($p < 0.01$). Co was higher in women than men, and Pb was higher in men than women.

	Elements				
	B (μg/g)	Mn (μg/g)	**Co (μg/g)**	Cu (μg/g)	Zn (mg/g)
Male	1.71[a] (1.61)	0.68[a] (1.52)	**0.94 (±0.11)**	1.28[a] (1.30)	0.42 (±0.07)
Female	1.54[a] (2.16)	0.70[a] (1.30)	**0.99 (±0.10)**	1.33[a] (1.29)	0.44 (±0.08)
p value	0.40	0.64	**<0.01**	0.39	0.32

	Elements					
	Rb (μg/g)	Sr (mg/g)	Mo (μg/g)	Cd (μg/g)	**Pb (μg/g)**	Ca (%)
Male	0.51 (±0.13)	0.20[a] (1.70)	0.26 (±0.06)	0.04[a] (2.17)	**5.47[a] (3.09)**	27.12 (±1.68)
Female	0.49 (±0.12)	0.18[a] (1.82)	0.26 (±0.05)	0.04[a] (2.48)	**2.95[a] (3.33)**	26.91 (±1.50)
p value	0.39	0.25	0.76	0.89	**<0.01**	0.48

The bold values show the element that significant difference was observed with sexual distinction.
[a] Geometric mean (geometric standard deviation).