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Determining trace metal elements in the tooth enamel from Hui and Han Ethnic groups in China using microwave digestion and inductively coupled plasma mass spectrometry (ICP-MS)

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ABSTRACT

The content of trace elements in teeth can provide information on oral health, environmental factors and diet habits. A method for human dental enamel digestion using microwave system with concentrated nitric acid and hydrogen peroxide is described for further determination trace elements of Mn, Fe, Cu, Zn, Ni, Sr, Cd, and Pb by inductively coupled plasma mass spectrometry (ICP-MS). The reference materials of bone ash (NIST SRM 1400) and bone meal (NIST SRM 1486) were used to evaluate the accuracy of the proposed method. The data were assessed statistically using t-tests. The results showed a significant difference ($P < 0.05$) in the trace element Fe as well as the trace elements Al, Mn, Cu, Zn, Ni, Sr, Cd, and Pb ($P < 0.01$). In healthy tooth enamel, the contents of the trace elements Cd, Ni, Mn, and Pb, which may promote or accelerate caries and its development, are higher in the Han than the Hui ethnic group. However, for healthy tooth enamel, the contents of the trace elements Cu, Fe, Zn, and Sr, which may prevent caries, are lower in the Han than the Hui ethnic group. Different tooth structure and diet habits for the Hui and Han ethnic groups in Xi'an, China may be the primary basis for the distinction in trace element content.

1. Introduction

Dental caries is a common oral disease and has an especially high incidence in children and adolescents. The World Health Organization (WHO) has listed it as the third largest non-communicable disease that endangers human health other than cancer and cardiovascular diseases [1]. To better prevent and treat dental caries, many countries consider dental caries an important research area in oral medicine [2].

Within countries, dental caries incidence can differ among distinct ethnic groups due to eating habits, culture, and geographical environment [3]. In China, the minority ethnic group Yi has the highest dental caries incidence rate (56.0%, mean DMFT (decayed missing filled teeth) 1.52), and the Hui ethnic group has the lowest dental caries incidence rate (18.2%, mean DMFT 0.3) [3]. The Han ethnic group which has the largest proportion of China has the middle dental caries incidence rate (37.4%). Therefore, the difference of caries between different ethnics at the same regional not only has important practical significance for the prevention of dental caries, but also provide a theoretical basis for regional caries prevention.

Trace elements are essential substances in a normal human body and are crucial for human growth, development, metabolism, immunity, and endocrine function [4,5]. Trace elements in teeth not only provide information on the living geography and eating habits of the inhabitants, but they also provide a basis for an oral health evaluation. Because teeth are easy to store and rarely contaminated, trace elements in teeth are often measured in scientific archaeology, forensic identification, paleoecology, and other disciplines [6–9].

The researchers shown that the content for Mg, Al, Mn, Fe, Cu, Zn, Ni, Sr, Cd, Pb, and other trace elements is closely related to dental caries. Among these metal elements, Zn, Fe, Cu and Sr had the effect to prevent dental caries, but Mg, Al, Ni, Mn, Cd and Pb has effect to facilitate or accelerate the occurrence and development of dental caries at some extent [10–16]. Therefore, detecting the content of trace elements in teeth may aid in determining trace element content in the body of a patient suffering from dental caries. Such data may provide a basis for methods for preventing caries and its development [10–16].

In this experiment, using microwave digestion and ICP-MS, the content of trace elements Mg, Al, Mn, Fe, Cu, Zn, Ni, Sr, Cd, and Pb in healthy tooth enamel from the Hui and Han ethnic groups in Xi'an, China were determined. The normal parameters for both males and females in Hui and Han ethnic groups were measured. This study contributes anthropology as well as ethnology. Additionally, this study provides scientific

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reference data for clinical oral preventive medicine as well as a future basis for methods for determining the content of trace elements relevant to dental caries as well as preventing its incidence and development in the Hui and Han ethnic groups from Xi'an, China.

2. Materials and Methods

2.1. Instruments and operating conditions

Inductively coupled plasma mass spectrometer: PE ICP-MS (Perkin Elmer-Nexion 300D, PerkinElmer Corporation, USA). The elements were determined by PE 300D with an S10 Auto sampler at the Division of Chemical Metrology & Analytical Science, National Institute of Metrology and the State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment in Xi'an, Chinese Academy of Sciences (IEECAS). The ICP-MS instrument operating parameters were established by automatically optimizing the instrument conditions. They met the instrument standard requirements, such as sensitivity, background values, and stability.

Ethos Touch Control microwave digestion system (Milestone, Italy); Milli-Q ultrapure water system (Millipore Corporation, USA).

2.2. Reagents

Single element standard solution: Mg, Al, Mn, Fe, Cu, Zn, Ni, Sr, Cd, and Pb, at concentrations of 1000 mg / L (National Analysis Center for Iron and Steel at Central Iron & Steel Research Institute).

Mass calibrants: Li (7), Co (59), Y (89), Ce (140), and Tl (205) at concentrations of 0.01 g / L (PE, USA); internal standard solutions: ^{6}Li, ^{45}Sc, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb, and ^{209}Bi at concentrations of 0.001 g/L (PE, USA); Milli-Q H_2O:18.2 MΩ at 25°C from Millipore (Elix-Millpore, USA). HNO_3: Obtained from the Beijing Institute of Chemical Reagent and purified using the SavillexTM DST-100 sub-boiling distillation system (Minnetonka, MN, USA). H_2O_2: 30 wt. % in H_2O (Sigma-Aldrich Co. LLC.).

2.3. Tooth sample collection and pretreatment

Two hundred permanent health teeth were collected from males and females in the Hui and Han ethnic groups (ages 20-69) who have lived in Xi'an for many years. The detailed procedures for teeth rinsed, soaked and ground in this study follow the methods of Li et al [13].

The microwave digestion conditions used in this study for teeth digestion were adapted to those previously used for biological samples following the method of He et al. [17] Tooth samples were weighed, ground to powder using mortar and pestle, and weighed again before the digestion (depending on the tooth type and size, they ranged from 0.0090 to 0.7350 g). In this study, powdered teeth samples were predissolved in 3 mL 50% HNO_3 solution and 2 mL 30% H_2O_2 solution using microwave digestion tank. The tank was then gently shaken. Approximately 3 mL Milli-Q water was added dropwise. For the certified reference material (NIST SRM 1400 and NIST SRM 1486), an amount of ca. 0.0500 g of sample was digested in microwave as described above.

3. Results

Ten metal elements, including Mg, Al, Mn, Fe, Cu, Zn, Ni, Sr, Cd, and Pb in tooth enamel from the Hui and Han ethnic groups in Xi'an, China were measured. The results were statistically analyzed and are shown in Table 1.

From Table 1, it found that the content of trace elements were different between two ethnic groups. The average content of trace element such as Pb, Cd, Ni, Mn, Cu, Fe, Sr and Zn was 144.258 μg/g,0.5361 μg/g,0.983 μg/g,5.20 μg/g,0.702 μg/g,31.052 μg/g,144.039 μg/g and 129.807 μg/g in Han ethnic groups, respectively. And was 129.172 μg/g, 0.4679 μg/g, 0.872 μg/g, 3.549 μg/g, 1.085 μg/g, 36.557 μg/g, 159.835 μg/g and 148.759 μg/g in Hui ethnic groups

Table 1
The content in tooth enamel (μg/g)

Element	Hui Ethnic Group ($\bar{x} \pm$ SD)	Han Ethnic Group ($\bar{x} \pm$ SD)	*U* tests
Pb	129.172 ± 15.034	144.258 ± 14.249	0.3891**
Cd	0.4679 ± 0.027	0.5361 ± 0.034	24.1542**
Mg	5533.887 ± 719.228	4562.3 ± 730.452	1.78
Mn	3.549 ± 1.157	5.202 ± 2.426	3.591**
Fe	36.557 ± 10.698	31.052 ± 9.693	4.742*
Sr	159.835 ± 36.156	144.039 ± 48.333	0.255**
Cu	1.085 ± 0.093	0.702 ± 0.057	7.057**
Zn	148.759 ± 68.230	129.807 ± 34.485	3.281**
Ni	0.872 ± 0.149	0.983 ± 0.201	10.027**
Al	28.368 ± 8.684	23.0758 ± 10.895	14.223**

*$P < 0.05$, **$P < 0.01$

Normality test results showed that Mg had a normal distribution ($P > 0.05$). The trace elements Al, Mn, Fe, Cu, Zn, Ni, Sr, and Cd had non-normal distributions ($P < 0.05$). U tests showed a statistically significant difference ($P < 0.05$) for the Fe content from the tooth enamel samples. A statistically significant difference for Al, Mn, Cu, Zn, Ni, Sr, Cd, and Pb was even more apparent ($P < 0.01$).

4. Discussion

4.1. The difference of trace element content between Hui and Han ethnic groups

Fig. 1 distinguishes the metal elements content in healthy tooth enamel from the Hui and Han ethnic groups in Xi'an, China, into two groups. One group comprises the trace elements Cd, Ni, Mn, and Pb, which can promote and accelerate dental caries and its development. In this group, the trace element content in healthy tooth enamel from the Han ethnic group is higher than in the Hui ethnic group. A second group comprises the trace elements Cu, Fe, Zn, and Sr, which have demonstrated certain anti-caries functions. In this group, the trace element content was higher for the Hui ethnic group than the Han ethnic group.

4.2. Factors that impact the trace element content in tooth enamel from the Hui and Han ethnic groups

The trace element content in tooth enamel is influenced by many factors for a variety of reasons [12]. The distinct living habits of the Hui and Han ethnic groups in China might be the primary basis for such difference trace element content and dental caries incidence. From a dietary perspective, the Hui group regularly eats crude food, including raw or undercooked beef and lamb. The crown surface regularly receives friction, and self-cleaning effects are enhanced. Dental hard tissue is quickly worn out. Areas, such as furrows, crevices and point gaps, which are prone to dental caries, are also worn out and thus less prone to dental caries. In addition, the Hui people enjoy tea and edible nuts (walnuts, hazelnuts and almonds), which may enrich trace elements, such as Cu, Al, Fe, Zn and Sr, in the tooth enamel. Other factors, such as cultural differences, economic income, oral hygiene habits, and oral saliva pH values for the two ethnic groups cannot be ignored, as they are important epidemiological factors that affect dental caries incidence and development.

5. Conclusions

Using microwave digestion and ICP-MS, the content of the trace elements Mg, Al, Mn, Fe, Cu, Zn, Ni, Sr, Cd, and Pb in the tooth enamel from the Hui and Han ethnic groups in Xi'an, China, was accurately determined. Experimental results showed the following:

1) There was a significant difference in the content of the trace elements Al, Mn, Fe, Cu, Zn, Ni, Sr, Cd, and Pb in the tooth enamel

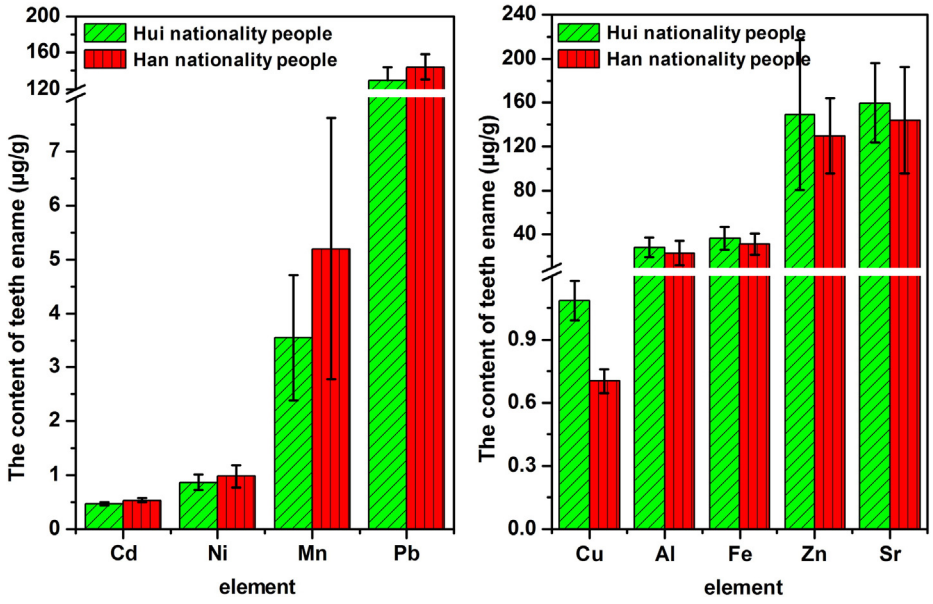

Fig. 1. The relationships for the trace elements in tooth enamel from the Hui and Han ethnic groups in Xi'an

from the Hui and Han ethnic groups in Xi'an China. The contents of trace elements Cu, Fe, Zn, and Sr, which have anti-caries properties, were significantly higher in the Hui ethnic group.

2) The different habits for the Hui and Han ethnic groups in Xi'an, China may be the primary basis for the distinction in trace element content and dental caries incidence.

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