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Introduction

In Japan, it has been reported that the mean birth weight is decreasing gradually and the babies with low birth weight are increasing during recent two decades[1]. These phenomena are related to maternal low dietary intake and/or cigarette smoking. There is a possibility that deficiency and/or excess of major elements and trace elements in fetus have occurred resulting from exposure to low dietary and cigarette smoking.

Deciduous tooth enamel commences calcification at the end of the first trimester in utero and continues to develop up to a year after birth. The neonatal line which is found in all deciduous teeth represents the time of birth and the border line between prenatal enamel and postnatal enamel. Mature enamel contains no active cells and its chemical composition reflects the status of metabolism of trace elements during formation period. Therefore, deciduous tooth enamel is a suitable indicator of trace element exposure in utero.

In previous studies, flame atomic absorption spectroscopy, inductively coupled plasma emission and mass spectrometry were often used to measure trace element concentration[2,3]. These analytical methods need bulk techniques in which whole teeth or some portion of enamel is digested in a suitable mineral acid. Because enamel tissue is destroyed in this technique, we cannot know the time-based elemental distribution profiles. However, in micro-PIXE analysis, tooth enamel samples are used without destruction, multiple trace elements concentration are measured at the same time, and the distribution of trace elements and their magnitudes can be seen on color maps of scanning area. In this study, trace elemental intake during pre- and post-natal development were measured on deciduous tooth enamel sections using micro-PIXE analysis.

Material and methods

Two deciduous canines from one healthy Japanese child were used for this study. These teeth were caries free and extracted at the age of eight years because of pre-shedding mobility. Each tooth crown was embedded in dental resin, and sectioned in the labio-lingual plane that passed through the cusp tip. The 100 μm thick un-decalcified section was cut from the one half-tooth section. The location of neonatal line was confirmed in the each thin section using an optical microscope. The sample for micro-PIXE analysis was prepared as an enamel section including both pre- and post-natal enamel which identified by neonatal line. The surface of section was coated with carbon.

Analysis of samples was carried out with the microbeam analysis system at Tohoku University[4,5]. Two X-ray detectors for high energy and low energy were used. Sample was set the enamel surface parallel to the direction of microbeam to reduce edge effect. A Mylar filter (600 μm thick) was attached in front of the high energy X-ray detector to reduce pile-up events. The beam scanning area was set to $0.4\times0.4 \sim 1\times1$ mm^2, and the beam spot size of 2×2 μm^2. Averaged beam current was ca. 150 pA. Measurement time was around 1 hour. The elemental maps were obtained using the GeoPIXE software[6]. For correct calculation, the major composition and density of the layer must be set. The layer is presumed to be hydroxyapatite ($Ca_5(PO_4)_3(OH)$) of 100 μm thickness, with a density of 3.15 g/cm^3 [7].

Ethics approval for this study was obtained from the Ethics Committee of Tohoku University Graduate School of Dentistry.

Results of analyses

Neonatal line (NNL) was identified on enamel section (Fig. 1). In Fig. 1, the enamel lying under the neonatal line was considered to be postnatal enamel and the upper part to be prenatal enamel. The embedding material layer is shown out of tooth sample.

Typical X-ray spectra measured by the detectors for low and high energies are shown in Fig. 2. Besides calcium (Ca) and phosphorus (P) which were main contents of enamel, sodium (Na), magnesium (Mg), chlorine (Cl), zinc (Zn), and strontium (Sr) were detected. The elemental maps obtained by scanning this section are shown in Fig. 3. X-ray maps of Na and Sr were uniform over an area of the tooth sample. Ca, P, Mg, Cl and Zn showed different distribution between enamel and dentin. Elemental concentrations of Ca and P were higher in enamel than in dentin. On the other hand, elemental concentration of Mg in dentin was slightly higher than in enamel. The concentrations of these three elements were uniform within each tissue (enamel and dentin). Whereas, distribution profiles of Cl and Zn were specific and showed higher concentration in surface enamel. Accumulation of Zn was observed in the most outer layer of enamel. Unfortunately, no elements showed different profiles of X-ray maps between prenatal enamel and postnatal enamel.

Discussion

Thirty-five trace elements have been reported to be present in sound enamel of permanent teeth with wide range[8]. Twelve elements (Ca, Cu, Mg, Zn, Sr, Al, Ba, Pb, U, Ce, La and Pr) have been found in the deciduous teeth from Ugandan and UK children[2]. Shashikiran et al.[3] showed the presence of 18 elements (F, Sr, K, Al, Si, Ni, B, Fe, Cu, Mn, Co, Cr, Zn, Mg, Se, Pb, Mo and V) in sound and carious enamel of primary and permanent teeth. All of elements detected in this study have been already found in sound deciduous enamel. Lead (Pb) which was found in the teeth from Ugandan and UK children[2] (1.21ppm and 1.33ppm, respectively), from Indian children[3] (<0.1ppm), and from Mexican children[9] was under detection limit in the enamel section from Japanese child in this study. The detection limit was ca. 9ppm in this study. The sensitivity of Pb in the present measurement was lower than that in the other measurements used in the previous studies. However, Pb concentrations shown in those studies were not measured in prenatal or postnatal enamel but in whole tooth crown or enamel powder.

There are few studies described about the distribution of trace elements in enamel. In this study, we obtained the specific distribution profiles of some elements by micro-PIXE analysis. The distribution of Zn, showed the accumulation in enamel surface area. This result was different from the result of Kang's study[10] using LA-ICP-MS in which zones of enhanced Zn intensities was seen along the neonatal line. Some elements including Pb have been demonstrated high levels in surface enamel area. However, it is needed to be considered that some events occurring at enamel surface, like oral exposure, de/remineralization, and chemical adsorption/absorption, complicates enamel chemistry in surface enamel area. Zn is considered to be one of important elements for estimating the environment in utero.

The location of neonatal line identified on the optical microscope image was useful to identify the location of neonatal line on the corresponding distribution map. Unexpectedly, the change of distribution profile around neonatal line was not obvious on all elemental concentration maps. The child in this study was healthy and had no special event around birth time. Therefore, the difference between prenatal enamel and postnatal enamel might not be detected.

Conclusions

This study showed the presence of 5 trace elements (Na, Mg, Cl, Zn, and Sr) in the sound enamel including pre- and post-natal enamel of deciduous teeth. Furthermore, we could obtain the distribution maps of these elements with specific profiles. Although no elements detected the difference of distribution profiles between prenatal enamel and postnatal enamel in this sample from healthy child, it was suggested that this analytical methods using micro-PIXE would be able to estimate the trace elements in prenatal and postnatal enamel, respectively.

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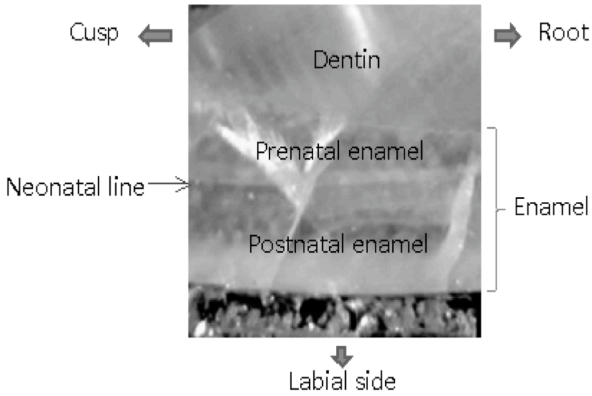

Figure 1.　Enamel section.

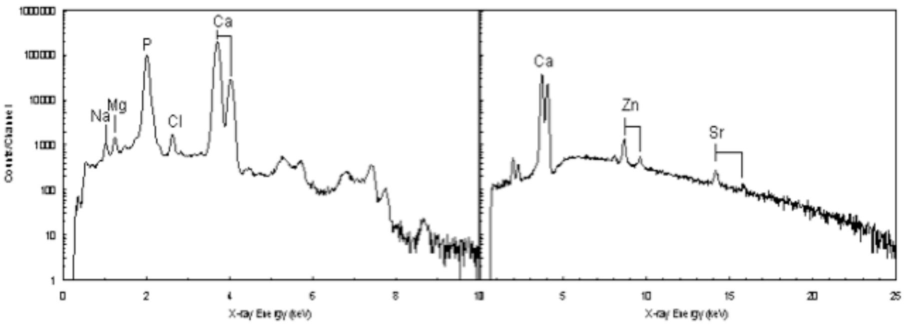

Figure 2.　Typical X-ray Spectra Measured by the Detectors for Low and High Energies.

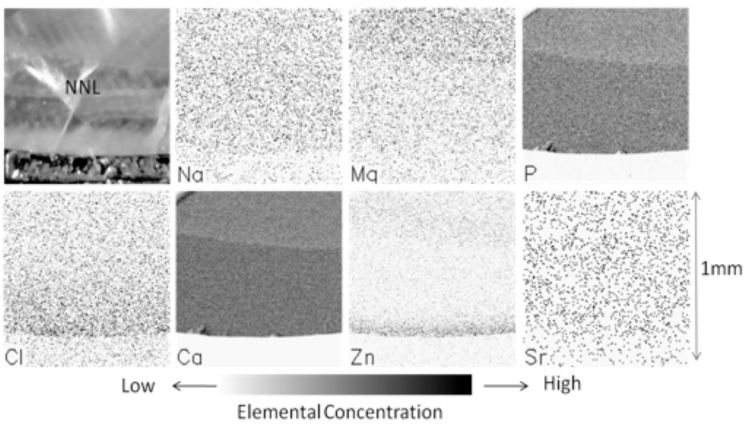

Figure 3.　Elemental maps of the elements.