Distribution of fluoride and magnesium concentrations in deciduous tooth enamel of children with cerebral palsy and Down syndrome

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Abstract
BACKGROUND: Systemic disturbance during the fetal and postnatal periods in disabled children may affect the formation and mineralization of deciduous tooth enamel. AIM: To reveal differences in the status of mineralization in the surface and inner layers of deciduous tooth enamel between children with cerebral palsy (CP), those with Down syndrome (DS), and controls. METHODS: Using extracted deciduous teeth obtained from CP children (5 teeth), DS children (5 teeth), and control children (11 teeth), fluoride and magnesium concentrations, used as mineralization parameters, were measured in three regions of the enamel at different depths: the enamel surface (ES), neonatal line (NL), and dentino-enamel junction (DEJ). RESULTS: Fluoride concentration was significantly higher in the ES region than in the NL or DEJ region in all three groups. There was no significant correlation between type of disability and fluoride concentration. Magnesium concentration was significantly higher in the DEJ region than in the NL or ES region. A significant correlation was seen between type of disability and magnesium concentration. CONCLUSIONS: Magnesium concentration was significantly higher in DS children than in control children, suggesting that DS children undergo poorer mineralization. Magnesium concentration tended to be higher in CP children than in control children.

Introduction
In disabled children, disturbances occurring during the fetal and postnatal periods lead to delays in physical and mental growth and development. In particular, systemic disturbance during these periods may affect the formation and mineralization of the deciduous tooth enamel, in which tooth crown formation starts during fetal life and continues until a few months after birth. Several previous studies have suggested a possible relationship between systemic disturbance and deciduous tooth enamel formation1–5). Children with cerebral palsy (CP) and Down syndrome (DS) encountered in dental practice often show different pathogenesis and pathology from each other. It has been reported that two-thirds of children with CP have poor mineralization of the deciduous tooth enamel, as determined macroscopically1). The amount of prenatal enamel formation in the mandibular second deciduous molars and deciduous canines (which are formed in different stages of fetal life) and the Ca/P ratios at these sites have been found to differ in children with CP and DS as well as typically developing children (control children)2,3). Compared to control children, children with CP and DS showed higher ratios of prenatal

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enamel formation in the deciduous canines, which are formed relatively early during fetal life, but a lower Ca/P ratio of prenatal enamel formation and a higher Ca/P ratio in mandibular second deciduous molars, which are formed later during fetal life. These results suggest that the amount and mineralization status of enamel formation varies depending on the type of dental germ, and that children with CP and DS tend to have poorer mineralization compared to control children.

A study on the effects of acid on the enamel of the mandibular second deciduous molar with regard to acid resistance and surface roughness following acid decalcification among the same populations of children revealed that children with CP and DS have lower acid resistance and 3–5 times higher surface roughness compared to control children, suggesting abnormal crystal morphology and crystal defects in the deciduous teeth of children with DS. Another study on the remineralization reaction of the deciduous tooth enamel following decalcification in children with DS versus control children found that remineralization occurred from the deep enamel in control children, whereas it occurred from the deepest layer of the decalcified enamel in children with DS, suggesting different mechanisms of remineralization reaction in the deciduous teeth between the two groups of children.

The enamel crystal is mainly composed of hydroxyapatite (Ca_{10}(PO_4)_6(OH)_2) and has a very unstable structure, with its constituent atoms and molecules susceptible to substitution. In addition, its chemical stability is substantially affected by the type of incorporated ions and defect of ions, and the substitution of trace elements affects its crystal structure. In particular, substitution with Mg$^{2+}$ results in unstable crystal structure, whereas substitution with F$^{-}$ results in stable crystal structure.

None of the previous studies of deciduous tooth enamel of disabled children have measured fluoride and magnesium concentrations in each layer of the deciduous enamel, from the surface through neonatal line to dentino-enamel junction (DEJ), in order to compare the overall mineralization status of the deciduous tooth enamel between disabled and typically developing children. This would be important to determine, because differences in mineralization may mean disabled children are more prone to deciduous tooth caries than typically developing children.

To this end, in this study, using the concentrations of fluoride and magnesium — which have opposite effects on enamel — as the parameters of the degree of mineralization, we measured these concentrations in the surface, neonatal line, and DEJ of the deciduous enamel in order to compare the mineralization status of deciduous tooth enamel in its surface and inner layers between typically developing children and children with CP or DS.

### Materials and Methods

#### Enamel samples and preparation

The distribution of teeth samples is presented in Table 1. Mandibular deciduous central and lateral incisors extracted from patients in need of tooth extraction at a dental hospital in Aichi Prefecture, Japan, were used as samples. The teeth samples were extracted with the consent of each child or guardian and were used with their agreement only for the purposes of the experiment. This study was carried out with approval from the Ethics Committee of the Department of Dentistry, Aichi-Gakuin University (approval No.176).

<table>
<thead>
<tr>
<th>Subjects and deciduous teeth samples</th>
<th>CP (cerebral palsy)</th>
<th>DS (Down syndrome)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (Male : Female)</td>
<td>5 (3 : 2)</td>
<td>5 (3 : 2)</td>
<td>5 (3 : 2)</td>
</tr>
<tr>
<td>Average age (± S.E.)</td>
<td>7.2 (± 0.7)</td>
<td>7.2 (± 0.6)</td>
<td>6.9 (± 0.3)</td>
</tr>
<tr>
<td>Minimum age</td>
<td>5.2</td>
<td>5.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Maximum age</td>
<td>9.7</td>
<td>8.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Number of teeth samples</td>
<td>5</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>(Central incisor : Lateral incisor)</td>
<td>(3 : 2)</td>
<td>(4 : 1)</td>
<td>(6 : 5)</td>
</tr>
</tbody>
</table>
as samples. Only those teeth without local fluoride application within the past 6 months or without macroscopic evidence of caries, white spots, or cracks were used. The teeth samples included 5 teeth obtained from 5 children with CP (age range: 5.2 to 9.7 years, mean ± S.E.: 7.2 ± 0.7 years), 5 teeth from 5 children with DS (age range: 5.6 to 8.8 years, mean ± S.E.: 7.2 ± 0.6 years), and 11 teeth from 11 control children (age range: 5.1 to 8.2 years, mean ± S.E.: 6.9 ± 0.3 years). There was no significant difference in age at teeth extraction between the three groups.

From the teeth samples, longitudinal abrasive sections with a thickness of 450 μm were cut from the mesiodistal center of the labial surface along the long labial-lingual axis. Then, the part of the longitudinal abrasive section in which the middle layer of the enamel contained the neonatal line (NL) and where the enamel surface (ES), NL, and DEJ were aligned in parallel was selected and cut into 1,000-μm-long sections ranging from the enamel surface to the pulp cavity.

Micro-sampling

Prepared tooth sections were attached and fixed to a dedicated metal rod so that the long axis of the rod was perpendicular to the enamel surface. Each tooth section fixed to a rod was mounted on a Mikrokator® (E. Johansson, Sweden) and abraded with a lapping film (15 μm grade, Imperial Lapping Film®, 3M, USA) to prepare powder samples for each 30-μm fraction, according to the abrasive micro-sampling method described by Weatherell et al.⁷ This procedure was repeated from the ES through the NL to the EDJ. Four microliters of 1 M HClO₄ was applied onto each powder sample on a lapping film and collected into a polyethylene capsule by aspiration. Then, 4 μl of 1 M sodium acetate buffer (pH 5.2) was applied 4 times and aspirated to collect a total of 20 μl of sample solution.

Determination of fluoride, phosphorus, and magnesium concentrations

Four microliters each of the collected sample solution was used to determine phosphorus and magnesium concentrations, and the remaining portion of the solution was used to determine fluoride concentration.

Phosphorus concentration was measured using a spectrophotometer (Spectrophotometer 150-02, Shimazu, Japan) according to the colorimetric method described by Chen et al.⁸ To measure magnesium concentration, 4 μl of the test solution was added to 2 ml of 3,000 ppm La(NO₃)₃·6H₂O and subjected to an atomic absorption spectrometer (Z-8200, Hitachi, Japan). Fluoride concentration was measured by the fluoride ion electrode method described by Hallsworth et al.⁹, using a fluoride ion electrode (Orion 9409BN, Thermo Electron, USA), a comparison electrode (Orion 900100, Thermo Electron), and an ion meter (Orion EA920, Orion Research, USA).

Fluoride and magnesium concentrations in each fraction of the deciduous teeth were calculated from
the F:P ratios obtained, on the assumption that the P concentration (dry weight concentration) was 18.5%.

**Enamel regions for evaluation**

The three enamel regions of the ES, NL, and DEJ were defined for evaluation purposes as follows. The ES region consisted of 2 fractions: the 30-μm fraction from the surface and the next 30-μm fraction toward the deep layer. The NL region consisted of 3 fractions: the 30-μm fraction containing the NL and 2 adjacent 30-μm fractions toward the deep and superficial layers. The DEJ region consisted of 2 fractions: the 30-μm fraction adjacent to the DEJ and the next 30-μm fraction toward the superficial layer (Fig. 1).

**Statistical analysis**

The data for fluoride and magnesium concentrations were analyzed using two-way analysis of variance (ANOVA) and Scheffe’s test, with factors of depth of the enamel and types of disability. Analysis results were considered to show statistically significance when P values were 0.05 or less.

**Results**

**Fluoride concentration**

Table 2 shows the fluoride concentration for the three subject groups. Two-way ANOVA was used to compare fluoride concentration at different depths of the enamel and for the different subject groups.

### Table 2 Fluoride concentrations of subjects

<table>
<thead>
<tr>
<th></th>
<th>ES (enamel surface)</th>
<th>NL (neonatal line)</th>
<th>DEJ (dentino-enamel junction)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>182.9 (± 17.7)</td>
<td>73.2 (± 9.3)</td>
<td>77.9 (± 14.6)</td>
<td>105.7 (± 11.1)</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
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<tr>
<td>DS</td>
<td>242.4 (± 31.2)</td>
<td>68.2 (± 10.0)</td>
<td>53.0 (± 6.9)</td>
<td>113.6 (± 17.1)</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>258.8 (± 31.7)</td>
<td>77.8 (± 12.5)</td>
<td>66.3 (± 8.9)</td>
<td>126.2 (± 14.4)</td>
</tr>
<tr>
<td>(n=11)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ppm: mean (± S.E.) **: P<0.01

Fluoride concentrations were highest in the ES region, followed by the NL and DEJ regions. ppm: mean (± S.E.), **: P<0.01
The fluoride concentration differed significantly between the different regions of the enamel ($P < 0.001$), showing that fluoride concentration was affected by the depth of the enamel (contribution rate, $37.5\%$). No significant differences were found between the three subject groups.

Figure 2 shows that fluoride concentration differed in different regions of the enamel. Fluoride concentration was highest in the ES region, followed by the NL and DEJ regions. Scheffe’s test showed that the concentration in the ES region was significantly higher than that in the NL or DEJ region ($P < 0.01$).

Fluoride concentration was highest in the control children, followed by children with CP and DS, with no significant difference between the all three subject group, where the latter includes the control children with no disorder.

**Magnesium concentration**

Table 3 shows the magnesium concentration for the three subject groups.

Two-way ANOVA was used to compare magnesium concentration at different depths of the enamel and for different subject groups. The magnesium concentration differed significantly between the different regions of the enamel ($P < 0.01$) and the different subject groups ($P < 0.01$), with contribution rates of $8.3\%$, and $6.4\%$, respectively.

Figure 3 shows that magnesium concentration differed according to the region of enamel. Magnesium concentration was highest in the DEJ region,
followed by the NL and ES regions. Scheffe’s test showed that the concentration was significantly higher in the DEJ region than in the NL or ES region \((P<0.05\) and \(P<0.01\), respectively). No significant difference was found between the NL and ES regions. Thus, magnesium concentration gradually decreases towards the outer enamel from the inner layers.

Magnesium concentration was highest in children with DS, followed by children with CP and control children, with a significant difference between children with DS and control children \((P<0.01)\). Magnesium concentration tended to be higher, although not significantly so, in children with CP than in control children.

**Discussion**

Tooth enamel contains various kinds of trace elements. The substitutions of \(\text{Ca}^{2+}\), \(\text{PO}_4^{3-}\), and \(\text{OH}^-\), the main components of hydroxyapatite, with these trace elements has been shown to alter the acid resistance of hydroxyapatite\(^6\). In particular, substitution of these ions with \(\text{Mg}^{2+}\) or \(\text{F}^-\) has been shown to cause opposite effects on the acid resistance of the enamel\(^6\). More specifically, substitution of \(\text{OH}^-\) with \(\text{F}^-\) results in the stabilization of hydroxyapatite crystal structure and increased crystallinity\(^6\), whereas substitution of \(\text{Ca}^{2+}\) with \(\text{Mg}^{2+}\), which has a smaller ionic radius than \(\text{Ca}^{2+}\), results in reduced stability of the crystal structure, decreased crystallinity, and structural defects\(^5\). Fluoride and magnesium show different distribution patterns in the enamel: low fluoride and high magnesium concentrations in low-enamel density regions and high fluoride and low magnesium concentrations in high-enamel density regions\(^10,11\). Previous studies have shown that in both deciduous and permanent teeth, fluoride concentration in the enamel exhibits a gradient that increases from the inner to superficial layers\(^12-15\). Our results that revealed a higher fluoride concentration in the ES region than in the NL and DEJ regions in control children and children with CP or DS are in good agreement.

In the present study, we found no significant difference in fluoride concentration, expressed as the mean of the fluoride concentrations in the ES, NL, and DEJ regions, between the three groups of children. Since fluoride concentration is significantly higher in the surface layer than in the inner layers, it is speculated that the fluoride concentration result for each group is determined largely from the concentration. Since the 60-\(\mu\)m layer from the enamel surface was regarded as the surface layer in this study, it was likely that a high fluoride concentration in this region greatly contributed to our results.

The present study also found that magnesium concentration in the deciduous tooth enamel differed significantly depending on depth of the enamel, as well as subject group, as determined by factorial analysis. In terms of depth of the enamel specifically, previous studies have shown that magnesium concentration in the enamel gradually increases from the superficial to inner layers\(^12,16\). Our results also showed a significantly higher magnesium concentration in the DEJ region than in the NL or ES region. The finding that magnesium, which has a different influence on caries susceptibility than does fluoride, showed a distribution pattern opposite to that of fluoride suggests incorporation of fluoride from the ES results in an improved crystal structure and reduced magnesium concentration, as previously reported.

Regarding Mg concentration in the outermost 20\(\mu\)m of the occlusal and cervical enamel surface of the deciduous molar teeth, there has been a report that the Mg concentration of the deciduous molar teeth of DS children was slightly higher than that of typically developing children\(^17\). In this study, the results that were in favor of the results of the report were obtained with the measurement of Mg concentration in the outermost layer, neonatal line, and dentino-enamel junction of the enamel. With regard to the permanent teeth of DS patients, previous report showed that Mg concentration of the whole enamel was higher than that of normal persons\(^18\). These findings suggest that DS affects the mineralization of the deciduous tooth enamel which starts in the prenatal period as well as the mineralization of the permanent tooth enamel which starts after birth.

Systemic disturbance occurring anywhere from conception to the neonatal period which results in CP has been shown to cause not only systemic effects but also local hypoplasia in the fetus\(^19,20\). An analysis of the chemical composition of deciduous tooth enamel in low-birth-weight infants and premature infants revealed a higher magnesium concentration in hypoplastic enamel than in healthy enamel\(^21\). CP is mainly caused by various systemic
disorders, and these disorders may also cause mineralization disorder of the enamel. The present finding of a relatively high magnesium concentration even in deciduous tooth enamel with no macroscopic evidence of hypoplasia suggests that systemic disturbance occurring anywhere from conception to the neonatal period may affect the formation of the deciduous tooth enamel, as tooth crown formation starts during fetal life and continues until a few months after birth. Thus, children with CP will tend to have insufficient mineralization of the deciduous tooth enamel. In this study, however, we did not examine each patient’s history of previous systemic disorder, and this should be fully examined in future studies before assessing the status of mineralization of the deciduous tooth enamel.

Acknowledgments

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References