Remineralization of Primary Tooth Enamel from Individuals With Down Syndrome

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ABSTRACT

Purpose: The purpose of this study was to clarify the characteristics of primary tooth enamel of Down syndrome patients (DSPs). We examined 9 primary teeth of Down syndrome children and 11 primary teeth of normally developed children to investigate the remineralization processes of enamel by transverse microradiography and X ray micro analyzer (XMA).
Methods: Mineral loss, lesion depth, maximum mineral value, minimum mineral value, depth of maximum mineral value, and depth of minimum mineral value were used to analyze transverse microradiography (TMR). In addition, we calculated the percentage of enamel remineralization.
Results: All the parameters in the 2 groups showed marked recovery. The results indicated that the Down syndrome group was significantly remineralized the same way as the control group. According to the comparison of mineral content distribution by XMA, the content distribution of magnesium was different between the 2 groups.
Conclusion: While recovery through remineralization of primary teeth was similar between Down syndrome children and normally developed children, the mechanism of remineralization process may be different between the 2 groups; consequently, magnesium may be considered as one of the factors affecting recovery.
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Dental characteristics in Down syndrome patients (**DSPs**) include the following: delayed timing of eruption[1-4]; microdontia[5]; conical teeth[6]; malalignment of teeth[7]; and higher prevalence of periodontal disease.[8-11] Researchers[3,12-23] who have reported on caries prevalence in DSPs are divided on the association between caries prevalence and Down syndrome.

Many epidemiological studies[12-19] have reported low caries prevalence in DSPs. Conversely, some have reported that DSPs did not necessarily show a low caries prevalence.[20,21] Other studies have reported polarization of caries prevalence in DSPs[10,22,23]: they belong to either the group with very serious dental caries or that with no caries at all.

Based on biochemical analysis of tooth enamel, Hideshima et al.[24] reported that the calcium (**Ca**) content was lower and the magnesium (**Mg**) content was higher in the permanent teeth of DSPs than healthy patients. Furthermore, Nakano[25] reported that in DSPs the fluoride (**F**) content was lower and the Mg content was higher in primary teeth vs healthy patients, which did not support lower caries prevalence in DSPs.

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When the balance between demineralization and remineralization of the enamel surface is disturbed, dental caries, and tooth lesions develop. This is why many studies[26-35] have been conducted on remineralization of demineralized enamel in the early stage for the purpose of dental caries prevention. The subjects of such studies, however, have been limited to normally developed children and/or adults. No known studies have been carried out on the enamel of DSPs regarding demineralization and remineralization.

Therefore, the purpose of this study was to clarify the demineralization and remineralization of primary tooth enamel from Down syndrome patients.

METHODS

Primary teeth examined in this study were obtained when they were replaced by permanent teeth. They were free from visible dental caries, white spot lesions, or fissures. A total of 20 primary teeth were examined: 9 primary teeth of the group of DSPs and 11 primary teeth of the group of normally developed children (control group). The t test showed no statistically significant difference between DSPs and the control group (subject ages=11.4±0.4 SD and 10.4±0.3, respectively) in the average age of permanent tooth replacements.

Approval for this study was obtained from the Ethics Committee of the Department of Dentistry, Aichi-Gakuin University, Nagoya, Japan.

SAMPLE PREPARATION

Enamel blocks were obtained from buccal surfaces of the teeth.[31] The tooth roots were removed using a diamond disk (Isomet, Buehler, USA). The crowns were sectioned to create 2 enamel blocks of the same size (2 x 3 mm). The samples were measured using a digital micrometer (MDC-25M, Mitutoyo, Kanagawa, Japan). Two blocks were obtained from each tooth: one for demineralization and the other for remineralization. The samples, excluding the enamel surface layer, were coated with nail varnish.

DEMINERALIZATION AND REMINERALIZATION PROCEDURES

Demineralization and remineralization procedures were carried out according to the method described by Lynch et al.[26]

One block was immersed in demineralizing solution for 7 days. The demineralizing solution contained 0.1 M lactic acid and 8% CMC (calboxymetylcellulose), and the solution was adjusted to a pH of 4.5 by using potassium hydroxide. A pH meter (F-52, HORIBA, Kyoto, Japan) was used for the adjustment.

The other block, which had been immersed in the demineralization solution for 7 days, was thoroughly rinsed with distilled water and immersed in remineralizing solution for 8 days. The remineralizing solution contained 1.5 mmol/l $CaCl_2$, 0.9 mmol/l KH2-PO_4, 130 mmol/l KCl, and 20 mmol/l HEPES (2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid), and the solution was adjusted to a pH of 7.0 by using potassium hydroxide. A pH meter was used for the adjustment.

The enamel blocks, which had been treated according to the aforementioned manner, were embedded in quick self-curing resin. The enamel blocks embedded in resin were cut buccolingually to obtain 300-μm-thick sections using a diamond disk (Isomet, Bueler, America). The sections were ground using abrasive waterproof paper (SC-1000, KOVAX, Tokyo, Japan) to a thickness of approximate 150 μm (Figure 1).

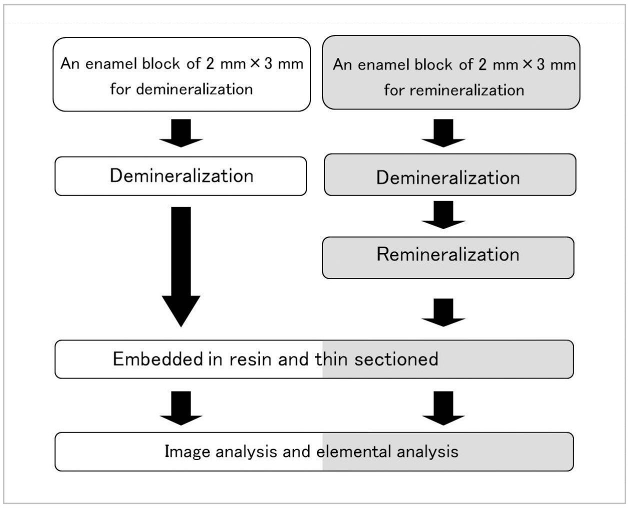

Figure 1. Flow diagram of the experimental procedure.

ASSESSMENT BASED ON TRANSVERSE MICRO-RADIOGRAPHY (TMR)

Immediately after the demineralization and remineralization procedures, respectively, transverse microradiographs of specimens were taken together with an A1 stepwedge on high precision photo plates (HRP-SN-2, Konica Minolta, Tokyo, Japan).

Microradiographs were taken at 10 kV and 2.0 mA by means of a soft X ray generator (TMR2, Softex, Tokyo, Japan); the exposure time was 5 minutes, and the focal length was 44.4 mm. Microradiographs were examined with a microscope (SZX9, Olympus, Tokyo, Japan), which was set at 100× magnification, and TMR-images were captured with a CCD camera (DP90, Olympus). Winroof image analysis software (Mitani Corp, Tokyo, Japan) was used.

According to the method described by Angmer et al.,[32] the parameters of lesion depth (**Ld**, μm), and mineral loss (**ΔZ**, vol %, μm) were used to evaluate lesion remineralization. Furthermore, according to the method referred by Iijima et al.[27] and Yamagishi et al.,[33] the parameters of maximum mineral value (**Vmax**), minimum mineral value (**Vmin**), depth of maximum mineral value (**Vmax** [**Ld**]), and depth of minimum

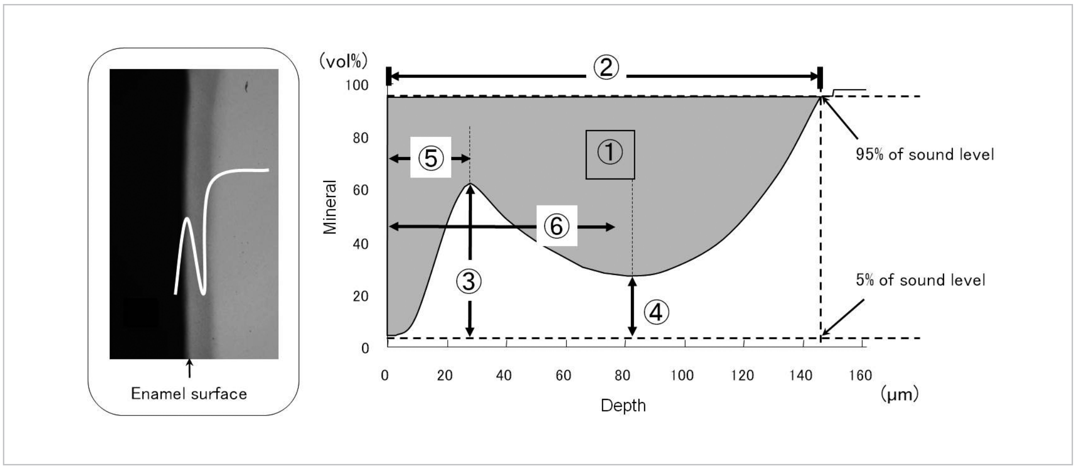

Figure 2. Schematic mineral distribution and 6 parameters; the parameters of mineral loss (ΔZ, vol %– μm; [1]), lesion depth (Ld, μm; [2]), maximum mineral value (Vmax, vol%; [3]), minimum mineral value (Vmin, vol%, [4]), depth of maximum mineral value (Vmax [Ld], μm; [5]), and depth of minimum mineral value (Vmin [Ld], μm; [6]), were also used to evaluate the remineralization of lesions.

mineral value (**Vmin** [**Ld**]) also were used to evaluate lesion remineralization (Figure 2).

In this study, the percentage of enamel remineralization was calculated concerning each of the aforementioned 6 items obtained via the image analysis. The calculation formula was as follows (according to the method described by Iijima[27]:

> Percentage of the enamel remineralization of each item (%) = {(value after demineralization procedure—value after remineralization procedure)/value after demineralization procedure} ×100.

ELEMENTAL ANALYSIS BY XMA (X RAY MICRO-ANALYZER)

An XMA (JXA-8900, Jeol, Tokyo) was used for line analysis of calcium (Ca), phosphorus (P), magnesium (Mg), and potassium(K) to examine the mineral concentration distribution from the enamel surface layer to deeper layers after the remineralization procedure.

Table.1 Comparison of demineralization and remineralization

(mean± S.E.)

	Micro radiographic parameter		Demineralization	Remineralization	De VS Re
The Down group (n=9)	ΔZ	(vol%·μm)	6036.04±192.15	3612.41±187.62	p<0.01
	Ld	(μm)	156.03±5.63	114.47±3.60	p<0.01
	Vmax	(vol%)	64.98±2.77	76.68±2.35	p<0.05
	Vmax (Ld)	(μm)	49.40±2.62	30.92±2.87	p<0.01
	Vmin	(vol%)	53.64±2.43	62.23±3.17	p<0.10
	Vmin (Ld)	(μm)	97.12±4.66	62.80±3.69	p<0.01
The Control group	ΔZ	(vol%·μm)	5290.59±326.11	2888.88±249.88	p<0.01
	Ld	(μm)	138.58±5.31	96.08±6.70	p<0.01
	Vmax	(vol%)	71.54±2.11	76.93±2.60	p<0.01
	Vmax (Ld)	(μm)	47.74±3.76	31.14±3.60	p<0.01
	Vmin	(vol%)	59.51±2.91	69.38±2.07	p<0.01
	Vmin (Ld)	(μm)	92.80±5.57	48.66±5.66	p<0.01

Analysis specimens were obtained by embedding the aforementioned specimens once more in quick self-curing resin and dry abrading them using lapping film sheets (nos. 1,000, 3,000, and 10,000). The specimens obtained were carbon-shadowed in a vacuum deposition chamber and analyzed by XMA under the following condition: accelerating voltage = 15.0 kV, beam current = 5 x 10^{-8}A, focused beam in diameter=2.50 μm.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS for Windows 11.0 (SPSS Inc, Tokyo, Japan). Mann-Whitney U-test was used to compare DSPs and the control group. Wilcoxon's signed rank test was used to compare the values and the statistical significance before and after the remineralization procedure in both DSPs and the control group.

RESULTS

When comparing between values after the demineralization and remineralization procedures based on TMR (Table 1), the ΔZ value after exposure to the remineralizing solution was statistically significantly lower in both groups than demineralization ($P<.01$).

As for Ld, the value after exposure to the remineralizing solution was significantly lower in both groups than demineralization($P<.01$), meaning that lesion depth after exposure to the remineralizing solution was significantly reduced compared with demineralization. Concerning all the other following parameters—V max, V min, V max (Ld), and V min (Ld)—Ld after exposure to the remineralizing solution were significantly reduced compared with demineralization in both groups ($P<.01$ - $P<.10$), and mineral contents after exposure to the remineralizing solution increased compared with the 2 groups after demineralization, meaning that there was marked recovery in both lesion depth and mineral content.

When comparing the percentage of enamel remineralization between DSPs and the control group based on TMR (Figure 3), there was no statistically significant difference in ΔZ or Ld between the 2 groups.

When examining specific parameters, however, DSPs showed a higher tendency percentage of enamel remineralization when it came to Vmax (~20±8%) than the control group (~8±2%) (P<.10). Concerning Vmin (Ld), however, DSPs showed a lower tendency value (~35±4%) than the control group (46±7%; P<.10).

Concerning Vmin and Vmax (Ld), on the other hand, there was no significant difference in the percentage of enamel remineralization between the 2 groups.

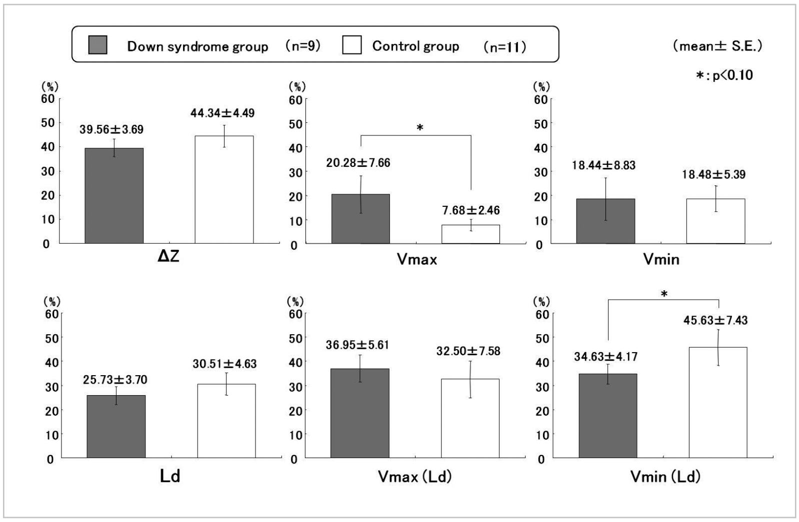

Figure 3. Comparison of percentage enamel remineralization in the Down syndrome group and control group; percentage of the enamel remineralization of each item (%) = value after demineralization procedure–value after remineralization procedure)/value after demineralization procedure x100.

CHEMICAL ELEMENT CONTENT DISTRIBUTION BASED ON XMA (FIGURES 4–5)

Figure 4 shows chemical element content distribution based on XMA after exposure to the remineralizing solution. The elements of Ca, P, and K did not show any difference between DSPs and the control group. On the other hand, DSPs showed a constant level of Mg content from the enamel surface layer through deeper layers (y=0.05X+23.97), while the control group showed a gradual increase in content from the enamel surface layer toward deeper layers (y=0.13X+28.93) in Figure 5. A significant difference was observed in the correlation coefficient between DSPs (r=0.31) and the control group (r=0.75). Their 95% confidence limits of the slope of lines were 0.01 to 0.10 and 0.10 to 0.15, respectively, resulting in the difference in content distribution of Mg between the 2 groups.

DISCUSSION

We carried out this study to clarify the characteristics of primary tooth enamel of DSPs. Specifically, we investigated changes found after demineralization and remineralization processes in primary tooth enamel in DSPs and the control group based on the image analysis using TMR and XMA and compared the changes between the 2 groups.

According to the comparison based on TMR, both groups showed marked recovery in all 6 parameters after remineralization vs after demineralization. This suggested that demineralized enamel was significantly remineralized after it had been immersed in the remineralizing solution.

To evaluate the effectiveness of remineralization, the percentage of enamel remineralization was calculated. There was no significant difference in Ld and ΔZ between the 2 groups. There was a slightly higher tendency toward Vmax in DSPs vs the control group. As for Vmin(Ld), DSPs showed a significantly lower tendency than the control group.

These suggested that the effectiveness of the remineralization process might be lower in deeper layers of enamel in DSPs. As the balance between tooth demineralization and remineralization tipped toward demineralization, we believe that demineralization might have progressed faster and reached deeper layers of enamel in DSPs than the control group.

This study's results also might have implied that the mechanism of remineralization in deeper enamel layers differed between the 2 groups: the mechanism of mineral loss and mineral gain might be different in demineralized deeper layers of enamel between both groups.

According to the comparison of element concentration distribution in enamel based on the XMA, there was a difference in the level of Mg content after exposure to the remineralizing solution between 2 groups. The Mg concentration from the enamel surface layer

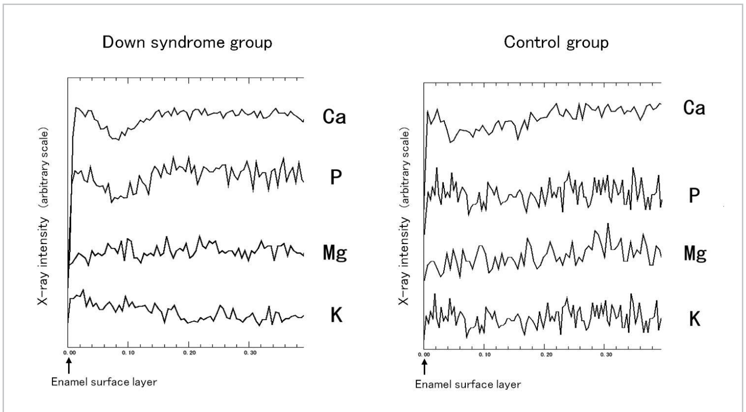

Figure 4. Line analysis of chemical element content distribution based on XMA after exposure to remineralization solution.

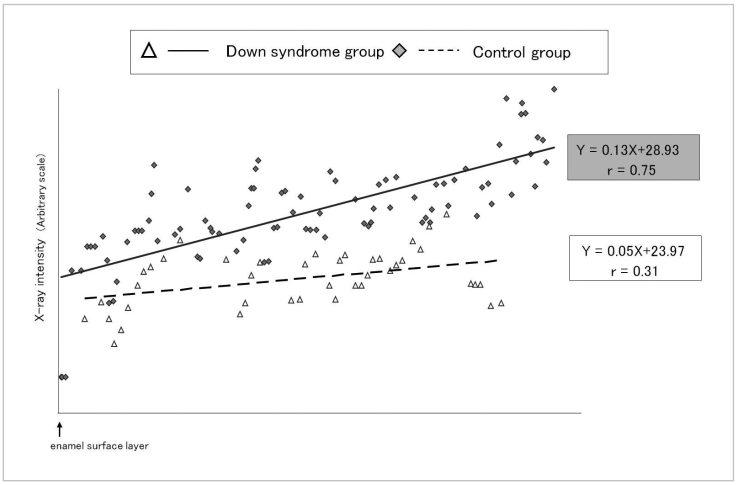

Figure 5. Comparison of a Mg content level from the enamel surface layer with least square method.

to deeper layers of primary teeth was at a constant level in DSPs but increased in the control group. According to Takashi,[25] acid demineralization of the enamel surface layer of primary teeth in DSPs progressed further compared to normally developed children. Takashi[25] argued that the lower degree of mineralization in primary tooth enamel of children with DSPs was due to the low content of F and the high content of Mg in the enamel surface.

Mg replaces Ca in the crystal lattice of hydroxyapatite, the main inorganic constituent of tooth enamel, and causes fragility in hydroxyapatite crystals, resulting in higher caries susceptibility. Remineralized enamel undergoes mineral ionic composition changes, leading to the lower contents of Mg^{2+} and CO_3^{2-} ions. This leads to higher acid resistance of remineralized enamel than sound enamel.[3] These facts show that Mg and caries susceptibility are closely related. In this study, based on the comparison of changes after exposure to the remineralizing solution between the control group and DSPs, we found a difference in Mg content distribution between the 2 groups. Mg content distribution in demineralization and remineralization processes should be carefully investigated to clarify caries prevalence in DSPs.

In future studies, Mg is an element which should be carefully investigated to better clarify the influence of Mg and to deepen the understanding of dental characteristics of DSPs. Biochemical analysis of enamel focusing on Mg should be carried out to observe the remineralization process over time using the following methods: (a) single thin section method[34,35] or (b) the structural evaluation of enamel using a scanning electron microscope or an atomic force microscope for the purpose of the in-depth analysis of the mechanism of demineralization and remineralization in DSPs.

CONCLUSIONS

1. While the primary teeth of Down syndrome patients (DSPs) showed the same level of remineralization as those of normally developed children, the remineralization mechanism may be different between the 2 groups.
2. Magnesium (Mg) seems to be a factor causing the difference in the mechanism.

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