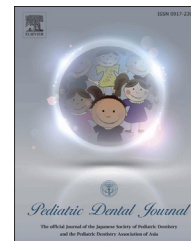


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Pediatric Dental Journal

journal homepage: www.elsevier.com/locate/pdj

Original Article

Element distribution and histological observation of enamel in deciduous canines of children with Down syndrome



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ARTICLE INFO

Article history:

Received 15 April 2015

Received in revised form

19 August 2015

Accepted 20 August 2015

Available online 15 November 2015

Keywords:

Enamel prism

Down syndrome

Deciduous canines

Electron probe microanalyses

ABSTRACT

Background: There have been many reports that caries susceptibility of children with Down syndrome is lower than that of unaffected children. However, the perspectives on the factor of caries susceptibility have not yet been unified due to its diversity. This study aimed at comparing children with Down syndrome with unaffected children regarding the biochemical analysis and the histological observations with increasing depths from the surface of deciduous teeth.

Methods: Atomic concentrations of calcium and phosphorus at the core regions of the enamel prism were analyzed using an electron probe microanalyzer. Histological observations were simultaneously conducted. In addition to the subjective comparisons, percentages of the enamel prism cores (interprismatic substance area ratio) were also calculated.

Results: Regarding calcium and phosphorus concentrations or calcium/phosphorus ratio, there were no significant differences between the two groups at any of the layers. Additionally, there was no significant change in either group with increasing depths from surface. The findings of enamel surface revealed three-dimensionality in unaffected children, while smoothness in children with Down syndrome. In children with Down syndrome, the outline of enamel prisms showed some of which formed incompletely. Enamel prism cores were similar to the interprismatic substance in terms of crystal structure. Interprismatic substance area ratio of children with Down syndrome was lower than that of unaffected children in all the layers except at 150 μm and 250 μm .

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<http://dx.doi.org/10.1016/j.pdj.2015.08.002>

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Conclusion: In this study, the deciduous canine enamel of children with Down syndrome was thought to include more organic material than that of unaffected children.

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1. Introduction

Down syndrome is a congenital disorder arising from an abnormality in chromosome 21, and is associated with intellectual disability and physical abnormalities such as short stature and dysmorphic facial features. Usually caused by an extra copy of the chromosome (trisomy 21), Down syndrome is a relatively common anomaly that occurs in one of every 600 live births. In recent years, the incidence of Down syndrome has been increasing in Japan [1].

Characteristic oral signs of patients with Down syndrome include decreased salivary secretion [2], difference in salivary composition [3,4], and fissured tongue. With regard to dental signs specifically, permanent teeth show a higher incidence of malformations such as dwarfed teeth and conical teeth [5–7], congenitally missing teeth [8,9], and malalignment [10,11].

Although a previous epidemiologic survey reported that caries are fewer in Down syndrome than in the general population [12], no studies have investigated this phenomenon in both deciduous [13,14] and permanent teeth [15–17].

Previous studies on the biochemical composition of deciduous tooth enamel in children with Down syndrome have found a high calcium concentration and low calcium/phosphorus ratio, which cause delayed enamel growth [18]. Nakano [19] reported that deciduous tooth enamel has a lower fluorine concentration and higher magnesium concentration at the surface and inside and outside of the neonatal line [20] in children with Down syndrome than in unaffected children.

In recent years, research on the relationship between the histological structure and acid resistance of deciduous tooth enamel has indicated that acid resistance in Down syndrome is low, due to coarse transections of the enamel prisms or the internal deletion and abnormal structure of the crystals present in enamel [21].

We previously compared the calcium concentration, phosphorus concentration, and histology, such as the form and arrangement of the enamel prisms [22], at the surface of deciduous canines between children with Down syndrome and unaffected children. The observed differences in these factors affect the reactivity of the tooth surface toward acid and lead to the higher incidence of caries in children with Down syndrome. However, the progression of caries is not well understood.

In this study, we used an electron probe microanalyzer (EPMA) to simultaneously observe the element distribution and histological structure of deciduous tooth enamel. By these methods, we compared the changes with depth in calcium concentration, phosphorus concentration, and fine structure of the enamel prisms in the teeth of children with Down syndrome and those of unaffected children.

2. Materials and methods

This investigation was performed on 12 exfoliated lower deciduous canines; six teeth were from children with Down syndrome (Down syndrome group) and six teeth were from unaffected children (normal group). All teeth were free of visible caries, white spot lesions, and fissures.

There was no significant difference in average age at extraction between the Down syndrome group (mean \pm standard deviation, 9.8 ± 0.9 years) and the normal group (9.7 ± 0.4 years). A summary of the study methodology is given in Fig. 1. Briefly, tooth roots were removed with a diamond disk (IsoMet; Buehler, Lake Bluff, IL, USA). Crowns were washed for 5 minutes in distilled water using an ultrasonic cleaner (Yamato1210, Branson, Kanagawa, Japan), dewatered with ethanol, and dried with acetone.

The area observed was the center of the tooth mesiodistal to the labial surface and widest buccolingual contour. An exposed window ($1 \times 1 \text{ mm}^2$) was produced in order to be able to perform measurement and observation of the same part with increasing depth since it is necessary to repeat observation and disposal. To ensure the samples were level during observation, the teeth were fixed with self-curing resin onto a glass board. The teeth were placed into an acrylic ring of 16 mm in diameter and embedded in epoxy resin (Epok812; Oken, Tokyo, Japan).

Mirror polishing of the surface of the samples was performed using 1500 and 2000 grit waterproof sandpaper (KOVAX, Kanagawa, Japan), and $1.0 \mu\text{m}$ and $0.3 \mu\text{m}$ alumina slurry (Refinotec, Tokyo, Japan). Acid treatment was performed by immersing the teeth in 0.5% hydrochloric acid for 30 second. Then, the samples were washed under running tap water 30 second and washed in distilled water using the ultrasonic cleaner for 20 minutes (Branson).

- 1) Removed tooth roots, dewatered with ethanol, and dried with acetone
- 2) Produced exposed window ($1 \times 1 \text{ mm}^2$) to the section for observation
- 3) Mirror polishing of the surface after embedding in epoxy resin
- 4) Acid treatment with hydrochloric acid, and washed in distilled water
- 5) Sputter coating with carbon
- 6) Biochemical analysis and histological observation by EPMA
- 7) Grind sample $50 \mu\text{m}$, and Steps 4 – 6 are repeated to $400 \mu\text{m}$

Fig. 1 – The flow chart of this study. EPMA = electron probe microanalyzer.

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