ปริมาณแร่ธาตุในฟันน้ำนมระหว่างกลุ่มที่มีประสบการณ์การมีฟันผุแตกต่างกัน การทดลองในห้องปฏิบัติการ

Mineral Comparisons of Primary Teeth Among Different Caries Experience Groups *in vitro*

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บทคัดย่อ

วัตถุประสงค์: การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบหาปริมาณแคลเซียม ฟอสฟอรัส ฟลูออรีน และแมกนีเซียมในฟันน้ำนมของเด็กที่มีประสบการณ์การมีฟันผุแตกต่างกันโดยใช้กล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด และสเปคโตรสโคปีแบบกระจายพลังงาน

วิธีการศึกษา: ฟันน้ำนมที่ถูกถอนจำนวน 30 ซี่ ถูกแบ่งออกเป็น 3 กลุ่มเท่ากันตามประสบการณ์การมีฟันผุต่ำ ปานกลาง และสูง ผิวฟันด้านเรียบที่ปราศจากรอยผุของฟันจะถูกใช้เป็นตัวแทนของฟันแต่ละซี่ในแต่ละกลุ่มฟันถูกตัดตามแนวยาว ทำความสะอาดผิวฟันที่ตัดด้วยการแช่ในสารละลายโซเดียมไฮโปคลอไรต์เข้มข้น ร้อยละ 5.25 เป็น

Abstract

Objective: The aim of this study was to investigate the calcium, phosphorus, fluorine and magnesium content of primary teeth among three caries experience groups using Scanning Electron Microscopy (SEM) equipped with Energy Dispersive Spectroscopy (EDS).

Materials and Methods: Thirty extracted primary teeth were equally divided into three groups: low, moderate and high caries experience. A caries-free smooth surface was used to represent the whole tooth in each group. Each tooth was

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เวลา 12 ชั่วโมง จากนั้นล้างด้วยเครื่องทำความสะอาดอัลตราโซนิกเป็นเวลา 10 นาที ปล่อยให้แห้งที่อุณหภูมิห้องนาน 3 วัน จากนั้นนำเข้าสู่กระบวนการศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด โดยศึกษาเคลือบฟัน และเนื้อฟันอย่างละ 2 ตำแหน่ง คือ นอก และใน วัดค่าร้อยละของอะตอมของธาตุแคลเซียม ฟอสฟอรัส ฟลูออรีน และแมกนีเซียมโดยเทคนิคอีดีเอส นำข้อมูลมาหาค่าเฉลี่ย และส่วนเบี่ยงเบนมาตรฐานของธาตุทั้งสี่นำมาวิเคราะห์ความแปรปรวนแบบสองทาง ($p \leq 0.05$)

ผลการศึกษา: ชั้นเคลือบฟันของกลุ่มที่มีประสบการณ์การมีฟันผุต่ำมีปริมาณของธาตุแคลเซียม และฟอสฟอรัสสูงกว่ากลุ่มที่มีประสบการณ์การมีฟันผุปานกลาง และสูงอย่างมีนัยสำคัญทางสถิติ อย่างไรก็ตามไม่พบความแตกต่างกันอย่างมีนัยสำคัญทางสถิติของปริมาณธาตุฟลูออรีน แมกนีเซียม และสัดส่วนของแคลเซียมต่อฟอสฟอรัสระหว่างทั้งสามกลุ่ม ในทางกลับกันพบว่าเคลือบฟันชั้นนอกมีปริมาณของธาตุแคลเซียม ฟอสฟอรัส และฟลูออรีนสูงกว่าเคลือบฟันชั้นใน เนื้อฟันชั้นนอกมีปริมาณธาตุฟลูออรีนสูงกว่าเนื้อฟันชั้นใน เป็นที่น่าแปลกใจที่เนื้อฟันชั้นในมีปริมาณธาตุแคลเซียม และฟอสฟอรัสสูงกว่าเนื้อฟันชั้นนอก ส่วนปริมาณธาตุแมกนีเซียมจะเพิ่มขึ้นเมื่อเข้าใกล้สู่ชั้นในของเนื้อฟัน

บทสรุป: การศึกษานี้พบความเป็นไปได้ที่ความต้านทานต่อการผุของเคลือบฟัน ขึ้นอยู่กับปริมาณแคลเซียมและฟอสฟอรัส แต่ไม่ได้ขึ้นอยู่กับฟลูออรีนหรือแมกนีเซียม อย่างไรก็ตามยังคงจำเป็นต้องมีการศึกษาเพิ่มเติมต่อไป

คำสำคัญ: ฟันน้ำนม องค์ประกอบธาตุในฟัน ประสบการณ์การมีฟันผุ สเปคโตรสโคปีแบบกระจายพลังงาน (อีดีเอส)

longitudinally sectioned. The cut surface was cleaned by immersion in 5.25% sodium hypochlorite for 12 hours, and placed in an ultrasonic cleanser for 10 minutes. It was left to dry at room temperature for three days and processed for SEM examinations. Two areas, outer and inner, of the enamel and dentin were examined. The atomic% (at.%) of calcium, phosphorus, fluorine and magnesium were measured using the EDS technique. The means and standard deviations of these four elements were analyzed using two-way ANOVA ($p \leq 0.05$).

Results: The enamel, in general, in the low caries experience group had a significantly greater calcium and phosphorus content than in the moderate and high caries experience groups. However, there was no significant difference in fluorine, magnesium or calcium-to-phosphorus ratio between any of the caries experience groups. On the other hands, the outer enamel had greater calcium, phosphorus and fluorine content than did the inner enamel. The outer dentin had greater fluorine content than did the inner dentin. Surprisingly, the inner dentin had greater calcium and phosphorus content than did the outer dentin. The deeper the measurement depth in the dentin, the greater was the magnesium content.

Conclusions: Our findings raise the possibility that the caries resistance of enamel depends upon calcium and phosphorus, but not fluorine or magnesium, content. However, further investigations are still needed.

Keywords: primary tooth, tooth elements, caries experiences, energy dispersive spectroscopy (EDS)

Introduction

Mineralized dental hard tissue, enamel and dentin, serve as protective layers for dental pulp, a soft tissue layer in the center of the tooth structure.[1] Enamel is the most highly mineralized tissue at the outermost of the tooth crown structure. It has mineral composition by weight about 96%[2] in the form of a nanocrystalline calcium phosphate crystal called hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) which incorporated with small amounts of essential trace elements,[3] such as Carbonate (CO_3), Magnesium (Mg), Sodium (Na), Fluorine (F), etc.[4] rendering it more or less resistant to acid. Dentin, composed of 70% inorganic material by weight, mainly in hydroxyapatite form, lies underneath the enamel.[1] Compared to enamel, the higher concentration of organic material in dentin renders it less resistant to acid than is the enamel.[5] Dentin is a porous structure with greater permeability than enamel, due to abundant dentinal tubules.[6]

It is widely agreed that hardness of dental hard tissue is highly affected by its mineral content.[7,8] Calcium and phosphorus are the principal contents of hydroxyapatite crystals in enamel, dentin, cementum, and bone, while small amounts of fluorine are crucial to help increase the acid resistance property of the crystals. The acid resistance properties of dental hard tissues may relate to their individual chemical compositions, their degree of mineralization and their mutual arrangement in the apatite crystal structure.[4] Primary teeth are more susceptible to caries than are permanent teeth,[9] due to their enamel and dentin being thinner, more permeable, less mineralized and less dense than permanent teeth.[10] However, dental caries susceptibility depends on several factors, such as mineral content, acidic diet, salivary flow rate, buffer capacity of saliva, frequency of water-drinking, microflora on the plaque, etc.[1]

Energy dispersive spectroscopy (EDS) analysis under a scanning electron microscope (SEM) can be used to assess both the chemical composition of human mineralized tissue and its morphology with high sensitivity (0.1%) without destroying or altering the specimens studied.[11]

Several authors examined the mineral composition in enamel, using various techniques, such as atomic absorption spectroscopy, spark source mass spectrometry, neutron activation analysis, etc. and demonstrated some variations in element concentrations between sound and carious teeth.[12-17] The mineralized composition of enamel and dentin may be a key factor in the caries development process. The total level of mineral concentration in the teeth may serve as a determined predictor of patients' susceptibility to the developing of dental caries. However, little is still known about the relationship between the elemental composition of enamel and dentin and caries experience. Therefore, the aim of this study was to investigate the calcium, phosphorus, fluorine and magnesium content of enamel and dentin in primary teeth among three caries experience groups, by using SEM equipped with EDS.

Materials and methods

This study has been approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (No.43/2017). The tooth specimens were collected with the consent of the patients and their parents.

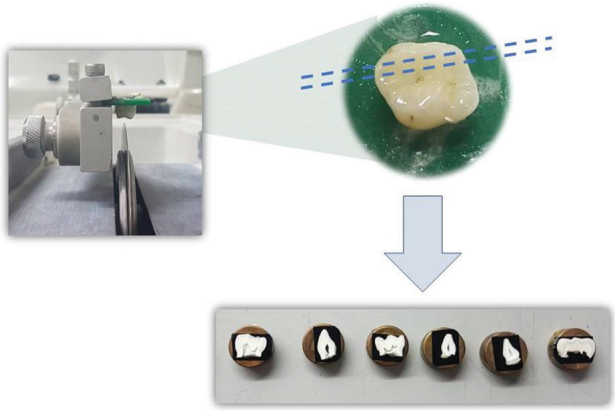
The exfoliated and/or extracted primary teeth were obtained from healthy children aged 4-12 years old. The teeth must have had at least one intact smooth surface and no evidence of developmental defects (fluorosis or hypoplasia), or have previously had root canal treatment. Teeth which had severe attrition on the occlusal surface, insufficient sound enamel or dentin, or had a history of restorations on smooth surfaces of the teeth selected for the study, were excluded from the study.

Individuals' caries experience was classified by modifying the World Health Organization (WHO) dmft/DMFT index in the mixed dentition.[18] The dmft index was used for primary teeth (d = decayed, m = missing due to caries, f = filled, t = teeth) and DMFT index was used for permanent teeth (D = decayed, M = missing due to caries, F = filled, T = teeth).

The teeth were cleaned with running water and stored in normal saline solution (NSS) with 0.1% thymol at 4°C.[19] The samples were grouped into three caries experience groups. Group 1 (low dmft/DMFT score between 0 and 2, Group 2 (moderate dmft/DMFT score between 5 and 6 and Group 3 (high dmft/DMFT score equal or more than 9). The score ranges 3-4 and 7-8 were excluded in order to amplify the differences in the element content among the groups.

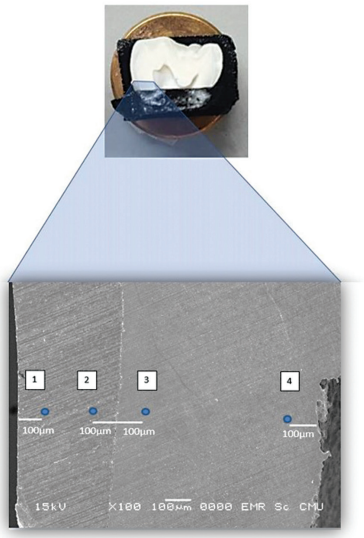
The roots were cut off from the tooth specimens at the cemento-enamel junction (CEJ) using a water-cooled high-speed cylindrical diamond bur (Intensive®, Swiss Dental Products, Montagnola, Switzerland). The remaining pulp tissue was removed with a barbed broach. One smooth surface (either buccal or lingual) of each tooth was selected and longitudinally sectioned, parallel to the long axis, into small pieces approximately 2 mm in width and less than 3 mm thick using a precision sectioning saw (Isomet®1000, Buehler, IL, USA).

The residual debris on the cut surface of the specimen was cleaned using the following processes. The specimens were immersed in 5.25% sodium hypochlorite for 12 hours, placed in an ultrasonic



รูปที่ 1 *แผนภาพแสดงการเตรียมตัวอย่างฟันด้วยเครื่องตัดฟันความเร็วต่ำ ควบคุมทิศทางการตัด และการเตรียมชิ้นตัวอย่างบนแท่นเพื่อการวัดด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด*

Figure 1 *Procedure for tooth specimen preparation by cutting with a precision sectioning saw, controlling the direction of cutting, and transferring the specimen onto the stubs for SEM examination.*



รูปที่ 2 *แผนภาพแสดงตำแหน่งของพื้นผิวฟัน (บริเวณกึ่งกลาง 1/3) ที่ใช้ในการศึกษาภายใต้กล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราดและสเปคโตรสโคปีแบบกระจายพลังงาน (1 = ชั้นนอกสุดของชั้นผิวเคลือบฟัน; 2 = ชั้นในของเคลือบฟัน; 3 = ชั้นนอกของเนื้อฟัน; 4 = ชั้นในของเนื้อฟัน)*

Figure 2 *Selected tooth surface area (middle 1/3 the tooth sample) for examination under scanning electron microscope with EDS analysis. (1 = outer enamel surface layer; 2 = inner enamel layer; 3 = outer dentin layer; 4 = inner dentin layer).*

cleanser for 10 minutes and rinsed with distilled water. The prepared samples were air-dried in a closed environment at room temperature for three days before being processed for examination under SEM and EDS analysis.

The morphology of the sample surfaces was observed under SEM (JSM-5910LV, JEOL Ltd., Tokyo, Japan) at 100x magnifications, voltage 15 keV, working distance 11 mm, spot size (as a percentage given by the equipment) 20-40%.

The four examination areas in the middle 1/3 of each sample were the outer enamel 100 microns beneath the enamel surface (Level 1), the inner enamel 100 microns from the dentino-enamel junction (DEJ) (Level 2), the outer dentin 100 microns from the DEJ (Level 3) and the inner dentin 100 microns from the pulp surface (Level 4), as illustrated in Figure 2. The atomic% (at.%) of calcium (Ca), phosphorus (P), fluorine (F) and magnesium (Mg) were measured using the EDS technique under SEM.

Data were input and statistically analyzed using SigmaPlot® version 12 (Systat Software, Erkrath, Germany). Descriptive data were reported in terms of mean±SD. The at.% of the test elements obtained from EDS analysis were compared using two-way ANOVA. A p value less than 0.05 was considered as a significant difference.

Results

Calcium, phosphorus, fluorine and magnesium were detected using the EDS technique in all samples, as illustrated in Figure 1. The ranges of percent of calcium, phosphorus, fluorine and magnesium were 14.05-21.99, 9.06-13.25, 0.10-1.29, 0.20-0.97, respectively (Figure 3). Enamel and dentin generally contained smaller amounts of fluorine and magnesium than of calcium and phosphorus.

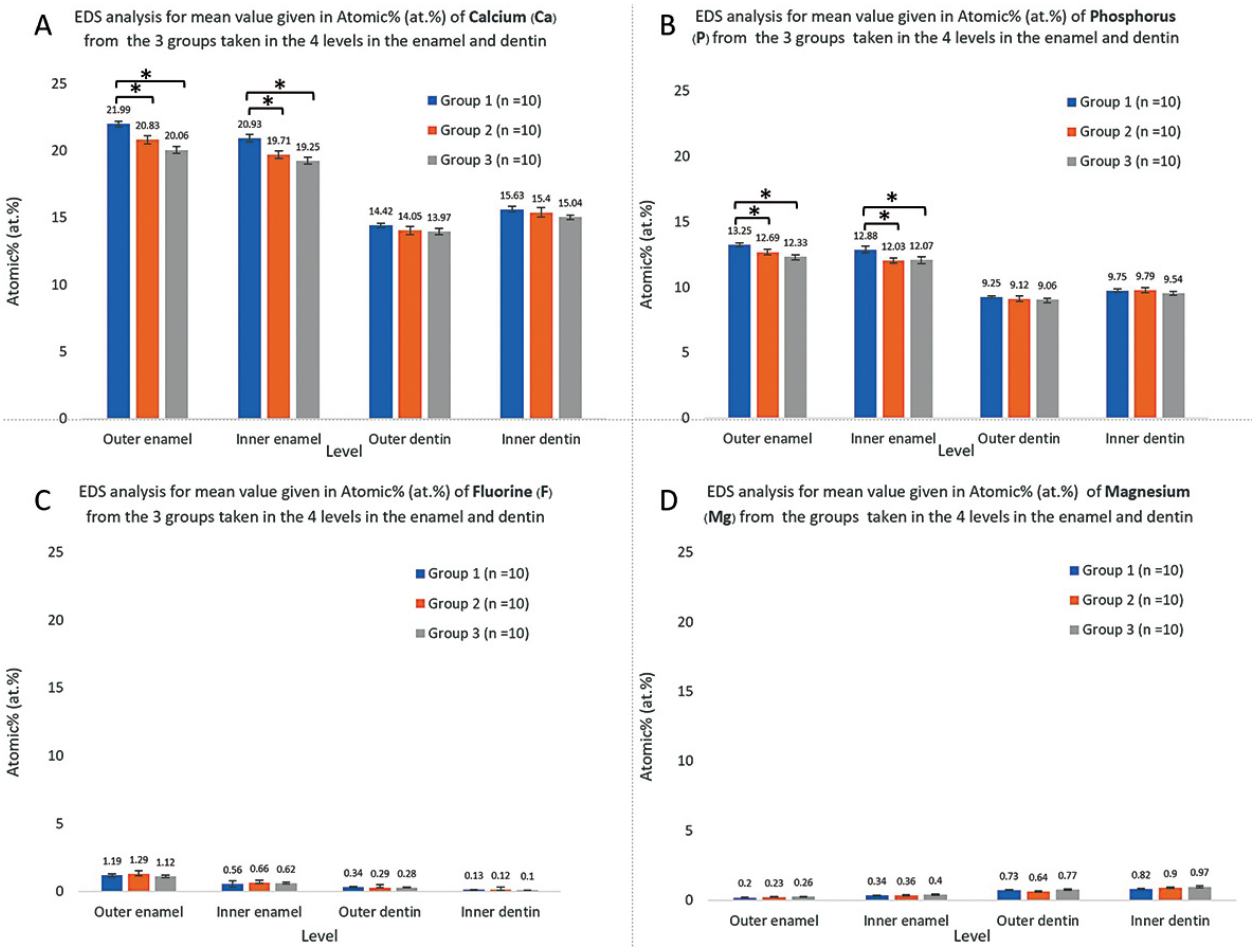
Two-way ANOVA tests suggested that there was statistically significant interaction between caries experience groups and tooth levels for calcium and phosphorus ($p < 0.05$).

The calcium content of the low caries experience group (18.24±3.38) was significantly ($p < 0.05$) greater than that of the moderate and high caries experience groups (17.56±2.93 and 17.08±2.75, respectively). The phosphorus content of the low caries experience group (11.28±1.89) was significantly ($p < 0.05$) greater than that of the high caries experience group (10.75±1.55). Fluorine and magnesium content were not significantly different between any of the caries experience groups.

Enamel contained significantly greater calcium, phosphorus and fluorine contents than did the dentin, whereas the dentin contained greater magnesium content. The content of all four elements was significantly different among all levels, except for phosphorus, which showed no significant difference between the two levels of enamel. There was no significant difference in calcium and phosphorus ratio between any of the tooth levels.

The calcium and phosphorus content significantly ($p < 0.05$) decreased in order from the outer enamel (20.92±1.13 and 12.70±0.68, respectively), to the inner enamel (19.99±1.13 and 12.34±0.73, respectively), the inner dentin (15.42±0.80 and 9.72±0.47, respectively) and the outer dentin (14.18±0.76 and 9.16±0.47, respectively). Fluorine content tended to decrease from the outer enamel (1.19±0.34), to the inner enamel (0.56±0.16), the outer dentin (0.29±0.08) and the inner dentin (0.12±0.05). On the contrary, magnesium tended to increase in order from the outer enamel, to the inner enamel, the outer dentin and the inner dentin (0.23±0.08, 0.36±0.12, 0.71±0.14, 0.90±0.18, respectively).

There were statistically significant differences ($p < 0.05$) in calcium contents among all four levels of the low, moderate and high caries experience groups, except between the outer and inner enamel of the high caries experience group.



รูปที่ 3 *รูปแสดงค่าเฉลี่ยร้อยละของอะตอมของ (A) ธาตุแคลเซียม, (B) ฟอสฟอรัส, (C) ฟลูออรีน และ (D) แมกนีเซียม โดยใช้การวิเคราะห์อีดีเอส 4 ตำแหน่งจากชั้นเคลือบฟันถึงชั้นเนื้อฟันจากตัวอย่างทั้ง 3 กลุ่ม (กลุ่มที่ 1 = ประสบการณ์การมีฟันผุต่ำ; กลุ่มที่ 2 = ประสบการณ์การมีฟันผุปานกลาง; กลุ่มที่ 3 = ประสบการณ์การมีฟันผุสูง), * แสดงความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อใช้สถิติวิเคราะห์ความแปรปรวนแบบสองทาง ($p \leq 0.05$) และการทดสอบเปรียบเทียบเชิงพหุคูณของทูคีย์*

Figure 3 *Chart of mean values, expressed in at.% of (A) calcium (Ca), (B) phosphorus (P), (C) fluorine (F) and (D) magnesium (Mg) from the EDS analysis at the four different levels from the enamel layer to the dentin layer of primary teeth from the 3 groups (Group 1 = Low caries experience group; Group 2 = Moderate caries experience group; Group 3 = High caries experience group).*

** significant difference at $p \leq 0.05$ when analyses by Two-Way Repeated Measures ANOVA and Tukey's multiple comparison test.*

When phosphorus content was compared within the same caries experience group, the result suggested significantly ($p < 0.05$) greater phosphorus content in enamel than in dentin.

The fluorine content within the same caries experience group was significantly different ($p < 0.05$) between all tooth levels, except between the outer and the inner dentin.

No significant difference was found when magnesium content was compared within the same caries experience group, except between the outer enamel (0.20±0.06) and the inner enamel (0.34±0.09)

in the low caries experience group, and between the outer dentin (0.64±0.17) and the inner dentin (0.90±0.19) in the moderate caries experience group and between the outer dentin (0.77±0.12) and the inner dentin (0.97±0.18) in the high caries experience group.

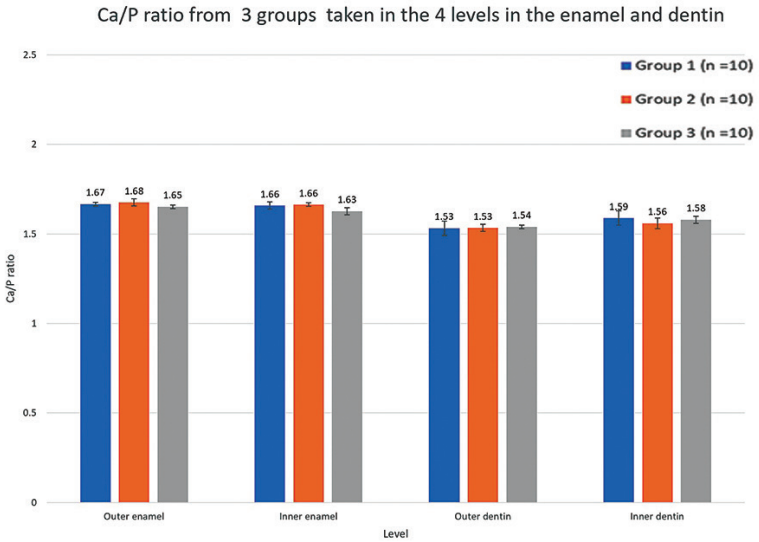
The calcium-to-phosphorus ratio in the outer enamel in the low and moderate caries experience groups, (1.67±0.04 and 1.68±0.07, respectively) and in the inner enamel in the same groups (1.66±0.08 and 1.66±0.08, respectively) was significantly ($p < 0.05$) greater than in the outer dentin (1.53±0.09 and 1.53±0.07, respectively) and in the inner dentin in the same groups (1.59±0.06 and 1.56±0.09, respectively), whereas the calcium-to-phosphorus ratio in the outer enamel in the high caries experience group (1.65±0.06) was significantly ($p < 0.05$) greater than in the outer dentin in the same group (1.54±0.06), as shown in figure 4.

Considering elemental content within the same tooth level, the calcium and phosphorus content in the outer enamel (21.99±0.70 and 13.25±0.51, respectively) and the inner enamel (20.93±0.94 and 12.88±0.79, respectively) in the low caries experience group were significantly ($p < 0.05$) greater than in the moderate (20.83 ±1.00 and 19.71±0.93, respectively) and high (20.06±0.81 and 19.25±0.83) caries experience groups.

However, there was no significant difference in the levels of fluorine and magnesium among any of the caries experience groups.

Discussion

The tooth susceptibility to caries progression depends upon calcium, phosphorus, and fluorine contents.[20] While magnesium plays an important role in regulating the crystal growth and is a predisposing factor for dental caries. The EDS technique



รูปที่ 4 *รูปแสดงค่าสัดส่วนของแคลเซียมต่อฟอสฟอรัสโดยใช้การวิเคราะห์อีดีเอส 4 ตำแหน่งจากชั้นเคลือบฟันถึงชั้นเนื้อฟันจากตัวอย่างทั้ง 3 กลุ่ม (กลุ่มที่ 1 = ประสบการณ์การมีฟันผุต่ำ; กลุ่มที่ 2 = ประสบการณ์การมีฟันผุปานกลาง; กลุ่มที่ 3 = ประสบการณ์การมีฟันผุสูง)*

Figure 4 *Chart showing calcium-to-phosphorus ratio from the EDS analysis of four different levels from the enamel layers to the dentin layers from the 3 groups (Group 1 = Low caries experience group; Group 2 = Moderate caries experience group; Group 3 = High caries experience group).*

used in this study gave similar findings of calcium, phosphorus, fluorine and magnesium content in the enamel and dentin of primary teeth to those of other studies.[21-25]

The elemental analysis by EDS in this study, expressed in at.%, indicated that the calcium, phosphorus and fluorine content was significantly greater in enamel than in dentin, and all showed the highest amounts at the surface of enamel in concordance with other studies.[26-29] The mean values of calcium and phosphorus in this study were greater than reported by Gutiérrez-Salzar and Reyes-Gasga,[26] who found greater calcium and similar phosphorus content than did Ortiz, *et al.*[27] but could not detect fluorine content.

Fluorine is found in the form of fluoroapatites and fluorohydroxyapatites in the hard dental tissues.[30] Low fluoride content may lead to decreased acid resistance in enamel, rendering it susceptible to caries. However, in this study, there was no significant difference in fluorine content between any of the caries experience groups at any level. Fluorine content in the outer enamel was significantly greater than that in the inner enamel. The small amount of fluorine measured in this study made it difficult to evaluate the difference. Using other techniques, such as chemical analysis,[31] laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS),[32] atomic absorption,[33] etc., may help to provide more definite fluorine content.

Calcium and phosphorus content were greater in enamel than in dentin, conforming to the findings of other studies.[13,26,28,29,34] Fluorine content was greater in enamel than in dentin, consistent with the findings of other studies.[35,36] Magnesium showed a similar pattern in all caries experience groups, with greater content in dentin than in enamel and greater content in the inner than the outer area, similar to the observations of other studies.[13,26,29]

It has been proposed that lower caries susceptibility is related to decreased porosity of enamel in primary teeth.[37] Some previous studies have found possible correlations in mineral composition corresponding to different caries experience.[38-41] The results of this study suggest that calcium and phosphorus content were lower in the enamel in the high caries experience groups compared to the low and moderate caries experience groups, possibly resulting in the chemical composition of enamel being altered, due to the demineralization process. The hydroxyapatite crystals are dissolved by the organic acid formed by bacteria through the anaerobic metabolism of sugars derived from the diet, resulting in loss of calcium and phosphate ions from the tooth surface. The high caries experience group may have had higher rates of demineralization than did the other groups. Nedosenko *et al.*[42] found the relative amounts of calcium and phosphorus in the enamel samples with a low resistance of caries were lower compared to the enamel samples with a high resistance of caries which is in agreement with this present study.

The EDS analysis indicated that the mean±SD of calcium-to-phosphorus ratio in enamel (1.63± 0.05) was higher than that in dentin (1.57±0.05), a finding which corresponds to the findings of other studies.[26,29] The greater calcium-to-phosphorus ratio in enamel than in dentin is probably due to calcium being bonded to other elements than phosphate.[29] The lower ratio in the dentin could be explained by the lower inorganic content in dentin than in enamel, as is widely known.[26] Although previous study suggested that the calcium-to-phosphorus ratio was significant differences between enamel of caries-affected teeth and that of healthy primary teeth,[17] under the different experimental area, no statistically significant difference were revealed on the smooth surface sample among difference caries experience groups in this study.

There is no specific analytical method for the estimation of the elemental content of human teeth. In this study, the estimation was done using SEM configured with the EDS technique, which is a non-destructive micro-analytical technique used to accurately estimate the elemental content of materials. It has high sensitivity (0.1%) for elements which have an atomic number equal to, or larger than, six, and is largely used in engineering and chemistry. Recently, this technique has been widely used in dental research.[11]

The different values of calcium, phosphorus, fluorine and magnesium between this study and previous studies may be due to several factors, such as the locations in which the studies were conducted, selection criteria, sample preparation, variations between individual teeth, the type, age and ethnicity of samples, and even the analytical method performed.[1,13,14,29,43,44]

Surprisingly, the low caries experience group had significantly greater calcium and phosphorus content than did the moderate and high caries experience groups. But there was no significant difference in fluorine or magnesium content between any of the caries experience groups.

Conclusions

The EDS elemental analysis showed greater calcium and phosphorus content in the low caries experience group than in the other groups, whereas similar fluorine and magnesium content was found among the three groups. This finding raises the possibility that the caries resistance of enamel depends upon greater calcium and phosphorus content, but is not related to fluorine or magnesium, content. However, further investigations are still needed.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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