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Original Article

# Microhardness and Cutting Resistance in Enamel of Primary Molars Among Various Caries Experience Groups *In Vitro*

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## Abstract

**Objectives:** To investigate cutting resistance, microhardness, and their correlations with primary teeth enamel, from different caries experience groups.

**Methods:** Forty-five extracted primary molars were divided equally into three groups using the dmft/dmft+DMFT index: low, moderate, and high caries experience groups. Each tooth was divided into 2 parts to test cutting resistance and microhardness. All data were compared statistically between groups with different caries experiences using the one-way ANOVA. The correlations were investigated using the Spearman's and the Pearson's correlation.

**Results:** The high caries experience group had significantly lower microhardness of enamel (295.8±12.73 Vickers Hardness Number (VHN)) than the moderate and low caries experience groups (315.01±16.13 VHN; $p$=0.001 and 325.96±9.91 VHN; $p$<0.001, respectively). The cutting resistance of enamel from the high caries experience group (87.23±15.06 grams) was also significantly less than those from the moderate and low caries experience groups (112.78±16.02 grams; $p$=0.002, and 111.67±24.75 grams; $p$=0.003, consecutively). There were negative correlations between caries experience and cutting resistance (r=-0.46; $p$=0.002) and between caries experience and microhardness (r=-0.71; $p$<0.001) but a positive correlation between cutting resistance and microhardness (r=0.39; $p$=0.009).

**Conclusions:** Enamel of primary teeth from the high caries experience group had less microhardness and cutting resistance than those of the moderate and low caries experience groups.

**Keywords:** caries experience, cutting resistance, enamel, hardness, primary tooth

## Introduction

Enamel, the hardest tissue in the body[(1)], contains the highest proportion of mineralization in its composition[(2)] that makes it highly resistant to acid from dental caries[(3)] and acidic drinks.[(4,5)] The microstructure orientation of enamel rods and hydroxyapatite crystals also enhance the mechanical properties of enamel.[(1,6-8)] Within the same tooth, enamel at buccal and lingual surfaces can be easier to cut than occlusal surfaces, due to their relatively lower hardness and Young's modulus.[(6)]

The enamel hardness gradually decreases from surface towards the dentin, as the mineral deposition of calcium and phosphorus decreases.[(9)] At the enamel surface, the Vickers Hardness Number (VHN) in permanent teeth ranges from 316.0 to 328.4[(10)] and in primary teeth range, from 299.54 to 374.06.[(11,12)] Our previous studies suggest that the enamel from low caries experience teeth has greater hardness than moderate and high caries experience groups[13,14], corresponding to higher concentrations of calcium and phosphorus composition.[15] Acidic beverages cause reduction of enamel hardness and increased tooth wear.[16] Few studies[13-15] have so far investigated enamel properties of teeth with different caries experience.

The tooth cutting process for a final restoration requires a gentle approach, especially in children, to make patients feel most comfortable. Most studies of tooth preparation focus on cutting devices including handpiece type, rotation speed, dental bur, advancement rate and temperature change during cutting, in order to improve the tooth preparation procedure.[17-22] The literature suggests that the application force on an airotor for cutting enamel ranges from 0.01 to 2.94 N.[23-26] However, dentists clinically adjust the applied force to achieve the desired form of preparation intuitively[27], and find that some teeth are more easily cut than others when using the same airotor handpiece and new diamond bur. This suggests that the individual tooth might have other properties affecting the cutting procedure.

The cutting resistance, a reaction force from the tooth itself which counteracts the dental bur during tooth preparation, might play a role in specifying the dentist's application force. The cutting resistance has never been investigated in addition to the tooth hardness. To avoid excessive force and minimize patient discomfort while maintaining the efficiency of the tooth cutting process, it would be interesting to study the cutting resistance, and microhardness of primary teeth in different caries experience groups.

The aims of the present study, therefore, are to investigate the microhardness and cutting resistance of enamel among various caries experience groups in extracted primary teeth, and to investigate the correlations of microhardness and enamel cutting resistance to caries experience groups.

## Materials and Methods

This study was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (No. 12/2020). Forty-five extracted primary teeth from 45 subjects with intact buccal and lingual surfaces were collected from healthy children aged 4-12 years with parental consent. Teeth with buccal or lingual surface caries, developmental defects or any restoration were excluded.

In order to indicate patients' caries experience, the dmft/dmft+DMFT scores were evaluated on the day of tooth extraction. The score indicated a number of teeth which met the following categories. Upper case letters are used for permanent teeth and lower case letters for primary teeth.

d or D: decay or caries tooth and recurrent caries (secondary caries).

m or M: missing tooth due to extraction from carious origin.

f or F: filled tooth including any form of restorations of carious lesion.

t or T: number of teeth.

The difficulty of collecting samples from each dmft/dmft+DMFT index scores such as 0, 1, 2, in the children made it difficult to directly correlate the variables with each dmft/dmft+DMFT index scores. Therefore, dmft/dmft+DMFT index scores were categorized into three groups. Scores 0 to 2 were categorized in the low caries experience group while scores 5 to 6 and ≥ 9 were classified into the moderate and high caries experience groups, respectively. The dmft/dmft+DMFT scores of 3, 4, 7, and 8 were excluded to amplify the difference between groups. Finally, there was a total of 45 samples, 15 samples for each caries experience group.

After sectioning the root off, the crown was cut along a central groove divided into the buccal part and the lingual part. The samples were stored in normal saline solution with 0.1% thymol solution at 4°C, until used for testing cutting resistance and microhardness. Any carious lesions or restorations were removed with diamond bur on an airotor handpiece.

The enamel of the lingual part (Figure 1A) was used for the microhardness test using Vickers hardness tester (STARTECH SMV-1000, Guiyang Sunpoc International Trade Co., Ltd., Guiyang, China) with 200 grams load on the indenter (a square based pyramid with 136° between opposite faces) for 15 seconds. The Vickers hardness testing machine was regularly calibrated according to ISO 6507[28] with a reference block to accept the 3% error. The enamel surface was painted with red permanent marker (Figure 1B) with the painted area facing down into a cylindrical polyvinylchloride (PVC) mold of 20 mm diameter (Figure 1C), filled with epoxy resin. After the epoxy resin was cured hard, the bottom surface was turned up (Figure 1D) and the red marked area was rubbed with 800 grits silicon carbide abrasive paper until approximately 1x1 mm enamel was exposed, followed by polishing with 1000 and 1200 grits abrasive papers (Figure 1E). Only 0.1–0.2 mm of each specimen was polished off to obtain a flat surface for microhardness testing. Then, the whole lingual sample was immersed in an ultrasonic cleanser for 5 minutes and left at room temperature until the enamel surface had less moisture.

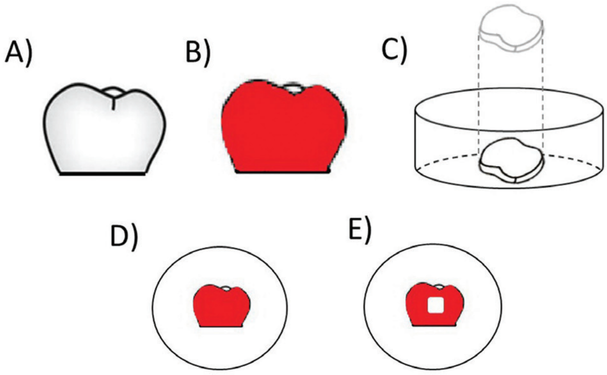



**Figure 1:** (A) Lingual part sample, (B) Enamel surface of lingual part sample painted in red color, (C) Lateral view of cylindrical PVC mold, the painted area facing down into the mold, (D) Bottom view of PVC mold with the sample, (E) Polished surface with exposed enamel

Five indentations were tested on surface of the lingual part, 100 $\mu$m apart in the occluso-gingival direction, according to Badr and Ibrahim, 2010[12] (Figure 2). The different hardness value of each test mark was due to the different type of mineral, the degree of mineralization and mineral particle size in each tested area.[29] The VHN of each indentation was defined as follows:

$$VHN = 1.8544\,\frac{F}{D^2}$$

Where F is load in kilogram force (kgf), and D is the mean of two diagonals in mm. The mean of these 5 hardness values was calculated to represent the hardness of each individual tooth sample.

The cutting resistance was tested by cutting enamel of the buccal part using a cylinder diamond bur and the same airotor handpiece with the same setting. The schematic diagram of the cutting resistance experimental setup is shown in Figure 3.

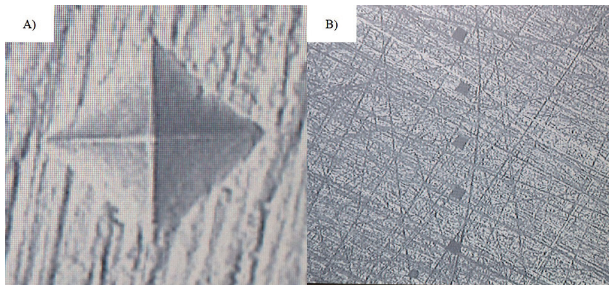



**Figure 2:** Light microscope images of enamel after being tested with Vickers microhardness indentation. (A) A well-defined indentation in the enamel showing diagonal lengths, x400 magnification, (B) A line of 5 indentations along occluso-gingival direction and 100 µm apart, x100 magnification.

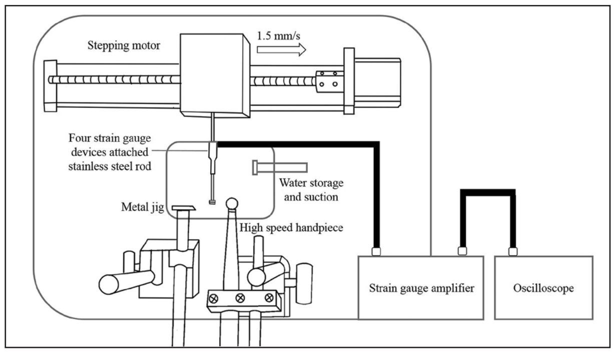
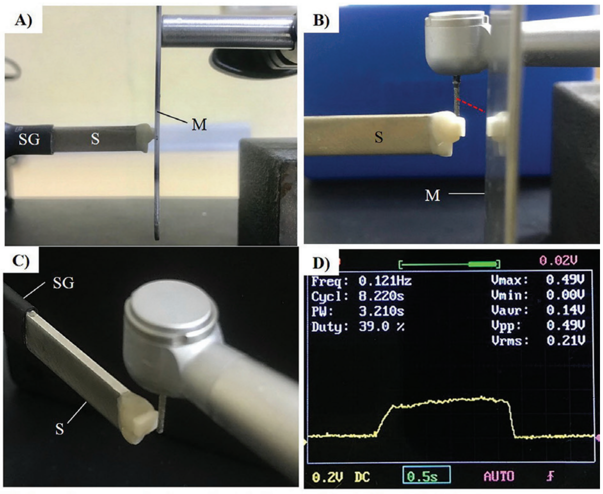



**Figure 3:** Schematic diagram of the cutting resistance experimental setup.

A single used 1 mm medium grain size (90-106 µm) cylinder diamond bur (ISO: 8063141115240101, JOTA, Switzerland), mouthed onto the high speed airotor handpiece (Optima BA535FM, B.A. international, UK) rotated at 300,000 rpm with a fixed stationary water spray coolant. The buccal half of the tooth specimen was trimmed into 2.5 x 3 mm block and mounted with composite resin (3M™ Filtek™ Z350 XT Universal Restorative, 3M ESPE, USA) onto the tip of a rectangular stainless steel rod equipped with a full bridge circuit of strain gauge devices (RS PRO Wire Lead Strain Gauge 3.5mm, RS components Ltd., UK). The bur and the tooth sample were precisely positioned to cut off 0.5 mm of enamel thickness using a metal jig (Figure 4). The constant-speed stepping motor (42BYGH48, HT, Malaysia) was used to drive the stainless steel rod at 1.5 mms toward the rotating diamond bur. The changes in resistance of the strain gauge devices caused by the deformation of stainless steel rod were amplified and monitored on the oscilloscope (DSO138, JYE Tech Ltd., China).




SG: strain gauge devices, S: stainless steel rod, M: metal jig,

**Figure 4:** The images demonstrated the setting of the cutting resistance test. (A, B) Set buccal part sample at the same position by parallel the flat plane of metal jig and aligned to the cylinder diamond bur at 0.5 mm (the red dashed line), (C) 0.5 mm of enamel was cut while tooth sample on the stainless steel rod and strain gauge devices was driving (1.5 mm/s) toward a rotating bur, (D) Monitor cutting resistance force on an oscilloscope.

Changes of the electrical signal for each sample displayed on the oscilloscope with a baseline in volts (X axis) was converted to the cutting resistance in grams (Y axis) by comparing the measured values with the calibration graph (Y= 418.01X-0.5076) obtained from applied commercial standard weights (TMS Co., Ltd., Thailand) of 20 grams, 50 grams, 100 grams and 200 grams on the stainless steel rod equipped with strain gauge devices, and values were read out to plot the graph.

The one-way ANOVA with Tukey's multiple comparisons was used to compare the microhardness of enamel and cutting resistance among the three caries experience groups. The microhardness and the cutting resistance of enamel among caries experience groups were tested with the Spearman's correlation. The association between enamel microhardness and cutting resistance was investigated using the Pearson's correlation, where a $p<0.05$ value was considered as significantly different.

## Results

The mean score of dmft/dmft + DMFT for low, moderate, and high caries experience groups were 1.2±0.86, 5.33±0.49, and 12.07±3.49, respectively.

The mean±SD of microhardness for all primary teeth enamel was 312.26±18.00 VHN. Enamel from the high caries experience group had the lowest microhardness (295.83±12.73 VHN), significantly lower than those of the moderate (315.01±16.13 VHN; $p=0.001$) and low (325.96±9.91 VHN; $p<0.001$) caries experience groups (Figure 5A). The low caries experience group had slightly greater microhardness of enamel than the moderate caries experience group, but not significantly statistically different.

The cutting resistance of the low, moderate and high caries experience groups showed a similar trend to microhardness. Enamel from the high caries experience group had the lowest cutting resistance (87.23±15.06 grams), significantly lower than those of the moderate (112.78±16.02 grams; $p=0.002$) and low (111.67±24.75 grams; $p=0.003$) caries experience groups (Figure 5B). The overall cutting resistance value was 103.89±22.16 grams, equivalent to 1.02±0.22 N.

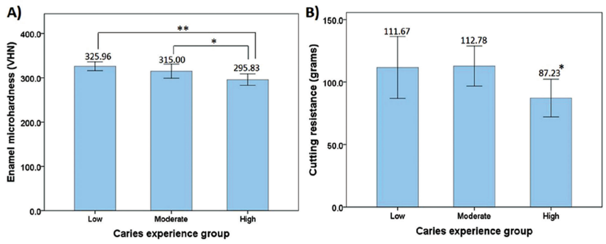



**Figure 5:** Bar chart of mean values between the various caries experience groups, (A) Enamel hardness, (B) Cutting resistance. *Significant difference at $p<0.01$, ** significant difference at $p<0.001$ when analyzed by the one-way ANOVA using Tukey's multiple comparisons.

The caries experience groups had negative correlations with enamel microhardness (r=-0.71; $p<0.001$) Figure 6A, and also cutting resistance (r=-0.46; $p=0.002$) in Figure 6B. The correlations between the individual dmft/dmft+DMFT scores and enamel microhardness, and between the individual dmft/dmft+DMFT scores and cutting resistance also showed similar trends. However, the enamel microhardness showed a positive relationship with cutting resistance (r=0.39; $p=0.009$) (Figure 6C).

## Discussion

A microhardness Vickers indenter in this study used a square based pyramid because it is the most commonly used.[30] The elongation of diagonals of indentations give advantages in detecting errors during hard-

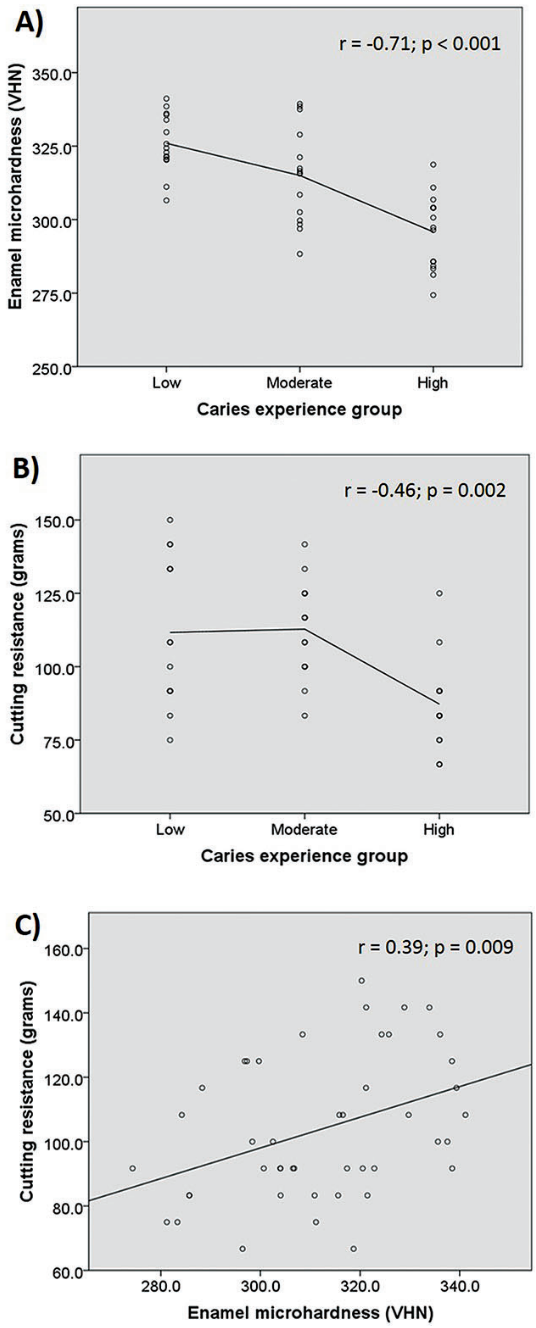



**Figure 6:** The correlation among caries experience groups, cutting resistance and enamel microhardness were tested. (A) Spearman's correlation test showing the line joining the median values of enamel hardness for each of the caries experience groups. These indicate a very strong negative correlation between enamel microhardness and caries experience (r=-0.71; *p*<0.001), (B) Spearman's correlation test showing the line joining the median values of cutting resistance for each of the caries experience groups. Indicating a strong negative correlation between cutting resistance and caries experience (r=-0.46; *p*=0.002), (C) The Pearson's correlation test showing a scatter plot of the relationship between enamel hardness and cutting resistance for all of the samples. The linear regression line (Y= 0.48x - 44.86) showed a moderate positive correlation between cutting resistance and enamel microhardness (r=0.39; *p*=0.009).

ness measurement. The average enamel microhardness of primary molars from all specimens in this study was 312.26±18.00 VHN, equivalent to 3.06±0.18 GPa (by multiplied 0.0098). It was slightly higher than previous outer enamel nanohardness studies of 2.65±0.54 GPa that used the same caries experience group categories(13) and microhardness tests(11,12) which ranged between 299.54 and 374.06 VHN. Apart from bio-variability of enamel, the differences in indenter size and shape between nano-hardness and microhardness tests yield small differences in the hardness values.(31)

The results of this study agreed with the literature, in which enamel from the low caries experiences group had higher microhardness values(14) and nanohardness values(13) than the high caries experience group. The negative correlation between caries experience groups and microhardness in primary teeth indicated that the high caries experience group had lower microhardness values, which might be due to less mineral content than in the low caries experience group. Higher calcium and phosphorus concentrations increased enamel hardness and decreased enamel porosity, which could lower caries susceptibility by reducing permeability.(9) The Energy Dispersive X-Ray Spectroscopy (EDS) technique discovered higher calcium and phosphorus concentrations in the enamel of primary teeth from the low caries experience group.(15) These high concentrations of calcium and phosphorus promote greater strength of enamel with the principal composition of hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) in enamel.(32) The ultrastructure study found that the lower caries experience enamel which had greater hardness contained a higher number of enamel prisms, greater prism density, and smaller protein gaps.(33)

The mineralization process of enamel is regulated by amelogenin, ameloblastin, and enamelin.(34) The Knoop hardness tests showed that enamel from over-expressing amelogenin had higher microhardness values and was more resistant to acid dissolution.(35) Abnormal protein function or decreased amounts of protein from genetic variation such as tuftelin 1 (TUFT1), amelogenin X-Linked (AMELX) and ameloblastin (AMBN) could lead to abnormal mineralization(34,36), resulting in lower microhardness of smooth surface enamel.(37) These genetic variations which impact enamel development make it prone to demineralization under acidic conditions, making it more susceptible to caries formation.(35,38)

The mineral compositions and microstructure of enamel not only reflected the hardness of the tooth, but also played an important role on acid dissolubility and caries resistance of the tooth[1,6,8,39], as well as strengthening the enamel and making it harder to cut. The literature suggested that the forces applied on the airotor for cutting permanent teeth vary from 0.1 to 2.94 N.[23-26]

The average cutting resistance of primary molar in this study was 1.02±0.22 N, which concurred with the range of cutting forces in the literature.[23-26] Importantly, all cutting equipment was controlled including the fixed stationary airotor handpiece, the new single used dental bur rotating at the same speed, and the constant stepping motor which drove the tooth sample toward the rotation bur; thus the different cutting resistance value of each tooth sample was dependent on only the tooth itself. Cutting resistance, one of enamel's properties, was a variable that provided reaction to the constant cutting force of driving the tooth sample toward an airotor at 1.5 mm/s. A positive correlation between enamel microhardness and cutting resistance was found (r=0.39; $p$=0.009), suggesting that higher concentrations of mineral-containing enamel would be difficult to cut. The caries experience groups had negative correlation with cutting resistance, indicating that a tooth in the high caries experience group was easier to cut than in the low caries experience group. These would explain the clinical perception that a tooth with aggressive caries is cut much easier than a sound tooth, and that a molar incisor hypomineralized (MIH) tooth was much easier to cut, as it had significant lower enamel hardness (144.30±106.54 VHN) compared to a normal tooth (350.70±30.15 VHN).[40]

The overall average cutting resistance value of primary teeth in this study was 103.89±22.16 grams. With this resistance value, dentists do not need to use a lot of force to achieve enamel cutting, as long as it is in an appropriate cutting environment such as new dental burs, and adequate water coolant. Importantly, in patients with high caries experience, less force is needed. If the excessive force is applied, it will transform into heat and vibration. Increasing the temperature will harm the adjacent dental pulp and periodontal tissues[41,42], and the vibration affects patients' comfort, with a result on the level of patient cooperation.

Enamel microhardness and cutting resistance might be affected by environmental factors including fluoride exposure, tooth-brushing frequency, sweet preferences and frequency of routine dental checkups[43,44], which were not evaluated and thus considered the limitations of this study. Exposure to fluoride, oral health care and dietary habits may influence the oral acidic environment that might result in different mineral content, enamel hardness and cutting resistance. Further investigation is required to correlate other physical properties of enamel to caries experience in both dentitions.

## Conclusions

This study showed that enamel microhardness and cutting resistance of primary molars are related to a child's caries experience. Dentists should be aware when applying force during primary tooth preparation, particularly for children in the high caries experience group. Other enamel properties might also vary in different caries experience groups, so further investigations are still needed.

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## Conflicts of interest

The authors declare no conflicts of interest.

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