Original Article

Nanohardness and Elastic Modulus Properties of Enamel and Dentin of Primary Molars *In Vitro*, in People with Various Caries Experiences, Using a Nano-indentation Technique

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Abstract

Objective: The aim of this study was to investigate the nanohardness and elastic modulus properties of enamel and dentin in extracted human primary teeth in people with various caries experiences, using a nano-indentation technique.

Materials and methods: Forty-five primary molar teeth were equally divided among three groups, low, moderate, and high caries experience, using the dmft/DMFT index. Non-carious and carious teeth, with intact buccal surfaces, from healthy children aged 4-12 years, were included in this study. Two-millimetre-thick specimens were prepared using two longitudinal sections. Nanohardness and elastic modulus in four zones, outer enamel, inner enamel, outer dentin and inner dentin were obtained using a nano-indentation technique. The mean nanohardness and elastic modulus between groups were analyzed and compared using two-way ANOVA at the $p \leq 0.05$ level of significance.

Results: In general, both nanohardness and elastic modulus were significantly different among the four tested zones with greater values in enamel than dentin and outer than inner. Outer enamel and outer dentin in the low caries experience group had significant greater nanohardness (2.88±0.46 GPa, 0.86±0.08 GPa, respectively) than in the high caries experience group (2.37±0.56 GPa, 0.72±0.12 GPa, respectively), whereas only the outer enamel in the low caries experience group had greater elastic modulus (76.46±10.46 GPa) than that of the high caries experience group (61.29±13.33 GPa).

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Conclusions: The outer enamel of the low caries experience group had greater mechanical properties than did that in the high caries experience group. This finding raises the possibility that the caries susceptibility of individuals depends on tooth mechanical properties.

Keywords: deciduous tooth, hardness, elastic modulus, dental enamel, dentin, tooth demineralization

Introduction

Knowledge of dental tissue hardness and elastic modulus is important, as dental tissue acts as a mechanical device in chewing and cutting food. Hardness evaluation is a universally-accepted indirect method used to define the quantity of mineral content in the tooth.(1) Many studies have shown the relationship between tooth mechanical properties and tooth elemental composition.(2-6)

Enamel, mainly composed of hydroxyapatite crystals, is the hardest substance in the body and can withstand mechanical forces of mastication.(7) The actual hardness of enamel can contribute to its fragility; however enamel hardness can be maintained by the elastic property of the underlying dentin.(8,9) The nanohardness of primary tooth enamel is 4.5-4.9 GPa, whereas its elastic modulus is 80.4-103.2 GPa.(10,11) Dentin has more organic and less inorganic material than enamel.(12,13) This composition makes dentin a rigid but elastic tissue.(13,14) Investigations of nanohardness of primary tooth dentin have demonstrated nanohardness of 0.52–0.92 GPa and elastic modulus of 11.59–19.89 GPa.(10,15)

Dental caries is a multifactorial disease.(16,17) The Decayed, Missing and Filled tooth (DMFT or dmft) index is commonly used, and is a well-established measurement for reporting the caries experience of individuals.(18) A previous study by our colleagues demonstrated a relationship between the caries experience of individuals and tooth trace-element composition.(19) High calcium and phosphorus content resulted in low caries experience. However, only a few researchers have investigated the relationship between the mechanical properties of teeth and individual caries experience, especially in primary teeth.(20,21) The nanohardness of enamel and dentin in primary teeth in young patient has not previously been studied using nano-indentation investigation. Therefore, the nanohardness of enamel and dentin in human primary teeth in children with various caries experiences were investigated in this study, using the nano-indentation technique.

Materials and methods

Extracted primary molar teeth were collected from healthy children aged 4-12 years living in the northern region of Thailand. This study was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (No. 4/2019). Non-carious and carious teeth, with intact buccal surfaces were obtained for this study. The size of cavity in the selected carious teeth must not have extended more than half of bucco-lingual width of the crown. Teeth with developmental defects, previously treated root canals or initial white spot lesions on the buccal surface were excluded.

Individual dmft/DMFT data were recorded by dentist on the day of extraction or on the dental visit before tooth was expected to self-exfoliate. Forty-five samples were equally classified into three caries-experience groups according to modified dmft/

DMFT values: Group 1 (low caries experience) had dmft/DMFT between 0 and 2, Group 2 (moderate caries experience) had dmft/DMFT value 5 or 6 and Group 3 (high caries experience) had dmft/DMFT greater than 9. Teeth that did not fit the categories were excluded from this study.

The teeth were washed under running tap water and stored in normal saline solution with 0.1% thymol solution at 4°C until use. The root was cut off at the cemento-enamel junction (CEJ). Two-millimetre-thick specimens were prepared by cutting the teeth into two longitudinal sections using a low-speed diamond saw with water irrigation. The cut specimens were immersed in a solution of ultrasonic cleanser for 10 minutes to remove debris during the cutting process and left dry at room temperature for 10 minutes. The specimens were embedded, using epoxy resin, in a 15 mm-diameter circular brass mold, with one cut surface face down and the other surface left uncovered for further testing. The surface to be tested was sequentially polished with 800, 1000, 1200 and 1500 grits sandpaper. All specimens were air-dried at room temperature for 48 hours before being tested using the nano-indentation technique. Four zones were tested using a nano-indentation machine (iMicro Indentation System, Nanomechanics, Inc., Oak Ridge, Tennessee, USA) at the height of contour of the crown: outer enamel (at 100 µm depth from surface), inner enamel (100 µm from the dentinoenamel juction; DEJ), outer dentin (100 µm from the DEJ), and inner dentin (100 µm from the pulp surface).

Nanohardness and elastic modulus were tested at six indentation points in each zone, 50 nm apart, by applying 5 mN maximum indentation force, at a 0.2 mN/s loading rate for a 1000 ms holding time using a Berkovich diamond tip (Nanomechanics, Inc.). The indenter tip was pressed in a perpendicular direction to the enamel rods and the dentinal tubules. Mean nanohardness and mean elastic modulus were obtained from three indentation points which had productive values.

Two-way ANOVA with multiple comparisons was used to compare the differences between the three caries-experience groups and between both the nanohardness and the elastic modulus in the four zones of the tooth samples. Significance was established at $p \leq 0.05$.

Results

Mean±SD of nanohardness and elastic modulus were 2.36 ± 0.60 GPa and 64.32±13.62 GPa, respectively, for the enamel, and 0.70 ± 0.18 GPa and 20.12±3.78 GPa, respectively, for the dentin. From the outer enamel to the inner enamel, the mean±SD of nanohardness and elastic modulus were significantly decreased, respectively, from 2.65±0.54 GPa and 69.05±13.19 GPa to 2.07±0.52 GPa and 59.60±12.45 GPa, whereas those of the outer dentin (0.80±0.15 GPa, 22.23±3.18 GPa, respectively) were decreased to 0.59±0.13 GPa, 18.01±3.13 GPa, respectively, for the inner dentin.

However, overall mean±SD of nanohardness and elastic modulus in the three caries-experience groups were not significantly different. In detail, the outer enamel and the outer dentin of the high caries experience group (2.37±0.56 GPa, 0.72±0.12 GPa, respectively) had significantly lower nanohardness than did the low caries experience group (2.88±0.46 GPa, 0.86±0.08 GPa, respectively), whereas the nanohardness in the moderate caries experience group (2.70±0.50 GPa, 0.82±0.20 GPa, respectively) was not significantly different from that in the other groups. There were no significant differences in nanohardness the caries experience groups for the inner enamel and the inner dentin.

The mean values of elastic modulus in the low caries experience group were higher than in the other groups in all zones, but with no significant differences. For the outer enamel, elastic modulus in the low

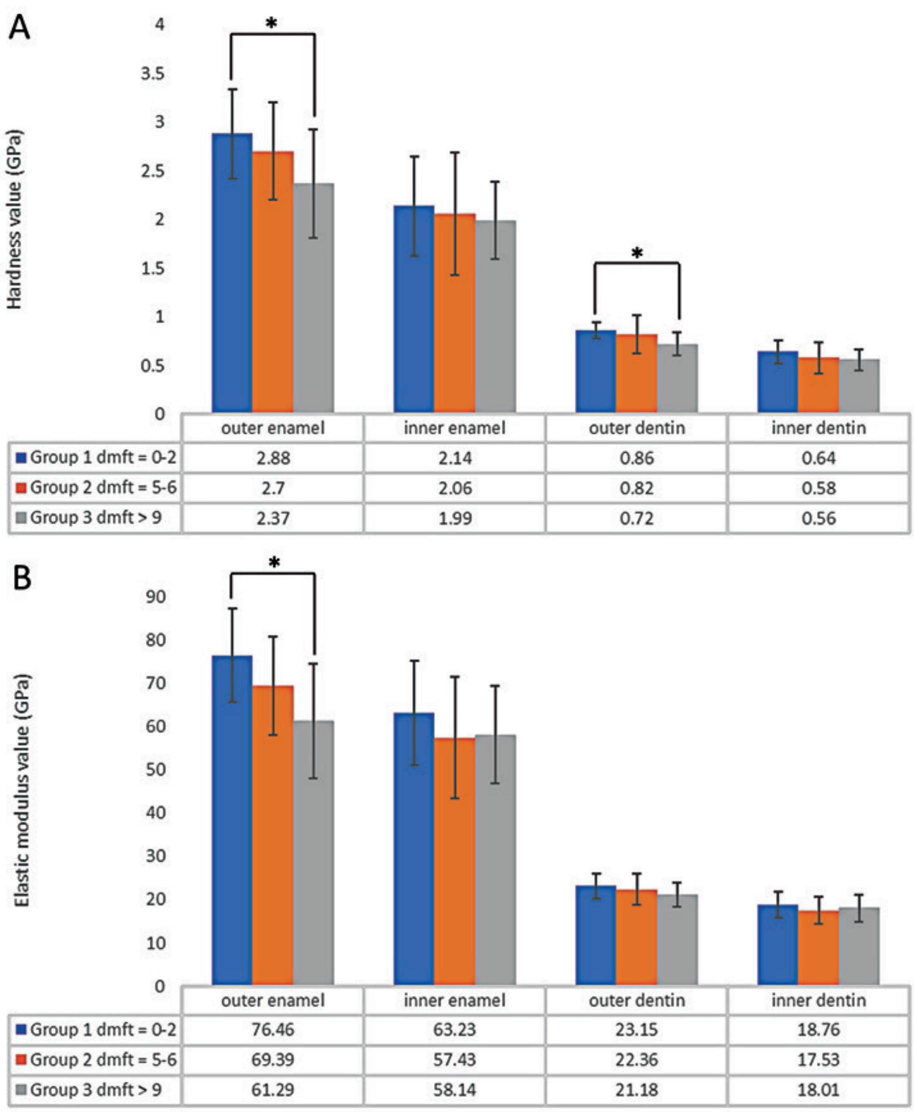

Figure 1 *Bar chart of mean nanohardness (A) and elastic modulus (B) values between the various caries experience groups in the four zones of the teeth*

caries experience groups (76.46±10.46 GPa) was significantly greater than in the high caries experience group (61.29±13.33 GPa).

Discussion

The overall mean nanohardness and elastic modulus in the enamel of primary teeth were lower than in previous nano-indentation studies, which showed nanohardness and elastic modulus values between 4.5-4.9 GPa and 80.4-103.2 GPa, respectively.[10,11] Differences in the direction of the indenter to the alignment of the enamel rods and different indentation forces yielded different results.[11,22] Interpretation of the results of nano-indentation may not be comparable across studies.

There was significant reduction of nanohardness and elastic modulus with depth from the outer enamel to the inner enamel by 21.89% and 13.68%, respectively. This finding conformed to the findings of other studies on permanent molars that found 26% reduction of hardness and elastic modulus of the inner enamel near the DEJ compared with the outer enamel.[23,24] The results of one microindentation and Micro-CT study showed a direct correlation between the hardness of the enamel and the HA crystal density, both of which decrease with the distance from the enamel surface to the DEJ.[3] Enamel rods, a hard organic component, decrease in size toward the DEJ, whereas porosity and water content increase and the softer enamel interrods double in volume.[25,26] Moreover, an elemental composition study suggests that the outer enamel has greater calcium, phosphorus and fluorine content than the inner enamel.[19]

The dentin of primary teeth has less nanohardness than the enamel.[13,14] The lower the inorganic composition, the greater are the organic composition and the percentage of water content. The overall mean nanohardness and elastic modulus of the dentin found in this study were 0.70±0.18 GPa and 20.12±3.78 GPa, respectively. Mahoney *et al.*[10] found slightly greater nanohardness 0.92±0.11 GPa than did this study. However, the method of sample preparation, the indentation force and the direction of indentation varied among the studies.

In the dentin layer, depth also affected the nanohardness and elastic modulus values. Nanohardness and elastic modulus were reduced from the outer dentin (0.80±0.15 GPa and 22.23±3.18 GPa, respectively) toward the inner dentin (0.59±0.13 GPa and 18.01±3.13 GPa, respectively). This finding conforms with the findings of other studies.[15,26-28] Angker *et al.*[15] found significantly decreased dentin hardness in primary teeth from the outer (0.91±0.15 GPa), and middle (0.85±0.19 GPa) to the inner layers (0.52±0.24 GPa). The decrease in hardness and elastic modulus values may result from the increase in the volume of the dentinal tubules from 4% in the outer dentin to 16% in the inner dentin, 1 mm from the pulp[29] the outer dentin has thicker peritubular dentin and fewer dentinal tubules than does the inner dentin.[13]

In general, no significant differences were found in either nanohardness or elastic modulus between the three caries-experience groups. However, the outer enamel in the low caries experience group had significantly greater nanohardness and elastic modulus than in the high caries experience group. This study is the first to report the correlation between dmft/DMFT values and nanohardness and elastic modulus of the primary tooth. The results of this study support those of Gutíérrez-Salazar and Reyes-Gasga,[20] who reported greater microhardness of enamel in low caries risk than in high risk caries teeth. In contrast, Kelly *et al.*[21] reported no relationship between caries experience and the microhardness of enamel. Instead, they found several correlations of caries experience with the number and density of prisms and the size of protein gaps. However, no study has shown a correlation between nanohardness and caries experience in primary teeth.

The relationship between the quantity of trace elements in primary teeth and caries experience was investigated by Wongyai *et al.*[19] They found that the outer enamel of the low caries experience teeth contained significantly higher atomic percentages of calcium and phosphorus content than did that (meaning the outer enamel) of the moderate and the high caries experience teeth. Higher levels of calcium and phosphorus, the principle components of the HA crystal, may yield greater strength of enamel and dentin.

The outer enamel of the samples may have been affected by the acidity of the food consumed and individual oral hygiene care, but not the inner enamel. The low mechanical properties of samples in the high caries experience group compared to those in the low caries experience group may be the result of high individual consumption of acidic food or poor oral hygiene care.

The outer dentin in the low caries experience group also had significantly greater nanohardness than did the outer dentin in the high caries experience group, whereas there was no significant difference in nanohardness in the inner dentin or in elastic modulus values in all dentin depths among caries-experience groups. Corresponding with one trace element study, no significant difference was found between either the outer or inner dentin in any caries experience group.(19)

Most caries experience and caries susceptibility research published indicates that enamel contributes greatly to caries defense. However, some studies have described the role of dentin in caries progression.(19,29) The hardness of the outer dentin depends on increased thickness of peritubular dentin and reduced density of dentinal tubules.(29) The inner dentin has the opposite properties. Further study of the relationship between physical and chemical composition of dentin and the caries susceptibility of individuals is needed.

Conclusions

The low caries experience group had significantly greater nanohardness than did the high caries experience group in the outer enamel and the outer dentin layers. However, only the outer enamel had significantly greater elastic modulus in the low caries experience group than in the high caries experience group. This finding raises the possibility that the caries susceptibility of individuals depends on tooth mechanical properties. However, further investigation is needed.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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