Measurement of surface hardness of primary carious lesions in extracted human enamel —Measurement of Knoop hardness using Cariotester—

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The clinical feasibility of a novel device called a Cariotester was investigated by measuring the Knoop hardness (KHN) of white spot lesions diagnosed as ICDAS code 1, 2 or 3. To obtain an equation for converting the Cariotester indentation depth into the KHN, a regression analysis was performed between the depth and measured KHN for human enamel. The Cariotester was then used to measure the indentation depth for white spots (ICDAS code 1, 2 or 3) in extracted teeth, and the KHN values were determined using the above equation. The KHN was 219.9±19.7, 162.4±24.0 and 31.7±17.5 for code 1, 2 and 3 lesions, respectively, which was 30, 49 and 90% lower than that for healthy enamel. Using the formula reported in the literature, the mineral density was calculated to be 87.7 vol.% for healthy enamel, and 75.1, 66.1 and 35.5 vol.% for code 1, 2 and 3 lesions, respectively.

Keywords: White spot, ICDAS code, Cariotester, Knoop hardness, Mineral density

INTRODUCTION

The recently-proposed International Caries Detection and Assessment System (ICDAS) code is on its way to becoming the international standard for the assessment of dental caries. One distinctive feature of ICDAS is that it considers non-cutting methods (remineralization) for evaluating early-stage carious enamel, enabling the assessment of caries progression using methods that are feasible in standard clinical practice. ICDAS classifies enamel caries (white spots) according to codes 1 to 3. Code 1 is visible early change in enamel. When seen wet, there is no evidence of any change in color, but after air drying a carious opacity is visible. Code 2 is a distinct visual change in enamel when viewed wet. There are clear enamel changes that appear as white or brown spots. Code 3 has localized enamel breakdown without visible signs of dentin lesions. The higher the code number, the more severe the extent of dental caries. The hardness of white spot surfaces is also predicted to decrease accordingly but the specific level of hardness corresponding to each code remains unclear.

Dental hardness is typically measured using a Knoop hardness tester, but this device cannot be used in clinical settings. However, a new device known as a Cariotester (SUK-971, SaneiME Corp., Yokohama, Japan), which was developed in Japan in 2010, enables simple intraoral measurement of carious dentin (Fig. 1). The principle of the Cariotester is that when an indenter coated with poster paint is pressed into an object, the width of lost paint corresponds to the depth of the indentation (Fig. 1). Several in vitro and in vivo studies have examined the hardness of dentin using the Cariotester.

The first objective of this study is to reveal the relationship between the indentation depth determined by a Cariotester with an enamel indenter and the Knoop hardness (KHN) determined by a microhardness tester. The second objective is to measure the KHN value using the Cariotester on the surface of white spot lesions diagnosed as ICDAS codes 1, 2 and 3 on extracted teeth.

MATERIALS AND METHODS

Relationship between enamel indentation depth and Knoop hardness

The Cariotester is a device that tests indentation hardness, so an indent must be made on the surface being measured. The enamel measured in our test is approximately 5 times harder than dentin, so assuming that we would not obtain the desired indentation depth using a conventional dentin indenter (tip curvature radius of R=18 μm, cone angle of 50°), we developed a new enamel indenter with a reduced tip curvature radius (R=10 μm, cone angle of 50°). This enamel indenter is manufactured using the same tungsten carbide as the conventional dentin indenter, and its indentation load is the same as that used to measure dentin hardness at 150 gf.

The extracted human molars were preserved in 10% neutral formalin after extraction. For this study, we selected 2 molars with extensive white spots on the occlusal surface (Hyogo College of Medicine Ethical Review Board approval No. 586). The occlusal surface was polished under running water using 120–1,500 silicon carbide (SiC) abrasive paper to produce a
Extracted human molars with white spots widely distributed on the occlusal surface were used. The occlusal surface was flattened and polished, and measurement regions with diameters of about 0.7 mm were designated. A smooth surface in the white spot areas. Multiple small regions with diameters of approximately 0.7 mm were then designated within the white spots under a stereomicroscope at a magnification of 10 to 20× (Fig. 2). In each small region, the depth of indentations and the KHN values were measured using the Cariotester and Knoop indentions in a white spot region.
a microhardness tester, respectively. (MVK-E, Akashi Seisakusho) (Fig. 3). First, we used the Cariotester to measure the depth of indentations at 5 arbitrary sites within the small region and calculated the mean depth. We then used the microhardness tester to measure the KHN values at 5 arbitrary sites within the same small region, and calculated the mean KHN value. The KHN indentations were made with a 25-, 50-, 100-, or 200-gf load, selected according to enamel hardness, for 15 s. We obtained mean paired data for indentation depth and KHN within the same small region. In this way, many pairs of corresponding data sets were obtained at small regions containing white spots. Next, we repolished the white spots until the indentations were completely removed, and measured paired data for indentation depth and KHN value at other regions containing white spots using the methods described above. By repeating this procedure, we obtained paired data for 19 white spots. We also used the same method to measure the indentation depth and KHN value at 3 healthy unwhitened enamel regions. With the resulting 22 sets of paired data, we prepared a scatter plot and performed a regression analysis.

Measuring Knoop hardness for ICDAS code 1, 2 and 3 white spots
From the extracted human molars preserved in 10% neutral formalin, we selected 16 molars with either white or brown spots on the smooth surface (Hyogo College of Medicine Ethical Review Board approval No. 586). There were 25 sites assessed as ICDAS code 1, 30 sites assessed as code 2, and 22 sites assessed as code 3.

Using the Cariotester with the enamel indenter, we measured the indentation depth at each site under a 150-gf load. For the code 3 white spots, we measured white spot areas where the enamel surface layer had decayed, confirming these sites with a stereomicroscope at a magnification of 10×. After measuring each site 5 times, we converted the indentation depths to KHN values and calculated the means. To assess the extent of softening in each white spot, we identified healthy enamel areas in 16 molars (ICDAS code 0) and measured their KHN values with the Cariotester based on the method described above. We used one-way analysis of variance (ANOVA) with a significance level of 1% to statistically compare the KHN values obtained for the code 1, 2 and 3 white spots and the code 0 enamel.

RESULTS
Figure 4 shows the relationship between the indentation depths measured with the Cariotester and the KHN values determined with the microhardness tester for a total of 22 sites, specifically 19 enamel white spot regions and 3 unwhitened healthy regions of extracted teeth. The regression equation was determined to be:

\[ y = 6839.2x^{-1.4087} \]  

wherein \( y \) denotes the KHN value and \( x \) denotes the indentation depth (μm). The correlation coefficient (\( R^2 \)) was 0.9879.

Figure 5 shows typical examples of code 1 and 2 white spots and their corresponding KHN values. Figure 6 shows the KHN values for code 0 healthy enamel and code 1, 2 and 3 white spots measured with the Cariotester. The mean KHN value was 316.2±18.4 (\( n=16 \)) for code 0 enamel, and 219.9±19.7 (\( n=25 \)), 162.4±24.0 (\( n=30 \)) and 31.7±17.5 (\( n=22 \)) for code 1, 2 and 3 white spots, respectively. One-way ANOVA revealed significant differences in the KHN values for each code level, with the hardness declining significantly as the code level moved from 0 to 2 (\( p<0.01 \)).

DISCUSSION
Early-stage carious enamel with clinically-diagnosed white spots is generally classified as ICDAS code 1 or 2, while localized enamel decay within white spots is assessed as code 3. The ICDAS proposed a clinically-feasible method of caries assessment because it considers the use of non-cutting methods (remineralization) for evaluating early-stage caries. We therefore believe that ICDAS will gain international acceptance in the future.

Previous studies on early-stage enamel caries (white spots) in extracted teeth investigated depth-related changes in mineral content by creating mineral profiles based on transverse microradiography (TMR) and micro computed tomography (CT) images. These studies demonstrated that surface mineral levels only decreased slightly but subsurface mineral levels declined considerably, while comparison of active and inactive white spots failed to show any significant differences in surface mineral levels (g/cm³). However, when the white spots are continuously exposed to plaque accumulation and other factors that trigger caries progression, the surface mineral level declines until the lesions become porous, resulting in decreased enamel surface hardness followed by partial surface decay classified as ICDAS code 3 caries. To prevent this white spot surface decay, it is therefore essential to maintain a certain level of hardness on the outermost surface.

The Knoop hardness test is a conventional method for determining the hardness of teeth. Performing measurements with the microhardness tester requires that the surfaces to be measured have a mirror-polished smoothness. However, natural enamel surfaces consist of a series of uneven grooves known as perikymata which prevent hardness measurements in their unmodified state. Measurement of hardness must therefore be performed after a slight amount of polishing to produce a smooth surface, or at the polished cross section perpendicular to the white spot surface as close as possible to the enamel surface. In any case, both of these methods have precluded the use of a microhardness tester to measure white spot surface hardness. The Cariotester, on the other hand, uses a novel method in which the indentation depth is determined by measuring the width of lost paint from the indenter, thus enabling...
measurement of hardness on the outermost surface of the white spot.

In the present study, we investigated the relationship between the indentation depth and KHN value by measuring these variables on white spot surfaces using the Cariotester and a microhardness tester, respectively. Consequently, we obtained formula (1) for converting the Cariotester indentation depth to the KHN value. Using the Cariotester to measure the indentation depth of white spot and healthy enamel surfaces thus made it possible to determine the KHN value. The respective KHN values for the ICDAS code 1, 2 and 3 white spot surfaces were 219.9±19.7, 162.4±24.0 and 31.7±17.5. The markedly lower KHN value for code 3 white spots was attributed to the fact that the hardness of code 3 was measured at the demineralized subsurface area where the enamel surface layer had decayed. Since the mean KHN value for healthy enamel (code 0) was 316.2±18.4, the KHN values for code 1 and 2 caries were 30% and 49% lower, respectively.

Featherstone et al.3) investigated the relationship between the enamel KHN value and the mineral density. Their study yielded the following equation:

\[ \text{Mineral density (vol.\%)=4.3 \times KHN^{0.5}+11.3} \quad (2) \]

Using equation (2) to calculate the mineral density from the Cariotester KHN values, we found that the mineral density was 34.7±6.4 vol.% for code 3 white spots. These findings show that the mineral density decreases with decreasing hardness (Fig. 7). In this way, enamel mineral densities can be determined from KHN values so Cariotester-based measurements of white spot hardness over time make it possible to quantitatively assess whether white spot lesions are in a state of progression, arrestment or regression in terms of mineral density.

The question of whether subsurface demineralization can be estimated from white spot surface hardness is one of considerable interest. In the present study, the maximum KHN value for code 1 white spots (n=25) was 247.0 and the minimum KHN was 173.1. For code 2 white spots (n=30), the maximum KHN value was 199.3 and the minimum was 118.6. The difference between the maximum code 1 KHN value and the minimum code 2 KHN value is approximately 130, indicating major variations in white spot surface hardness. If the hardness of white spot surfaces is in fact related to subsurface demineralization, then measuring the enamel surface hardness using the Cariotester could allow us to estimate the degree of this subsurface demineralization.

The mean indentation depth using the Cariotester was 11.6±0.8 μm for code 1 white spots and 14.5±1.7 μm for code 2 white spots (Table 1). The thickness of the white spot surface layer is presumed to be 35 to 130 μm10), so these indentations would not be expected to penetrate this layer and reach the demineralized subsurface layer. However, even though Cariotester indents are minute, they still cause damage to the enamel. It is therefore necessary to somehow observe the prognosis of these indents clinically, including whether they recover with time. The indents could be filled with the remineralization material that is used to coat the white spots. In this case, even if the material covering the enamel was worn and removed over time from eating and brushing, the coating material filling the indent would remain and provide sustained fluoride release. Furthermore, if the material filling the indent plays the role of a reservoir for slow-release fluoride, it will be effective in remineralizing the white spot, and this matter warrants further investigation in the future.

**CONCLUSIONS**

After creating a smooth, polished surface on white spots with early stage carious enamel, we measured the indentation depth using a Cariotester and the Knoop hardness (KHN) using a microhardness tester. Our findings demonstrated a markedly strong correlation...
between the indentation depth and the KHN value, given by the following regression equation:

\[ \text{KHN} = 6839.2D^{-1.4087} \]

where \( R^2 = 0.9879 \) and \( D \) denotes the indentation depth (\( \mu \text{m} \)).

Next, we measured the indentation depth for white spots on extracted teeth using the Cariotester, and determined the KHN value using the regression equation. Our results showed that the KHN value was 219.9 and 162.4 for ICDAS code 1 and 2 white spot surfaces, respectively, and 31.7 for the decayed surfaces of code 3 white spots. Compared to healthy enamel which had a KHN value of 316.2, the hardness was 30%, 49%, and 90% lower for code 1, 2 and 3 white spots, respectively. Moreover, the mineral density calculated from the KHN values obtained using the Cariotester showed that code 0 healthy enamel had a mineral density of 87.7 vol.%, while the code 1, 2 and 3 white spots had a density of 75.1, 66.1 and 35.5 vol.%, respectively. In conclusion, our findings suggest that the Cariotester is a promising instrument for testing and monitoring early-stage carious enamel.

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