Correlation Between Fissure Discoloration, Diagnodent Measurements, and Caries Depth: An In Vitro Study

Paola Francescut, DMD Adrian Lussi, Dipl chem, DMD, PD

Dr. Francescut is assistant professor and Dr. Lussi is head, Department of Pediatric Dentistry and Histology, School of Dental Medicine, University of Bern, Bern, Switzerland. Correspond with Dr. Francescut at paola.francescut@zmk.unibe.ch

Abstract

Purpose: This in vitro study was performed to correlate the presence of discoloration on occlusal surfaces with its histological depth and assess its influence on Diagnodent measurements in a group of permanent and primary teeth.

Methods: Ninty-five primary and 95 permanent third molars were randomly selected from a pool of macroscopically intact teeth. One site of the fissure on each occlusal surface was selected and categorized according to its discoloration. Each site was measured 3 times with Diagnodent. The teeth were prepared histologically and evaluated according to their caries extent under a microscope (final magnification ×12.8).

Results: In the group of permanent teeth with dark brown or black discoloration (N=23), 13% showed dentinal caries, 57% were sound or had an initial enamel lesion, and 30% had a deep enamel lesion. In the group of primary teeth with dark brown and black discoloration (N=19), 42% presented dentinal caries, 42% showed deep enamel caries, and 16% had an initial enamel lesion. The difference between permanent and primary teeth was statistically significant ($P<.05$). Discolored fissures showed higher Diagnodent values than nondiscolored or opaque fissures in both groups (independent of their caries status).

Conclusions: The presence of brown or dark spots on fissures were not useful for the prediction of dentinal caries for permanent teeth. In primary teeth, however, a higher correlation between fissure discoloration and dentinal lesions was found. Diagnodent tends to overscore discolored surfaces. (*Pediatr Dent*. 2003;25:559-564)

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The accurate evaluation of the extent or activity of caries is a difficult task dependent on several factors like conditions during the inspection (eg, light and drying), patient's caries risk, tooth age and morphology, and training and experience of the dentist. Thus, developing a confident and objective caries detection method has become an important aim for many researchers in the last few years.[1-6] Although the most frequently used diagnostic methods (visual inspection, visual inspection with probe, visual inspection with a magnifying glass, and bitewing radiographs) offer a relatively good diagnostic performance, there are still difficulties associated with them. These methods showed good to excellent ability to recognize sound surfaces (specificities ranging from 0.63-1) but unsatisfactory capacity to recognize caries (sensitivities ranging from 0.01-0.67).[2-3,7,9,13-14,28] Considering these limitations, research is needed to find other useful methods or parameters that, combined with the traditional methods, can improve caries detection ability and avoid their disadvantages.

Today, some devices such as the electrical caries monitor (ECM) and the laser-based device (Diagnodent) are available for the practitioner to assist in the detection of caries. All of them offer heightened objectivity in caries assessment.[3,6,17,22,24,28,31] Although they mostly perform the same or better than the current conventional methods, they also show a range of false-positive, and false-negative answers that make them not absolutely trustworthy.[2,17-18,22]

A complex morphology of the fissure (especially I-type and V-type fissures)[7,16] and the presence of microcavities[8-10] or brown discolored fissures[11-12] can be considered aids in the diagnostic process. These parameters have been proposed, alone or combined, as predictors of dentinal caries or as factors that make fissures more susceptible to develop caries. There are controversial opinions about the value of brown discolored fissures. Some authors have found this is not a useful parameter for the prediction of occlusal caries because it could lead to a large amount of false-positive answers. Such a feature should be carefully analyzed before making a treatment decision.[5,7,13-14] On the other hand, some authors suggest that brown or black stained fissures indicate caries or are more likely to be decayed than nonstained fissures after some years.[11-12,15]

Furthermore, the presence of discoloration and exogenous stain, as well as plaque, calculus, or composite filling materials, may produce fluorescence that, when measured with Diagnodent, lead to false-positive answers.[17-19,30]

The literature available about caries diagnosis in the primary dentition is limited.[9,23,28-29] Therefore, the aim of this study was to evaluate and compare in vitro the presence of different levels of fissure discoloration on occlusal surfaces in a group of primary teeth compared to a group of permanent teeth and correlate those levels with their histological depths of caries.

To determine in which degree the response of the Diagnodent laser-based device can be influenced by the presence of such discoloration, its performance was also analyzed in both groups of teeth.

Methods

The sample consisted of 190 teeth. Of these, 95 were primary molars that also had been used for a previous study (11 first lower molars, 39 second lower molars, 14 first upper molars, 31 second upper molars).[28] The other 95 were permanent molars (most of them third molars). All teeth were randomly selected from a pool (stored in 5% buffered formalin). They were macroscopically intact to the naked eye and presented no restorations. All the occlusal surfaces were rinsed and cleaned thoroughly with distilled water and a toothbrush. When calculus was present, a hand scaler was gently used to remove it.

Finally, the teeth were photographed and drawings of the occlusal surfaces were also sketched. One examiner randomly selected a site of the fissure in each occlusal surface and recorded its precise location in the occlusal surface. The site was then marked on each drawing to recognize it easily during the measurements and observations. Each sample was then stored in individual plastic containers with paper towels soaked in saline solution with Thymol (0.15%) and stored in the refrigerator.

Visual examination

All 190 teeth were photographed under the same conditions with a Leica microscope (model Z 45 V) using a Kodak Ektachrome slide film of 64 ASA at an exposure time of 0.125 seconds. Lighting was provided by 2 optic fibers connected to a light source of 220 V, Hz 50/60, 1 A (Intralux 150 H, Volpi Ag, Switzerland). All photographs were taken at the same overall final magnification (×2). The slides were projected with a slide projector (Leitz Pradovit S AF, Biel-Bienne, Switzerland) and 2 dentists examined them separately by visual inspection. The slides were viewed by the examiners at a final magnification of ×100. In only 9 out of 109 cases, where the agreement was not reached, a third operator similarly trained and experienced gave the definitive answer to achieve a consensus.

Prior to this evaluation, all participating dentists had a training session to reach an agreement in the criteria used to evaluate discoloration. The dentists had to categorize the test site according to the following criteria: (1) no discoloration; (2) opaque spot; (3) yellow to light brown discoloration; or (4) dark brown to black discoloration. Additional demineralization (opacities), rough surfaces, or dark shadows under the enamel related to the selected site were also recorded as additional discoloration.

Diagnodent measurement

The Diagnodent (KaVo, Biberach, Germany) consists of a battery-operated laser device capable of detecting fluorescence emitted by carious lesions and differentiating healthy from diseased tissues. Its properties, principles, and operation have been already described in detail in previous works.[2,32] The values obtainable with Diagnodent range between 0 and 99. A value of 0 represents no fluorescence signal (no caries). The higher the value, the deeper or more infected the caries. To distinguish dentinal caries in vivo, Diagnodent values up to 20 were recommended in a previous study.[17] However, in vivo and in vitro situations cannot be compared. Storage solutions change the caries' fluorescence response, and, therefore, the in vivo scales must not be transferred to the in vitro situation.[17,33]

One operator measured the sites with the laser fluorescence device. Prior to every measurement, the device was calibrated using the ceramic standard provided by the manufacturer. After drying the sample with air for 2 seconds, the fluorescence of a sound smooth surface of the teeth was assessed. This baseline value was afterwards subtracted from the value found at the selected site. There, the tip was rotated around it, and to identify the area where the caries was most advanced, the highest value was recorded. To evaluate intrarater reproducibility, a total of 3 measurements were performed at each site following the aforementioned procedure. At least 1 week elapsed between each measurement. The average value of the 3 different measurements obtained was used for further analysis.

Histological evaluation

Once all measurements were completed, the teeth were histologically prepared. They were serially sectioned perpendicularly to their occlusal surfaces, and the extent of caries was assessed under a microscope. The teeth were ground longitudinally from buccal to lingual to the periphery of the

Table 1. Correlation Between Discoloration and Histological Assessment

Histology	No discoloration		Opaque		Yellow to light brown		Dark brown to black	
	Primary	Permanent	Primary	Permanent	Primary	Permanent	Primary	Permanent
D0, D1	14 (93%)	14 (93%)	5 (42%)	6 (40%)	16 (33%)	18 (43%)	3 (16%)	13 (57%)
D2	1 (7%)	1 (7%)	7 (58%)	4 (27%)	24 (49%)	17 (40%)	8 (42%)	7 (30%)
D3, D4	0	0	0	5 (33%)	9 (18%)	7 (17%)	8 (42%)	3 (13%)
Total	15	15	12	15	49	42	19	23
	NS		NS		NS		$P<.05$	

NS=no statistical significance (Pearson chi-square test).
D0=no caries.
D1=initial enamel caries.
D2=deep enamel caries (up to the dentino enamel junction).
D3=superficial dentinal caries (extending to the outer half of dentine).
D4=deep dentinal caries.

test site and periodically colored with rhodamine for 5 seconds. The excess color was removed with ethanol 70%. For further evaluation, the teeth were dehydrated in a solution of increasing alcohol concentrations with added basic fuchsin (0.5%) to achieve block staining.

Afterwards, the alcohol was removed with acetone and the samples were embedded in methylmethacrylate and polymerized. Then, the specimens were sectioned in the center of the spot to 40 μm (section thickness) and counterstained with light green (0.25%) in acetic acid (0.2%) for 2 minutes.[20]

Once the slices were prepared, they were inspected and photographed with a microscope (Leica ZOOM 2000 model No. Z45V) at different final magnifications: ×3, ×2, ×8, and ×12.8. The deepest caries detected at the 3 sections observed was assessed and recorded as follows:

1. D0=no caries;
2. D1=enamel caries confined to the outer half of enamel;
3. D2=enamel caries extending into the inner half of enamel up to the dentino enamel junction;
4. D3=dentinal caries extending into the outer half of dentin;
5. D4=deep dentinal caries.

Data analysis

The performance of Diagnodent was evaluated by means of sensitivity (proportion of diseased teeth recognized as diseased by the test or diagnostic method) and specificity (proportion of sound teeth recognized as sound by the diagnostic method). The best cutoff for Diagnodent (value at which the maximum sensitivities and specificities are achieved) at D2 and D3 levels were established for permanent and primary teeth separately, as described in previous works.[17,28]

Interrater reproducibility (agreement between both participant dentists) as well as intrarater reproducibility (agreement between the 3 individual measurements of Diagnodent) were analyzed by means of Cohen's kappa tests. A kappa value higher than 0.75 implies excellent agreement, a value between 0.4 and 0.75 means good agreement, and a kappa value smaller than 0.4 implies marginal agreement.[21] Relative variation between the 3 measurements was assessed for each sample. The influence of discoloration on Diagnodent results was plotted as box diagram graphs. The Kruskal-Wallis test was applied to assess statistical significance between these results. For multiple comparisons, the *P* values were corrected by the Bonferroni adjustment procedure (Systat 1995, Evanston, Ill). All categorical data were analyzed by the chi-square test. The ratio of the additional discoloration (opacities and/or shadows under the enamel associated to the observed site) as well as the ratio of the location of the evaluated site were calculated.

Results

The correlation between type of discoloration and histological result for primary and permanent teeth can be seen in Table 1. The results show that, among dark brown to black discolored fissures, 57% of the permanent teeth showed no caries or had an initial enamel lesion (D0, D1), 30% showed a deep enamel lesion (D2), and only 13% were dentine decayed (D3, D4). On the other hand, 16% of the primary teeth with dark brown to black discolored fissures showed initial enamel lesion (D1), 42% indicated a deep enamel lesion (D2), and another 42% showed a deep dentinal caries (D3, D4). None of the histologically sound primary teeth (D0) presented such discoloration. The difference between these results was statistically significant ($P<.05$; chi-square test). For Diagnodent, the best cutoffs were set at a value in which the maximal sensitivity and specificity were obtained. Table 2 shows the resulting sensitivity and specificity values found in each group of teeth.

The highest specificity and sensitivity for primary teeth were found at a cutoff of 5 for enamel caries detection (D2; specificity=68% and sensitivity=75%) and a cutoff of 13 for dentinal caries detection (D3; specificity=85% and sensitivity=82%). The highest specificity and sensitivity for permanent teeth were found at a cutoff value of 6 for enamel caries detection (D2; specificity=49% and sensitivity=77%)

Table 2. Sensitivity and Specificity for Primary and Permanent Teeth at D2 (Enamel Caries) and D3 (Dentin Caries) Levels

	D2	D3
	Cutoff≥5	**Cutoff≥13**
Primary teeth		
Sensitivity	0.75	0.82
Specificity	0.68	0.85
	Cutoff≥6	**Cutoff≥10**
Permanent teeth		
Sensitivity	0.77	0.73
Specificity	0.49	0.65

and a cutoff of 10 for dentinal caries detection (D3 level; specificity=65% and sensitivity=73%). The Cohen's kappa value for intrarater reproducibility for Diagnodent (agreement between first, second, and third Diagnodent measurements) at the D2 and D3 level ranged between 0.76 and 0.86 for primary teeth and between 0.81 and 0.98 for permanent teeth (excellent reproducibility). In 31% of primary teeth and 33% of permanent teeth with high Diagnodent values (>10), variations of 5 to 26 units between individual measurements were registered. Small Diagnodent values (<10) underwent variations of a maximum of 5 units between measurements. The Cohen's kappa value for interrater reproducibility (agreement between both operators) was 0.70 for primary teeth and 0.74 for permanent teeth (good reproducibility).

Box plots in Figures 1 and 2 represent the correlation between each type of discoloration (nondiscolored, opaque fissures, and discolored samples) and Diagnodent value. According to the Kruskal-Wallis test, Diagnodent values obtained at discolored fissures with histological levels of D0 and D1 were statistically significantly higher than those obtained at nondiscolored fissures at the same histological levels in both primary and permanent teeth (P<.01). The same results were obtained at D2 for primary teeth (P=.01) and at D3 and D4 for permanent teeth (P<.05). Additional staining was present in 65% of the dentine-decayed/stained fissures in primary teeth and in 40% of the same fissures in permanent teeth. Moreover, 10 samples of 33 total analyzed distal fossas of upper and lower second primary molars (30%) showed dentinal caries. All of them were associated with a yellow, light brown, or black staining, while central and mesial fossa as well as developmental grooves showed a dentinal caries prevalence of 9%, 8%, and 1%, respectively.

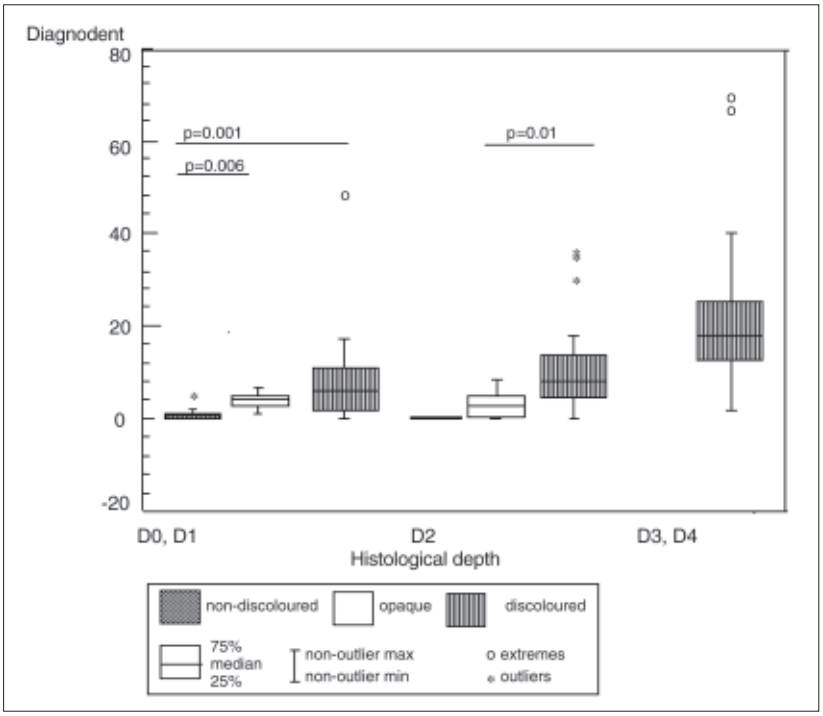

Figure 1. Correlation between Diagnodent measurements and type of discoloration at each histological level for primary teeth. Significantly different results are joined by a line (Kruskal-Wallis test).

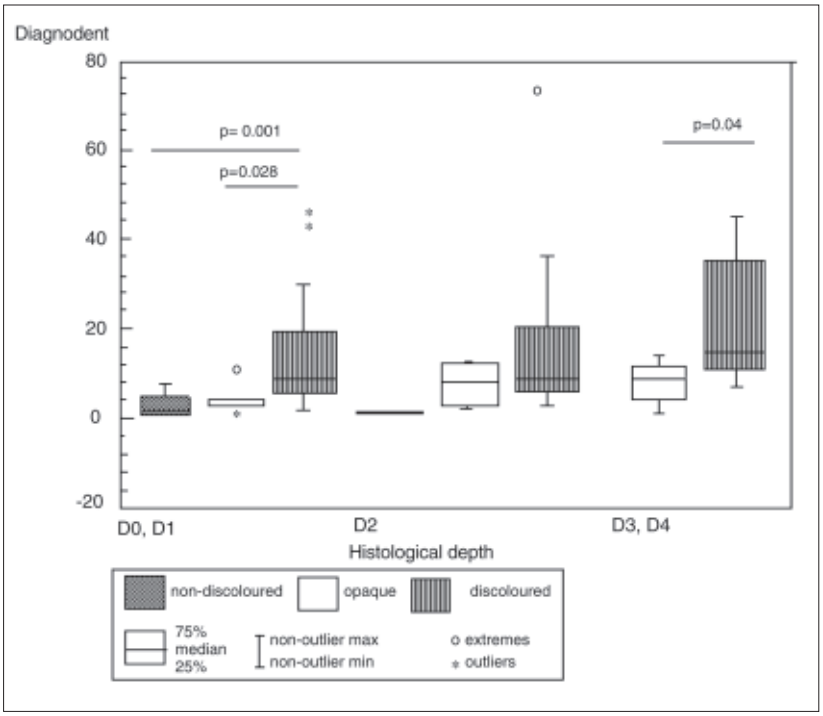

Figure 2. Correlation between Diagnodent measurements and type of discoloration at each histological level for permanent teeth. Significant differences are joined by a line (Kruskal-Wallis test).

Discussion

This study showed that most of the dark brown to black stained fissures on primary teeth showed deep caries in enamel (42%) or that it already spread into dentin (42%). On the other hand, only 3 permanent teeth (13%) showed dentin caries. The other dark brown and black discolored fissures showed mostly no caries or superficial enamel lesion or a deep enamel caries (57% and 30%, respectively). According to this distribution, dark discolored fissures could be suspected as dentin decay in primary teeth but not necessarily in permanent teeth. Due to certain dietary habits in adults (eg, certain foods, tea, coffee, or cigarettes), occlusal fissures of permanent teeth become pigmented. Hence, staining in the permanent dentition is not always related to pathological changes. The authors' results confirm the conclusions of previous studies. Staining on occlusal fissures on permanent teeth are of limited value in the diagnostic of dentinal caries.[8] This feature should be carefully considered to avoid overtreatment.[5,7,13-14]

On the other hand, these external factors are not to be expected in the primary dentition. Therefore, a higher probability for such discoloration to be a pathological sign on primary teeth could be suspected. Moreover, a considerable proportion of additional discoloration (roughness, opacities, and shadow under the enamel) has been found in association with dentin-decay/stained fissures in both types of teeth (40% and 65% in permanent and temporary teeth, respectively).

Photographs were preferred to direct visual examination to assess discoloration since they provide a constant magnification and assure a higher reproducibility.

The presence of microcavities on the occlusal surface also represents a valuable criterion for identification of dentinal lesions.[8,25-27] Although the samples used in the present work were all carefully selected to include only those with intact occlusal surfaces and an accurate inspection was carried out, no systematic assessment of the presence or absence of microcavities was performed. Neither was the location of the test site categorized in the authors' sample of permanent teeth since they were mostly third molars whose history (position in the arch, stage of eruption) was unknown. Thus, no conclusions can be drawn about fissure location and caries distribution on the authors' group of permanent teeth.

Regarding primary teeth, a higher proportion of dentinal caries was detected on the distal fossa of upper and lower second molars (30%). It can be speculated that the distal fossa of second molars is difficult to reach and keep clean. Therefore, a higher incidence of caries could be expected. More research is needed to confirm this hypothesis. The best sensitivity and specificity for primary and permanent teeth were found at a cutoff of 5 (for primary teeth) and 6 (for permanent teeth) for enamel caries detection and at 10 (for permanent teeth) and 13 (for primary teeth) for dentinal caries detection. Although the highest sensitivities and specificities were detected at these cutoffs, it is also convenient to express this in terms of a range of cutoff values rather than of only 1 cutoff value. These ranges varied from 5 to 8 for enamel caries detection and from 9 to 13 for dentinal caries detection in both dentitions. These cutoffs are similar to those found in previous in vitro investigations.[22] In this work, Diagnodent showed a tendency to overscore discolored sites (yellow, light brown, dark brown, and black fissures).

As can be seen in Figures 1 and 2, at similar caries depths discolored fissures resulted in Diagnodent median values of about 5 to 7 units higher compared to opaque or nondiscolored fissures. A total of 46% of stained fissures in primary teeth and 42% in permanent teeth showing enamel caries (D1, D2) were falsely detected as dentin decay. The tendency of Diagnodent to overscore discolored fissures was also reported in another in vivo study.[18] To minimize the false positive readings, it could be necessary to establish higher cutoffs for the detection of dentinal caries. This also leads to a reduction in the sensitivity, but it increases the specificity, which means also a reduction of false-positive results and a smaller risk of overtreatment. The chances of obtaining false-positive results and a smaller and the operative advice safer.[17]

Conclusions

1. Brown or dark spots on fissures are not useful as a predictor for dentinal caries on permanent teeth. In primary teeth, however, there is a higher correlation between these discolorations and dentinal lesions was found. A discolored fissure on primary teeth could hide dentinal caries underneath.
2. The presence of staining on the fissure is associated with higher Diagnodent readings and, therefore, with an increase in the risk of false-positive answers.

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