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ORIGINAL ARTICLE

Mutans streptococci colonization and longitudinal caries detection with laser fluorescence in fissures of newly erupted 1st permanent molars

PERNILLA LUNDBERG[1,2], MAJ-LIS MORHED-HULTVALL[2] & SVANTE TWETMAN[1,3]

[1]*Department of Odontology, Faculty of Medicine, Umeå University, Umeå, Sweden,* [2]*Teg Public Dental Clinic, Umeå, Sweden,* [3]*School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark*

Abstract

Objective. To longitudinally apply a laser fluorescence (LF) device (DIAGNOdent) in newly erupted 1st permanent molars over a 3-year period and to relate the findings to mutans streptococci (MS) colonization, fissure morphology, and caries development. ***Material and methods.*** The material consisted of 101 consecutive 5 to 6-year-old children attending a Public Dental Clinic and who volunteered after ethical approval and informed consent had been given. Only fully erupted molars with clinically sound fissures were included. At baseline, the fissures were subjectively categorized as "shallow" or "deep", and, prior to the LF readings, a plaque sample was collected and cultivated for MS using a chair-side kit. The registrations were repeated annually and the microbial samplings after 2 years. The total drop-out rate was 12%. ***Results.*** The mean LF values increased significantly ($p < 0.05$) with increasing age from 8.2 to 12.4 in the teeth that remained sound. Thirty-five teeth were decayed or filled during the follow-up and their mean LF values increased from 13.4 to 40.7. The LF readings were significantly higher in molars with "deep" fissures ($p < 0.05$) at all visits. MS colonization at baseline was associated with an increased risk for caries (OR = 11.6, $p < 0.05$) and significantly elevated LF readings. Baseline LF readings ≥ 12 were not diagnostic for dentin caries or fillings over the study period (sensitivity 0.57; specificity 0.86). ***Conclusion.*** LF readings could be used to some extent to monitor fissure morphology and caries development in fissures of newly permanent molars over time, but elevated initial values were not predictive for caries development.

Key Words: *Caries detection, 1st molars, fissure morphology*

Introduction

The early diagnosis of caries in 1st permanent molars is a key element in childhood caries prevention, but detection of non-cavitated lesions in the fissures is a complex issue. Traditionally, this is carried out through a visual-tactile examination, often combined with bitewing radiographs. During the past decade, a laser fluorescence (LF) method has emerged as an adjunct to the clinical examination [1]. A systematic review has concluded that LF is more sensitive than traditional diagnostic methods but at the expense of poorer specificity, which limits its clinical usefulness [2]. However, the review was mostly based on *in vitro* studies, with very little evidence available from *in vivo* reports, and it pointed out that longitudinal studies were lacking [3]. Anttonen and co-workers [4,5] examined a group of children at a 1-year interval and demonstrated that increased LF values correlated positively with increased caries lesions and that the tested device could be used to monitor caries progression in both permanent and primary molars. Other reports suggest that the LF technique can also reflect caries regression and may be used as an intermediate or surrogate endpoint in clinical intervention trials [6,7]. To expand our knowledge on the use of the LF method under field conditions, we investigated 1st permanent molars over an extended period of time in parallel with clinical and microbial findings. The aim of this study was to apply a LF device (DIAGNOdent) in the fissures of newly erupted 1st permanent molars and to follow-up longitudinally over a 3-year period and relate the findings to mutans streptococci (MS) colonization, fissure morphology, and caries development. The null hypothesis was that the LF readings would not change over time.

Correspondence: Svante Twetman, Department of Cariology and Endodontics, School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Nørre Allé 20, DK-2200 Copenhagen N, Denmark. Tel: +45 35326810. E-mail: stw@odont.ku.dk

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Material and methods

Subjects

The material comprised 101 consecutive and healthy 6-year-old children (54 boys and 47 girls) attending a Public Dental Clinic in the City of Umeå, Sweden. The children volunteered and their parents gave consent after receiving verbal and written information. All the children had been receiving free comprehensive preventive-oriented dental care at the clinic with annual recalls since the age of 3 years. The inclusion criteria were at least one newly erupted and clinically intact 1st permanent molar present. "Newly erupted" was defined as a new occlusal surface present since the last examination or a surface under eruption with the entire fissure system available for visual inspection. Teeth with obvious signs of enamel hypo-mineralization or hypoplasia in the fissure system were excluded. Daily toothbrushing with fluoride toothpaste was reported by all parents and the piped community water supply had a low fluoride content (<0.3 mg/l). The reasons for 12 children dropping out during the follow-up period were: relocation ($n = 8$), not showing-up at the clinic ($n = 2$), did not want to continue participation ($n = 2$).

Study design

The longitudinal study design was approved by the Regional Ethics Review Board in Umeå, Sweden. At the baseline inclusion, a microbial sample collected from the fissures with the aid of a micro-brush was transferred to a site-specific plastic strip as described below. Prior to the LF readings, the fissures were thoroughly cleaned with pumice paste and rubber cups and, after drying, subjectively characterized as "shallow" or "deep" based on visual and tactile examination with a blunt explorer. The LF readings were then carried out at three different sites on each tooth, as detailed below. The LF readings were repeated annually and the microbial sampling was renewed after 2 years. The patients' general dentist was informed about the objective of the study but maintained the full responsibility for diagnosis and treatment decisions during the study period. Data on caries development, fissure sealants, and conservative treatment were extracted from the digitized dental records.

Laser fluorescence readings

The diagnostic LF recordings were performed with DIAGNOdent, a chair-side laser device from KaVo (Biberach, Germany), in which the tooth was illuminated with a red laser light with a wavelength of 655 nm allowing reading values from 0 to 99 [1]. First, the device was calibrated using a ceramic standard provided by the manufacturer. The measurements were carried out after 5 s of drying with air spray and a reference value from sound facial enamel was obtained. The tip was then applied in the most mesial part of the fissures (M1) and the instrument was slightly tilted along its own axis. A second site in the central occlusal fissure (M2) was then recorded in the same way. All sites were measured twice and the single peak value was recorded. Two authors (PL and ML M-H) carried out all readings and, with few exceptions, the same clinician performed the measurements within the same patient over the years. The examiners were calibrated prior to the trial and were blinded at each follow-up to the outcome of the previous registrations.

Microbiological cultivation and enumeration

The bacterial samples of the central fissures (M2) were collected with a micro-brush and transferred directly to a specially prepared plastic strip (Dentocult SM – Strip Mutans; Orion diagnostica, Helsinki, Finland) in accordance with the previously described site-specific method [8,9]. After cultivation in a selective agar broth at 37°C for 48 h, the number of colony forming units (CFU) of MS, identified by morphology characteristics, was counted in a stereo-microscope with 10–25 times magnification. The following scores were used: **0** = no detectable growth; **1** = 1–10 CFU: **2** = 11–100 CFU; **3** = >100 CFU.

Statistical methods

All data were processed using the SPSS software, version 14.0 (SPSS Inc., Chicago, Ill., USA). The relationship between variables was calculated with the Pearson correlation coefficient. The follow-up LF readings were compared with baseline values with the aid of Student's paired two-tailed *t*-test. The categorized data were compared by odds ratio calculation and chi-square tests. The level of significance was set to 5% ($p < 0.05$).

Results

A total of 326 occlusal surfaces in 89 children registered at baseline could be followed during the 3-year study period. Additionally, 30 first molars erupted and were included during the first year of the study. The distribution and drop-outs are given in Table I. Sixteen teeth fissure-sealed during follow-up were excluded. The mean LF values of the teeth that remained sound and in those that were decayed/filled are presented in Table II. In the sound teeth, there was a non-significant tendency to increased reference values over the study period, but this seemed not be to the case for the mesial part (M1) of the fissure system. In the central fissure (M2),

Table I. Number of occlusal surfaces assessed with laser fluorescence in 89 patients and distribution of drop-outs

		Missing		
Time	Total number assessed	Non-erupted	Sealed	Filled, decayed
Baseline	326	30	–	–
1st year	340	1	–	15
2nd year	319	–	12	25
3rd year	305	–	16	35

however, the mean LF values increased significantly ($p < 0.05$) with increasing age from 8.2 at 6 years to 12.6 at 9 years. The mean LF readings were significantly higher in sound molars with deep fissures ($p < 0.05$) at all visits (Figure 1) compared to sound molars with shallow fissures. In total, 35 teeth were decayed or filled during the follow-up, and their mean M2 LF values increased from 13.4 at baseline to 40.7 ($p < 0.05$) at the last measurement before restoration. Table III presents a statistically significant positive correlation ($p < 0.05$) between the LF readings and bacterial scores registered at baseline and after 2 years, respectively. High MS counts (score 3) were more prevalent in deep fissures (29%) than in the shallow fissures (15%). The baseline MS score in relation to the fate of the fissure and LF readings are given in Table IV. The presence of MS (score 1–3) in the fissures at baseline was associated with an increased risk for caries (odds ratio 11.6; 95% CI: 4.4–31.4; $p < 0.05$) and significantly elevated LF readings ($p < 0.05$). With a baseline cut-off point set at MS score 1–3 for a positive test, the sensitivity for "disease" (dentin caries or filling) was 0.86 with a specificity of 0.66. High LF readings (≥ 12) in the central fissure (M2) at baseline, however, were not predictive for dentin caries or fillings over the study period, as shown in the two-by-two Table V. With that scenario, the sensitivity was 0.57 with a specificity of 0.86. The likelihood ratio for a positive test (LR+) was 4.1.

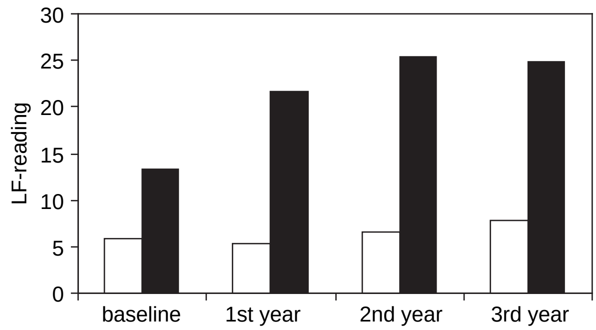

Figure 1. Mean laser fluorescence readings (M2) at baseline and during 3 years in 1st permanent molars with fissure systems characterized as "deep" (filled bars, $n = 55$; 15.3%) or "shallow" (unfilled bars, $n = 301$; 84.7%).

Discussion

The present study was undertaken as a field trial with the overall aim of evaluating use of the DIAGNOdent device in longitudinal occlusal caries detection and to see whether or not it could be of predictive value. The unique features were the large size of the material timed to the molar eruption, the long follow-up period, and the fact that the LF readings could be related to the MS colonization.

Table II. Longitudinal laser-fluorescence readings (mean (SD)) of a labial reference site (ref) and in the mesial (M1) and central fissures (M2) of newly erupted 1st permanent molars that remained sound during the 3-year observation period ($n = 305$) and in those that were decayed or filled ($n = 35$)

Event	Site	Baseline (mean (SD))	1st year (mean (SD))	2nd year (mean (SD))	3rd year (mean (SD))
Sound	ref	2.9 (1.6)	2.4 (2.3)	2.4 (1.9)	4.1 (2.6)
	M1	4.6 (3.2)	3.9 (2.6)	4.2 (3.6)	4.6 (3.2)
	M2	8.2 (5.9)	9.9 (8.1)	11.0* (11.7)	12.6* (9.0)
Diseased	ref	3.2 (1.3)	3.2 (1.9)	2.7 (1.1)	5.4 (1.9)
	M1	6.3 (5.2)	5.8 (3.6)	6.6 (5.9)	10.2* (8.1)
	M2	13.4 (10.6)	30.8* (23.4)	31.5* (27.6)	40.7* (22.5)

*Significantly different from baseline ($p < 0.05$).

Table III. Mean laser fluorescence readings (M2) in relation to mutans streptococci counts at baseline and after 2 years in 89 children

	Baseline		2nd year	
MS score	n (%)	LF, mean (SD)	n (%)	LF, mean (SD)
0 (no growth)	198 (61.3)	7.0 (4.0)	120 (37.0)	6.8 (4.1)
1 (1–10 CFU)	53 (15.9)	10.8 (7.8)	100 (30.6)	9.5 (6.4)
2 (11–100 CFU)	35 (10.6)	11.6 (8.7)	51 (15.6)	13.4 (9.5)
3 (>100 CFU)	40 (12.2)	11.1 (10.6)	55 (16.8)	24.0 (19.5)

Statistically significant positive correlations ($p < 0.05$): baseline $r = 0.49$; second year $r = 0.56$.

Table IV. The 3-year fate of the occlusal surfaces and mean LF readings in the central fissure (M2) in relation to the baseline mutans streptococci (MS) counts. *n* denotes the number of teeth

		Sound after 3 years		Diseased after 3 years	
Baseline MS score	Total (*n*)	*n* (%)	LF, mean (SD)	*n* (%)	LF, mean (SD)
0	198	193 (97.5)	10.1 (6.4)	5 (2.5)	24.1 (4.4)
1	53	46 (86.8)	13.3 (8.1)	7 (13.2)	26.7 (10.7)
2	35	26 (74.3)	16.3 (11.7)	9 (25.7)	43.0 (20.7)
3	40	26 (64.9)	20.5 (14.4)	14 (35.1)	43.0 (20.7)

The two examiners were experienced dentists, trained and calibrated on a number of patients prior to the start of the study in order to perform the clinical procedures in a standardized way. Caries diagnosis on dentin level and treatment decisions were made by the patients' regular team, which was instructed to act in accordance with the guidelines of the Public Dental Service in the region. It is important to stress that the diagnostics efforts were not validated at the time of filling and that the fate of the occlusal surfaces presented here was based entirely on the dental records. It should be underlined, however, that no proximal fillings on 1st molars were present in the material. Approximately 5% of the teeth were sealed during the course of the study and, interestingly, no relationship to the baseline morphology scores was disclosed (data not shown). Since it was not clear from the records whether the fissure sealant therapy had been performed as a true preventive measure or was due to initial caries, these surfaces were excluded from the final calculations. One single DIAGNOdent device was used throughout the trial, but, despite this, the LF readings exhibited considerable intra- and inter-individual variations, as indicated by the wide standard deviations. A notable observation was that the intra-individual LF readings in the "deep" central fissures (M2) exhibited greater variation compared with the less deep mesial fissures (M1), not to mention the labial reference sites. In further interpreting the results, it is important to bear in mind that the calculations were made with the single tooth as unit in spite of the fact that they could not strictly be considered as independent.

The clinical findings of the present study concurred with those of a similar study from Finland [5], and significantly increased LF values were disclosed in the 1st molars that were diagnosed as decayed on a dentin level or required restorative treatment. For these surfaces, the null hypothesis could be rejected. Although the mean LF reading at baseline was significantly higher in the diseased teeth, an elevated value was not predictive for dentinal caries or fillings with a calculated sensitivity of 0.57. This was lower than values reported from previous cross-sectional *in vivo* studies [4,10–12], but it should be noted that the present threshold level for a "positive test" was set lower here than in previous trials (>20–30) because very few measurements reached such a high baseline level in the present material. The cut-off point at LF $\geq$12 was chosen in order to have a reasonable and realistic number of positive tests (19%). The general caries incidence was relatively low and it should be stressed that the study design did not allow any conclusions on the predictive ability of early caries lesions. Finally, the calculation of the specificity values reported here must be regarded with caution, since it was assumed that the sound fissures did not have dentin caries.

A novel and interesting finding was the close interrelationship between bacterial counts, caries development, and LF recordings. The clear positive correlation between occlusal MS colonization and LF readings has not been described previously. This finding was not unexpected in light of the assumption that a more intense fluorescence is emitted from demineralized enamel than from a sound hard tissue due to its organic content of bacteria and their metabolites [1,13, pers. comm.]. The greater likelihood of trapped organic material was probably also one reason behind the association between deep fissures and LF values. The higher the baseline MS score, the higher the LF readings were found after 3 years, which, in turn, was associated with a higher proportion of diseased teeth. The present data clearly suggest that an early MS colonization of the fissures has a better sensitivity for caries and restorations than the LF method, but neither of them exceeded the sum of sensitivity and specificity (160%), which indicates that such a test would be meaningful. The microbial sampling procedure and cultivation is more time-consuming than LF readings, however, and requires basic laboratory

Table V. Two-by-two table showing the caries predictive ability of elevated baseline laser fluorescence readings in young 1st permanent molars. A baseline laser fluorescence value $\geq$12 was considered as a "positive test". Values denote the number of teeth

	Outcome after 3 years		
Baseline LF reading (M2)	Diseased	Sound	Total
≥12	20	41	61
<12	15	250	265
Sum	35	291	326

facilities, which may imply a higher cost for each true positive diagnosis.

In conclusion, the novel finding was a clear positive correlation between LF readings and MS colonization in young 1st permanent molars. Furthermore, the present findings reinforce previous studies that a LF caries detection device can be helpful in longitudinal monitoring of caries development in young 1st permanent molars, but there are no clear cut-off levels predictive for dentinal caries development.

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