Assessment of dental caries activity using quantitative light-induced fluorescence and microbial distribution in primary molars

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Assessment of dental caries activity using quantitative light-induced fluorescence and microbial distribution in primary molars

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감사의 글

박사 학위 과정을 마치고 학위논문을 완성하기까지 지도해주시고 도움을 주신 많은 분들께 감사드립니다.

우선, 바쁘신 와중에도 연구의 방향을 제시해주시고, 연구가 올바르게 진행될 수 있도록 지도해 주신 김성오 교수님께 감사드립니다. 또한 소아치과 의사로서 귀감을 보여주신 최병재 교수님, 열정적인 모습으로 소아치과 의사가 나아가야 할 방향을 알려주신 이제호 교수님, 임상과 연구에 있어 보다 깊고 세심하게 생각할 수 있게끔 조언해주신 최형준 교수님, 다양한 주제에 대하여 관심을 넓히고, 접근해 볼 수 있는 기회를 갖게끔 도움을 주신 송제선 교수님, 연구를 진행하고 논문을 완성하는데 있어 격려해주시고 많은 도움을 주신 강정민 교수님께도 감사드립니다.

지금의 제가 있기까지 멀리서 응원해주시고 고생하신 부모님께 감사드리며, 격려를 보내준 동생에게 감사하다고 전하고 싶습니다. 그리고 어렵고 힘든 상황속에서 묵묵히 곁에서 힘이 되어준 아내 윤지에게 감사하며, 제가 나아가는 길에 대해 관심 갖고 응원해주신 장인어른과 장모님께도 감사드립니다.

여러가지 부족한 가운데 많은 분들의 도움과 격려로 학위를 마무리 지을 수 있었습니다. 도움을 주신 많은 분들께 진심으로 감사드립니다. 많은 분들의

도움과 가르침을 잊지 않고 늘 노력하며, 헌신하는 삶을 살아갈 수 있게끔 노력하겠습니다. 감사합니다.

2022년 12월

김치훈 드림

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Assessment of dental caries activity using quantitative light-induced fluorescence and microbial distribution in primary molars

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Evaluation of the activity status of dental carious lesions, a fundamental factor in deciding the course of treatment, is a subjective and challenging clinical task. If the activity status of a lesion can be distinguished through quantitative measurements, and the difference in the microbiological distribution of the lesion or the surrounding environment can be identified, the activity status can be determined more objectively and easily. Therefore, this study aimed to assess differences in quantitative measures obtained from the quantitative light-induced fluorescence method and microbial composition of carious

dentin and saliva according to the activity status of caries lesions in primary molars.

A total of 34 teeth from 34 children were evaluated in this study. The activity status of carious lesions was classified using the International Caries Classification and Management System criteria (active or inactive). Images of the carious lesions were captured using a quantitative light-induced fluorescence device for quantitative analyses. Carious dentin and saliva were collected to detect and quantify selected bacterial species (S. mutans, S. sobrinus, Lactobacillus species, F. nucleatum, P. nigrescence, P. intermedia) and C. albicans by quantitative polymerase chain reaction. Mann–Whitney U tests were performed to evaluate differences in quantitative measures from quantitative light-induced fluorescence, the microbial composition of carious dentin, and saliva according to the activity status of carious lesions.

Red fluorescence values (ΔR, ΔRmax) from the quantitative light-induced fluorescence method were significantly higher in active lesions (ΔR, $p = 0.009$; ΔRmax, $p = 0.014$). The quantitative mean levels of Lactobacillus species ($p = 0.010$) in carious dentin and S. sobrinus ($p = 0.017$) in saliva were significantly higher in the active-lesion group.

Quantitative measures related to red fluorescence from the quantitative light-induced fluorescence method, levels of Lactobacillus species from carious dentin, and levels of S. sobrinus from saliva were associated with caries lesion activity.

Keywords: dental caries, caries activity, quantitative light-induced fluorescence, microbial distribution, primary molars

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I. Introduction

Dental caries is one of the most prevalent chronic infectious diseases affecting 2.3 billion people globally and is highly prevalent among underprivileged children (Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016, 2017; Peres et al., 2019). It is now commonly accepted that dental caries is a dynamic process covering the continuum from the atomic level of demineralization to dentinal involvement and eventual cavitation. The dynamic balance

between demineralization and remineralization determines the result (Featherstone, 2004). Demineralization can be arrested by adequate oral biofilm control, limited sugar consumption, and the use of fluoridated agents. Thus, a carious lesion can be active (demineralization process in progress) or inactive (interrupted demineralization process) (Ekstrand et al., 2009; Nyvad and Fejerskov, 1997). In diagnosing a carious lesion and determining a treatment plan, it is vital to determine its activity and evaluate the detection and severity assessment of carious lesions (Braga et al., 2010b).

Currently, visual inspection is the only validated method for evaluating caries activity status (Ekstrand et al., 2007; Guedes et al., 2014; Nyvad et al., 2003). Longitudinal monitoring of the lesions and evaluation of etiological factors of the disease, such as biofilm formation and other clinical parameters of the lesion, should also be considered (Braga et al., 2010a). Although visual inspection is a comfortable and easy method, it depends on a subjective evaluation and has a few shortcomings, such as low sensitivity and reliability (Gimenez et al., 2015). Thus, its association with quantitative diagnostic methods is of interest to lend objectiveness to a diagnosis strategy for caries, aid in treatment-related decision-making, and in monitoring the evolution or inactivation of the lesions.

A few fluorescence-based methods have been developed for the quantitative diagnosis of carious lesions. A fluorescence tool named quantitative light-induced fluorescence (QLF, Inspektor Research Systems, Amsterdam, the Netherlands), which emits blue light of a wavelength around 405 nm, uses a camera to capture fluorescence, both in the red region of the visible spectrum (exogenous to the tooth and emitted by bacterial products) and in

the green region of the spectrum (intrinsic to the tooth and related to the de- and remineralization processes) (de Josselin de Jong et al., 2009; Heinrich-Weltzien et al., 2003). Caries lesion activity results in changes in the microstructure of the teeth, and bacterial metabolites appear (Ando et al., 2006; de Josselin de Jong et al., 2009; Heinrich-Weltzien et al., 2003), which results in differences in fluorescence. In our previous study, the results of QLF analysis for dental caries of primary teeth were reliable in detecting the progression of dental caries (Cho et al., 2021). Several clinical studies have differentiated active and inactive carious lesions using QLF (Felix Gomez et al., 2016; Meller et al., 2012; Novaes et al., 2017), but different correlations have been reported between QLF parameters and lesion activity depending on the study.

Dental caries is a cariogenic bacteria-mediated process that results from complex interactions and dysbiosis (Marsh, 2018; Tanner et al., 2018). To understand the lesion activity of dental caries, it is vital to understand the microbial perspective of carious lesions. Decades of caries-related microbiological research have shown that acidogenic and aciduric bacteria, such as *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus* species, are significant pathogens involved in disease development (Loesche, 1986). More recent discoveries on caries-associated microbiota using advanced molecular techniques have suggested that there exists a wide variety of acid-tolerant bacteria, including *Actinomyces, Fusobacterium, Scardovia, Bifidobacterium, Atopobium, Prevotella, Veillonella*, and fungi like *Candida* (Beighton et al., 2010; Dige et al., 2014; Falsetta et al., 2014; Gross et al., 2012; Obata et al., 2014; Takahashi and Nyvad, 2008; Tanner et al.,

2016). Regarding active and inactive caries lesions, it was understood that the more active the caries lesion, the more acid-producing the environment, and accordingly, acid-resistant bacteria selectively become the dominant species (Takahashi and Nyvad, 2008). However, few studies have directly compared the differences in microbial composition between clinically evaluated active and inactive lesions.

Thus, this study aimed to determine whether there is an association between the quantitative measurements obtained from QLF parameters, the microbial composition of carious dentin and saliva, and the activity status of carious lesions in primary molars.

II. Materials and Methods

1. Study Population

The study protocol was approved by the institutional review board (approval no. IRB 2-2019-0056) of the Yonsei University Dental Hospital and was carried out in accordance with institutional guidelines. A total of 44 teeth from 44 children with no systemic diseases and diagnosed with dentin caries in the primary molars were eligible for inclusion in this study. However, six children dropped out because of difficulties in collecting sufficient saliva for microbiological analyses, and four were excluded because of insufficient carious dentin tissue, leaving a population of 34 participants (34 teeth). The exclusion criteria were as follows: 1) individuals who had experienced fevers or other infectious diseases and taken antibiotics during the past 3 months; 2) individuals who had a serious systemic disease, mental disorder, or failure to cooperate with treatment; 3) teeth with any detectable enamel or dentin hypoplasia, or restorative treatments; 4) teeth with radiographic signs of infection or pathologic resorption; and 5) teeth with caries that could not be isolated with a rubber dam. Informed consent was obtained from all the participants and their parents.

2. Clinical examination

Two calibrated examiners performed all the clinical examinations before treatment. The following basic sociodemographic and clinical variables were recorded: age, sex (boys or girls), arch type (upper or lower), type of tooth (first primary molar or second primary molar), and position of the caries lesion (occlusal or proximal). Caries experience of subjects was measured using the decayed, missing, and filled teeth (dmft) index. Caries lesions were categorized using the International Caries Detection and Assessment System (ICDAS) criteria (ICDAS codes 0–6) (Ismail et al., 2007), and the activity status of carious lesions was evaluated using the International Caries Classification and Management System criteria (active or inactive, Table 1) (Pitts et al., 2014). Caries lesion activity status of the teeth adjacent to the tooth selected for analysis was also evaluated. If the caries lesion activity between the selected tooth and adjacent teeth was different, the subject was excluded from this study.

Table 1. International Caries Classification and Management System criteria for caries lesion activity

	Active caries lesion	Inactive caries lesion
Initial and Moderate Caries stage	Surface of enamel is whitish/yellowish; opaque with loss of luster, feels rough when the tip of the ball-ended probe is moved gently across the surface. The lesion may be covered by thick plaque prior to cleaning	Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the ball-ended probe is moved gently across the surface. The lesion may not be covered by thick plaque prior to cleaning
Extensive caries stage	Dentin feels soft or leathery on gentle probing	Dentin is shiny and hard on gentle probing

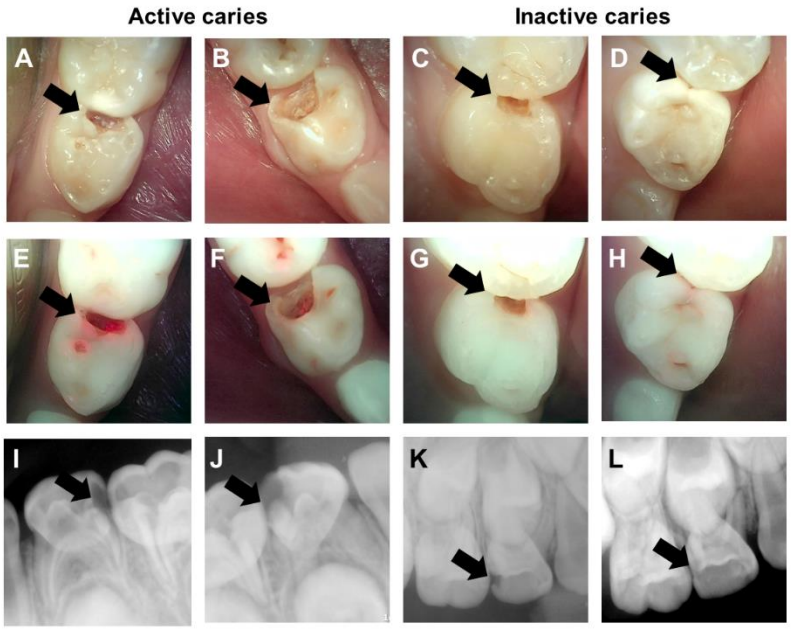
3. QLF image acquisition and assessment

Two trained examiners captured occlusal fluorescence images using a Qraypen (AIOBIO, Seoul, Korea). The lips and soft tissues were retracted, and images were captured in a darkened room maintained under the same lighting conditions to maximize the quality of the QLF images.

The obtained fluorescence images were analyzed using QA2 software (v. 1.25, Inspektor Research Systems BV, Amsterdam, The Netherlands), and QLF parameters (ΔF, ΔFmax, A, ΔQ, ΔR, and ΔRmax) were calculated (Table 2). Examples of white light, fluorescence, and radiographic images and measurement values obtained through the QLF are shown in Figure 1.

Table 2. Description of QLF parameter terminologies

Terminology	Definition
ΔF (%)	The average loss of fluorescence in the carious surface (the depth of the lesion) compared to the fluorescence in the sound tooth area
ΔFmax (%)	The maximum loss of fluorescence in the carious surface
A (px^2)	The area of the carious lesion surface
ΔQ (%·px^2)	The percentage of green fluorescence loss (ΔF) times the area of the lesion (A)
ΔR (%)	The change in the ratio of red and green pixels in the lesion area or area of interest compared to the sound area of the tooth
ΔRmax (%)	The maximum change in the ratio of red and green fluorescence in the lesion area



Tooth	#74	#84	#54	#54
ΔF (%)	-38.6	-29.6	-29.7	-12.7
ΔF max (%)	-97.1	-72.0	-56.8	-37.6
ΔQ (%·px^2 /10^4)	-41.7	-25.9	-12.3	-12.68
ΔR (%)	122	54	37	25
ΔR max (%)	2962	238	57	39

Figure 1. Examples of white light images (A-D), fluorescence images (E-H), radiographic images (I-L), and QLF images scores according to caries activity status. Active caries (A and B) and inactive caries (C and D) were classified by the International Caries Classification and Management System criteria. The size of the dental cavity was larger in (A) than (B), but the mean and maximum values of ΔF and ΔR were higher in (A). (A) had higher caries activity than (B), and it could be seen that the fluorescence intensity of (E) was stronger than that of (F). The cavitation size in (A) and (C) were similar, but (A) was in an active state and showed high ΔR and ΔRmax values.

4. Microbial analysis

1) Saliva Collection

Unstimulated whole saliva was collected from each subject by direct expectoration into 50 mL sterile falcon conical tubes for 5–10 min. Collections were performed at least 1 h after food intake to avoid contamination with non salivary components. The tubes were then transported on ice to the laboratory and processed within 1 h. The saliva was clarified by centrifugation at 10,000 rpm at 4°C for 10 min. The supernatant was stored at −70°C for future analysis, and the resulting pellet was used for DNA extraction and quantitative polymerase chain reaction microbial analysis.

2) Carious dentin sampling

One clinician examined the cases and collected samples under aseptic conditions. The selective removal of carious tissue (Schwendicke et al., 2016) was performed to collect the samples. The mouth was rinsed with hydrogen peroxide before caries collection. Following local anesthesia, the carious teeth were isolated using a rubber dam. Undermined enamel, debris, and superficial carious tissue were removed using sterile high-speed burs (H7 314 008; Brasseler, Lemgo, Germany) under water cooling. The cavity was irrigated with 5 mL of 0.9% sterile saline. The lift-draw method was used to remove the superficial layer of caries using a low-speed carbide bur (No. 4, Prima Classic RA4, Prima Dental Group, Gloucester, UK). The samples of deepest layer of caries were excavated either using a sterile spoon excavator or new sterile low-speed round bur (in the cases of inactive caries hard to remove spoon excavator) and immediately placed in a 1.5-mL microcentrifuge tube containing TE buffer (10 mM Tris-HCl, 1 mM EDTA, molecular grade, pH 8.0), transported to the laboratory, and stored at −70 °C until further analysis. (Becker et al., 2002; Liu et al., 2020; Zheng et al., 2019)

3) DNA extraction and quantitative polymerase chain reaction analysis

DNA extraction was performed using an ExgeneTM Cell SV kit (GeneAll Biotechnologies, Seoul, South Korea), according to the manufacturer's instructions. The samples were treated with 180 μL of lysozyme at 30 mg/mL and incubated at 37°C for 30 min. Proteinase K solution (20 μL at 20 mg/mL) and 200 μL of buffer BL were added to each sample, followed by incubation at 56°C for 30 min and 95°C for 15 min. Subsequently, 200 μL of absolute ethanol was added, and the mixture was transferred to a column and centrifuged at 14,000 rpm for 1 min. After washing the column with 600 μL of buffer BW, 700 μL TW buffer was added. Next, 100 μL of AE buffer was used to elute the DNA. DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). All DNA samples were stored at − 20 °C before use.

Targeted oral microorganisms and primer/probe set or a primer set sequence used for quantitative polymerase chain reaction in this study are listed in Table 3 (*S. mutans, S. sobrinus, Lactobacillus* species*, F. nucleatum, P. nigrescence, P. intermedia, C. albicans*). Polymerase chain reaction amplification was performed in a reaction volume of 20 μL (Bioneer Inc., Daejeon, South Korea). Polymerase chain reaction cycling was performed using the CFX96 Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The cycling consisted of an initial denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, primer annealing at 60°C for 40 s, and primer extension

at 72°C for 30 s. After completing the cycling steps, a final extension step was performed at 72°C for 5 min.

Table 3. Primers used for bacterial quantification in samples from caries lesion in teeth by using quantitative polymerase chain reaction

Species	Primer Sequence (5' to 3')	Fragment length (base pairs)
Streptococcus mutans	F: GCCTACAGCTCAGAGATGCTATTCT R: GCCATACACCACTCATGAATTGA P: TGGAAATGACGGTCGCCGTTATGAA	114
Streptococcus sobrinus	F: GTGTCTCAGTCCCAGTGTGG R: GATAGCTAATACCGCATAAGAGGAGT P: TCAGATGGACCTGCGTTGTATTA	174
Lactobacillus species	F: TGGAAACAGRTGCTAATACCG R: GTCCATTGTGGAAGATTCCC	232
Fusobacterium nucleatum	F: GACATCTTAGGAATGAGACAGAGATG R: CAGCCATGCACCACCTGTCT P: TEXAS RED-CAGTGTCCCTTCGGGGAAACCT-BHQ-2	73
Prevotella nigrescence	F: GCAAGAACGTGATGACGGGA R: ATTTCGCAGTCTTTGGGATCTTT P: Cy5-TTGCCAGGAAAACTTGCCGA-BHQ-2	79
Prevotella Intermedia	F: CCACCAACGACAACCTTCCA R: TCTACTGCTTCGAGCGCAC P: HEX-CAAGACAATCTCCGACGGAACGTT-BHQ-1	103
Candida albicans	F: GGTCAAGGTCATACTTTCTATGTTAC R: TTGACCGTTAGCGTAGCTC P: CAACGCCAAGATTTCTGTTCCA	132
Total species	F: TGGAGCATGTGGTTTAATTCGA R: TGCGGGACTTAACCCAACA P: CACGAGCTGACGACARCCATGCA	161

F: Forward primer, R: Reverse primer, P: Probe.

5. Data management and statistical analysis

Data management and statistical analyses were performed using R software (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria), considering the two groups of children according to the activity status of caries lesions (active and inactive) as the dependent variables. The abundance of each species was calculated as the percentage of total bacteria in each sample. The Kolmogorov–Smirnov test was used to verify the data distribution. Mann–Whitney U tests were applied to compare data from the fluorescence-based method to detect and quantify bacterial differences between active and inactive groups. A significance level of 5% was set for all analyses.

III. Results

1. Description of study populations

A total of 34 teeth from 34 children were evaluated in this study. The study population's characteristics are shown in Table 4. The mean (standard deviation) age of the children was 5.5 (1.2) and 5.5 (1.7) years in the inactive and active groups. The mean (standard deviation) dmft score of children was 7.8 (1.8) and 8.0 (3.3) in the inactive and active groups respectively, with no significant between-group difference. The active group had more girls and included more samples from the occlusal surfaces of primary lower second molars than the inactive group, but there was no significant between-group difference.

Table 4. Clinical characteristics of study samples according to the caries lesion activity status

Variables	Caries lesion activity status	
	Inactive (n = 17)	Active (n = 17)
	n	n
Age	5.5 ± 1.2 [a]	5.5 ± 1.7 [a]
Gender		
Boys	7	8
Girls	10	9
Arch type		
Upper	9	6
Lower	8	11
Type of tooth		
1st primary molar	13	7
2nd primary molar	4	10
dmft score	7.8 ± 1.8 [a]	8.0 ± 3.3 [a]
ICDAS code		
4	3	5
5	12	10
6	2	2
Position of caries lesion		
Occlusal	4	5
Proximal	13	12

[a] Values are presented as mean ± standard deviation

2. QLF measurements

The median (interquartile range) values from the QLF measurements according to caries lesion activity are shown in Table 5. The red fluorescence values (ΔR and ΔRmax) were significantly higher in active lesions than in inactive lesions; however, there were no statistical differences in the green fluorescence values (ΔF, ΔFmax, and ΔQ).

Table 5. Relationship between the measures from the QLF methods and the detection of the caries lesion activity status

Fluorescence measures	Inactive lesion (n = 17) Median ± IQR	Active lesion (n = 17) Median ± IQR	*p* value
ΔF (%)	-21.6 ± 6.8	-25.5 ± 16.2	0.482
ΔFmax (%)	-49.3 ± 14.7	-64.8 ± 35.7	0.474
ΔQ (%·$px^2/10^4$)	-15.3 ± 26.7	-37.0 ± 46.1	0.107
ΔR (%)	30.0 ± 15.0	45.0 ± 35.0	0.009
ΔRmax (%)	67.0 ± 45.0	91.0 ± 134.0	0.014

p values from Mann-Whitney U test

IQR: Interquartile range

3. Quantification of caries dentin bacteria

The quantitative PCR results for each carious dentin bacterial species, according to the activity status of the caries lesions, are shown in Figure 2. The levels of *Lactobacillus* species were significantly higher in the active group than in the inactive group; however, there was no statistical difference in the levels of *S. mutans, S. sobrinus, F. nucleatum, P. nigrescens, P. intermedia,* and *C. albicans*.

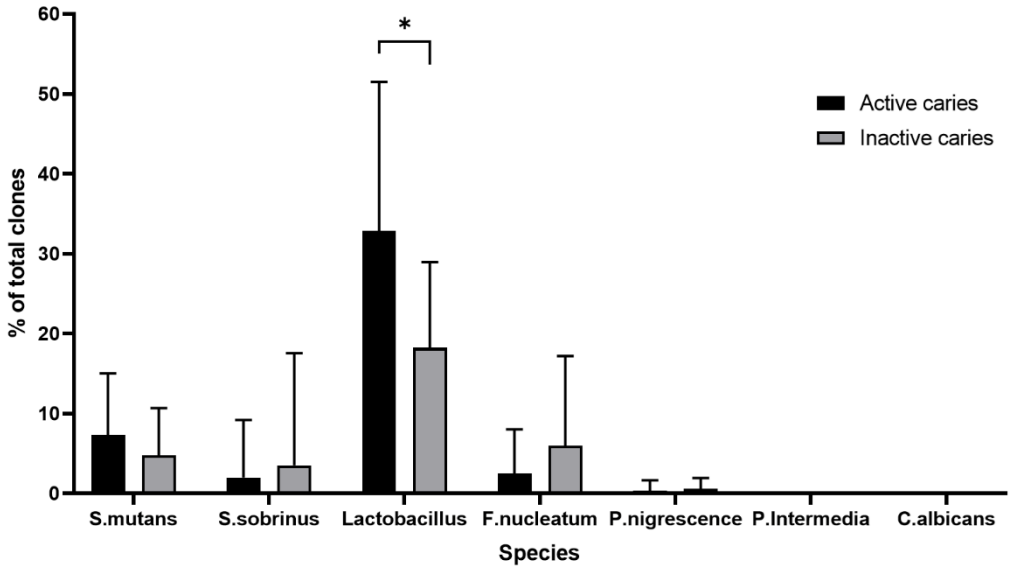


Figure 2. Mean levels of the species in carious dentin according to caries lesion activity status. *$p < 0.05$

4. Quantification of salivary bacteria

The quantitative PCR results for each salivary bacterial species, according to the activity status of carious lesions, are shown in Figure 3. The levels of *S. sobrinus* species were significantly higher in the active group than in the inactive group; however, there was no statistical difference in the levels of *S. mutans, Lactobacillus* species*, F. nucleatum, P. nigrescens, P. intermedia,* and *C. albicans*.

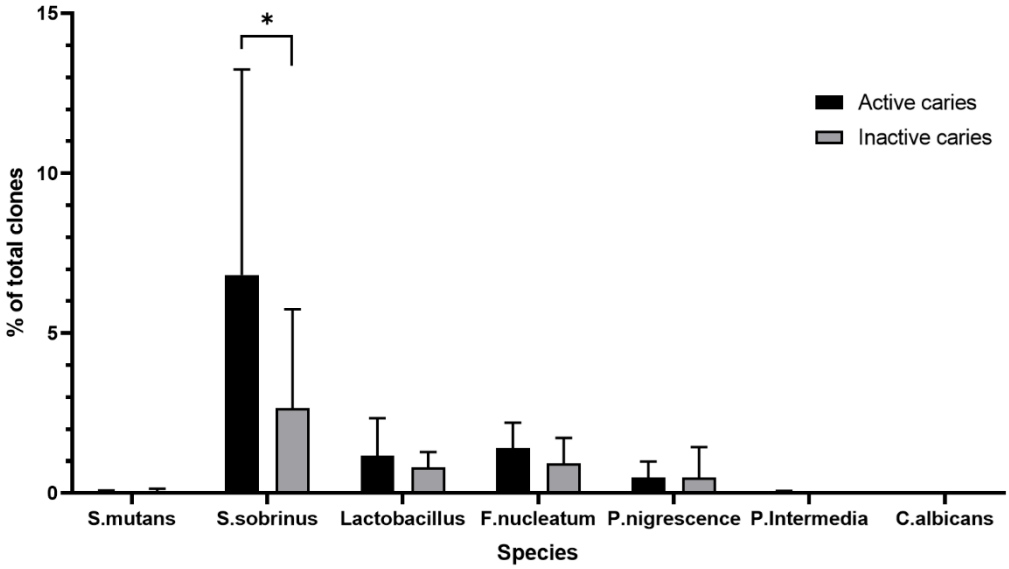


Figure 3. Mean levels of the species in saliva according to caries lesion activity status.

*$p < 0.05$

IV. Discussion

Evaluation of the activity status of dental carious lesions, a fundamental factor in deciding the course of treatment, is a subjective and challenging clinical task (Braga et al., 2010b). If the activity status of a lesion can be distinguished through quantitative measurements, and the difference in the microbiological distribution of the lesion or the surrounding environment can be identified, the activity status can be determined more objectively and easily. Therefore, in this study, carious lesions in the primary molars were classified as active or inactive according to the International Caries Classification and Management System criteria, and the difference between the values measured using the QLF method of active and inactive lesions was examined. The difference in the distribution of caries-related bacteria in carious dentin and saliva obtained from the patients was investigated.

In this study, a caries lesion of one tooth per person was selected, and its lesion activity and carious dentin tissue were analyzed. However, in patient with multiple caries lesions, the caries activity may be different depending on each tooth, which may affect the grouping the sample and analysis of the results of this study. To prevent the influence of these factors, the caries lesion activity of the teeth adjacent to the tooth selected for analysis was also evaluated. If the caries lesion activity between the selected tooth and adjacent teeth was different, the subject was excluded from this study. Through this process, the selected tooth

can more clearly represent each caries lesion activity group (active or inactive), which can be useful in analyzing differences according to groups.

When analyzing bacteria in carious dentin and saliva in this study, specific bacterial species (*S. mutans, S. sobrinus, Lactobacillus* species*, F. nucleatum, P. nigrescence, P. intermedia)* and *C. albicans* were selected and used for analysis. This was done to examine the differences in lesions more efficiently through the key microorganisms involved in the progression of caries in the caries model generated through decades of caries studies. *S. mutans, S. sobrinus*, and *Lactobacillus* species, a group of acidogenic and aciduric bacteria, are the major pathogens involved in caries development (Loesche, 1986). *Fusobacterium* and *Prevotella* species are acid-tolerant bacteria known to be important in caries (Dige et al., 2014; Takahashi and Nyvad, 2008; Tanner et al., 2016). *F. nucleatum* is known to act as a bridging organism by binding to both early and late colonizers (Kolenbrander et al., 2006). *P. nigrescence* has been reported as a risk marker for early childhood caries (Kanasi et al., 2010), and *P. intermedia* has been reported to be highly correlated with red fluorescence in QLF measurements (Han et al., 2016; Lennon et al., 2006). *C. albicans* plays an important role in early childhood caries (de Carvalho et al., 2006; Jean et al., 2018; Yang et al., 2012).

Regarding the values measured using QLF, only the values related to red fluorescence (ΔR, ΔRmax) were significantly higher in the active lesion than in the inactive lesion. The correlation between red fluorescence measurements and the activity of carious lesions has been reported in several previous studies (Felix Gomez et al., 2016; Lee et al., 2013). Red

fluorescence emission seen in QLF images is proposed to result from the excitation of bacterial metabolites (endogenous porphyrins) by violet-blue light. Metabolite production can be affected by differences in metabolic ability resulting from signaling interactions between bacteria (Astvaldsdóttir et al., 2010). As the emission of red fluorescence is caused by bacterial porphyrins, it can be correlated with the activity of the lesion (Lee et al., 2013).

Bacterial analysis of carious dentin revealed that the level of *Lactobacillus* species was significantly higher in the active lesion than in the inactive lesion. From the perspective of the caries process, according to the ecological hypothesis, when a lesion changes from an inactive to an active state, its environment gradually becomes an acid-producing environment. Once an acidic environment has been established, microbial acid adaptation and subsequent acid selection occur and so *S. mutan*s and other aciduric bacteria increase in number and promote lesion development by sustaining the aciduric environment characterized by 'net mineral loss' (Takahashi and Nyvad, 2008). *Lactobacillus* species are representative non-mutans aciduric bacteria, and the results of this study were consistent with these caries process models.

Bacterial saliva analysis revealed that the level of *S. sobrinus* was significantly higher in active lesions than in inactive lesions. The prevalence of *S. sobrinus*, another predominant species of the mutans streptococci group, is associated with high caries activity (Hirose et al., 1993; Hughes et al., 2012; Loyola-Rodriguez et al., 2008). It has been speculated that the combination of *S. mutans* and *S. sobrinus* leads to increased caries activity (Babaahmady et al., 1998). However, several studies have reported that patients with

elevated levels of *S. sobrinus* maintain high caries activity regardless of the level of *S. mutans* (Gross et al., 2012; Hughes et al., 2012). These results suggest that *S. sobrinus* may be a potentially useful pathogen for evaluating caries activity.

The main limitation of this study was that only the distribution of specific bacterial species was examined by analyzing the bacteria in carious dentin and saliva. It is known that some bacterial species play an important role in the progression of dental caries. However, dental caries is not caused by a few specific bacterial species. With the development of molecular microbiological analysis methods, numerous bacterial species have been reported to be involved in the development of dental caries (Nyvad et al., 2013). In addition, this study found a difference in the QLF measurements and levels of bacteria in carious dentin and saliva between active and inactive lesions, but it did not provide reference points to differentiate between active and inactive lesions. More samples are needed to present a reference point for classification.

Despite these limitations, the significance of this study is that it confirmed a correlation between the conventional method for classifying the activity of carious lesions using the visual and tactile senses and red fluorescence measurement through QLF. This can be the basis for an objective and quantitative method of evaluating caries activity using the QLF. In addition, we confirmed a difference in the distribution of bacteria in the carious dentin and saliva based on the activity status of the carious lesion. This can be the basis for demonstrating that microbial factors may be important in predicting the activity of carious lesions.

V. Conclusion

It can be concluded that quantitative measurements related to red fluorescence using QLF, levels of Lactobacillus species in carious dentin, and levels of S. sobrinus in saliva are associated with caries lesion activity status. The significance of this study is that it presented the possibility of more objectively and quantitatively evaluating the activity of carious lesion through QLF. It may also be important to evaluate a patient's overall caries risk in predicting the progression of carious lesions. However, since the actual progression of caries proceeds site-specifically, it is also important to evaluate the activity of individual lesions. Based on these results, if it is possible to prepare a quantitative reference point to distinguish the carious lesion activity, it will be helpful in establishing a more objective and rational treatment plan for dental caries.

VI.References

Ando M, Stookey GK, Zero DT (2006). Ability of quantitative light-induced fluorescence (QLF) to assess the activity of white spot lesions during dehydration. *Am J Dent* 19(1): 15-18.

Astvaldsdóttir A, Tranæus S, Karlsson L, Holbrook WP (2010). DIAGNOdent measurements of cultures of selected oral bacteria and demineralized enamel. *Acta Odontol Scand* 68(3): 148-153.

Babaahmady KG, Challacombe SJ, Marsh PD, Newman HN (1998). Ecological study of Streptococcus mutans, Streptococcus sobrinus and Lactobacillus spp. at sub-sites from approximal dental plaque from children. *Caries Res* 32(1): 51-58.

Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, et al. (2002). Molecular analysis of bacterial species associated with childhood caries. *J Clin Microbiol* 40(3): 1001-1009.

Beighton D, Al-Haboubi M, Mantzourani M, Gilbert SC, Clark D, Zoitopoulos L, et al. (2010). Oral Bifidobacteria: caries-associated bacteria in older adults. *J Dent Res* 89(9): 970-974.

Braga MM, Martignon S, Ekstrand KR, Ricketts DN, Imparato JC, Mendes FM (2010a). Parameters associated with active caries lesions assessed by two different visual scoring systems on occlusal surfaces of primary molars - a multilevel approach. *Community Dent Oral Epidemiol* 38(6): 549-558.

Braga MM, Mendes FM, Ekstrand KR (2010b). Detection activity assessment and diagnosis of dental caries lesions. *Dent Clin North Am* 54(3): 479-493.

Cho KH, Kang CM, Jung HI, Lee HS, Lee K, Lee TY, et al. (2021). The diagnostic efficacy of quantitative light-induced fluorescence in detection of dental caries of primary teeth. *J Dent* 115: 103845.

de Carvalho FG, Silva DS, Hebling J, Spolidorio LC, Spolidorio DM (2006). Presence of mutans streptococci and Candida spp. in dental plaque/dentine of carious teeth and early childhood caries. *Arch Oral Biol* 51(11): 1024-1028.

de Josselin de Jong E, Higham S, Smith P, van Daelen C, van der Veen M (2009). Quantified light-induced fluorescence, review of a diagnostic tool in prevention of oral disease. *Journal of Applied Physics* 105(10): 102031.

Dige I, Grønkjær L, Nyvad B (2014). Molecular studies of the structural ecology of natural occlusal caries. *Caries Res* 48(5): 451-460.

Ekstrand KR, Martignon S, Ricketts DJ, Qvist V (2007). Detection and activity assessment of primary coronal caries lesions: a methodologic study. *Oper Dent* 32(3): 225-235.

Ekstrand KR, Zero DT, Martignon S, Pitts NB (2009). Lesion activity assessment. *Monogr Oral Sci* 21: 63-90.

Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, et al. (2014). Symbiotic relationship between Streptococcus mutans and Candida albicans synergizes virulence of plaque biofilms in vivo. *Infect Immun* 82(5): 1968-1981.

Featherstone JD (2004). The continuum of dental caries--evidence for a dynamic disease

process. *J Dent Res* 83 Spec No C: C39-42.

Felix Gomez G, Eckert GJ, Ferreira Zandona A (2016). Orange/Red Fluorescence of Active Caries by Retrospective Quantitative Light-Induced Fluorescence Image Analysis. *Caries Res* 50(3): 295-302.

Gimenez T, Piovesan C, Braga MM, Raggio DP, Deery C, Ricketts DN, et al. (2015). Visual Inspection for Caries Detection: A Systematic Review and Meta-analysis. *J Dent Res* 94(7): 895-904.

Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. (2017). *Lancet* 390(10100): 1211-1259.

Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL (2012). Beyond Streptococcus mutans: dental caries onset linked to multiple species by 16S rRNA community analysis. *PLoS One* 7(10): e47722.

Guedes RS, Piovesan C, Ardenghi TM, Emmanuelli B, Braga MM, Ekstrand KR, et al. (2014). Validation of Visual Caries Activity Assessment: A 2-yr Cohort Study. *J Dent Res* 93(7 Suppl): 101s-107s.

Han SY, Kim BR, Ko HY, Kwon HK, Kim BI (2016). Assessing the use of Quantitative Light-induced Fluorescence-Digital as a clinical plaque assessment. *Photodiagnosis Photodyn Ther* 13: 34-39.

Heinrich-Weltzien R, Kühnisch J, van der Veen M, de Josselin de Jong E, Stösser L (2003). Quantitative light-induced fluorescence (QLF)--a potential method for the dental

practitioner. *Quintessence Int* 34(3): 181-188.

Hirose H, Hirose K, Isogai E, Miura H, Ueda I (1993). Close association between Streptococcus sobrinus in the saliva of young children and smooth-surface caries increment. *Caries Res* 27(4): 292-297.

Hughes CV, Dahlan M, Papadopolou E, Loo CY, Pradhan NS, Lu SC, et al. (2012). Aciduric microbiota and mutans streptococci in severe and recurrent severe early childhood caries. *Pediatr Dent* 34(2): e16-23.

Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. (2007). The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dent Oral Epidemiol* 35(3): 170-178.

Jean J, Goldberg S, Khare R, Bailey LC, Forrest CB, Hajishengallis E, et al. (2018). Retrospective Analysis of Candida-related Conditions in Infancy and Early Childhood Caries. *Pediatr Dent* 40(2): 131-135.

Kanasi E, Johansson I, Lu SC, Kressin NR, Nunn ME, Kent R, Jr., et al. (2010). Microbial risk markers for childhood caries in pediatricians' offices. *J Dent Res* 89(4): 378-383.

Kolenbrander PE, Palmer RJ, Jr., Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI (2006). Bacterial interactions and successions during plaque development. *Periodontol 2000* 42: 47-79.

Lee ES, Kang SM, Ko HY, Kwon HK, Kim BI (2013). Association between the cariogenicity of a dental microcosm biofilm and its red fluorescence detected by

Quantitative Light-induced Fluorescence-Digital (QLF-D). *J Dent* 41(12): 1264-1270.

Lennon AM, Buchalla W, Brune L, Zimmermann O, Gross U, Attin T (2006). The ability of selected oral microorganisms to emit red fluorescence. *Caries Res* 40(1): 2-5.

Liu G, Wu C, Abrams WR, Li Y (2020). Structural and Functional Characteristics of the Microbiome in Deep-Dentin Caries. *J Dent Res* 99(6): 713-720.

Loesche WJ (1986). Role of Streptococcus mutans in human dental decay. *Microbiol Rev* 50(4): 353-380.

Loyola-Rodriguez JP, Martinez-Martinez RE, Flores-Ferreyra BI, Patiño-Marin N, Alpuche-Solis AG, Reyes-Macias JF (2008). Distribution of Streptococcus mutans and Streptococcus sobrinus in saliva of Mexican preschool caries-free and caries-active children by microbial and molecular (PCR) assays. *J Clin Pediatr Dent* 32(2): 121-126.

Marsh PD (2018). In Sickness and in Health-What Does the Oral Microbiome Mean to Us? An Ecological Perspective. *Adv Dent Res* 29(1): 60-65.

Meller C, Santamaria RM, Connert T, Splieth C (2012). Predicting caries by measuring its activity using quantitative light-induced fluorescence in vivo: a 2-year caries increment analysis. *Caries Res* 46(4): 361-367.

Novaes TF, Reyes A, Matos R, Antunes-Pontes LR, Marques RP, Braga MM, et al. (2017). Association between quantitative measures obtained using fluorescence-based methods and activity status of occlusal caries lesions in primary molars. *Int J*

Paediatr Dent 27(3): 154-162.

Nyvad B, Crielaard W, Mira A, Takahashi N, Beighton D (2013). Dental caries from a molecular microbiological perspective. *Caries Res* 47(2): 89-102.

Nyvad B, Fejerskov O (1997). Assessing the stage of caries lesion activity on the basis of clinical and microbiological examination. *Community Dent Oral Epidemiol* 25(1): 69-75.

Nyvad B, Machiulskiene V, Baelum V (2003). Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. *J Dent Res* 82(2): 117-122.

Obata J, Takeshita T, Shibata Y, Yamanaka W, Unemori M, Akamine A, et al. (2014). Identification of the microbiota in carious dentin lesions using 16S rRNA gene sequencing. *PLoS One* 9(8): e103712.

Peres MA, Macpherson LMD, Weyant RJ, Daly B, Venturelli R, Mathur MR, et al. (2019). Oral diseases: a global public health challenge. *Lancet* 394(10194): 249-260.

Pitts NB, Ismail AI, Martignon S, Ekstrand K, Douglas GV, Longbottom C (2014). ICCMS™ guide for practitioners and educators.

Schwendicke F, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, et al. (2016). Managing Carious Lesions: Consensus Recommendations on Carious Tissue Removal. *Adv Dent Res* 28(2): 58-67.

Takahashi N, Nyvad B (2008). Caries ecology revisited: microbial dynamics and the caries process. *Caries Res* 42(6): 409-418.

Tanner AC, Kressirer CA, Faller LL (2016). Understanding Caries From the Oral

Microbiome Perspective. *J Calif Dent Assoc* 44(7): 437-446.

Tanner ACR, Kressirer CA, Rothmiller S, Johansson I, Chalmers NI (2018). The Caries Microbiome: Implications for Reversing Dysbiosis. *Adv Dent Res* 29(1): 78-85.

Yang XQ, Zhang Q, Lu LY, Yang R, Liu Y, Zou J (2012). Genotypic distribution of Candida albicans in dental biofilm of Chinese children associated with severe early childhood caries. *Arch Oral Biol* 57(8): 1048-1053.

Zheng J, Wu Z, Niu K, Xie Y, Hu X, Fu J, et al. (2019). Microbiome of Deep Dentinal Caries from Reversible Pulpitis to Irreversible Pulpitis. *J Endod* 45(3): 302-309.e301.

국문요약

정량광형광기술을 활용한 유구치의 치아우식 활성도 및 세균 조성 분포 분석

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우식 병소의 활성도를 파악하는 것은 해당 병소의 치료 유무를 결정하는데 있어서 매우 중요하며, 이를 시각과 촉각으로 판단하는 과정은 다소 주관적이고 어려움이 따른다. 이러한 우식의 활성도를 정량적인 측정값을 통해 구분할 수 있고 병소 또는 주변 환경의 미생물학적 분포 차이를 파악할 수 있다면 우식병소의 활성도 파악은 좀 더 객관적이고 쉽게 판단이 가능할 것이다. 이에 본 연구에서는 유구치에 존재하는 우식병소들의 활성도에 따라 정량광형광기술을 이용한 우식병소의 정량적 측정값들, 해당 병소를 가지고 있는 환자에서 얻어진 우식조직과 타액의 세균 분포에 차이가 나타나는지를 확인하려고 한다.

이번 연구는 34명의 어린이로부터 총 34개의 유구치를 평가하였다. 우식병소의 활성도는 International Caries Classification and Management System의 분류기준에 따라 활성, 비활성 군으로 분류하였다. 우식병소의 정량적 분석을 위해 정량광형광기를 이용하여 우식병소의 영상을 채득하였다. 우식병소의 상아질과 환자의 타액을 채득하였고, 정량적 중합효소 연쇄반응 분석을 통해 특정 세균종 (*S. mutans, S. sobrinus, Lactobacillus* species, *F. nucleatum, P. nigrescence, P. intermedia, C. albicans*)의 분포를 정량적으로 분석하였다. 우식병소의 활성도에 따른 정량광형광기술을 통한 정량적 측정값, 우식병소 및 타액의 세균분포 차이를 통계적으로 확인하기 위하여 Mann-Whitney U test가 수행되었다.

활성 우식 병소에서 정량광형광기술을 통해 얻어진 정량적 측정값 중 적색 형광과 연관된 측정값들 (ΔR, ΔRmax)이 유의미하게 높은 값을 나타내었다 (ΔR, $p = 0.009$; ΔRmax, $p = 0.014$). 우식 병소 조직의 Lactobacillus species ($p = 0.010$) 및 타액의 S.sobrinus ($p = 0.017$) 비율 또한 활성 우식병소에서 유의미하게 높은 비율을 나타내었다.

본 연구를 통해 유구치의 우식병소의 활성을 파악하는데 있어 정량광형광 기술의 적색 형광 측정값과 우식병소의 Lactobacillus species 및 타액의 S.sobrinus의 분포비율이 연관성이 있음을 확인할 수 있었다.

핵심되는 말: 치아 우식증, 우식 활성도, 정량광형광, 미생물 분포, 유구치