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RESEARCH ARTICLE

Effects of Cariogenic Bacteria and Sealant Evaluated by International Caries Detection Assessment System

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Abstract:

Aim:

Evaluation of tooth surface level effect of cariogenic bacteria and sealant.

Background:

International Caries Detection Assessment System (ICADS) is a clinical scoring system that can assess the non-cavitated early stage of dental caries by surface level. Scores used in ICDAS are ordinary and each tooth within one individual is not statistically independent.

Objective:

In this study, by applying mixed effect modeling, the effect of cariogenic bacteria and fissure sealant for tooth surface-level caries progression was analyzed.

Methods:

Ninety-eight patients who had been regularly visited the dental hospital for the regular check-ups were enrolled in this study. Among them, patients who visited at baseline, after one and two years, were included for the analysis. Fifty-two patients were dropped out. The study population consisted of 25 boys and 21girls and their mean ages were 9.3 +/- 2.1. Salivary levels of cariogenic bacteria were measured by qPCR. Mixed effect modeling with repeated measures was applied for the analysis.

Results:

Salivary levels of *S. mutans* and *Lactobacilli* were affected by the progression of the ICDAS score. Maxillary teeth, molars and buccal and occlusal surfaces were tended to progress. Maxillary tooth, molar tooth and buccal, approximal, and occlusal surface were tended to be affected by both cariogenic bacteria.

Conclusion:

By applying mixed effect modeling, highly-detailed surface-level analysis can be available.

Keywords: ICDAS, Mixed effects modeling, Cariogenic bacteria, Fissure sealant, Follow up study, Dental caries.

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1. INTRODUCTION

The prevalence of dental caries has been decreasing throughout the world, especially in developed countries [1, 2]. It is attributed to advances of preventive strategies such as fluoride toothpaste and fissure sealant [3]. DMF Index, which measures the past caries experience, has been used as an epidemiological and clinical index. DMF Index misses the information of enamel caries, especially at the early stages and depth of the lesions. The etiology of dental caries is a dynamic process. Imbalance of mineralization and demineralization of

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enamel surface leads to excessive demineralization and subsequent dental caries. Initial enamel demineralization can be detected as white spot lesions. Early diagnosis becomes an important issue because white spot lesions can be treated in a non-operative manner [4]. Demineralized but non-cavitated enamel can be arrested and reconstitute, and the surgical approach to the treatment is no longer tenable [5]. Therefore, the need to evaluate the pre-cavitated enamel lesions raised. In addition, dental caries is site-specific in nature [6 - 8], however, DMF Index and DMFS Index cannot represent site-specific information.

Cariogenic bacteria are major agents of dental caries [9,10]. Especially, *Streptococcus mutans* has the characteristics of water-insoluble glucan production, biofilm formation ability and acid production. *Lactobacilli* has strong acid production ability [11]. Occlusal surfaces in children are affected by oral bacterial microbiome [12]. However, teeth are consisted of four or five surfaces. Most of the epidemiological studies did not show which surface tends to be affected by cariogenic bacteria [13 - 15]. In addition, as cavitation by dental caries takes a long time, the observational periods of epidemiological study take a long time. This characteristic of dental caries makes the clinical trials of dental caries difficult. In the clinical practice, knowledge of the tooth surface level risks by cariogenic bacteria is important. Clinicians need to find out initial and tiny changes of dental caries at the tooth surface level. Therefore, the evaluation system of the initial stage of dental caries should be established.

Pit and fissure sealants are a recommended procedure to prevent caries of the occlusal surfaces of permanent molars. However, we recommend that the caries prevalence level of both individuals and the population should be taken into account. The review by Cochrane Database Systematic Review concluded that the methodological quality of published studies concerning pit and fissure sealants was poorer than expected [16]. Therefore, there is a need to elucidate the effect of pit and fissure sealant in combination with the risk of dental caries by cariogenic bacteria at the tooth surface level.

An international group designed the International Caries Detection and Assessment System (ICDAS) to overcome these problems. It allows the assessment of carious lesions in the enamel and dentine [17 - 19], and it is not restricted to cavitated lesions [20]. ICDAS is proved to be feasible for epidemiological research with an acceptable degree of reliability to non-cavitated and cavitated lesions [21] offering increased sensitivity and accuracy in detection [22, 23].

The advantage of ICDAS is tooth surface level evaluation including non-cavitated carious lesions into the diagnostic criteria. However, diagnostic criteria consisted of ordinary scale and summary statistics are not available. Therefore, it lacks overall measures about caries status of individual tooth. These properties of ICDAS make the statistical analysis complex. Several reports are applied to ICDAS in epidemiological or clinical studies, however, some of them made statistical mistakes in handling ICDAS scores and some of them transformed ICDAS scores into DMF Index, which did not fully utilized the advantage of ICDAS. The data structure of ICDAS makes the analysis more complex. ICDAS scores of each tooth surface are not statistically independent. It nested in each tooth and each tooth nested in each individual.

In this study, we applied mixed effect modeling for the transition of ICDAS score and evaluated the salivary levels of cariogenic bacteria and the effect of fissure sealant at teeth surface level by two years follow up study for the subjects with mixed dentition age.

2. METHODS

2.1. Study Design

Two years follow up study for the patients who finished dental treatment at the dental hospital of Kyusyu Dental University was conducted. Dental caries were evaluated by the ICDAS score.

2.2. Setting

Patients who finished dental treatment at the department of pediatric dentistry dental of Kyusyu Dental University from August to September 2011 were recruited. Data by oral examination and salivary levels of cariogenic bacteria were collected at baseline after one and two years.

2.3. Patients

Patients aged 6 to 12 years were recruited. The inclusion criteria were systematically healthy, without any open dentin cavities. Exclusion criteria were patients with non-healthy, open dentin cavity, unrestored teeth, residual teeth or teeth fistula. Systemic health was decided by ASA physical status calcification system [24 - 26]. Healthy patients were of status 1 and non-healthy patients were of ASA status more than 2.

A total of 98 patients were recruited. Among them, 52 were dropped out. Subjects who finally completed the program were 46 (21 male and 25 female), and their mean age was 9 years 3.2 months ± 9 years 1.2 months.

2.4. Oral Examination

All clinical examinations were carried out by one examiner (Y.F.) using a dental unit (CREST NEO, Yoshida Co., Tokyo, Japan). The status of eruption and extraction of teeth was determined according to the WHO standard [27]. Prior to a professional tooth cleaning with the small rotating brush (PTC BRUSH, GC Co, Tokyo, Japan) and the polishing paste (PTC PASTE, GC Co., Tokyo, Japan), the entire buccal pits of the upper right and lower left teeth and the entire palatal or lingual pits of the upper left and lower right teeth were examined for plaque retentions and bleeding from Sulcus gingivae (0.5 mm). The visual evidence of plaque and bleeding were confirmed using a CPI probe (CP-11.5B6, Hu-Friedy, Chicago, IL, USA) [28, 29]. All the sealants were applied by one of the authors Y.F. before the start of the study.

After cleaning, the caries status, according to the WHO standard, was determined as decayed, filled and known extracted (def) in each surface [27]. Both intact and partially retained pit and fissure sealants were registered in all molars. Assessment of the pit and fissure sealants was scored with or without sealant visually and by palpation. Then, all the teeth

surfaces were examined according to ICDAS criteria by isolating and prolonged air-drying (5 seconds) [30].

2.5. Measurement of Cariogenic Bacteria

Salivary levels of cariogenic bacteria were measured in accordance with our previous report [31]. Genomic DNAs were isolated from saliva samples using a QuickGene DNA tissue kit S (Kurabo Industries Ltd., Osaka, Japan) in accordance with the manufacturer's instructions. 5' Nuclease-based real-time PCR assay was performed according to previously described method [24]. For real-time PCR assay, 20 μL of a mixture containing 1 μL of template DNA, 1×TaqMan Fast Universal PCR Master Mix (Life Technologies Japan Ltd., Tokyo, Japan), 200 nM forward and reverse primers, and 100 nM TaqMan probe was placed in each well of a 96-well plate. Amplification and detection were performed using the 7500 Fast Real-Time PCR System (Life Technologies Japan Ltd.) with the following cycle profile; 95ºC for 20 s, and 40 cycles of 95ºC for 3 s, and 60ºC for 30 s.

Sequences of *S. mutans*-specific and primers are as follows; the forward primers of *S. mutans* and Lactobacilli ssp. were 5'-GCCTACAGCTCAGAGATGCTATTCT-3', 5'-ATGAATGCTAGGTGTTGGAGGG-3', and reverse primers were 5'-GCCATACACCACTCATGAATTGA-3', 5'-TGCGGTCGTACTCCCCAG-3'. Amplicon sizes were 114 bp and 81 bp. TaqMan fluorescent probes (Smut3423T) were 5'-FAM-TGGAAATGACGGTCGCCGTTATGAA-TAMRA-3' and 5'-FAM- CCCTTCAGTGCCGCAGCTAACGC-TAMRA -3'.

Standard curves for each organism were plotted for each primer-probe set using *Ct* values obtained from the amplification of genomic DNA extracted from the samples of *S. mutans* strain. A number of bacteria through PCR were determined by comparison with plating culture dilutions on Brain Heart Infusion agar (Difco Laboratories, USA).

2.6. Sample Size

The optimal sample size required to detect statistically significant differences in the percent of patients with or without progression dental caries during the follow-up period was determined [32]. We calculated that 46 patients were necessary for each group ($\alpha = 0.05$ and $\beta = 0.80$). As it is known that around 60% of patients do not continue check-ups for more than two years, we recruited for all the patients who finished treatment for one month and we set 100 patients as a goal. As a consequence, a total of 98 patients were examined at baseline.

2.7. Drop Out

During the two years follow up, 54 patients were dropped out. Six were relocated for other areas of Japan. Twenty-six failed to comply with visits and 22 dropped out for personal reasons. In total, 46 subjects were included in the analysis.

2.8. Statistical Analysis

The change of ICDAS score was used as a major outcome. Descriptive analysis was carried out without statistical tests. As ICDAS scores nested in each tooth and each individual, simple statistical tests have the risk of Type I error. The transitions of ICDAS score, their associated factors and their interactions were analyzed by generalized mixed effect modeling with repeated measures. As the distributions of ICDAS scores were skewed, Poisson distribution was used for the probability distribution. Patients and teeth were used as a grouping variable. Autoregressive 1(AR 1) was used for the covariance structure. *P*-values less than 0.05 were considered statistically significant. These analyses were carried out by SPSS Statistics Ver 24.0 (IBM Japan, Tokyo, Japan) and the models were specified in Supportive information 1.

3. RESULTS

The descriptive analysis of the characteristics of the subjects participated in this study and ICDAS scores is shown in Table **S1** and Fig. **S1**. The prevalence of dental caries evaluated by ICDAS was rare.

During the one and two years follow up, ICDAS scores of most of the tooth surfaces were stable Table **1**. Only improved tooth surfaces were in a minority. The number of changes of ICDAS score from baseline to one year was stable: 2658 (91.2%), improved 107 (3.7%), and progressed 148 (5.1%). The number of changes of ICDAS score from baseline to two years was stable: 2644 (91.2%), improved 111 (3.8%), and progressed 158 (5.4%), respectively. For the calculation above, unerupted teeth at baseline were excluded. The number of changes of ICDAS scores was summarized against characteristics of the subjects, tooth type and tooth surface in Table **S2**. *P*-values were not calculated because the ICDAS scores were nested in teeth and subjects. For further analysis, mixed effect modeling (multilevel analysis) was necessary.

Then, factors that affect the changes of ICDAS scores were investigated. Table **2** shows the results of multilevel adjusted mixed effect modeling with repeated measures by all the factors investigated in this study. All the factors investigated in this study statistically significant correlations for the ICDAS score, with only one exception. The approximal surface had not statistically significant risk when compared with the lingual or palatal surface.

We investigated which type of tooth or tooth surface was affected by cariogenic bacteria. Table **3** shows the results of mixed effect modeling with repeated measures by cariogenic bacteria and tooth or tooth surface type and their interactions. When *S. mutans* or LB were positive in stimulated saliva, maxillary tooth was at risk when mandibular tooth set as a reference, molar tooth was at risk when anterior tooth set as a reference, and occlusal surfaces were at risk when lingual surfaces set as reference. The coefficient of maxilla tooth, molar tooth and occlusal surface was higher than other classifications by the interaction with both *S. mutans* and LB. These results indicated that the risks of dental caries are different between tooth type and tooth surface and the effects of cariogenic bacteria are dependent on the type of tooth and tooth surface.

Fissure sealant was applied only on the occlusal surface. The effects of interaction sealant and tooth type are shown in Table **4**. The coefficient of maxillary teeth with sealant was significant when mandibular teeth without sealant were used for reference. The coefficient of molar teeth without sealant

Table 1. Cross-tabulation of the ICDAS score at baseline, after one and two years.

–		ICDAS Score (Baseline)			
–		**0**	**1**	**2**	**3**
ICDAS Score (After one year)	0	2563	85	20	0
	1	76	80	1	0
	2	27	43	14	1
	3	2	0	0	1
ICDAS Score (After two year)	0	2550	103	6	0
	1	68	65	1	0
	2	49	41	28	1
	3	0	0	0	1

During the one and two years follow up, ICDAS scores of most of the tooth surfaces were stable and only improved tooth surfaces were in a minority.
Form baseline to one year; stable: 2658(91.2%), improved 107 (3.7%), and progressed 148(5.1%).
From baseline to two years; stable: 2644 (91.2%), improved 111 (3.8%), and progressed 158(5.4%).
For the calculation, unerupted teeth at baseline were excluded.

Table 2. Results of multilevel adjusted mixed effect model with repeated measures for the changes of ICDAS score (Model 1).

–	–		**Coefficient**	**95%CI**		***P*-value**
Intercept			-0.268	(-0.309 - -0.227)		<0.001
Gender	Male		Reference			
	Female		-0.091	(-0.104 - -0.079)		<0.001
Age by month			0.001	(0.001 - 0.001)		<0.001
Stimulated saliva volume			-0.007	(-0.009- -0.005)		<0.001
Cariogenic Bacteria	*S. mutans*	-	Reference	–	–	–
		+	0.148	(0.128 - 0.168)		<0.001
	LB	-	Reference	–	–	–
		+	0.060	(0.050 - 0.070)		<0.001
df			0.009	(0.007 - 0.012)		<0.001
Maxilla Mandibular	Mandibular		Reference	–	–	–
	Maxilla		0.058	(0.048- 0.068)		<0.001
Tooth type	Anterior		Reference	–	–	–
	Premolar		-0.028	(-0.040- -0.015)		<0.001
	Molar		0.064	(0.052 - 0.076)		<0.001
Tooth surface	Lingual or Palatal		Reference	–	–	–
	Buccal		0.177	(0.156 -0.198)		<0.001
	Approximal		0.004	(-0.006 - 0.014)		0.397
	Occlusal		0.254	(0.222 - 0.285)		<0.001

The model is specified in S1 File. All the factors investigated in this study showed statistically significant correlations for the ICDAS score, with only one exception. The approximal surface had not statistically significant risk when compared with the lingual or palatal surface.

Table 3. Effect of interaction of tooth surface and cariogenic bacteria on the changes of ICDAS score (Model 2 and 3).

–	***S. mutans***			**LB**		
–	–	**Coefficient (95% CI)**	***P*-value**	–	**Coefficient (95% CI)**	***P*-value**
Mandibular Maxilla						
Intercept	–	0.013 (0.005 - 0.021)	0.002	–	0.054 (0.044 - 0.065)	<0.001
Mandibular	*S. mutans* -	Reference	–	LB -	Reference	–
	S. mutans +	0.095 (0.079 - 0.112)	<0.001	LB +	0.081 (0.060 - 0.101)	<0.001
Maxilla	*S. mutans* -	0.022 (0.006 - 0.038)	0.006	LB -	0.015 (-0.001 - 0.031)	0.071
	S. mutans +	0.122 (0.103 - 0.141)	<0.001	LB +	0.120 (0.093 - 0.147)	<0.001
Tooth Type						
Intercept	–	0.027 (0.014 - 0.039)	<0.001	–	0.043 (0.034 - 0.053)	<0.001

(Table 3) contd.....

–		***S. mutans***			**LB**		
–	–	**Coefficient (95% CI)**	***P*-value**	–	**Coefficient (95% CI)**	***P*-value**
Anterior	*S. mutans* -	Reference	–	LB -	Reference	–
	S. mutans +	0.055 (0.038-0.073)	<0.001	–	0.060 (0.042 - 0.077)	<0.001
Premolar	*S. mutans* -	-0.011 (-0.028 - 0.007)	0.237	LB -	-0.004 (-0.019 - 0.012)	0.645
	S. mutans +	0.061 (0.038 - 0.083)	<0.001	LB +	0.091 (0.059 - 0.124)	<0.001
Molar	*S. mutans* -	-0.002 (-0.020 - 0.017)	0.876	LB -	0.069 (0.047 - 0.091)	<0.001
	S. mutans +	0.194 (0.162 - 0.225)	<0.001	LB +	0.216 (0.177 - 0.255)	<0.001
Tooth Surface						
Intercept	–	<0.001 (-0.001 - 0.002)	0.767	–	0.019 (0.010 - 0.028)	<0.001
Lingual	*S. mutans* -	Reference	–	LB -	Reference	–
	S. mutans +	0.054 (0.039 - 0.069)	<0.001	LB +	0.056 (0.033 - 0.078)	<0.001
Buccal	*S. mutans* -	0.060 (0.035 - 0.085)	<0.001	LB -	0.071 (0.050 - 0.092)	<0.001
	S. mutans +	0.204 (0.175 - 0.233)	<0.001	LB +	0.271 (0.225 - 0.317)	<0.001
Approximal	*S. mutans* -	0.005 (0.000 - 0.010)	0.069	LB -	<0.001 (-0.011 - 0.011)	0.950
	S. mutans +	0.050 (0.040 - 0.060)	<0.001	LB +	0.048 (0.030 - 0.066)	<0.001
Occlusal	*S. mutans* -	0.054 (0.020 - 0.088)	0.002	LB -	0.222 (0.176 - 0.269)	<0.001
	S. mutans +	0.418 (0.358 - 0.478)	<0.001	LB +	0.460 (0.373 - 0.548)	<0.001

Models were specified in S1 File. When *S. mutans* or LB was positive in stimulated saliva, maxillary tooth was at risk when mandibular tooth set as a reference, molar tooth was at risk when anterior tooth set as a reference, and occlusal surfaces were at risk when lingual surfaces set as reference.

The coefficient of maxillary tooth, molar tooth and occlusal surface was higher than other classifications in both *S. mutans* and LD. The effects of both *S. mutans* and LB on the ICDAS score of these tooth properties were stronger than other classifications. *S. mutans* and LB also significantly affected the ICDAS score of all other classifications.

Table 4. Effect of interaction of fissure sealant and tooth on the changes of ICDAS score (Model 4).

–	–	**Coefficient (95% CI)**	***P*-value**
Mandibular Maxilla			
Intercept	–	0.366 (0.288 - 0.444)	<0.001
Mandibular	Sealant -	Reference	–
	Sealant +	-0.098 (-0.200 - 0.005)	0.062
Maxilla	Sealant -	-0.033 (-0.264 - 0.199)	0.782
	Sealant +	-0.345 (-0.503 - -0.188)	<0.001
Tooth Type			
Intercept	–	0.125 (0.080 - 0.171)	<0.001
Premolar	Sealant -	Reference	–
	Sealant +	-0.071 (-0.237 - 0.094)	0.399
Molar	Sealant -	0.233 (0.042 - 0.508)	<0.001
	Sealant +	0.366 (-0.270 - 0.462)	0.096

Sealants were applied only on the occlusal surface. Applied sealant on maxillary teeth was effective when mandibular teeth without sealant were used for reference. Molar teeth without sealant were at risk when premolar teeth without sealant were used for reference. The coefficient of molar teeth with sealant was positive. Though it was not statistically significant, the results indicated that the risks of molar teeth were higher than premolars even with sealant.

was significant and positive when premolar teeth without sealant were used for reference. The coefficient of molar teeth with sealant was also positive, however, it was not statistically significant.

4. DISCUSSION

The advantages of ICDAS are tooth surface levels evaluation including uncavitated enamel lesion in diagnostic criteria. Several reports have been published on the availability of ICDAS and its applications. However, most of the reports did not utilize these advantages of ICDAS. A statistical method to analyze the ICDAS score is complex. ICDAS score consisted of an ordinary scale, therefore addition, subtraction, multiplication and division were not allowed. Summary statistics like mean or standard deviation of each tooth and each individual have no meaning [33, 34]. The statistically allowed summary statistics were a maximum value like Community Periodontal Index or minimum value. These statistics may not be clinically or epidemiologically useful. Therefore, several reports re-categorized ICDAS score like 2-6 and 5-6 [35], 0-4 and 5-6 [36], and 0-3 and 4-6 [37]. Individual levels summary statistics were presented by the percentage of these re-categorized scores [34], or subjects were dichotomized with or without at least one tooth surface with more than cut off points [36 - 38]. The methodology of these reports was no mistake, however, these reports did not utilize the property of ICDAS that can evaluate dental caries at the initial stage and

resulted in information loss. Other reports used ICDAS together with DMF Index. These reports used ICDAS as diagnostic criteria only. For the analysis, ICDAS scores were transformed into DMF Index [39 - 41]. In addition to information loss, these reports also did not utilize the properties of ICDAS. These previous reports did not apply tooth surface levels analysis. To analyze the changes of ICDAS score at tooth surface-level analysis, conventional regression models such as generalized linear model including logistic regression analysis, ordinary regression analysis were not available. As the ICDAS scores were nested in each teeth and each individual. By using these models, results have the risks of containing Type I error. The only solution to this problem is applying mixed effect modeling (multilevel analysis) that allows analyzing the nested data.

There are two previous reports that applied multilevel analysis for the ICDAS score at tooth or tooth surface-level analysis. These reports had shown that incisors, canines [42], first molars [42, 43], occlusal surface [42], and upper teeth [43] were at risk for the progression of dental caries when second molars, smooth surface, and mandibular teeth were used as references, respectively. Our results are shown in Table **2** substantially consistent with the results of these two studies though there were differences in classification. When compared to the mandibular and maxilla teeth, maxilla teeth were at risk. Similarly, molar teeth were at risk. These tendencies were consistent with the National survey in Japan [44]. For the tooth surface, occlusal surfaces were at higher risk. However, approximal surfaces were at lower risks when compared with the buccal surface. This may be because some of the approximal surfaces may be free from the adjacent approximal tooth surfaces for the subject with mixed dentitions.

S. mutans and Lactobacilli as cariogenic bacteria are known to be strong risk factors of dental caries [45 - 47]. For early childhood caries, *S. mutans* levels were a strong risk indicator [48]. However, little information is available about which tooth surface is tended to be affected by these cariogenic bacteria. By the recent advances of metagenome analysis, microbiome analysis had shown that *S. mutans* was detected on all surfaces in all patients and *Lactobacilli* were predominant at order levels [49]. High levels of *S. mutans* in saliva were correlated with proximal caries and high levels of LB were correlated with smooth-surface caries, and correlated significantly with occlusal caries [50]. Our results had shown that *S. mutans* and LB significantly affected all teeth. As shown in Table **3**, coefficients of maxillary teeth, molar teeth and occlusal surface were highest when mandibular teeth, anterior teeth and lingual surface were used as a reference, respectively. Coefficient of the approximal surface was almost the same level with anterior teeth. This may be because many of the subjects investigated in this study had mixed dentition and eruption of the premolar teeth was not completed. Therefore, cariogenic bacteria affected on ICDAS score of all types of teeth. However, susceptibility may differ between tooth and surface.

A systematic review from the Cochrane Database revealed that resin-based sealants reduced caries by between 11% and 51% as compared to no sealant when measured at 24 or 48 months [16]. Our results had shown that the coefficient of maxillary teeth with fissure sealant was minimum. That of the occlusal surface with fissure sealant was positive, however it was not significant. These results indicated that fissure sealant was more effective on maxillary teeth than mandibular teeth. Even by applying fissure sealant, the risk may remain at molar teeth when premolar teeth are used as a reference. These surface-level analyses could not be applied by the conventional DMFS index because the DMFS index could not evaluate the recalcification or decalcified enamel under the surface layer. As described above, by the application of ICDAS, information on tooth surface levels of susceptibility for dental caries, the effect of cariogenic bacteria and tooth levels protective effect of fissure sealants can be obtained.

CONCLUSION

By applying mixed effect modeling, the ICDAS score can analyze at the tooth surface level. This method fully utilizes the properties of ICDAS. The effect of cariogenic bacteria and fissure sealant may depend on the kind of tooth and tooth surface.

LIST OF ABBREVIATIONS

ICDAS	=	International Caries Detection and Assessment System
ASA Physical Status Classification	=	The American Society of Anesthesiologists' (ASA) Physical Status Classification
LB	=	Lactobacillus

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Human Investigations Committee of Kyushu Dental University, Japan (Approval number: 11-25).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. The reported experiments on humans were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent was obtained from all participants before the beginning of the study.

AVAILABILITY OF DATA AND MATERIALS

The data sets analyzed during the current study are available from the corresponding author upon request.

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CONFLICTS OF INTEREST

Information about the primer sequence and PCR conditions

was provided by GC Corporation.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

REFERENCES

[1] Misra S, Tahmassebi JF, Brosnan M. Early childhood caries-A review. Dent Update 2007; 34(9): 556-558, 561-562, 564. [http://dx.doi.org/10.12968/denu.2007.34.9.556] [PMID: 18087926]

[2] Jones CM, Davies GM, Monaghan N, Morgan MZ, Neville JS, Pitts NB. The caries experience of 5 year-old children in Scotland in 2013-2014, and in England and Wales in 2014-2015. Reports of cross-sectional dental surveys using BASCD criteria. Community Dent Health 2017; 34(3): 157-62. [PMID: 28872810]

[3] Himida T, Promise U. School-based dental sealant programmes may be effective in caries prevention. Evid Based Dent 2017; 18(1): 13-4. [http://dx.doi.org/10.1038/sj.ebd.6401218] [PMID: 28338038]

[4] Murdoch-Kinch CA, McLean ME. Minimally invasive dentistry. J Am Dent Assoc 2003; 134(1): 87-95. [http://dx.doi.org/10.14219/jada.archive.2003.0021] [PMID: 12555961]

[5] Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry: A review. FDI Commission Project 1-97. Int Dent J 2000; 50(1): 1-12. [http://dx.doi.org/10.1111/j.1875-595X.2000.tb00540.x] [PMID: 10945174]

[6] Carvalho JC, Ekstrand KR, Thylstrup A. Dental plaque and caries on occlusal surfaces of first permanent molars in relation to stage of eruption. J Dent Res 1989; 68(5): 773-9. [http://dx.doi.org/10.1177/00220345890680050401] [PMID: 2715469]

[7] Carvalho JC. Caries process on occlusal surfaces: Evolving evidence and understanding. Caries Res 2014; 48(4): 339-46. [http://dx.doi.org/10.1159/000356307] [PMID: 24577073]

[8] Vehkalahti MM, Solavaara L, Rytömaa I. An eight-year follow-up of the occlusal surfaces of first permanent molars J Dent Res 1991; 70(7): 064-1067.

[9] Abranches J, Zeng L, Kajfasz JK, *et al.* Biology of oral *streptococci.* Microbiol Spectr 2018; 6(5): 941-54. [PMID: 30338752]

[10] Seow WK. Early Childhood Caries. Pediatr Clin North Am 2018; 65(5): 941-54. [http://dx.doi.org/10.1016/j.pcl.2018.05.004] [PMID: 30213355]

[11] Takahashi N, Nyvad B. The role of bacteria in the caries process: Ecological perspectives. J Dent Res 2011; 90(3): 294-303. [http://dx.doi.org/10.1177/0022034510379602] [PMID: 20924061]

[12] Ribeiro AA, Azcarate-Peril MA, Cadenas MB, *et al.* The oral bacterial microbiome of occlusal surfaces in children and its association with diet and caries. PLoS One 2017; 12(7)e0180621 [http://dx.doi.org/10.1371/journal.pone.0180621] [PMID: 28678838]

[13] Tamaki Y, Nomura Y, Katsumura S, *et al.* Construction of a dental caries prediction model by data mining. J Oral Sci 2009; 51(1): 61-8. [http://dx.doi.org/10.2334/josnusd.51.61] [PMID: 19325201]

[14] Nishikawara F, Katsumura S, Ando A, *et al.* Correlation of cariogenic bacteria and dental caries in adults. J Oral Sci 2006; 48(4): 245-51. [http://dx.doi.org/10.2334/josnusd.48.245] [PMID: 17220624]

[15] Nomura Y, Senpuku H, Hanada N, Kumagai T. Mutans *streptococci* and *Lactobacillus* as risk factors for dental caries in 12-year-old children. Jpn J Infect Dis 2001; 54(1): 43-5. [PMID: 11326133]

[16] Ahovuo-Saloranta A, Forss H, Walsh T, *et al.* Sealants for preventing dental decay in the permanent teeth. Cochrane Database Syst Rev 2013; 28(3)CD001830 [http://dx.doi.org/10.1002/14651858.CD001830.pub4] [PMID: 23543512]

[17] Pitts N. "ICDAS": An international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dent Health 2004; 21(3): 193-8. [PMID: 15470828]

[18] Ismail AI, Sohn W, Tellez M, *et al.* The International Caries Detection and Assessment System (ICDAS): An integrated system for measuring dental caries. Community Dent Oral Epidemiol 2007; 35(3): 170-8. [http://dx.doi.org/10.1111/j.1600-0528.2007.00347.x] [PMID: 17518963]

[19] Pitts NB, Ekstrand KR. International Caries Detection and Assessment System (ICDAS) and its International Caries Classification and Management System (ICCMS) - methods for staging of the caries process and enabling dentists to manage caries. Community Dent Oral Epidemiol 2013; 41(1): e41-52. [http://dx.doi.org/10.1111/cdoe.12025] [PMID: 24916677]

[20] Ericson D. The concept of minimally invasive dentistry. Dent Update 2007; 34(1): 9-10, 12-14, 17-18. [http://dx.doi.org/10.12968/denu.2007.34.1.9] [PMID: 17348554]

[21] Nelson S, Eggertsson H, Powell B, *et al.* Dental examiners consistency in applying the ICDAS criteria for a caries prevention community trial. Community Dent Health 2011; 28(3): 238-42. [PMID: 21916361]

[22] Braga MM, Oliveira LB, Bonini GA, Bönecker M, Mendes FM. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization criteria. Caries Res 2009; 43(4): 245-9. [http://dx.doi.org/10.1159/000217855] [PMID: 19439944]

[23] Mendes FM, Braga MM, Oliveira LB, Antunes JL, Ardenghi TM, Bönecker M. Discriminant validity of the International Caries Detection and Assessment System (ICDAS) and comparability with World Health Organization criteria in a cross-sectional study. Community Dent Oral Epidemiol 2010; 38(5): 398-407. [http://dx.doi.org/10.1111/j.1600-0528.2010.00557.x] [PMID: 20636415]

[24] Saklad M. Grading of patients for surgical procedures. Anesthesiology 1941; 2: 281-4. [http://dx.doi.org/10.1097/00000542-194105000-00004]

[25] Enneking FK, Radhakrishnan NS, Berg K, Patel S, Wishin JM, Vasilopoulos T. Patient-centered anesthesia triage system predicts ASA physical status. Anesth Analg 2017; 124(6): 1957-62. [http://dx.doi.org/10.1213/ANE.0000000000001712] [PMID: 28257309]

[26] Mak PH, Campbell RC, Irwin MG. American Society of Anesthesiologists. The ASA physical status classification: Inter-observer consistency. Anaesth Intensive Care 2002; 30(5): 633-40. [http://dx.doi.org/10.1177/0310057X0203000516] [PMID: 12413266]

[27] World Health Organization Oral health surveys: Basic methods: In; 4th ed. 1997; pp. 21-52.

[28] Nomura Y, Morozumi T, Nakagawa T, *et al.* Site-level progression of periodontal disease during a follow-up period. PLoS One 2017; 12(12)e0188670 [http://dx.doi.org/10.1371/journal.pone.0188670] [PMID: 29206238]

[29] Nomura Y, Okada A, Kakuta E, Gunji T, Kajiura S, Hanada N. A new screening method for periodontitis: An alternative to the community periodontal index. BMC Oral Health 2016; 16(1): 64. [http://dx.doi.org/10.1186/s12903-016-0216-x] [PMID: 27388493]

[30] International Caries Detection and Assessment System (ICDAS) Coordinating Committee. Criteria Manual - International Caries Detection and Assessment System (ICDAS II). In. Scotland Dental Health Services Research Unit. 2005.

[31] Ishihara Y. A three-year follow up study for levels of salivary *Streptococcus mutans* and *Streptococcus sobrinus* from Japanese adolescents by quantitative real-time PCR Jpn J Cons Dent 2014; 57(5): 463-71.

[32] Piva F, Pereira JT, Luz PB, Hashizume LN, Hugo FN, Araujo FB. A longitudinal study of early childhood caries and associated factors in Brazilian children. Braz Dent J 2017; 28(2): 241-8. [http://dx.doi.org/10.1590/0103-6440201701237] [PMID: 28492756]

[33] ElSalhy M, Honkala S, Söderling E, Varghese A, Honkala E. Relationship between daily habits, *Streptococcus mutans*, and caries among schoolboys. J Dent 2013; 41(11): 1000-6. [http://dx.doi.org/10.1016/j.jdent.2013.08.005] [PMID: 23948392]

[34] Kirschneck C, Christl JJ, Reicheneder C, Proff P. Efficacy of fluoride varnish for preventing white spot lesions and gingivitis during orthodontic treatment with fixed appliances: A prospective randomized controlled trial. Clin Oral Investig 2016; 20(9): 2371-8. [http://dx.doi.org/10.1007/s00784-016-1730-6] [PMID: 26832780]

[35] Rodríguez G, Ruiz B, Faleiros S, *et al.* Probiotic compared with standard milk for high-caries children: A cluster randomized trial. J

Dent Res 2016; 95(4): 402-7.
[http://dx.doi.org/10.1177/0022034515623935] [PMID: 26747421]

[36] Hilgert LA, Leal SC, Mulder J, Creugers NH, Frencken JE. Caries-preventive effect of supervised tooth brushing and sealants. J Dent Res 2015; 94(9): 1218-24.
[http://dx.doi.org/10.1177/0022034515592857] [PMID: 26116491]

[37] Piovesan C, Ardenghi TM, Mendes FM, Agostini BA, Michel-Crosato E. Individual and contextual factors influencing dental health care utilization by preschool children: A multilevel analysis. Braz Oral Res 2017; 31(31)e27
[http://dx.doi.org/10.1590/1807-3107bor-2017.vol31.0027] [PMID: 28380090]

[38] Pinto-Sarmento TC, Abreu MH, Gomes MC, *et al.* Determinant factors of untreated dental caries and lesion activity in preschool children using ICDAS. PLoS One 2016; 11(2)e0150116
[http://dx.doi.org/10.1371/journal.pone.0150116] [PMID: 26900846]

[39] Melgar RA, Pereira JT, Luz PB, Hugo FN, Araujo FB. Differential impacts of caries classification in children and adults: A comparison of ICDAS and DMF-T. Braz Dent J 2016; 27(6): 761-6.
[http://dx.doi.org/10.1590/0103-6440201600990] [PMID: 27982192]

[40] Laajala A, Karhatsu P, Pesonen P, *et al.* Association of indirect restorations with past caries history and present need for restorative treatment in the Northern Finland Birth Cohort 1966. Clin Oral Investig 2018; 22(3): 1495-501.
[http://dx.doi.org/10.1007/s00784-017-2236-6] [PMID: 29038961]

[41] Alves LS, Susin C, Damé-Teixeira N, Maltz M. Impact of different detection criteria on caries estimates and risk assessment. Int Dent J 2018; 68(3): 144-51.
[http://dx.doi.org/10.1111/idj.12352] [PMID: 29313946]

[42] Guedes RS, Piovesan C, Ardenghi TM, *et al.* Validation of visual caries activity assessment: A 2-yr cohort study. J Dent Res 2014; 93(7)(Suppl.): 101S-7S.
[http://dx.doi.org/10.1177/0022034514531017] [PMID: 24713370]

[43] Braga MM, Martignon S, Ekstrand KR, Ricketts DN, Imparato JC, Mendes FM. 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 2010; 38(6): 549-58.
[http://dx.doi.org/10.1111/j.1600-0528.2010.00567.x] [PMID: 20735448]

[44] https://www.mhlw.go.jp/toukei/list/62-17b.html2019.

[45] Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of *Streptococcus mutans* and the ability to form biofilms. Eur J Clin Microbiol Infect Dis 2014; 33(4): 499-515.
[http://dx.doi.org/10.1007/s10096-013-1993-7] [PMID: 24154653]

[46] Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol 2015; 5(5): 10.
[http://dx.doi.org/10.3389/fcimb.2015.00010] [PMID: 25763359]

[47] Caufield PW, Schön CN, Saraithong P, Li Y, Argimón S. Oral lactobacilli and dental caries: A model for niche adaptation in humans. J Dent Res 2015; 94(9)(Suppl.): 110S-8S.
[http://dx.doi.org/10.1177/0022034515576052] [PMID: 25758458]

[48] Parisotto TM, Steiner-Oliveira C, Silva CM, Rodrigues LK, Nobre-dos-Santos M. Early childhood caries and mutans *streptococci*: A systematic review. Oral Health Prev Dent 2010; 8(1): 59-70.
[PMID: 20480056]

[49] Ribeiro AA, Azcarate-Peril MA, Cadenas MB, *et al.* The oral bacterial microbiome of occlusal surfaces in children and its association with diet and caries. PLoS One 2017; 12(7)e0180621
[http://dx.doi.org/10.1371/journal.pone.0180621] [PMID: 28678838]

[50] Chedid NR, Bourgeois D, Kaloustian H, Baba NZ, Pilipili C. Caries prevalence and caries risk in a sample of Lebanese preschool children. Odontostomatol Trop 2011; 34(134): 31-45.
[PMID: 22003807]