

## Three months variation of the incipient tooth caries in Japanese school children

Hidenori Yamada, DDS, PhD,<sup>a</sup> Yoshiaki Nomura, DDS, PhD,<sup>a</sup> Shinpei Tsuge, DDS, PhD,<sup>b</sup> and Nobuhiro Hanada, DDS, PhD<sup>a</sup>

<sup>a</sup>Department of Translational Research, Tsurumi University School of Dental Medicine, Yokohama, and

<sup>b</sup>Tsuge Dental Clinic, Gifu, Japan

**Purpose:** To confirm the incipient caries so called white spot can be reversible process of demineralization and remineralization.

**Materials and Methods:** A total of 128 pupils were selected for the analysis. Dentists conducted oral examinations under light using dental mirrors. Images of the labial surface of the maxillary right and left central incisors of all the subjects captured using the quantitative light-induced fluorescence (QLF) systems. Saliva samples were obtained by having subjects chew a gum base for at least 3 minutes that contained no taste or flavor additives. Salivary pH value was evaluated using pH testing paper.

**Results:** In this study, we revealed the 3 months variation of the incipient caries in Japanese school children. Even DMF/DMFT was a low level, over one third of all pupils (37.5%) had incipient caries detected by the quantitative light-induced fluorescence (QLF). After 3 months, 11 pupils (8.6%) had disappeared the incipient caries in central incisors. However, newly incipient caries were appeared in residuary pupils. Finally, a half of all pupils had incipient caries detected.

**Conclusion:** In this study, we revealed the three months variation of the incipient caries in Japanese school children using the quantitative light-induced fluorescence (QLF). We found that 8.6% of the subjects had disappeared the incipient caries. This data shows that the incipient caries is a reversible process of tooth demineralization and remineralization. (*Asian Pac J Dent 2013; 13: 5-10.*)

**Key Words:** demineralization, incipient caries, quantitative light-induced fluorescence, remineralization

### Introduction

With the advances of the knowledge of the dental caries, incipient caries so called white spot or early caries can be reversible disease process of demineralization and remineralization.<sup>1</sup> Dental caries is now considered being as a dynamic process in which multiple factor determine whether process begins, progresses, stops or reverses.<sup>2</sup> The demineralization and remineralization of incipient caries are intimately related and occur based upon the dental biofilm and the availability of the refined carbohydrates for the bacterial fermentation to organic acid. There are varied biological factors in saliva that protect the tooth surface from caries development.<sup>3</sup>

It is now accepted that several strategies can be used for facilitate the remineralization process. It is including treatment such as the concentrated use of topical fluoride, improved oral hygiene, and reducing the number of exposures to the fermentable carbohydrate.<sup>2,3</sup> The prevention, control, and treatment should be based on the appropriate diagnosis and detection of the disease in its earliest stages. Under these conditions, it has been advocated that highly sensitive and accurate method for the detection of dental caries is necessary. In these concepts, the light or fluorescence applied method had been developed.<sup>4,5</sup> Quantitative laser/light-induced fluorescence (QLF) is a technique for the detection, quantification, and longitudinal monitoring of incipient caries. This technique is noninvasive and nondestructive. Some studies have shown that QLF is a highly sensitive method that is suitable for the detection of visually undetected initial caries lesions.<sup>6-9</sup> However, the clinical or epidemiological data by the QLF is not sufficient, and few data is available for the evaluation of the dental caries progression especially without any intervention.

Thus, we conducted an observational survey for the school children during 3 months without intervention. After 3 months, we evaluated the progression or recovery of the incipient caries. We also analyzed the so-called

risk factors for the dental caries including salivary cariogenic bacterial levels.

## Materials and Methods

### Study design

Pupils received a first routine dental examination with QLF and the saliva test. Then, 3 months later the second dental examination was performed. The study population was pupils from 4th grade elementary school children (nine or ten years old) residing in the Gifu Prefecture, Japan. The fluoride concentration of the drinking water in this area is less than 0.8 ppm. Children and parents were informed of the survey in advance at parent-teacher's meeting, and we also obtained informed consent. Among the 147 pupils, 128 pupils (71 males, 55.5% and 57 females, 45.5%) were selected for the analysis. This study was approved by the ethical committee of the Tsurumi University School of Dental Medicine (No. 430, 2007).

### Clinical examination

Dentists conducted oral examinations under light using dental mirrors. Teeth conditions were scored as sound, decayed or filled. The decayed or filled teeth were identified according to the standard method and criteria of Japanese Association of School Dentists. Saliva samples were obtained by having subjects chew a gum base for at least 3 minutes that contained no taste or flavor additives. Salivary pH value was evaluated using pH testing paper (Toyo Roshi, Tokyo, Japan).

### Fluorescence image acquisition

Images of the labial surface of the maxillary right and left central incisors of all the subjects captured using light-induced fluorescence systems. For acquisition of the images, QLF system (QLF/cin 1007, Inspektor Research system B.V. Amsterdam, Netherland) was used. The images of the tooth surface were acquired using a miniature CCD camera inside the hand piece through a 520 nm high pass filter, transmitting only light at wavelength less than 520 nm.

### Image analysis

Average fluorescence loss (%) and size (cm<sup>2</sup>) of white spot lesions were determined by QLF in a manner described by Ando et al. 2001.<sup>10</sup> Fluorescence loss ( $\Delta F$ ; %) and area of the lesion ( $A$ ; mm<sup>2</sup>) and fluorescence loss integrated over the lesion area ( $\Delta Q$ ;  $\Delta F \times A$ ; % x mm<sup>2</sup>) were determined. The presence or absence of initial caries lesions was diagnosed by QLF. Fluorescence radiance level less than 95% of sound surface were considered to be white spot lesions. Another variable  $\Delta Q$  (% , cm<sup>2</sup>) that defined as fluorescence loss integrated over the lesion size was determined.

### Microbial procedures

The saliva samples were vortexed for 10 s and inoculated onto Mitis-Salivarius agar (MS agar, Difco, Tokyo, Japan) medium for total streptococci. We used modified MSB agar medium, which is MS agar (Difco) supplemented with 20% sucrose (Wako Pure Chemicals Co., Osaka, Japan), 20 mg/mL Yeast Extract (Becton Dickinson MD, USA), 0.25 U bacitracin (Sigma, Inc., St. Louis, MO, USA), 10 mg/mL Colistin (Wako Pure Chemicals Co., Osaka, Japan), 10 mg/mL nalidixic acid (Wako), 4 mg/mL gramicidin (Sigma), and 1% tellurite solution for the mutans streptococci. We used Rogosa SL (Nippon Becton Dickinson Company, Ltd. Tokyo, Japan) agar medium for lactobacilli. These media were inoculated using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo, Japan). MS agar and modified MSB agar media were incubated for 48 hours anaerobically. After the anaerobic incubation, we counted the colonies to determine the number of bacteria per ml whole-saliva

on each agar medium using a spiral systems counting grid.

### Statistical Analysis

The descriptive analysis of the baseline characteristic of the subjects participated in this study were calculated separately by male and female. The p-values were calculated by Mann-Whitney U test. The statistically significance of the changes of  $\Delta F$  and  $\Delta Q$  values was calculated by Wilcoxon signed-rank test. To investigate the co-relation of the salivary cariogenic bacterial levels and changes of the  $\Delta F$  values, the subjects were classified into four groups by the changes of  $\Delta F$  values. The p-values were calculated by Kruskal-Wallis test. These analyses were carried out by SPSS 14.0 (SPSS, Tokyo, Japan).

### Results

The baseline characteristics of the oral conditions and the salivary levels of the cariogenic bacteria of the subjects were given in Table 1. The decayed or filled teeth in Table 1 were identified by school dentists. Caries prevalence and mean DMFT in the permanent dentition between male (DMFT 0.23) and female (DMFT 0.26) were remarkably low. The subjects who had at least one DMF were 18 pupils (14.0%).

**Table 1.** Baseline characteristics of the subjects participated in this study

	Female (n=57)		Male (n=71)		p-value*
	Mean	SD	Mean	SD	
Number of remaining teeth	15.51	3.69	14.13	2.44	0.02
Number of sound teeth	15.25	3.51	13.90	2.49	0.02
Number of decayed teeth	0.05	0.23	0.04	0.20	0.78
Number of filled teeth	0.21	0.73	0.18	0.64	0.90
Number of missing teeth	0.00	0.00	0.00	0.00	1.00
DMF	0.26	0.74	0.23	0.70	0.51
Total streptococci (log <sub>10</sub> CFU/mL)	6.56	0.30	6.42	0.84	0.28
Mutans streptococci (log <sub>10</sub> CFU/mL)	3.25	2.00	3.29	1.83	0.66
Lactobacilli (log <sub>10</sub> CFU/mL)	2.39	1.63	2.45	1.68	0.85
5 min stimulated saliva volume	5.49	2.90	6.88	3.79	0.06
Salivary pH	7.49	0.32	7.60	0.30	0.05

\*Mann-Whitney U test

**Table 2.** The cross tabulation of incipient caries at baseline and after 3 months by QLF evaluation

Baseline	After 3 months				Total
	None	Right central incisor (+)	Left central incisor (+)	Both incisors (+)	
None	58 (45.3%)	9 (7.0%)	9 (7.0%)	4 (3.1%)	80 (62.5%)
Right central incisor (+)	4 (3.1%)	23 (18.0%)	0 (0%)	0 (0%)	27 (21.1%)
Left central incisor (+)	1 (0.8%)	0 (0%)	9 (7.0%)	0 (0%)	10 (7.8%)
Both incisor (+)	1 (0.8%)	2 (1.6%)	3 (2.3%)	5 (3.9%)	11 (8.6%)
Total	64 (50%)	34 (26.5%)	21 (16.4%)	9 (7.0%)	128 (100%)

The cross tabulation of with or without incipient caries at baseline and after 3 months were shown in Table 2. Among 128 subjects, eleven pupils (8.6%) had incipient caries in both central incisors which examined, and 37 pupils (28.9%) had incipient caries either in central incisors and 80 pupils (62.5%) had no incipient caries in central incisors. Even DMF/DMFT was a low level, over one third of all pupils (in right 21.1% and in left 7.8%,

in both 8.6%: total 37.5%) had incipient caries detected by QLF.

After 3 months, second dental examination was performed. Eleven pupils (8.6%) had disappeared the incipient caries in central incisors. However, newly incipient caries were appeared in residuary pupils. Finally, a half of all pupils (in right 26.5% and in left 16.4%, in both 7.0%) had incipient caries detected by QLF.

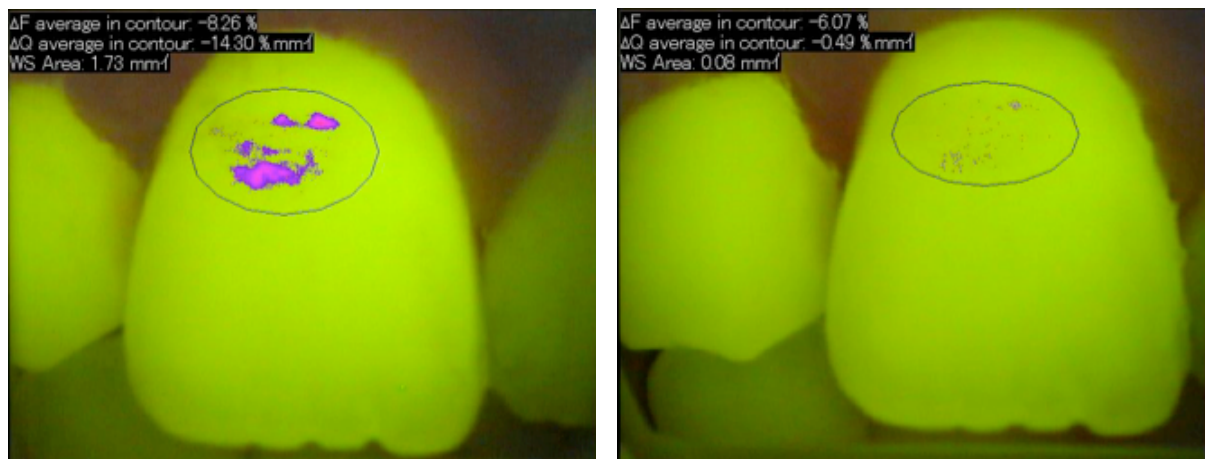
The distribution of the  $\Delta F$  and  $\Delta Q$  values were given in Table 3. The distributions of the both values were highly skewed. The changes of the  $\Delta F$  and  $\Delta Q$  values when compared with the baseline and after 3 months, the changes were statistically significant by Wilcoxon signed-rank test.

**Table 3.** The distribution of  $\Delta F$  and  $\Delta Q$

$\Delta F$	n	$\Delta Q$	n
0	197	0	197
6	7	<0.5	10
7	27	1	10
8	13	2	10
9	2	3	8
10-20	5	4-10	11
<20	5	10<	10

**Table 4.** The relationship between incipient caries and salivary bacteria

Number of bacteria	Cured incipient caries (n=4)	No incipient caries (n=60)	With incipient caries (n=44)	Incidence of incipient caries (n=22)	Total (n=130)	p-value
Lactobacilli (log <sub>10</sub> CFU/mL)	1.63 +/- 1.88	2.62 +/- 1.51	2.38 +/- 1.72	2.11 +/- 1.92	2.42 +/- 1.66	0.469
Mutans streptococci (log <sub>10</sub> CFU/mL)	1.60 +/- 1.94	3.65 +/- 1.64	3.18 +/- 1.89	2.80 +/- 2.31	3.28 +/- 1.89	0.072



**Fig. 1.** Three months variation of the incipient tooth caries. In some cases, drastically reduction of the  $\Delta F$  and  $\Delta Q$  values were observed. **a**, QLF image photographed in a first dental examination. **b**, QLF image photographed in a second dental examination.

The changes of the individual data were photographed and reserved by QLF computer apparatus. In some cases, drastically reduction of the  $\Delta F$  and  $\Delta Q$  values were observed (Fig. 1). Then the subjects classified into four groups: the incipient caries cured in either teeth, no incipient caries in the baseline and after 3 months, with

incipient caries at baseline in either teeth but not cured, and new incidence of incipient caries in either teeth. The effects of the salivary levels of cariogenic bacteria for the changes of the  $\Delta F$  values were investigated. Table 4 showed the relationship between incipient caries and salivary bacteria. Statistically significant effects of the mutans streptococci and lactobacilli were not observed for the changes of  $\Delta F$  value.

## Discussion

The demineralization process of enamel starts when the acids are generated by biofilm bacteria. These acids led to lower the biofilm pH to below pH 5.5. Then, begin the loss of calcium and phosphates from the surface enamel, which creating incipient caries, so called a white spot lesion. According to the school dental health criteria of Japanese Association of School Dentists, a visible white spot lesion is identified as caries for observation (CO). The incipient caries (white spot or CO) is characterized by low calcium and phosphate content and is the initial detectable lesion of enamel demineralization in the surface or subsurface of the tooth. The incipient caries will progress into cavitation if the biofilm is not removed from the tooth surface for a long time.<sup>11</sup> The rate of carious progression is relatively slow, with enamel lesions confined to outer enamel taking 5-6 years to progress through to dentine.<sup>12</sup> Another research shows that progression of caries from the surface of the enamel into dentine, sometimes taking up to four years, and that preventive efforts can reduce the carious lesion progression.<sup>13</sup>

However, the demineralization process is reversible. Saliva, a supersaturated solution of calcium and phosphates, plays a critical role in the process of remineralization. Remineralization of the incipient caries effectively occurs when the biofilm is removed. After removal of biofilm, supersaturated saliva can be easily access to the damaged enamel surface. Treatment of the incipient caries by professional mechanical tooth cleaning<sup>14</sup> or dental drug delivery system (3DS)<sup>15</sup> and their subsequent remineralization has the potential to significantly advance noninvasive clinical management of dental caries.

In this study, we revealed the three months variation of the incipient caries using QLF. We found that 8.6% of the subjects had disappeared the incipient caries within three months. Certainly, fluoride supplies strong and significant effects of the remineralization. In Japan, more than 85% of commercially available toothpaste contained the fluoride. Therefore, without special intervention, proper number of incipient caries will be repaired.

However, new incipient caries were also observed, and number of incipient caries were higher than before. In order to control the incipient caries, three-monthly dental check-ups with QLF inspection will be preferable.

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**Correspondence to:**

Dr. Nobuhiro Hanada

Department of Translational Research, Tsurumi University School of Dental Medicine

2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan

Fax: +81-45-573-2473 E-mail: hanada-n@tsurumi-u.ac.jp

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