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Evaluation of risk factors for dental caries from 6 to 8 years old children

Seiko Katsumura*[1], Fusao Nishikawara*[2], Yoh Tamaki*[2,3,4], Hidenori Yamada*[3], Yoshiki Nakamura*[2,5], Keita Sato*[6], Shinpei Tsuge*[7], Yoshiaki Nomura*[2] and Nobuhiro Hanada*[2]

*[1] Department of Anatomy, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[2] Department of Oral Health, National Institute of Public Health
2-3-6 Minami, Wako, Saitama 351-0197, JAPAN
*[3] Department of Preventive Dentistry and Public Health, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[4] Division of Oral Health, Department of Health Science, Kanagawa Dental College
82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, JAPAN
*[5] Department of Orthodontics, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[6] Department of Forensic Medicine and Dentistry, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN
*[7] Tsuge Dental Clinic
3384-5 Nakanohou-cho, Ena, Gifu 509-8231, JAPAN

Abstract The aim of this study is to evaluate the risk factors for dental caries in young children in Japan. We studied 645 children attending 13 of 30 elementary schools in two areas in Japan. Total salivary level of mutans streptococci and lactobacilli were evaluated at pre-school medical check-ups. Other factors we evaluated included use of fluoride containing dentifrices, administration of fluoride varnish in a private dental office, having regular check-ups, use of mouthwash with fluoride, and daily intake of sweet juice or snacks. Oral examinations were carried out at annual medical check-ups with 2.5 years follow-up and finally 585 children were analyzed. By multivariate logistic regression analysis, only three factors; gender, salivary levels of mutans streptococci and the presence or absence of dental caries in deciduous teeth had a statistically significant correlation with the incidence of dental caries after 2.5 years of follow-up. At age 8, odds ratios were 1.821 for female gender, 1.259 for mutans streptococci ($\log_{10}$cfu/m*l*) and 2.262 for dental caries in deciduous teeth. Although the prevalence of dental caries has declined in Japan, mutans streptococci remain a risk factor.

Key words
Lactobacilli,
Risk factors,
Streptococcus mutans,
Total streptococci

Introduction

The prevalence of dental caries has declined worldwide. Age-related prevalence does not fit a normal distribution because most subjects, especially children, have no dental caries, and because for those with dental caries, most have only one or two. However, there are children with many dental caries and children at high risk[1,2]. Therefore, risk factors for dental caries should be re-examined in order to inform public health strategies for the prevention of dental caries[3].

The etiology of dental caries has been investigated and risk factors have been proposed from both cross-sectional and longitudinal studies[4,5]. Most have been confirmed by epidemiological studies[6,7] which indicate that the presence of oral bacteria such as mutans streptococci (**MS**) and lactobacilli (**LB**) correlate with the prevalence of dental caries[8,9].

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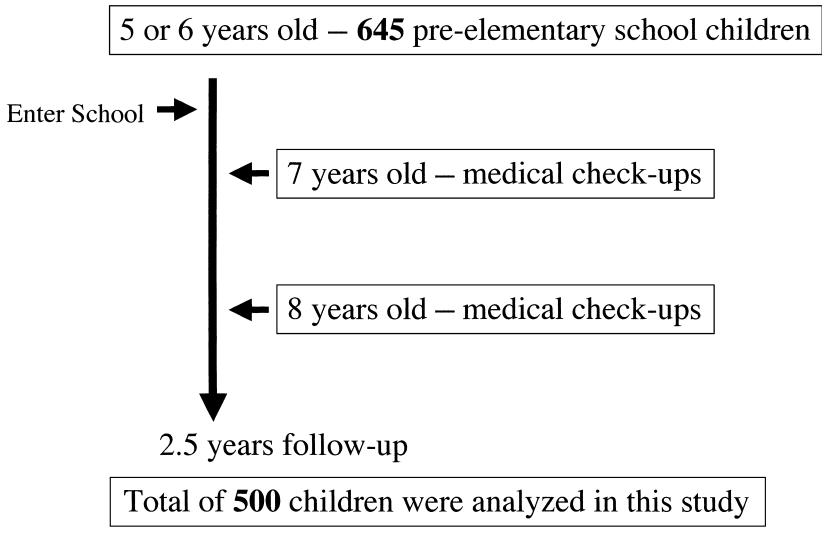

Fig. 1 The design for children was participated in this study

We started the study for 645 pre-school children. There were 125 children dropped out because the moving out or illnesses while for 2.5 follow-ups, total of 500 children were finally analyzed in this study.

Many published reports based on cohort studies demonstrate that MS and LB are risk factors for dental caries[10–12]. Some studies indicate that a history of dental caries and oral levels of MS and their combinations are the best predictors of the incidence of dental caries[5]. Moreover, our previous study showed that salivary levels of MS and LB correlate with the prevalence of dental caries[13]. This may be the reason why cariogenic bacteria may exist in the carious cavity[14] and are released into the saliva. In the present study, we evaluated school children at their annual medical check-up at school. We investigated the incidence of dental caries in each tooth and evaluated risk factors. The aim of this study was to analyze the contribution of these factors to the incidence of dental caries over 2.5 years of follow-up.

Materials and Methods

We planned the prospected cohort study to follow-up the elementary school children for 3 years to investigate the new incidence of the dental caries. This study design was approved by Ethic Committee of Tsurumi University School of Dental Medicine, Approval No. 408.

Study population

The study population was a sample from pre-elementary school children (5 or 6 years old) residing in the Ena and Nakatsugawa areas of Gifu Prefecture, Japan. The fluoride concentration of the drinking water in this area is less than 0.8 ppm based on the water quality standards of Japan Waterworks Law[15]. Thirteen of the 30 elementary schools, nine from the Ena area and four from the Nakatsugawa area were sampled. Children and parents were informed of the survey in the mail that announced their entry into elementary school. A total of 645 children participated in this study. Twelve children dropped out before the base line examinations. After enter school, they had the dental examination in medical check-ups once a year. Finally, we analyzed the results for 500 children were checked-up at all of three times for 2.5 years (Fig. 1). The primary reasons for drop out were relocation and missed check-ups due to illness. We obtained clinical samples and completed questionnaires during the pre-school medical check-ups; we also obtained informed consent. Details are described in our previous report[16].

Clinical examination and clinical samples

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 based on WHO standard method and criteria[17]. Saliva samples were obtained by having subjects chew a gum base for 3 min that contained

Table 1 Descriptive analysis of the prevalence of dental caries at each age

		Age	Male		Female		*P*-value	Total	
			mean	SD	mean	SD		mean	SD
Deciduous teeth	Number of remaining teeth	6	17.000	2.485	16.390	2.476	0.001	16.690	2.491
		7	14.160	2.231	13.220	2.336	<0.001	13.670	2.325
		8	11.890	2.042	10.920	2.498	<0.001	11.400	2.329
	d	6	1.010	1.678	1.110	2.093	0.762	1.060	1.894
		7	0.970	1.651	1.040	1.834	0.796	1.000	1.741
		8	0.980	1.624	0.940	1.722	0.458	0.960	1.671
	f	6	2.680	2.968	2.330	2.745	0.203	2.510	2.872
		7	2.830	2.836	2.450	2.547	0.215	2.640	2.698
		8	3.020	2.723	2.680	2.589	0.154	2.840	2.658
Permanent teeth	Number of remaining teeth	6	4.510	3.247	5.670	3.291	<0.001	5.100	3.313
		7	8.770	2.732	9.660	2.591	<0.001	9.240	2.689
		8	11.300	1.960	12.280	2.468	<0.001	11.790	2.279
	D	6	0.010	0.141	0.030	0.220	0.322	0.020	0.185
		7	0.050	0.300	0.090	0.464	0.594	0.070	0.392
		8	0.090	0.463	0.170	0.606	0.021	0.130	0.540
	M	6	0.000	0.000	0.000	0.000	1.000	0.000	0.000
		7	0.000	0.000	0.000	0.000	1.000	0.000	0.000
		8	0.000	0.000	0.000	0.000	1.000	0.000	0.000
	F	6	0.040	0.298	0.030	0.261	0.790	0.030	0.279
		7	0.030	0.198	0.070	0.373	0.302	0.050	0.300
		8	0.090	0.358	0.130	0.446	0.287	0.110	0.404

D(d): decayed teeth in the permanent teeth (in deciduous teeth), M: missing teeth, F(f): filling teeth
P-values were calculated the significant difference between male and female by Mann-Whitney U tests.

no taste or flavor additives, and salivary pH was evaluated using pH-testing paper (Toyoroshi, Tokyo, Japan).

Microbial procedures

To estimate total streptococci, MS and LB in saliva, we performed microbial procedures according to the method described previously[10,18]. The obtained saliva samples were vortexed for 30 seconds and diluted $1:10^2$–10^4 in phosphate-buffered saline (PBS). Fifty microliters of samples were spread onto Mitis-Salivarius agar (MS: Difco, Tokyo, Japan) medium for total streptococci. We also used modified MSB agar medium[19] which is MS agar (Difco, Tokyo, Japan) supplemented with 20% sucrose (Wako Pure Chemicals Co., Osaka, Japan), 20 mg/m*l* Yeast Extract (Becton Dikinson MD, USA), 0.25 U/m*l* Bacitracin (Sigma, Inc., St. Louis, MO, USA), 10 mg/m*l* Colistin (Wako), 10 mg/m*l* Nalidixic Acid (Wako), 4 mg/m*l* Gramicidin (Sigma) for selective culture of MS, and Rogosa SLagar medium (Nippon Becton Dickinson Company, Ltd., Tokyo, Japan) for LB. These media were inoculated using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo, Japan). After anaerobic incubation for MS and modified MSB agar media for 48 hours, LB for 72 hours, we counted the colonies to determine the number of bacteria per m*l* whole-saliva on each agar medium using a spiral systems counting grid (Spiral Colony Counter; Yoshikawa Kogyo Co. Ltd., Fukuoka, Japan).

Questionnaires

Questionnaires were distributed by mail along with an announcement of the requirement for school participation; questionnaires were collected at pre-school medical check-ups. The questionnaires consisted of five items concerning fluoride use and diet. Fluoride use was evaluated by daily use of fluoride containing dentifrices (yes or no), a experience of

Table 2 Descriptive analysis of the DMF for each age

		Teeth	7			6			5			4			3			2			1		
		Age	6	7	8	6	7	8	6	7	8	6	7	8	6	7	8	6	7	8	6	7	8
Right	Upper	Not erupted	500	500	500	246	62	19	500	498	492	499	492	454	500	500	486	490	364	138	362	110	21
		Sound	0	0	0	250	430	465	0	2	8	1	8	45	0	0	14	10	136	362	137	389	478
		Decayed	0	0	0	2	4	12	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
		Filled	0	0	0	2	4	4	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	Lower	Not erupted	500	500	500	162	37	7	500	500	494	500	497	468	496	496	454	351	125	22	115	17	3
		Sound	0	0	0	332	448	450	0	0	5	0	3	30	4	4	46	149	375	478	385	483	496
		Decayed	0	0	0	3	8	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Filled	0	0	0	3	7	20	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1
Left	Upper	Not erupted	500	500	500	253	69	19	491	366	133	500	499	485	498	496	463	499	499	493	253	69	19
		Sound	0	0	0	240	417	464	9	134	367	0	1	15	2	4	37	1	1	7	240	417	464
		Decayed	0	0	0	1	7	9	0	0	0	0	0	0	0	0	0	0	0	0	1	7	9
		Filled	0	0	0	6	7	8	0	0	0	0	0	0	0	0	0	0	0	0	6	7	8
	Lower	Not erupted	500	500	500	157	40	7	352	118	23	496	496	447	499	493	458	500	498	490	361	119	23
		Sound	0	0	0	336	434	450	148	382	477	4	4	53	1	7	42	0	2	10	139	381	475
		Decayed	0	0	0	3	17	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
		Filled	0	0	0	4	9	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

n = 500, total of children were check-up for 2.5 years. By the result of Table 1, subjects have no missing teeth at each age.

having had a fluoride varnish at a private dental office or at usual health care check-ups (yes or no) and daily use of mouthwash with fluoride (yes or no). The questionnaire on dietary sugar intake consisted of two items: the number instances of daily intake of sweet soft drink, and the daily intake of sweet snacks (once, twice, three times or more than four times).

Statistical analysis

Before the analysis for every check-up results, patients were divided into two groups; those free from dental caries, and those with at least one decayed or filled tooth (df teeth). As with microbiological factors, the bacteriological counts were $\log_{10}$-transformed prior to statistical analysis to normalize the variances. Logistic regression analysis was used to evaluate the crude or adjusted odds ratios and their associated 95% confidence intervals (95% CI). To eliminate confounding factors, multiple logistic regression analysis was used.

Results

In this study, the DMFT (D; Decayed, M; Missing, F; Filled, T; Teeth) index at ages 6, 7 and 8 years were 0.050, 0.120 and 0.230, respectively. The descriptive analysis of the dental caries conditions for both deciduous and permanent teeth is shown in Table 1. At ages 6 and 7, the incidence of dental caries showed no statistically significant differences between male and female ($P = 0.303$ and $P = 0.206$ in permanent teeth). However, at age 8, females had more dental caries compared to males in permanent teeth and the difference was statistically significant ($P = 0.021$). When we compared dental caries in deciduous teeth of females at age 8, index of caries in d, f and df teeth were lower than in males. Table 2 shows a descriptive analysis for dental caries conditions for each tooth. As can be seen in this table, dental caries in incisors, canines and premolars rarely occurred until age 8. Dental caries mainly occurred in the first molar teeth.

To investigate the risk factors for the incidence of dental caries, subjects were divided into two groups: the presence or absence of dental caries for their age. Logistic regression analysis was then carried out to calculate the crude and multivariate adjusted odds ratios. As shown in Table 3, at age 6, no factors were associated with the incidence of new dental caries. At age 7, using the crude odds ratios, oral bacteria such as the salivary level of LB, total streptococci and MS had statistically significant correlations with the incidence of dental caries. At age 8, using the crude odds ratios, salivary pH, the salivary level of LB, total streptococci, MS,

Table 3 Crude odds ratios for the incidence of dental caries by logistic regression analysis

	6 years old				7 years old				8 years old			
	Crude odds ratio	95.0% CI		*P*-value	Crude odds ratio	95.0% CI		*P*-value	Crude odds ratio	95.0% CI		*P*-value
Saliva volume	1.034	0.786	1.361	0.809	1.000	1.000	1.000	0.058	0.887	0.760	1.035	0.129
Salivary pH	0.420	0.044	3.986	0.450	0.858	0.696	1.058	0.152	0.315	0.098	1.009	0.052
Lactobacilli (log)	1.223	0.975	1.534	0.081	1.217	1.048	1.413	0.010	1.171	1.049	1.308	0.005
Total streptococci (log)	2.551	0.376	17.282	0.337	5.129	1.386	18.989	0.014	3.064	1.137	8.252	0.027
S. mutans (log)	1.402	0.983	1.530	0.062	1.259	1.038	1.526	0.019	1.387	1.175	1.637	0.000
S. mutans ratio	1.032	0.986	1.081	0.177	1.027	0.993	1.062	0.116	1.045	1.018	1.072	0.001
Fluoride dentifrice	2.394	0.895	6.401	0.082	0.676	0.335	1.363	0.273	0.882	0.526	1.481	0.636
Fluoride varnish	0.830	0.481	1.430	0.501	0.952	0.672	1.347	0.780	1.055	0.812	1.371	0.688
Fluoride mouth rinse	1.125	0.393	3.220	0.827	1.175	0.583	2.371	0.652	0.936	0.541	1.619	0.812
Juice intake	1.098	0.598	2.017	0.763	1.092	0.726	1.643	0.672	1.370	1.013	1.855	0.041
Sweet snack intake	1.019	0.477	2.177	0.961	0.822	0.486	1.390	0.465	0.991	0.670	1.466	0.965
Regular check-ups	0.217	0.028	1.657	0.141	0.612	0.250	1.499	0.283	0.748	0.394	1.421	0.375
Dental carries in deciduous teeth +/−	0.943	0.326	2.728	0.914	1.944	0.839	4.503	0.121	3.193	1.543	6.608	0.002
Gender	1.370	0.513	3.660	0.529	1.468	0.760	2.835	0.253	1.799	1.080	2.996	0.024

Table 4 Multivariate adjusted odds ratios for the incidence of the dental caries by logistic regression analysis

	6 years old				7 years old				8 years old			
	Adjusted odds ratio	95.0% CI		*P*-value	Adjusted odds ratio	95.0% CI		*P*-value	Adjusted odds ratio	95.0% CI		*P*-value
Saliva volume	1.213	0.881	1.670	0.236	0.990	0.782	1.253	0.933	1.007	0.839	1.209	0.940
Salivary pH	2.222	0.190	25.641	0.524	6.369	1.178	34.483	0.031	2.331	0.604	9.009	0.220
Lactobacilli (log)	1.142	0.864	1.511	0.351	1.152	0.964	1.376	0.119	1.013	0.887	1.158	0.847
Total streptococci (log)	1.594	0.188	13.494	0.669	1.859	0.438	7.893	0.401	1.095	0.352	3.406	0.875
S. mutans (log)	1.337	0.868	2.060	0.188	1.096	0.880	1.364	0.413	1.259	1.045	1.517	0.015
Fluoride dentifrice	2.402	0.863	6.688	0.093	0.614	0.293	1.288	0.197	0.919	0.529	1.596	0.764
Fluoride varnish	0.803	0.451	1.428	0.455	0.914	0.631	1.323	0.633	0.994	0.749	1.318	0.965
Fluoride mouth rinse	1.122	0.369	3.409	0.839	1.184	0.583	2.403	0.641	0.892	0.511	1.556	0.687
Juice intake	1.162	0.600	2.254	0.656	1.092	0.699	1.706	0.699	1.353	0.969	1.890	0.076
Sweet snack intake	0.751	0.314	1.796	0.519	0.686	0.380	1.237	0.210	0.778	0.499	1.213	0.268
Regular check-ups	0.300	0.037	2.432	0.260	0.803	0.307	2.101	0.655	1.024	0.497	2.112	0.948
Dental carries in deciduous teeth +/−	0.454	0.130	1.588	0.216	1.117	0.435	2.870	0.818	2.262	1.024	4.996	0.043
Gender	1.193	0.418	3.402	0.741	1.247	0.624	2.492	0.533	1.821	1.048	3.162	0.033

juice intake, the presence or absence of dental caries in deciduous teeth, and gender, had statistically significant correlations with the incidence of new dental caries. Table 4 shows the multivariate-adjusted odds ratios by results of logistic regression analysis of the risk factors for dental caries. At age 6, no factors were associated with the incidence of new dental caries. At age 7, salivary pH had a statistically significant correlation with the incidence of new dental caries. At age 8, by multivariate adjusted odds ratios, the salivary level of MS, the presence or absence of dental caries in deciduous teeth and gender, had statistically significant correlations with the incidence of new dental caries.

Discussion

Mutans streptococci have been regarded as a key factor in the incidence of dental caries[20]. Previous studies showed that subjects having high MS and LB levels in saliva are at high risk for dental caries[21]. In our study, we confirmed the importance of MS

and LB as a risk factor for dental caries in children and our results are consistent with other reports[1,8] although a part of the results might be due to the more skewed distribution of subjects in df or DMF indexes for MS and LB levels in salivary level.

Two of epidemiological studies have used the commercially available kit, Dentocult SM™ [22,23], this method is very convenient. However, accuracy for detecting MS is questionable. In our study, we used modified MSB agar medium, because combinational used Dentocult SM was semi quantitative. The precise revaluation was necessary for our analysis. Growth of MS is higher than that in conventional MS containing 0.25 U Bacitracin (MSB) agar medium[24]. In young children, early colonization of MS and plaque accumulation on anterior buccal surfaces[25,26] have been shown to be strongly associated with caries development. Similar research has found that MS in plaque using Dentocult SM™ and past caries experiences at base line were the best predictors for the incidence of new dental caries in children[11]. Information about fluoride use at age 2 years was thought to measure habits affecting dental health within the family, and consequently, to be associated with development of caries. Our finding that fluoride has no predictive value for caries is in line with results found for 1 to 3 year olds[27]. The correlation between fluoride usage and the new incidence of dental caries is not statistically significant. Although this result disagrees with findings on fluoride-induced prevention of dental caries in some reports[5,28] it is consistent with other studies[29,30].

By the results in this study, we agree the suggest that high consumption of carbonated soft drinks is a risk indicator for dental caries in young children and should be discouraged[31]. Prediction models have also been based on other factors such as daily candy intake or sugar-containing soft drinks[32]. However, the ability of fluoride to prevent dental caries was definite.

And in this study, gender has statistically significant correlations with the incidence of new dental caries, its reason was that girls grow up earlier than boys, and permanent teeth of girl have early the risk for dental caries. The caries in deciduous teeth had also the correlations with the incidence of new dental caries by this results, it's necessary the prevalence of dental caries in young children.

In conclusion, the results of our study are consistent with many longitudinal studies on caries and salivary MS and our results suggest that these tests may be useful for predicting caries in primary and mixed dentition.

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