

A survey on the risk factors for the prevalence of dental caries among preschool children in Japan

Yoshiaki Nomura^{1,2}, Shinpei Tsuge³, Masaki Hayashi³, Masahiro Sasaki³, Tetsuya Yamauchi³, Nobuyoshi Ueda³ and Nobuhiro Hanada¹

¹) Department of Oral Health, National Institute of Public Health
1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, JAPAN

²) Department of Preventive Dentistry and Public Health, Tsurumi University School of Dental Medicine
2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, JAPAN

³) Gifu Dental Association
1-8 Kano-Jounan-dori, Gifu-shi, Gifu 500-8486, JAPAN

Abstract The etiology of dental caries has been suggested to be multifactorial. We conducted a cross-sectional study to investigate the contribution of the risk factors for dental caries, surveying a total of 645 preschool children at medical check-ups. Among 10 factors investigated in this study, salivary flow, amount of Lactobacillus, amount of total Streptococci, amount of mutans streptococci, and daily number of times of sweet soft drinks correlate with the prevalence of dental caries. Multivariate Logistic regression analysis using the five factors that correlated produced only two factors, salivary levels of mutans streptococci and Lactobacillus, which correlated with the prevalence of dental caries. Furthermore, clear dose-response relationships were observed in these two factors. We therefore suggest that cariogenic bacteria are the most important risk factor for dental caries among preschool children in Japan.

Key words

Lactobacilli,
Mutans streptococci,
Preschool children,
Risk factors,
Saliva

Introduction

Dental caries has been suggested to be a multifactorial disease. Keyes has proposed three main factors in the etiology of dental caries: host, substrate, and microflora¹. Furthermore, the mechanism of dental caries was theoretically explained². Some clinical trials have shown that controlling these factors suppresses the incidence of dental caries. For the host factors, sodium fluoride has been suggested as useful for preventing dental caries both clinically and economically³. For the substitute factors, diet sugar consumption is correlated with the incidence of new dental caries^{4,5}, and restricting the intake of diet sugar has been suggested to reduce the incidence of dental caries⁶. Recently, xylitol has been shown to be useful as an alternative sucrose, and its efficiency was confirmed⁷. For microflora

factors, plaque control has conventionally been used as the primary preventive method. Many clinicians have used plaque control as a tool for fighting both dental caries and also periodontal disease. Furthermore, application of anti-microbial drugs could reduce the number of mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*) in saliva or plaque for a few months⁸.

These factors are all correlated with the etiology of dental caries. However, the weight of these factors contributing to the prevalence or incidence of dental caries has not been clarified. These factors may confound each other in attempts to explain the etiology of dental caries, and some studies have shown that controlling only one of these factors could not suppress the incidence of dental caries completely.

In this study, we obtained clinical samples and information about the etiology of dental caries by questionnaires at preschool medical check-ups. The aim of this study was to analyze the contribution

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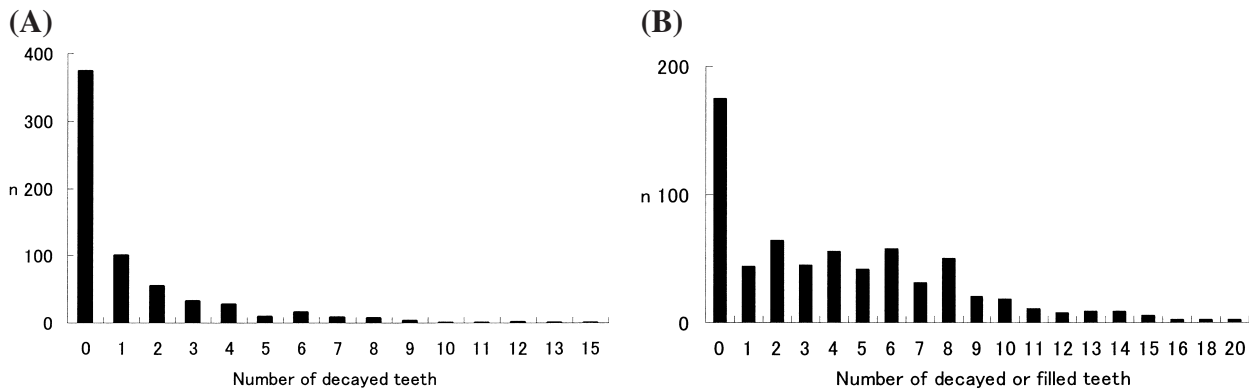


Fig. 1 The distribution of dt and dft

The distribution of the number of decayed deciduous teeth (A) and decayed or filled teeth (B) in this study. The median of the decayed teeth was 0 and decayed or filled teeth was 3. With the erupted permanent teeth, no decayed or filled teeth was observed.

of these factors in children by multiple Logistic regression analysis to clarify their importance.

Materials and methods

Study population

The study population was sampled from pre-elementary school children (five or six years old) residing in the Ena and Nakatsugawa areas of Gifu Prefecture, Japan. The fluoride concentration of drinking water in this area is less than 0.8 ppm. Thirteen of the thirty elementary schools, 9 from Ena area and 4 from Nakatsugawa, were selected to sample the population. Children were informed of the survey by the letter from the municipal office announcing their entry into elementary school and a total of 645 children participated in this study. Twelve children dropped out, primarily because of relocation and missing check ups due to illness. We obtained clinical samples and questionnaires during the pre-school medical check-ups; we obtained informed consent at the collection of the questionnaires.

Clinical examination and clinical samples

Dentists conducted oral examinations under a light and using dental mirrors. Teeth examined for dental caries were scored as sound, decayed or filled. The decayed or filled teeth were identified according to the WHO standard method and criteria⁹.

Saliva samples were obtained by having subjects chew a gum base that contains no taste or flavor additives for 3 mins. The 3-min stimulated salivary flow and salivary buffering capacity were evaluated by pH testing paper (Toyoroshi, Tokyo, Japan).

Microbial procedures

To quantify the total Streptococci, mutans streptococci, and Lactobacilli in saliva, we performed microbial procedures according to the method described previously¹⁰. Saliva samples (50 μ l) were sonicated by ultrasonic dispersion (60 power output) for 10 seconds and poured onto Mitis-Salivarius agar (MS, Gibco, Tokyo, Japan) plates for total Streptococci, improved Mitis-Salivarius agar plates containing 0.02 M bacitracin (Wako, Osaka, Japan) (MSB) and 2 μ g/ml of Gramidine¹¹ for mutans streptococci and Rogosa SL agar plates (Nippon Becton Dickinson Company, Ltd., Tokyo, Japan) for Lactobacilli using an EDDY JET spiral system (Gunze Sangyo, Inc., Tokyo, Japan). In the improved MSB plate, the growth of the mutans streptococci are higher than that in the conventional MSB plate¹². All samples were then incubated for 48 hours anaerobically. After the anaerobic incubation, we counted the colonies on each agar plate and calculated the number of bacteria per ml whole-saliva.

Questionnaires

Questionnaires were distributed by mail with the announcement of school participation and collected at preschool medical check-ups. The questionnaires consisted of five items concerning fluoride usage and diet. Fluoride usage was evaluated by daily usage of fluoride containing dentifrices (yes or no), the experience of fluoride varnish at private dental office or usual health care check-ups (yes, experienced or never) and daily use of mouthwash with fluoride (yes, experienced or never). The questionnaire on

Table 1 Descriptive analysis of each factors

	This study		National average in Japan
	Mean	SD	
Number of decayed teeth	1.25	2.19	2.02
Number of filled teeth	2.90	3.26	2.37
Number of df teeth	4.15	4.01	4.38
Salivary flow (ml/3 mins)	2.96	1.74	—
Salivary pH	7.276	0.198	—
Total Streptococci (CFU/ml, log ₁₀ count)	6.887	0.261	—
Lactobacillus (CFU/ml, log ₁₀ count)	2.561	2.35	—
mutans streptococci (CFU/ml, log ₁₀ count)	4.208	2.305	—

Mean and standard deviation of the data from the oral examination and bacterial cultures obtained in this study. The mean number of decayed or filled teeth was below the average of a national survey in Japan. The data of the national average of Japan were obtained from The Survey of Dental Diseases by Health Policy Bureau Ministry of Health and Welfare Japan (1999).

diet sugar intake consisted of two items, the number of daily intakes of sweet juice and the daily intake of sweet snacks (once, twice, three times or more than four times).

Statistical analysis

Before the analysis, patients were divided into two groups: subjects free from dental caries and subjects with at least one decayed or filled tooth (df teeth). For the high risk children, subjects were divided into two groups by the 75th percentile of the distribution by of the df teeth. As with microbiological factors, the bacteriological counts were log₁₀-transformed prior to statistical analysis to normalize the variances. After evaluation of the distribution, amount of the mutans streptococci and Lactobacillus were categorized into four groups by the 25th, 50th and 75th percentile of the distribution.

Logistic regression analysis was used to evaluate the crude or adjusted odds ratios and their associated 95 percent confidence intervals. To eliminate the confounding factors, multiple Logistic regression analysis was used for factors correlated to the prevalence of dental caries. To confirm the dose-response relationships, the final factors correlated with the prevalence of the dental caries were classified according to the distribution.

Then two-way ANOVA was used to investigate the co-effect for the mutans streptococci and Lactobacillus for the df teeth.

Results

Forty-two percent of the children participating in

Table 2 Results of the data obtained from questionnaires in this study

	n	%
Usage of fluoride containing dentifrice		
Yes	404	61.1%
No	245	37.1%
Experience of fluoride varnish		
Regularly	382	57.8%
Experienced	235	35.6%
Never	33	5.0%
Experience of fluoride mouth rinse		
Regularly	38	5.7%
Experienced	108	16.3%
Never	495	74.9%
Sweet soft drink intake (daily)		
Once	325	49.2%
Twice	228	34.5%
Three times	79	12.0%
Four or more times	17	2.6%
Sweet snack intakes (daily)		
Once	34	5.1%
Twice	393	59.5%
Three times	198	30.3%
Four or more times	24	3.7%

this study had decayed teeth, and 73.1% had df teeth. The distribution of results is shown in Fig. 1. Table 1 shows the mean and SD of the number of the decayed, filled, and df (decayed or filled) teeth, and the salivary flow, salivary pH and salivary levels of the bacteria investigated in this study. Table 2 shows the categorized results from questionnaires. Salivary levels of mutans streptococci were not detected

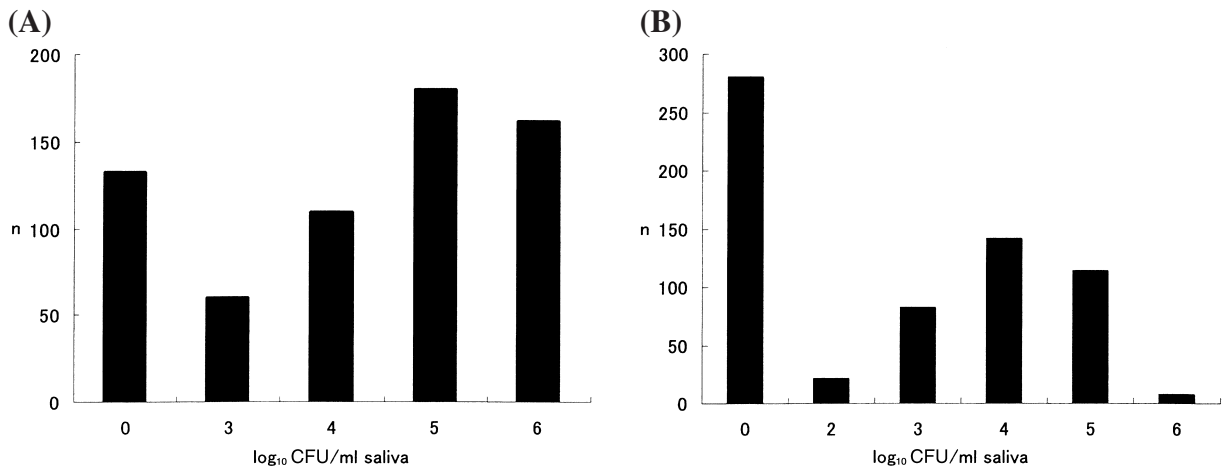


Fig. 2 The distribution of the microflora

The distribution of the mutans streptococci (A) and Lactobacillus (B) in this study. 20.6% of children could not detect mutans streptococci from their saliva, however, from 25.1% of children, mutans streptococci could be detected more than 10⁶ CFU/ml saliva.

Table 3 The odds ratios for the dental caries

(A)	Crude odds ratio	95% CI	P-value	Adjusted odds ratio	95% CI	P-value
Salivary flow rate	0.753	0.605–0.936	0.011	0.866	0.677–1.107	0.249
Salivary pH	0.607	0.250–1.477	0.272			
Lactobacillus (log ₁₀ count)	1.643	1.449–1.864	<0.001	1.405	1.222–1.617	<0.001
Total Streptococci (log ₁₀ count)	1.835	1.334–2.523	<0.001	1.156	0.806–1.658	0.431
mutans streptococci (log ₁₀ count)	1.406	1.297–1.525	<0.001	1.246	1.135–1.368	<0.001
Dentifrice containing fluoride	0.837	0.585–1.196	0.328			
Fluoride varnish	1.343	0.570–3.164	0.500			
Fluoride mouth rinse	0.500	0.205–1.219	0.127			
Juice intake	1.409	1.107–1.794	0.005	1.236	0.950–1.607	0.114
Sweet snack intake	1.106	0.839–1.459	0.475			

(B)	Crude odds ratio	95% CI	P-value	Adjusted odds ratio	95% CI	P-value
Salivary flow rate	0.640	0.410–0.999	0.049	0.651	0.402–1.054	0.081
Salivary pH	0.323	0.086–1.206	0.093			
Lactobacillus (log ₁₀ count)	1.566	1.290–1.900	<0.001	1.342	1.082–1.663	0.007
Total Streptococci (log ₁₀ count)	1.821	1.158–2.864	0.009	1.197	0.723–1.981	0.485
mutans streptococci (log ₁₀ count)	1.771	1.354–2.315	<0.001	1.378	1.029–1.874	0.032
Dentifrice containing fluoride	0.879	0.506–1.527	0.647			
Fluoride varnish	1.031	0.666–1.595	0.892			
Fluoride mouth rinse	0.521	0.215–1.256	0.150			
Juice intake	1.247	0.915–1.700	0.163			
Sweet snack intake	1.251	0.830–1.884	0.284			

Crude and multivariate adjusted odds ratios for subjects with df teeth or not (A) and for the high risks (B). Odds ratios were calculated by Logistic regression analysis. P-values were calculated by the Wald test. Among the 10 factors investigated in this study, only two factors such as salivary levels of mutans streptococci and Lactobacillus had statistically significant correlation with the dental caries conditions.

Table 4 Dose-response relationships of the odds ratios

		with or without dental caries				high risks		
		n	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
(A)								
	0	280	reference group			reference group		
	0<log ₁₀ CFU/ml<10 ⁴	103	3.112	1.809–5.355	<0.001	1.938	1.003–3.746	0.049
	10 ⁴ <log ₁₀ CFU/ml<10 ⁵	141	4.057	2.429–6.774	<0.001	4.331	2.515–7.457	<0.001
	10 ⁵ <log ₁₀ CFU/ml	121	6.812	3.587–12.938	<0.001	6.327	3.665–10.992	<0.001
(B)								
	0	133	reference group			reference group		
	0<log ₁₀ CFU/ml<10 ⁵	170	2.824	1.753–4.549	<0.001	1.139	0.552–2.315	0.725
	10 ⁵ <log ₁₀ CFU/ml<10 ⁶	180	3.651	2.248–5.929	<0.001	2.063	1.060–4.015	0.033
	10 ⁶ <log ₁₀ CFU/ml	162	11.746	6.162–22.390	<0.001	5.649	2.981–10.706	<0.001

Dose-response relationships of the salivary levels of Lactobacillus (A) and mutans streptococci (B) for dental caries. Clear dose-response relationships were observed in this study.

Table 5 Cross table of the number of df teeth by the salivary levels of mutans streptococci and Lactobacillus

		mutans streptococci				Total	
		0	0<log ₁₀ CFU/ml<10 ⁵	10 ⁵ <log ₁₀ CFU/ml<10 ⁶	10 ⁶ <log ₁₀ CFU/ml		
Lactobacillus	0	mean ± SD	1.943 ± 2.917	2.471 ± 2.917	3.303 ± 3.371	4.048 ± 3.498	2.586 ± 3.148
		n	107	85	67	21	280
	0<log ₁₀ CFU/ml<10 ⁴	mean ± SD	3.250 ± 2.990	3.447 ± 3.244	4.108 ± 3.478	7.077 ± 5.327	4.147 ± 3.820
		n	8	45	37	13	103
	10 ⁴ <log ₁₀ CFU/ml<10 ⁵	mean ± SD	3.636 ± 4.501	4.516 ± 4.007	4.578 ± 3.621	7.250 ± 3.832	5.434 ± 4.058
		n	11	32	47	51	141
	10 ⁵ <log ₁₀ CFU/ml	mean ± SD	2.857 ± 2.642	6.000 ± 3.640	5.103 ± 3.880	7.080 ± 4.341	6.277 ± 4.276
		n	7	8	29	75	121
	Total	mean ± SD	2.212 ± 3.070	3.280 ± 3.410	4.096 ± 3.610	6.726 ± 4.312	4.139 ± 3.989
		n	133	170	180	162	645

Dose-response relationship was observed by the mutans streptococci and Lactobacillus for df teeth. By two-way ANOVA analysis, P-values for the mutans streptococci was <0.001 and Lactobacillus <0.001. However, P-values for the mutans streptococci* Lactobacillus was 0.769, so interaction of the mutans streptococci and Lactobacillus for the number of df teeth was not observed in this study.

from 20.6% of the children, and salivary levels of Lactobacillus were not detected from 43.4% of the children (Fig. 2). However, more than 10⁶ CFU/ml salivary mutans streptococci were detected from 25.1% of children.

To correlate the prevalence of the number of df teeth and risk factors investigated in this study, we

performed Logistic regression analysis to calculate the odds ratio for df teeth. Table 3 shows the result of the crude odds ratios. Among 10 factors investigated in this study, salivary flow, amount of Lactobacillus, amount of total Streptococci, amount of mutans streptococci and daily number of sweet soft drinks taken were correlated with the prevalence

of dental caries. To eliminate the confounding factors, we performed multivariate Logistic regression analysis using the six factors that correlated with the dental caries. Table 3 shows the results of multivariate Logistic regression analysis. Only two of the five factors, amounts of mutans streptococci and Lactobacillus, were correlated with the prevalence of dental caries. For the medians of the decayed teeth, multivariate adjusted odds ratio were 1.259 by Lactobacillus and 1.105 by mutans streptococci; for the medians of the decayed or filled teeth, were 1.236 by Lactobacillus and 1.160 by mutans streptococci. The same tendencies were observed in the crude odds ratio if the children were divided into groups with or without decayed teeth or divided into two groups by the 75th percentile of the decayed or filled teeth (Table 3-B). To confirm the dose-response relationships, we categorized these factors by the distribution and then performed Logistic analysis again. Table 4 shows the dose-response relationships for these factors. Clear dose-response relationships were observed for the salivary levels of mutans streptococci and Lactobacillus. Then to check the co-effect of the mutans streptococci and Lactobacillus for dental caries, two-way ANOVA analysis was carried out. As shown in Table 5, no co-effects were found in mutans streptococci and Lactobacillus for dental caries.

Discussion

The etiology of dental caries has been suggested to be classified into three main categories, and this has been confirmed by laboratory investigation and clinical studies. In particular, mutans streptococci and Lactobacillus have been intensively studied as microflora factors. The nature of the acid production and biofilm formation by cariogenic microorganism has been studied^{13,14}. However, clinical studies have shown that some subjects have decayed teeth even though these bacteria were below detection levels in the oral cavity^{15,16}. These results have shown that the etiology of the dental caries cannot be explained easily. In contrast, some studies have shown that, as a substrate factor, dietary intervention to restrict the sucrose consumption has reduced the prevalence or incidence of the dental caries⁶. However, in this study, no correlation was found in daily sucrose consumption and the prevalence of the decayed teeth, filled teeth or df teeth. This may be because the method of surveying sucrose consumption by

questionnaire does not reflect the actual conditions for the substitute factors. However, in present, there is no other method to survey for these factors.

The difference in the results may thus be due to the method of surveillance.

This study found no correlation in fluoride usage by dentifrice and mouth rinse and varnish. Clearly, fluoride has been used to reduce the prevalence of dental caries. In this study, the institutions that applied the fluoride were varied. This may also be due to the methods of the investigation. The method of the fluoride application and intervals of application may be reflected in the result.

Some studies have shown that salivary flow rate and salivary buffering capacity or salivary pH contribute to the incidence of dental caries^{17,18}, while others found no correlation between these factors and dental caries^{16,19}. In this study, we could not find any statistically significant correlation between dental caries prevalence and salivary factors. It is generally considered that salivary flow rate was affected by the side effects of medication or systemic diseases. In general, the salivary flow rate of children is high, and few children take drugs affecting the sympathetic nerve system and reducing the salivary flow rate. Sgan-Cohen *et al.* found a significant correlation of the salivary flow and dental caries, however the correlation was weaker for other factors such as microflora²¹. This may be the main reason for the contradictory result.

In conclusion, of the three main factors suggested by Keyes, the microflora factors are strongly correlated with the prevalence of dental caries for the preschool children in Japan. In the future, controlling this factor for the cohort may lead to strong strategies for preventing dental caries through community-based prevention programs.

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