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Case Report

Correlation of cariogenic bacteria and dental caries in adults

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Abstract: Many studies suggest that mutans streptococci (MS), Lactobacillus (LB), and salivary buffering capacity are important risk factors for dental caries. However, target populations for most studies were children. In adult patients, the same risk factors affect the number of fillings or prostheses or secondary caries. It is therefore important to investigate these risk factors as predictors of caries in adults. In the present study, we evaluated the oral conditions of adult subjects at private dental offices using bite-wing radiographs. Detection of salivary LB level using Dentocult LB had a statistically significant correlation with the number of flat surface caries and approximal caries ($P < 0.001$). Detection of salivary MS level using Dentocult MS and salivary buffering capacity did not predict dental caries. Thus, detection of salivary LB level using Dentocult LB may be a useful tool for detecting approximal and secondary caries. (J. Oral Sci. 48, 245-251, 2006)

Keywords: adults; dental caries; mutans streptococci; Lactobacillus.

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Introduction

Epidemiological studies of risk factors for dental caries have focused mainly on salivary levels of cariogenic bacteria as predictors (1-3). It has been suggested that salivary levels of mutans streptococci (MS) and Lactobacillus (LB) correlate with the number of decayed teeth or the number of decayed, filled or missing teeth (DMF) (4). The group MS includes *Streptococcus mutans (S. mutans)* and *Streptococcus sobrinus (S. sobrinus)* and their virulence factors are well known as they exist within an oral biofilm on the tooth surface. Key virulence factors are acidogenicity, acid tolerance and synthesis of water insoluble glucan from sucrose (5-7). Meanwhile, LB does not avidly colonize teeth surfaces, but they may be transiently found in the oral cavity even before teeth erupt. They preferentially colonize the dorsum of the tongue and are carried into saliva by the sloughing of the tongue epithelium (8). Their numbers in saliva appear to reflect the consumption of simple carbohydrates by the host (9,10). LB is highly acidogenic in the presence of carbohydrates, they are acid tolerant (11), and they are often isolated from established carious lesions (12). Some LB strains are cariogenic in experimental animals. Their cariogenicity is dependent upon the consumption of carbohydrate rich diets by the animal (13). It is also known that large amounts of MS and LB inhabit caries lesions (14). However, a previous study reported that the detection of MS was site specific, while LB was rarely isolated, in

early caries (15). Commercially available chair side kits, Dentocult SM and Dentocult LB, have often been used to estimate levels of these bacteria to evaluate their ability to serve as a predictor of dental caries (16,17).

Based on the results of these kits, many studies have predicted the future incidence of dental caries (18-20). However, few studies have shown a correlation between the results of these kits and presence of dental caries (4).

Most study populations for prediction were children (21-23), while few studies included adults (24,25). This could be due to the fact that the mechanisms of pathogenesis in adults were so much more complicated than children. In adults, secondary caries and root caries occurred more frequently than primary caries. Moreover, smoking and medication which affect the salivary flow, may increase the risk of dental caries (26,27). Most studies evaluating oral levels of these bacteria were carried out as field studies. Under such conditions as mass check-ups, precise evaluation of dental caries is impossible. In children and adults, there was no difference in the proportion of LB in saliva (8). Therefore, adults may have a similar caries risk to that of children. Moreover, root caries in elderly people are due to their having prosthetic crowns, or non-use of interdental brushes and dental floss (19). Also, in adults, oral conditions are affected by restorations and prostheses. This may make it difficult to evaluate the incidence of new dental caries and dental caries already existing as secondary caries. In our study, dental caries were more precisely evaluated at dental offices with the aid of radiographs. The aim of this study was to evaluate the determination of salivary MS level using Dentocult MS, salivary LB level using Dentocult LB and buffering capacity as clinical indicators of the present dental caries condition, especially in adult patients with complicated oral conditions.

Materials and Methods

Study population and oral examination

The study population consisted of patients attending private dental offices for the treatment of caries. We enrolled 152 adult subjects (67 males and 85 females), who were generally healthy and had good oral conditions. The mean age was 36.1 ± 12.6 years old (age range: 20 to 63 years old). The subjects were informed about the aim of this study well in advance and signed consent forms. At the first visit, the number of decayed, missing and filled teeth (number of DMF teeth for adults) was recorded for each subject after oral examination using light and exploration using dental mirrors according to WHO criteria (28). Levels of dental plaque were evaluated by O'Leary's Plaque Control Record (PCR) with the aid of disclosing agents. Full mouth oral radiographs were taken using the bitewing method to precisely detect approximal caries (29,30). In Japan, conventionally, dental caries are classified according to their progression. We used these criteria and assigned subjects into one of two groups; CA: those who showed demineralization of enamel surfaces and dentinal caries CB: those who showed dental caries extending into the pulp and tooth stumps. We classified dental caries into flat surface caries and approximal caries. The number of teeth involved and the number of surfaces analyzed were correlated with the oral levels of cariogenic bacteria.

Evaluation of cariogenic bacteria and buffering capacity

Stimulated whole saliva samples were obtained from subjects by chewing for 5 min on wax blocks contained in the commercial kits. Salivary levels of MS and LB were estimated by Dentocult SM and Dentocult LB (Orion Diagnostica Co. Ltd, Epsom, Finland), respectively. The time of incubation for Dentocult SM was 48 hours and that for Dentocult LB was 96 hours. Evaluation of MS and LB levels was done using the model chart in the instruction manual. Salivary buffering capacity was evaluated using Dentobuff strips (Orion Diagnostica Co. Ltd, Epsom, Finland).

Statistical analysis

Kruskal-Wallis tests were used to evaluate the correlation between oral conditions and levels of cariogenic bacteria or salivary buffering capacity. We classified dental caries into several groups: primary caries or secondary caries, flat surface caries or approximal caries. Logistic regression analysis was used to evaluate the odds ratios of these factors for dental caries. Multiple logistic regression analysis was carried out to simultaneously evaluate the correlation of each dental caries condition. All of these analyses were carried out using SPSS software Ver. 14.0 (SPSS Co. Ltd Tokyo, Japan).

Results

The correlation between salivary levels of MS, LB, salivary buffering capacity and the number of decayed teeth, missing teeth, or teeth with fillings (DMFT) was determined. As shown in Table 1, determination of salivary MS level using Dentocult MS was significantly correlated with PCR (P = 0.016). We observed a tendency for the DMFT score to increase with an increase in the salivary levels of MS. Salivary LB level determined using Dentocult LB showed statistically significant correlations with PCR (P = 0.007), DMFT (P = 0.020), number of decayed teeth (P = 0.018) and number of approximal caries (P = 0.005) (Table 2). Similar to MS, we observed a tendency for the

DMFT score to increase with an increase in LB. Salivary buffering capacity had a significant correlation with the number of flat surface caries ($P = 0.004$) and approximal caries ($P = 0.026$) (Table 3).

Table 1 Correlation of oral conditions and detection of salivary MS level using Dentocult MS

MS	0 (n=31)		1 (n=40)		2 (n=56)		3 (n=30)		Total (n=157)		
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	*P*-value
Age at the first visit	36.770	11.514	33.670	11.439	38.360	13.145	37.670	13.535	36.720	12.518	0.385
D	3.700	4.268	3.900	3.078	4.050	3.009	4.900	3.913	4.110	3.450	0.381
M	2.300	2.959	1.130	1.870	1.840	2.192	1.550	2.501	1.680	2.343	0.221
F	8.520	5.381	10.600	6.054	11.180	5.809	11.760	5.540	10.660	5.795	0.069
DMFT	14.520	6.606	15.620	7.228	17.000	6.697	18.210	7.078	16.420	6.943	0.129
Total of flat surface caries	2.000	4.405	2.400	4.528	2.790	4.111	2.330	4.727	2.450	4.367	0.398
Total of approximal caries	3.920	6.202	6.100	6.476	6.480	7.527	5.970	6.184	5.780	6.771	0.232
PCR	32.160	18.664	34.410	14.700	42.280	20.838	44.560	21.578	38.710	19.623	0.016

Scores were evaluated for salivary levels of MS using the chart of the Dentocult SM kit.
Score 0 or 1: $< 10^5$ CFU/ml, Score 2: 10^5-10^6 CFU/ml, Score 3: $10^6 <$ CFU/ml
No statistically significant correlations were found for primary and secondary caries.
DMFT scores increased with increases in salivary MS level determined using Dentocult MS. Detection of salivary MS level using Dentocult MS was significantly correlated with PCR record but not other variables.

Table 2 Correlation of oral conditions and detection of salivary LB level using Dentocult LB

LB	0 (n=73)		1 (n=32)		2 (n=40)		3 (n=12)		Total (n=157)		
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	*P*-value
Age at the first visit	35.790	11.976	38.590	14.036	36.980	13.077	36.500	10.282	36.720	12.518	0.882
D	3.590	3.453	3.940	3.043	4.210	3.019	7.250	4.393	4.110	3.450	0.018
M	1.570	2.097	1.550	1.997	2.050	2.964	1.420	2.392	1.680	2.343	0.929
F	10.320	5.669	10.000	4.796	11.180	6.117	12.670	7.750	10.660	5.795	0.481
DMFT	15.420	6.959	15.480	5.732	17.440	7.319	21.330	6.679	16.420	6.943	0.020
Total of flat surface caries	1.120	2.273	2.190	3.042	2.720	3.651	10.250	9.206	2.450	4.367	0.000
Total of approximal caries	4.510	5.513	5.000	5.105	5.950	6.683	15.080	10.604	5.780	6.771	0.005
PCR	33.300	18.674	42.520	24.204	43.080	16.866	46.880	11.821	38.710	19.623	0.007

Scores were evaluated for salivary levels of LB using the chart of the Dentocult LB kit.
Score 0: 10^3 CFU/ml, Score 1: 10^4 CFU/ml, Score 2: 10^5 CFU/ml, Score 3: $10^6 <$ CFU/ml
There were no statistically significant correlations between LB levels and primary and secondary caries.
DMFT scores increased with increases in detection of salivary LB level using Dentocult LB. Determination of salivary LB level using Dentocult LB showed significant correlations with PCR, DMFT, number of decayed teeth, and total number of approximal caries.

Table 3 Correlation of oral conditions and salivary buffering capacity

Salivary buffering capacity	0 (n=12)		1 (n=70)		2 (n=50)		3 (n=25)		Total (n=157)		
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	*P*-value
Age at the first visit	37.830	13.849	38.640	13.349	35.280	11.137	33.680	11.817	36.720	12.518	0.300
D	2.800	2.573	3.670	3.291	4.780	3.699	4.500	3.514	4.110	3.450	0.213
M	0.800	1.033	1.790	2.603	1.540	1.982	2.000	2.654	1.680	2.343	0.853
F	12.200	7.146	10.520	5.814	10.720	5.771	10.290	5.465	10.660	5.795	0.807
DMFT	15.800	9.065	15.990	6.354	17.000	6.999	16.710	7.777	16.420	6.943	0.907
Total of flat surface caries	0.580	1.505	1.810	3.906	3.180	4.797	3.640	5.155	2.450	4.367	0.004
Total of approximal caries	2.750	2.958	4.640	6.249	7.900	7.833	6.200	6.212	5.780	6.771	0.026
PCR	42.060	18.596	41.340	21.442	36.000	19.599	35.150	13.566	38.710	19.623	0.434

Scores were evaluated for salivary buffering capacity using the chart of Dentobuff.
Score 0: immediately blue, Score 1: blue, Score 2: green, Score 3: orange
There were no statistically significant correlations for primary and secondary caries. The salivary buffering capacity showed a significant correlation with the number of flat surface caries and approximal caries.

To investigate the correlation of each dental caries condition and salivary levels of these bacteria, dental caries were divided into subgroups: primary caries and secondary caries; flat surface caries and approximal caries.

Table 4 Logistic regression analyses of MS

MS	Odds ratio	95% CI	*P*-value
Primary or Secondary			
Primary caries	1.100	0.94-1.30	0.230
Secondary caries	1.130	0.81-1.58	0.460
Flat or Approximal			
Flat surface caries	1.210	0.77-1.92	0.410
Approximal caries	1.090	0.76-1.55	0.650
Flat surface caries			
CA	1.520	0.84-2.74	0.170
CB	0.900	0.42-1.91	0.780
Approximal caries			
CA	1.420	0.73-2.73	0.300
CB	1.180	0.62-2.25	0.620
Multivariate Analyses			
Flat CA	1.390	0.71-2.70	0.330
Flat CB	0.650	0.21-1.99	0.450
Approximal CA	1.180	0.57-2.48	0.650
Approximal CB	1.490	0.56-3.95	0.420

CA: from the demineralization of enamel surface to dentinal caries
CB: dental caries extending into the dental pulp and tooth stump
There were no significant correlations for primary and secondary caries vs. salivary levels of MS.

Table 5 Logistic regression analyses of LB

LB	Odds ratio	95% CI	*P*-value
Primary or Secondary			
Primary caries	1.070	0.91-1.25	0.410
Secondary caries	1.300	0.90-1.84	0.170
Flat or Approximal			
Flat surface caries	2.100	1.29-3.41	0.000
Approximal caries	0.930	0.64-1.34	0.690
Flat surface caries			
CA	2.010	1.09-3.70	0.030
CB	2.760	1.17-6.55	0.020
Approximal caries			
CA	1.490	0.75-2.93	0.250
CB	3.150	1.54-6.42	0.000
Multivariate Analyses			
Flat CA	1.870	0.93-3.74	0.080
Flat CB	1.250	0.36-4.31	0.720
Approximal CA	1.090	0.50-2.35	0.830
Approximal CB	2.570	0.92-7.20	0.070

CA: from the demineralization of enamel surface to dentinal caries
CB: dental caries extending into the dental pulp and tooth stump
There were no statistically significant correlations found for primary and secondary caries.

Some teeth had both flat surface caries and approximal caries. To obviate the problem of confounding, multivariate logistic regression analysis was carried out. As shown in Tables 4, 5 and 6, there were no statistically significant correlations for salivary MS level using Dentocult MS, salivary LB level using Dentocult LB and buffering capacity with primary and secondary caries. Statistically significant correlations were observed between salivary LB level using Dentocult LB and the number of flat surface caries ($P < 0.001$). Furthermore, to confirm dose response relations, flat surface caries and approximal caries were divided based on their progression (CA or CB). Logistic regression analysis was again carried out. For salivary LB level using Dentocult LB and approximal caries, CB had a statistically significant correlation ($P < 0.001$). However, in the CA groups the correlation was not statistically significant. For salivary buffering capacity and flat surface caries, CA had a statistically significant correlation ($P < 0.030$). In contrast, for MS, there were no statistically significant correlations in any group.

Discussion

The primary role of the salivary MS level, determined using Dentocult MS, salivary LB level using Dentocult LB and buffering capacity, is to predict the future incidence of dental caries. Many epidemiological studies have shown the utility of these tests (18-20,31). MS and LB inhabitant

Table 6 Logistic regression analyses of salivary buffering capacity

Salivary buffering capacity	Odds ratio	95% CI	*P*-value
Primary or Secondary			
Primary caries	1.149	0.90-1.35	0.085
Secondary caries	0.980	0.71-1.33	0.870
Flat or Approximal			
Flat surface caries	1.410	0.89-2.23	0.140
Approximal caries	1.250	0.87-1.79	0.230
Flat surface caries			
CA	1.930	1.07-3.48	0.030
CB	1.730	0.80-3.73	0.170
Approximal caries			
CA	1.370	0.70-2.65	0.360
CB	1.850	0.97-3.54	0.060
Multivariate Analysis			
Flat CA	1.890	0.97-3.69	0.060
Flat CB	1.210	0.40-3.64	0.740
Approximal CA	1.000	0.47-2.12	1.000
Approximal CB	1.530	0.60-3.93	0.370

CA: from the demineralization of enamel surface to dentinal caries
CB: dental caries extending into the dental pulp and tooth stump
There were no statistically significant correlations for primary and secondary caries vs. salivary buffering capacity.

caries lesions and are released in saliva, thus we evaluated the salivary MS level using Dentocult MS, salivary LB level using Dentocult LB and buffering capacity as clinical indicators of the present caries condition.

MS play an important role in the development and progression of dental caries (32). LB also plays a role in progression of dental caries (32). These bacteria are found in carious teeth. Previous studies have suggested that MS inhabit both deep regions of decayed teeth and the surface of teeth (33). In contrast, LB colonizes various parts of the oral cavity such as the oral mucosa, tongue dorsum, saliva and tooth surfaces (34). LB has been detected in high numbers in both superficial and deep caries (35). It was reported that many species of lactobacilli have been detected in carious dentine (36). Although these tests have been mostly evaluated in children, in this study we used them to study adults. A previous study reported that factors such as plaque scores, higher counts of MS, and lower buffering capacity contributed significantly to the higher risk profiles for adults compared to children (25). Based on the results of these studies, we evaluated MS, LB and buffering capacity as clinical indicators of the present dental caries condition in adult patients.

As the results of this study indicate, salivary MS level determined using Dentocult MS had no significant correlation with the number of approximal caries or flat surface caries. This may be because this bacterium is not always released into saliva on chewing and salivary levels of MS may not always represent actual tooth conditions. The salivary buffering capacity had no significant correlation with the number of caries (Tables 3 and 6). It is conceivable that salivary buffering capacity depends on the acidogenicity of bacteria including MS. On the other hand, salivary LB level determined using Dentocult LB did correlate significantly with the number of both flat and approximal caries. Some studies reported that the salivary level of LB is an important factor that predicts the incidence of root caries in elderly populations (37,38). In our study, the ages of the subjects ranged from 20 to 63. There are also reports that the prevalence and incidence of root caries in elderly people are higher than in younger people (39,40). For adults, it is necessary to evaluate caries differently from children. For example, in adults one needs to consider the prosthetic restorations and periodontal status. In the present study, we substantiated the finding that LB detected in various parts of the oral cavity has a bearing on caries.

In conclusion, we found a correlation between salivary LB level determined using Dentocult LB and flat surface caries for several stages of caries. LB is not required for the development of lesions. Nonetheless, they may potently contribute to the demineralization of teeth once lesions are established on teeth. Salivary levels of LB may correspond to the stage of caries. In addition, clinically, secondary caries and approximal caries often remained unnoticed. Determination of the salivary LB level using Dentocult LB could be a useful tool to aid the detection of approximal and secondary caries.

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