Dentists' practice patterns regarding caries prevention: results from a dental practice-based research network

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ABSTRACT

Objective: The purposes of this study were to (1) quantify dentists' practice patterns regarding caries prevention and (2) test the hypothesis that certain dentists' characteristics are associated with these practice patterns.

Design: The study used a cross-sectional study design consisting of a questionnaire survey.

Participants: The study queried dentists who worked in outpatient dental practices who were affiliated with the Dental Practice-Based Research Network Japan, which seeks to engage dentists in investigating research questions and sharing experiences and expertise (n=282).

Measurement: Dentists were asked about their practice patterns regarding caries preventive dentistry. Background data on patients, practice and dentist were also collected.

Results: 38% of dentists (n=72) provided individualised caries prevention to more than 50% of their patients. Overall, 10% of the time in daily practice was spent on caries preventive dentistry. Dentists who provided individualised caries prevention to more than 50% of their patients spent significantly more time on preventive care and less time on removable prosthetics treatment, compared to dentists who did not provide individualised caries prevention. Additionally, they provided oral hygiene instruction, patient education, fluoride recommendations, intraoral photographs taken and diet counselling to their patients significantly more often than dentists who did not provide individualised caries prevention. Multiple logistic regression analysis suggested that the percentage of patients interested in caries prevention and the percentage of patients who received hygiene instruction, were both associated with the percentage of patients who receive individualised caries prevention.

Conclusions: We identified substantial variation in dentists' practice patterns regarding preventive dentistry. Individualised caries prevention was significantly related to provision of other preventive services and to having a higher percentage of patients interested in caries prevention, but not to the dentist's belief about the effectiveness of caries risk assessment. (Clinicaltrials.gov registration number NCT01 680 848).

ARTICLE SUMMARY

Strengths and limitations of this study

- To our knowledge, this is the first study to clarify dentists' practice patterns regarding caries prevention in Japan has substantial variation in the Dental Practice-Based Research Network.
- This study suggested possible reasons for the variation of practice patterns regarding caries prevention.
- This study has relatively wide diversity of participants, with respondents from all seven regions in Japan. Given the cross-sectional nature of the study, causative relationships between factors and the provision of individualised caries prevention were difficult to assess.

INTRODUCTION

Dental caries is a largely preventable disease,^{1–3} but it continues to affect 60–90% of school children and almost 100% of adults, constituting the most common chronic disease among children and adolescents.^{4 5} Oral health is essential to general health and optimal quality of life and the high prevalence of dental caries highlights the importance of public health approaches to its prevention.⁵ According to Zero *et al*⁶, dental caries is a dynamic dietomicrobial disease involving cycles of demineralisation and remineralisation. The early stages of this process are reversible by modifying or eliminating aetiological factors (such as plaque biofilm and diet) and increasing protective factors (such as fluoride exposure and salivary flow).⁶ Axelsson *et al*^{7 8} noted that improved self-performed oral hygiene, the daily use of fluoridated dentifrice, regularly repeated professional tooth cleanings and plaque control effectively prevented the recurrence of dental caries.^{7 8} Caries risk assessment is the first step in preventive treatment.⁹ Risk assessment is the determination of the person's

probability of developing new carious lesions during a specific time period and the probability of a change in the size or activity of existing lesions across time.^{2 10 11} Our previous studies revealed that dentists' perception of each potential caries risk factor and the administration of diet counselling varied between dentists.^{12 13} However, dentists' practice patterns regarding caries prevention and factors that affect these patterns remain unclear. The recent establishment of the Dental Practice-Based Research Network Japan (JDPBRN) created an opportunity for international comparisons. JDPBRN is a consortium of dental practices with a broad representation of practice types, treatment philosophies and patient populations and it has a shared mission with the DPBRN,¹⁴ now called the National DPBRN (<http://NationalDentalPBRN.org>). The network regions of the JDPBRN represent all seven districts in Japan (Hokkaido, Tohoku, Kanto, Chubu, Kansai, Chugoku-Shikoku and Kyushu). The studies conducted in the USA and Japan shared the same purpose of clarifying practice patterns regarding caries diagnosis and treatment.

The purposes of this study were to (1) quantify dentists' practice patterns regarding caries preventive dentistry and (2) test the hypothesis that certain dentists' characteristics are associated with these practice patterns.

MATERIALS AND METHODS

Study design

We conducted a cross-sectional study consisting of a questionnaire survey, which was administered in Japan between May 2011 and February 2012.¹² This study followed the World Association's Declaration of Helsinki. All participants provided written informed consent prior to participation. We used the same questionnaire that was used in the US DPBRN study 'Assessment of Caries Diagnosis and Caries Treatment'¹⁵ and the 'DPBRN Enrollment Questionnaire'.¹⁶ Four dentists and clinical epidemiologists collaboratively translated these questionnaires into Japanese. The translated version of these questionnaires is available at [http://www.dentalpbrn.org/uploadeddocs/Study%201\(Japanese%20Version.pdf\)](http://www.dentalpbrn.org/uploadeddocs/Study%201(Japanese%20Version.pdf)). (Original English version: <http://nationaldentalpbrn.org/pdf/Study%201%20questionnaire%20FINAL%20after%20pre-testing%20021306.pdf>). The questionnaires used in this study were validated by expert consultation and focus groups on potential subjects.¹⁷ Dentists were asked about their practice patterns regarding caries preventive treatment. Background data on patients, practice and participating dentists were also requested.

Questionnaires were distributed, answered and returned through the method described in the previous study.^{12 15 18}

Participants

We queried dentists working in outpatient dental practices who were affiliated with JDPBRN (n=282). Participants who indicated that they perform restorative dentistry at their practice were recruited from the JDPBRN website and mailings.

Variables

Dentists' practice patterns regarding individualised caries preventive dentistry

Practice patterns regarding individualised caries preventive regimens were measured with the following question: for what per cent of patients do you administer individualised caries preventive treatment specifically for their needs?

Items used to measure practice patterns regarding time spent doing prevention-related care and percentage of patients who receive specific dental services

Practice patterns regarding time spent doing prevention-related care and the percentage of patients who received specific dental services was measured using the questions listed in [table 1](#).

Variable selection

To identify the characteristics of the dentist, patient and practice that were associated with the use of individualised caries prevention, theoretical models were discussed and identified in accordance with previous studies.^{12 15 19 20} In addition, explanatory variables were extracted, consisting of four categories shown in [table 1](#).

Statistical analysis

Description and comparison of practice patterns by the use of individualised caries prevention

We examined the relationship between dental practice patterns and the use of individualised caries prevention. χ^2 tests were performed to assess the association between practice patterns and the use of individualised caries prevention.

The use of individualised caries prevention was categorised dichotomously: 'less preventive' (1–49%) and 'more preventive' (50–100%), according to a previous study.⁹ To compare our data with the US data, we calculated the mean percentages of patients who received individualised caries prevention according to a previous study.⁹

Factors affecting the decision to provide individualised caries prevention

Descriptive analysis was conducted through univariate regression analysis for explanatory variables associated with dentists' practice patterns of individualised caries prevention. Subsequently, multiple logistic regression analysis was conducted to examine the relationship between explanatory variables and the prevalence of patients receiving individualised caries prevention. Odds ratios (ORs) and 95% confidential intervals (CIs) were calculated. All statistical analyses were performed with STATA/SE (V.10; STATA Corporation, College Station, Texas, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Demographic information of participants

Questionnaires were distributed to 282 dentists and valid responses were collected from 189 (67%). The

Table 1 Outcomes and explanatory variables

Variables	Details
Percentage of patient contact time	What percentage of patient contact time do you (excluding your hygienist or other office staff) spend in a typical month performing the following procedures? If you always refer these procedures to other practitioners, please record 0% (1) Non-implant restorative (amalgams, composites, crowns, bridges, posts, foundations, etc); (2) implants (prosthetic and surgical procedures for implants); (3) removable prosthetics (full and partial dentures); (4) extractions (surgical and non-surgical); (5) periodontal therapy (surgical and non-surgical; includes scaling/root planing that you personally do); (6) endodontic therapy (root canals and endo surgery); (7) other (sealants, periodic and hygiene examinations, preventive dentistry, diagnostic or other; please specify)
Percentage of patients who received specific dental services	On what percentage of patients do you or your staff perform the following services at some time while they are patients in your practice (1) Diet counseling; (2) blood pressure screening, (3) oral cancer screening examination, (4) oral hygiene instruction, (5) in-office fluoride application, (6) fluoride gel/rinse prescribed or recommended for home use, (7) patient education from written pamphlets, (8) patient education from videos or slides, (9) intraoral photographs taken (conventional, non-video photography), (10) intraoral video images taken (usually performed with fiberoptic), (11) in-office whitening (usually performed with hydrogen peroxide), (12) at-home whitening (usually performed with carbamide peroxide)
Explanatory variables	(1) Dentists' individual characteristics Years since graduation from dental school, gender and efficacy of caries risk assessment (2) Practice setting Type of practice, practice busyness and city population (government ordinance-designated city with population over 700 000 or not) (3) Patients' characteristics Percentage of patients interested in caries prevention, patient age distribution and per cent of patients who self-pay (4) Procedure-related characteristics Whether caries risk is assessed as a routine part of treatment planning and the percentage of patients receiving hygiene instruction Percentage of patients who receive individualised caries prevention

demographic characteristics of the study participants are shown in table 2.^{12 13} The mean number of years ($\pm$ SD) elapsed since graduation from dental school was 18.5 ± 9.9 and the participants were predominantly men ($n=154$, 82%). With regard to practice setting, 40% ($n=76$) of practices were established in government ordinance-designated cities of over 700 000. The percentage of dentists who performed caries risk assessment as a routine part of treatment planning was 26% ($n=49$). The percentage of dentists who agreed that caries risk assessment is effective was 67% ($n=127$).

Dentists' practice patterns according to provision of individualised caries prevention

Seventy-two participants (38%) answered that 50% or more of their patients received individualised caries prevention (ie, were 'more preventive'). Eleven participants

(6%) answered that 100% of their patients received individualised caries prevention (figure 1).

Table 3 shows the practice patterns of dental procedures and the differences of practice patterns by use of individualised caries prevention. Participants spent 29% of their time on non-implant restorative, 19% on endodontic therapy and 18% on removable prosthetics. Participants spent 10% of their time on prevention-related care. Participants who were 'more preventive' ($n=72$) spent significantly more time on preventive dentistry ($p=0.0007$) and less time on removable prosthetics ($p=0.0159$).

Table 4 shows the relation among certain procedures performed in general dental practice and the percentage of time that patients receive prevention-related care. The mean percentage of patients who receive oral hygiene instruction was 67%, while 37% received patient

Table 2 Summary of dentists', practices' and patients' characteristics and certain dental procedures performed^{12 13}

	Number (%) or mean±SD
Dentist's individual characteristics	
Years since graduation from dental school (year)* (n=185)	18.5±9.9
Gender (male), n (%) (n=187)	154 (82)
Belief about the effectiveness of caries risk assessment, n (%) (n=189)	
Agree	127 (67)
Disagree or neutral	62 (33)
Practice setting	
Type of practice, n (%) (n=182)	
Employed by another dentist	77 (41)
Self-employed without partners and without sharing of income, costs or office space	105 (56)
Practice busyness, n (%) (n=181)	
Too busy to treat all people requesting appointments	19 (11)
Provided care to all, but the practice was overburdened	72 (40)
Provided care to all, but the practice was not overburdened	59 (33)
Not busy enough	31 (17)
City population (government ordinance-designated city), n (%) (n=189)	76 (40)
Patients' characteristics	
Percentage of patients interested in caries prevention, n (%) (n=189)	
0 (none)	16 (8)
1–24	80 (42)
25–49	38 (20)
50–74	46 (24)
75–99	8 (4)
100	1 (1)
Patient age distribution (years)*	
1–18 (%) (n=183)	16.1±13.2
19–44 (%) (n=188)	24.8±11.0
45–64 (%) (n=183)	30.4±11.2
65+ (%) (n=183)	28.5±17.4
Percent of patients who self-pay (%)* (n=183)	8.6±16.6
Dental procedure characteristics	
Percentages of patients who receive individualised caries prevention, n (%) (n=189)	
0 (none)	9 (5)
1–24	68 (36)
25–49	40 (21)
50–74	37 (20)
75–99	24 (13)
100	11 (6)
Caries risk is assessed as a routine part of treatment planning, n (%) (n=189)	49 (26)
Percentage of patients who received hygiene instruction (%)* (n=183)	67.3±34.8

*Mean±SD.

education from written pamphlets. Participants who were 'more preventive' (n=72) administered significantly more oral hygiene instruction ($p<0.0001$), gave written pamphlets ($p<0.0001$), either prescribed fluoride gels/-rinses or recommended for home use ($p<0.0001$), had patient education available from videos or slides ($p=0.0011$), obtained intraoral photographs ($p=0.0021$), had in-office fluoride applications ($p<0.0001$) and provided diet counselling ($p=0.0004$).

Factors associated with providing individualised caries prevention

The results of multiple logistic regression analysis are shown in table 5. Two factors were significantly associated with whether or not the practitioner reported providing

individualised caries prevention to 50% of patients or more. The ORs (95% CIs) were as follows: the percentage of patients interested in caries prevention, 5.81 (3.15 to 10.70); and the percentage of patients who received hygiene instruction, 1.02 (1.01 to 1.04).

DISCUSSION

Seventy-two participants (38%) answered that the percentage of patients who received individualised caries prevention was 50% or more ('more preventive'). Overall, 10% of the time in daily practice was spent on prevention-related care. Dentists who provided individualised caries prevention to 50% or more of their patients spent significantly more time on preventive care and provided less removable prosthetics treatment than

Figure 1 Distribution of the percentages of patients who receive individualised caries prevention, n. Seventy-two participants (38%) answered that 50% or more of their patients received individualised caries prevention. Eleven participants (6%) answered that 100% of their patients received individualised caries prevention.

those who did not. Additionally, they provided oral hygiene instruction, patient education, fluoride recommendations and diet counselling to their patients significantly more often than dentists who provided individualised caries prevention to less than 50% of their patients. The results of the multiple logistic regression analysis suggested that several variables were associated with whether or not dentists provide individualised caries prevention to 50% or more of their patients. Specifically, the percentage of patients interested in caries prevention and the percentage of patients

who received hygiene instruction were significantly associated with high percentages of patients who receive individualised caries prevention.

According to the results of the same questionnaire survey by the US DPBRN, 52% of patients received individualised caries prevention.⁹⁻²¹ The results of this study possibly suggest that dentists in the DPBRN and JDPBRN (41.3%) have similar tendencies in providing individualised caries prevention, but the proportion was lower in Japan than in the USA. Additionally, dentists spent 10% of their time on prevention-related care in

Table 3 Percentage of patient contact time spent doing certain procedures in a typical month, overall and by the percentage of patients who receive individualised caries prevention

Variable	All*	Individualised caries prevention		Difference† (more preventive–less preventive)	p Value
		0–49%* (less preventive)	50–100%* (more preventive)		
Prevention-related care (sealants, periodic and hygiene examinations, preventive dentistry, diagnostic or other; N=183)	9.5 (11.2)	7.4 (6.6)	13.1 (15.8)	5.8 (1.7)	0.0007
Non-implant restorative (amalgams, composites, crowns, bridges, posts, foundations, etc; N=183)	28.7 (14.2)	27.7 (13.1)	30.6 (15.9)	2.9 (2.2)	0.1813
Implants (prosthetic and surgical procedures for implants; N=183)	2.8 (7.5)	2.2 (7.0)	3.9 (8.2)	1.6 (1.1)	0.1544
Periodontal therapy (surgical and non-surgical; includes scaling/root planning that you personally do; N=183)	12.9 (10.1)	13.2 (10.5)	12.5 (9.4)	–0.7 (1.5)	0.6377
Extractions (surgical and non-surgical; N=183)	8.8 (6.2)	9.3 (7.2)	7.9 (4.1)	–1.5 (0.9)	0.1274
Endodontic therapy (root canals and endosurgery; N=183)	19.2 (11.0)	20.3 (12.1)	17.4 (8.6)	–2.9 (1.7)	0.0856
Removable prosthetics (full and partial dentures; N=183)	17.6 (11.9)	19.2 (13.0)	14.8 (9.3)	–4.4 (1.8)	0.0159

*Mean (SD).

†Mean (SE).

Table 4 Dentists' reports of the percentage of patients who receive the procedure at some time in their practice, overall and by the percentage of patients in the practice who receive individualised caries prevention

Variable	All*	Individualised caries prevention		Difference† (more preventive–less preventive)	p Value
		0–49%* (less preventive)	50–100%* (more preventive)		
Oral hygiene instruction (N=183)	67.3 (34.8)	56.4 (36.2)	85.1 (23.2)	28.7 (4.9)	p<0.0001
Patient education from written pamphlets (N=183)	37.3 (38.3)	28.1 (34.0)	52.7 (40.3)	24.6 (5.6)	p<0.0001
Fluoride gels/rinses prescribed or recommended for home use (N=183)	29.3 (32.5)	21.8 (27.7)	41.9 (36.0)	20.1 (4.7)	p<0.0001
Patient education from videos or slides (N=182)	21.6 (35.3)	15.0 (28.9)	32.4 (41.9)	17.4 (5.2)	0.0011
Intraoral photographs taken (N=183)	30.3 (36.3)	24.0 (34.1)	40.8 (37.6)	16.9 (5.4)	0.0021
In-office fluoride application (N=183)	23.3 (26.3)	17.2 (21.8)	33.4 (29.9)	16.2 (3.8)	p<0.0001
Diet counseling (N=183)	21.4 (27.2)	16.0 (24.2)	30.3 (29.6)	14.4 (4.0)	0.0004
Oral cancer screening examination (N=182)	6.1 (21.0)	4.6 (17.7)	8.4 (25.6)	3.8 (3.2)	0.2364
Blood pressure screening (N=183)	9.7 (20.9)	8.6 (19.8)	11.6 (22.7)	3.0 (3.2)	0.3461
At-home whitening (N=183)	4.6 (12.6)	3.6 (13.3)	6.1 (11.3)	2.5 (1.9)	0.2035
Intraoral video images taken (N=183)	3.5 (16.2)	2.8 (15.6)	4.7 (17.3)	1.9 (2.5)	0.4538
In-office whitening (N=183)	4.4 (14.5)	4.3 (16.7)	4.5 (10.1)	0.2 (2.2)	0.9309

*Mean (SD).

†Mean (SE).

this study, which was less when compared with Northern European dentists. A previous study conducted in Norway reported that the mean caries preventive treatment time was 16.6% of the total treatment time (dentists who did not treat adult patients were excluded)²² and 22% of the total time for child patients.²³ In Denmark, Iceland and Norway, dental prevention consumes 18–50% of the dentist's total time in dental care for children and adolescents.²⁴ In the USA, the average time that general practitioners spent performing preventive procedures increased from 9.4% in 1981 to 12.4% in 1993.²⁵ The lower preventive treatment time in Japan may be due to the differences in the healthcare system between Northern Europe and Japan. In Finland in 1999, all inhabitants under the age of 19 were entitled to free comprehensive public dental care, with a utilisation rate of approximately 95%.²⁶ However, in Japan, dental insurance systems mainly cover dental treatment,²⁷ so that the percentage of time spent on preventive treatment might be restricted due to economic reasons. In addition, dentists' perception regarding prevention could be one of the reasons that explain variations in preventive practice in this population. Taylor-Gooby *et al*²⁸ pointed out that professional values for preventive care affect practice patterns of preventive dentistry and that British dentists valued a restorative paradigm as opposed to a preventive paradigm which devalued traditional restorative skills in a context of

'continuing care' payment in 1990s. Also Fox²⁹ reviewed dentists' perceptions of prevention and its application in practice and highlighted that recently, most dentists regarded aspects of prevention to be part of their professional work, a source of job satisfaction and of value to the practice, its image and a marker of quality of care. Further studies are needed to clarify associations between dentists' perception of dental prevention and its practice.

A detailed analysis of the practice patterns of prevention-related care revealed that the percentages of time spent on preventive care, which differed between 'more preventive' and 'less preventive' dentists, were significantly different in practices that administered preventive care more than 20% of the time. Dentists in this study or their dental auxiliaries, provided oral hygiene instruction to 67% of their patients at some point in the patient's course of treatment and this percentage differed significantly as 'more preventive' and 'less preventive' dentists. The percentage of patients who received hygiene instruction was also associated with the administration of individualised caries prevention in the multiple regression analysis.

Our study clarified that a positive patient perception of preventive dentistry (as measured by the percentage of patients in the practice who are interested in caries prevention) and a higher percentage of patients in the practice who received hygiene instruction were

Table 5 A multiple logistic regression of whether the dentist provides individualised caries prevention on 50% or more of patients (n=163)

Variable	OR	95% CI	p Value
Dentists' individual characteristics			
Years since graduation from dental school	1.00	0.95 1.06	0.883
Gender (reference: male)	0.39	0.09 1.69	0.211
Belief about effectiveness of caries risk assessment			
Disagree or neutral	1.00		
Agree	0.91	0.31 2.67	0.865
Practice setting			
Type of practice			
Employed by another dentist	1.00		
Self-employed without partners and without sharing of income, costs or office space	1.13	0.37 3.50	0.831
Practice busyness			
Too busy to treat all people requesting appointments	1.00		
Provided care to all who requested appointments, but the practice was overburdened	0.92	0.13 6.67	0.935
Provided care to all who requested appointments, but the practice was not overburdened	0.94	0.12 7.26	0.952
Not busy enough—the practice could have treated more patients	0.64	0.06 6.49	0.706
City population (reference: non-government ordinance designated city)	1.12	0.42 2.97	0.818
Patients' characteristics			
Percentage of patients interested in caries prevention (every 25%)	5.81	3.15 10.70	p<0.0001
Percentage of child and teenage patients (1–18 years old)	1.04	0.99 1.08	0.093
Percentage of practice revenue or charges from self-pay	1.02	0.99 1.06	0.209
Dental procedure characteristics			
Caries risk assessment is conducted as a routine part of treatment planning (reference: no)	1.54	0.46 5.23	0.486
Percentage of patients who received hygiene instruction	1.02	1.01 1.04	0.009

The outcome of interest (individualised caries prevention) was coded as follows: 1=provides individualised caries prevention on 50% or more of the practice's patients; 0=does not. Hosmer-Lemshow goodness-of-fit, 0.0503.

associated with the use of individualised caries prevention to a higher percentage of patients. According to the results of the same questionnaire survey by the US DPBRN, dentists' individual characteristics, practice settings and dental procedures were associated with providing individualised caries prevention to a greater percentage of patients.⁹ Additionally, Brennan and Spencer³⁰ noted that dentists' individual characteristics, practice settings and patient characteristics influenced the pattern of preventive care delivered. Our model also included dentists' individual characteristics, practice settings and dental procedures and the dentists' ratings of patients' preference for preventive care were related more strongly than those factors. In addition, a previous systematic review noted that potential barriers to the adherence to physicians' guidelines included dentists' and patients' preferences.^{31–32} As Cabana *et al*³¹ noted, potential barriers to the adherence to physicians' guidelines changed depending on the topic and it is possible that dentists' ratings of patient preference are strongly related to the practice of preventive dentistry.

However, our studies suggested that dentists' beliefs about the effectiveness of caries risk assessment were not related to their tendency to use individualised caries prevention. A possible reason for this is that the majority of participants (67%) agree that caries risk assessment is effective. This high percentage of agreement with the

effectiveness of caries risk assessment is consistent with previous studies. According to the results of the same questionnaire by the US DPBRN, 77% of dentists answered that they agree with the effectiveness of caries risk assessment. Further studies are needed to clarify the relationship between the use of preventive dentistry and their beliefs about its effectiveness.

The main strength of this study was its relatively wide diversity of participants, with respondents from all seven regions of Japan. The age and gender distribution of this study sample was similar to the actual distribution of dentists in Japan (80% male, average age in the 40s),³³ thereby enhancing the generalisability of the findings. However, the study results should be approached with caution. First, participants were not selected by random sampling, but rather by responding to the invitation to participate in the JDPBRN. Second, no objective standard for cut-off regarding an adequate prevalence of patients receiving individualised caries prevention has been established, although we used prior planned cut-offs with the mean from the previous US studies. Third, it is possible that the questionnaire's validity is influenced by the reimbursement/insurance system, which is quite different between the USA and Japan. Finally, given the cross-sectional nature of our study, causative relationships between factors and the provision of individualised caries prevention were difficult to assess.

CONCLUSION

We identified substantial variation in dentists' practice patterns regarding caries preventive dentistry in this study population. Individualised caries prevention was significantly related to provision of other preventive services and to the practice having a higher percentage of patients interested in prevention, but not to the dentist's belief about the effectiveness of caries risk assessment.

Contributors YY, NK, FS, YM, GHG and VVG contributed in arranging reagents/materials/analysis tools, conceived and designed the experiments and revised and reviewed the paper. NK, YM and FS performed the experiments. YY and NK analyzed the data and wrote the paper.

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