DOI: 10.1111/j.1365-263X.2010.01099.x

Association between interdental plaque acidogenicity and caries risk at surface level: a cross sectional study in primary dentition

MARIA GRAZIA CAGETTI[1], GUGLIELMO CAMPUS[2], SILVANA SALE[3], FABIO COCCO[4], LAURA STROHMENGER[5] & PETER LINGSTRÖM[6]

[1]WHO Collaborating Centre of Milan for Epidemiology and Community Dentistry, University of Milan, Milan, Italy, [2]Dental Institute, University of Sassari, Sassari, Italy, [3]Dental Institute, University of Sassari, Sassari, Italy, [4]Chemical Department, University of Sassari, Sassari, Italy, [5]WHO Collaborating Centre of Milan for Epidemiology and Community Dentistry, University of Milan, Milan, Italy, and [6]Department of Cariology, Institute of Odontology at Sahlgrenska Academy, University of Göteborg, Göteborg, Sweden

International Journal of Paediatric Dentistry 2011; 21: 119–125

Background. In schoolchildren the most commonly decayed primary teeth are molars affecting proximal adjacent surfaces especially.
Aim. To determine whether a more acidic plaque in response to sucrose challenge is detected in children with more carious lesions.
Design. Plaque pH measurements, using the microtouch technique, were carried out in interproximal spaces between primary molars, in 157 high caries risk children (314 sites and caries status of the 628 proximal surfaces recorded). The area under the curve ($AUC_{5.7}$ and $AUC_{6.2}$) was analyzed.
Results. The $AUC_{5.7}$ and the $AUC_{6.2}$ showed a statistically significant difference between plaque adjacent to proximal surfaces with or without caries. Differences for $AUC_{5.7}$ and $AUC_{6.2}$ were recorded between one decayed surface compared to two decayed surfaces ($P < 0.01$) whereas a statistical significant difference was only observed for $AUC_{5.7}$, when the areas under the curve were obtained near one decayed surface compared to two sound surfaces ($P = 0.04$).
Conclusions. The higher acidogenicity of the dental plaque found in presence of a proximal carious lesion in primary maxillary molars represents an additional risk factor for the adjacent surface. This finding may help clinicians in treatment decisions.

Introduction

In the last decades an improvement in children dental health has been described in many industrialized countries (Marthaler, 2004). In Italy, the percentage of caries-free children increased from 10% to 64%, while the percentage of untreated decayed teeth increased from 44% to 62% from 1989 to 2004 (Campus *et al.*, 2007). Dental caries is the net result of many dynamically related factors. In addition to the hard tissue, other factors include carbohydrates consumption, microbiological biofilm composition, plaque and saliva amount and composition. The result of the interaction among the different variables involved in the caries process can be studied by assessing the metabolic activity of the oral biofilm, i.e., plaque acidogenicity[1–6]. In schoolchildren the most commonly affected teeth are primary molars, at occlusal and proximal surfaces level[7,8]. Caries risk evaluation can help the dentist to make specific preventive and treatment recommendations in order to reduce the child's risk and improve oral health. Risk assessment also contributes to efficient delivery of care, by eliminating unnecessary interventions and prolonging the prognosis of treatments[9–11].

The final outcome is related not only to its fermentation activity, but also to factors such as the exact composition of the tooth surface underlying the actual plaque area and the interaction between the two. Plaque factors, as well as acidogenesis and acid tolerance of plaque microorganisms, may vary[12,13]. The time factor is important, as the development

Correspondence to:
Dr. Guglielmo Campus, Dental Institute, University of Sassari, Viale San Pietro 43/C, I-07100 Sassari, Italy.
Tel.: +39 079 228540 (508); Fax: +39 079 228541.
E-mail gcampus@uniss.it

of a lesion is the result of repeated acid attacks during a certain time period.

Caries risk assessment is determined by the balance among factors that may negatively impact and protective factors that may promote oral health[9–11]. Caries experience (dmft and DMFT > 0) is often used to assess the caries risk at individual level. In order to create a risk profile able to predict the development of new lesions, a combination of different factors have to be considered. The acid formation by cariogenic bacteria is however crucial event in caries lesion development: variation in plaque pH has been found in relation to caries activity at individual level[14], as well as, at surface level[6].

Since the Stephan-curve was firstly described[15], this technique has been frequently used for evaluation of food cariogenicity and/or individual caries risk status[2,3]. The currently, most often used method is the microtouch method. This consists of a hand-held microelectrode and a reference electrode connected through a salt bridge to one of the subject's finger, making it possible to perform intermittent pH readings of interdental plaque. The technique is simple and enables intermittent readings at different sites; however, criticism has been made that the repeated insertion of the electrode into the plaque may disturb the plaque structure and change its acidogenic properties[16].

The hypothesis tested in this clinical study was to evaluate if the *in vivo* plaque adjacent to carious teeth has a stronger pH response to a sucrose challenge than plaque not associated with caries. To verify this hypothesis, an *in vivo* study was designed, evaluating the relationship between plaque pH and tooth surface status by analyzing the interdental supragingival plaque pH before and after a sucrose rinse in a group of high caries risk schoolchildren.

Materials and methods

Selection of the sample

The study was conducted in Sassari (Italy), in a population aged 7–9 years. A total of 1120 children were recruited for the initial screening. A preliminary survey was carried out in order to identify and select high caries risk children eligible for the study, as previously described[17]. Briefly, the inclusion criteria were the presence of 2 or 3 dentinal carious lesions and a salivary concentration of mutans streptococci (ms) higher than 10^5 CFU/mL. Mutans streptococci (ms) counts in saliva was assessed using the dip-slide technique (CTR bacteria, Ivoclar Vivadent, Germany). Ms salivary class was scored following manufacturer's instructions. The study sample consisted of 157 children (74 boys and 83 girls). The study was approved by the Ethics Committee at the University of Sassari (registration no. 2006/24).

Study design

Children came to the Dental Institute at the University of Sassari for an assessment of plaque acidogenicity. No toothbrushing or other oral hygiene methods were allowed in the morning before the measurement. The subjects also refrained from eating, drinking and using chewing gum 1 h before the measurements. For each child, measurements of plaque pH were made in the interproximal spaces between the first and second primary molars, on the left and right upper jaw. A total of 314 sites were studied. Each interproximal space was surrounded by (a) two sound proximal surfaces, (b) one sound and one decayed surface or (c) two decayed surfaces. Thus, the caries status of 628 proximal surfaces was recorded. No restorations were present in the interproximal spaces examined. Figure 1 shows the flow chart of the study design.

Clinical examination

Clinical assessments were carried out by two examiners (GC and SS) under standardized conditions with optimal artificial lighting, following air drying and using a plain mirror and a WHO-CPI probe. Dental caries was only registered if the probe did enter the lesion, or if the lesion could be judged as clearly into the dentine, undermining the enamel, with the underlying carious dentine appearing as a

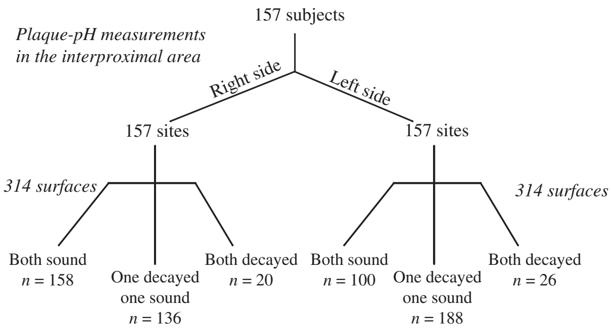

Fig. 1. Flow chart of the study design describing subjects, sides (left or right upper jaw) and sites (interproximal areas).

brownish or whitish spot. Arrested caries of dentine with a hard, smooth, 'non-sticky surface', were also registered as caries[18,19].

The two examiners received training and intra- and interexaminer reliability was assessed before the beginning of the survey[17,19]. Forty-five subjects were re-examined after 72 h by the two examiners. Interexaminer reliability was evaluated through analysis of variance for fixed effect[20,21], whereas intra-examiner reproducibility was assessed as percent agreement and Cohen's Kappa statistics. A good interexaminer reliability was found without significant differences ($P = 0.21$) and with a low value of mean squares for error (0.44). As regard intraexaminer reproducibility, the percent agreement was high (Cohen's Kappa 0.84).

Plaque pH measurements

Using the microtouch technique, plaque acidogenicity was assessed before and after a sucrose challenge. The pH of the plaque located in the left and right interproximal space between the first and second maxillary primary molars was measured in quintuplicate at six different times: at baseline (before sucrose rinse) and 2, 5, 10, 15 and 20 min after a 1-min rinse with 10 mL of 10% (w/v) sucrose using active movements. The interproximal spaces were exposed to the salivary secretion rate during the whole 20-min test period. An iridium touch microelectrode, diameter 0.1 mm (Beetrode NMPH-1®, World Precision Instruments, Sarasota, FL, USA) with a porous glass electrode as reference, was used (MERE 1, WPI, Sarasota, FL, USA)[2]. A salt bridge was created in a 3 M KCl solution between the reference electrode and one finger of the child. Before and after each pH evaluation, the electrode was calibrated using standard solutions with pH 7.00 and 4.00[2]. Plaque measurements in each interproximal space were made in randomized order for the different individuals; however, the pH of each child was always assessed in the same order.

Statistical methods and data analysis

For each subject and time point, the mean and standard error of the five pH readings, registered in the two interproximal spaces, were calculated. The pH-data were further analyzed separately for the left and right upper jaw as well as using the mean for the two sites. Area under the curve (AUC), described as the area between reference pH (6.2 or 5.7) and the pH curve, was calculated using a computer-based program[22]. The area under the curve at pH 5.7 and pH 6.2 ($AUC_{5.7}$ and $AUC_{6.2}$) was used as a measurement of the total discriminant ability of the presence or absence of carious lesions to play a role in plaque pH fall. Moreover, minimum pH and maximum pH fall were calculated. One-way analysis of variance (ANOVA) was performed to analyse statistically significant differences in plaque pH between plaque located near two sound, one sound and one decayed or two decayed proximal surfaces.

Instrument reliability (test-retest reliability) was measured by assessing the intraclass correlation coefficient (ICC), based on the five repeated measurements, in order to ensure the reproducibility of the instrument[21]. Validity tests were carried out. Data from baseline measurement were used to assess the validity of the instrument. Because there was no 'gold standard', construct validity was assessed. Statistical analysis was performed with Stata 10 for Mac. A *P*-value of <0.05 was considered statistically significant.

Results

The values for $AUC_{5.7}$ and $AUC_{6.2}$, minimum pH and maximum pH fall on the right and left upper jaw measured near sound and/or

Table 1. Area under the curve ($AUC_{5.7}$ and $AUC_{6.2}$), minimum pH and maximum pH fall (mean ± SE and 95% confidence intervals) obtained, after a sucrose rinse, for two sound (n = 258) and one or two decayed (n = 370) surfaces in the left and right upper jaw respectively.

	Decayed surfaces mean ± SE (95% CI)	Sound surfaces mean ± SE (95% CI)	*P*-value Anova one-way
Left upper jaw			
$AUC_{5.7}$	15.8 ± 0.8 (14.1–17.4)	10.6 ± 0.5 (9.5–11.7)	<0.01
$AUC_{6.2}$	25.3 ± 0.9 (23.4–27.1)	18.8 ± 0.6 (17.5–20.1)	<0.01
Minimum pH	4.4 ± 0.03 (4.3–4.6)	4.7 ± 0.04 (4.6–4.8)	0.20
Maximum pH fall	2.1 ± 0.05 (2.0–2.2)	1.6 ± 0.1 (1.5–1.7)	0.03
Right upper jaw			
$AUC_{5.7}$	16.6 ± 0.9 (14.8–18.4)	9.9 ± 0.5 (8.9–10.9)	<0.01
$AUC_{6.2}$	26.2 ± 0.9 (24.3–28.1)	18.2 ± 0.6 (17.1–19.4)	<0.01
Minimum pH	4.4 ± 0.05 (4.3–4.5)	4.7 ± 0.04 (4.6–4.9)	0.20
Maximum pH fall	2.0 ± 0.06 (1.9–2.1)	1.5 ± 0.1 (1.3–1.6)	0.03

carious surfaces are given in Table 1. After a 1-min 10% sucrose mouthrinse, the $AUC_{5.7}$ and the $AUC_{6.2}$ differed to a statistically significant degree between plaque adjacent to sound or decayed proximal surfaces, both on the right and left upper jaw. Minimum pH values were lower and maximum pH fall higher in plaque near a carious surface, although the statistical analysis only revealed significant differences in relation to caries presence for maximum pH fall. The minimum pH (mean ± SD) near two sounds, one sound and one decayed and two decayed surfaces was 4.7 ± 0.04, 4.5 ± 0.03 and 4.3 ± 0.03, respectively. The corresponding values for maximum pH fall was 1.6 ± 0.1, 1.8 ± 0.04 and 2.2 ± 0.06, respectively (data not in tables).

Figure 2 shows the mean plaque pH response curves after the sucrose rinse obtained in plaque from interproximal space near two sound surfaces (n = 258), one sound and one decayed (n = 324) or two affected surfaces (n = 46). A gradual increase in plaque acidogenicity was found in relation to increased caries status. Five minutes after the sucrose rinse, a quick fall in plaque pH was recorded for all spaces, but it was more marked in the presence of carious lesions. At 5 min, the plaque pH was 5.6 ± 0.7, when both surfaces were sound, 5.2 ± 0.8, when one surface was decayed and 4.7 ± 0.8, when both surfaces were decayed. The pH values showed a continuous drop until 15 min after the rinse and then began to rise again both in plaque located near a carious lesion and near a sound surface. Statistically significant differences ($P < 0.05$) were observed at each time point between plaque located in interproximal space surrounded by two sound and two affected surfaces. Significant differences ($P < 0.05$) were also recorded when comparing one affected surface and both affected surfaces 10, 15 and 20 min after the sucrose rinse.

In Table 2, $AUC_{5.7}$ and $AUC_{6.2}$ assessed in plaque near decayed or sound surfaces are listed (mean ± SE and 95% confidence intervals).

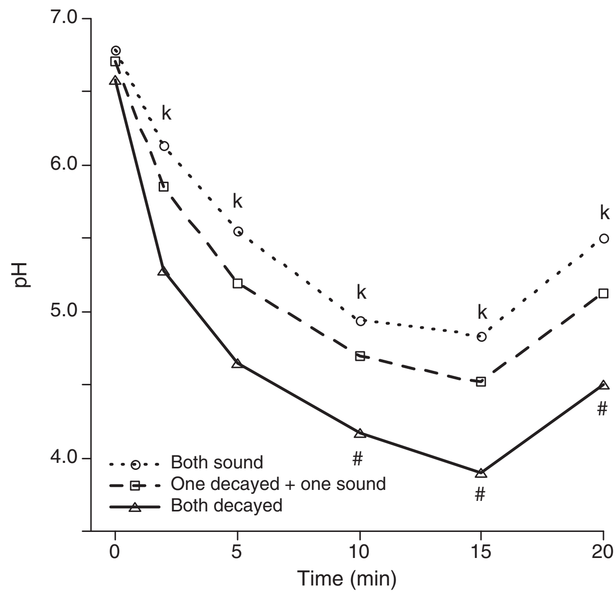

Fig. 2. Plaque pH response after 1-min sucrose rinse in relation to status of dental surface (both sound, one decayed + one sound, and both decayed) n = 314 sites. [k]Statistically significant difference ($P < 0.05$) between both decayed and both sound surfaces. [#]Statistically significant difference ($P < 0.05$) between both decayed and one decayed + one sound surface.

Table 2. $AUC_{5.7}$ and $AUC_{6.2}$ (mean ± SE and 95% confidence intervals) after a sucrose rinse, related to the caries status of the adjacent surfaces.

	$AUC_{5.7}$ mean ± SE (95% CI)	$AUC_{6.2}$ mean ± SE (95% CI)
One decayed and one sound surface	12.6 ± 0.9 (10.8–14.4)	23.5 ± 0.8 (20.1–26.9)
Both decayed surfaces	9.9 ± 0.5 (8.9–10.9)	18.2 ± 0.6 (17.1–19.4)
P-value Anova one-way	<0.01	0.01
One decayed and one sound surface	12.6 ± 0.9 (10.8–14.4)	23.5 ± 0.8 (20.1–26.9)
Both sound surfaces	17.4 ± 0.7 (14.3–20.5)	27.7 ± 0.4 (24.6–29.8)
P-value Anova one-way	0.04	0.05

Table 3. Random-effects regression model of binary sound *vs* decayed surfaces of dental plaque pH after a sucrose rinse.

Caries	Coefficient (SE)	*P*-value	Intraclass coefficient
pH 0 min	−0.28 (7.04)	0.69	0.82
pH 2 min	0.19 (6.22)	0.91	0.91
pH 5 min	−0.07 (7.02)	0.95	0.90
pH 10 min	−0.14 (6.54)	0.83	0.88
pH 15 min	0.61 (7.80)	0.71	0.92
pH 20 min	−1.39 (6.23)	0.82	0.90

Number of obs = 628; Group variable: caries/sound surface; Log likelihood = −158.71; Likelihood-ratio test of ICC = 129.78, $P < 0.01$.

The highest values for $AUC_{5.7}$ and $AUC_{6.2}$ were found for plaque located near two carious surfaces. The statistical analysis revealed significant differences in $AUC_{5.7}$ and $AUC_{6.2}$ between one decayed compared with two decayed surfaces ($P < 0.01$), whereas a statistically significant difference was observed for $AUC_{5.7}$ alone, when the areas under the curve were obtained in plaque near one decayed surface compared with two sound surfaces ($P = 0.04$).

No statistically significant relationship was found between the reproducibility of plaque pH measurements associated with the presence or absence of carious lesion in the interproximal space (Table 3). The high intraclass correlation values (range 0.82–0.91) showed how closely the single quantitative measurements in the same group resembled one another (data not in table).

Discussion

In this paper an *in vivo* association between interdental plaque acidogenicity and caries surface status in the interproximal space was studied. This finding can be useful to evaluate the caries risk status at surface level in order to establish an individualized caries treatment plan.

This study assessed the sucrose-induced plaque pH response of dental plaque using a microtouch electrode, which enables reliable measurements of pH fluctuations at discrete sites, even in field conditions[2]. In the present trial, the microtouch method has showed a good reproducibility when used in plaque covering both sound and carious surfaces.

The iridium oxide microelectrode used for the microtouch model enables fairly simple pH measurements. Moreover, there is in literature agreement regarding its ability to produce readings that are reasonably accurate and precise up to thirty minutes after standardization[16]. This study shows that the reliability of the microtouch technique for plaque pH measurements in interproximal maxillary primary molar/molar spaces was high and it was not affected by the presence of carious lesions in the interproximal space.

All the children included in the study were high caries risk subjects (mutans streptococci $>10^5$ CFU/mL in saliva and presented 2 or 3 dentinal carious lesions). Resting plaque pH values were generally lower near decayed tooth surfaces. The pH response of human dental plaque to sugar *in vivo* has indicated that the resting plaque pH, prior to sugar exposure, and the minimum pH, reached after sugar exposure, became increasingly lower with increasing caries activity[15,23]. In this study, data showed a small difference in plaque pH recorded in the three different space categories at baseline, whereas an accentuated difference took place after the sucrose exposure, which continued up to the maximum pH fall at 15 min. Moreover, increased caries activity is also clearly associated with higher dental plaque levels of mutans streptococci and lactobacilli[12], acid tolerance or acidogenesis at the acidic pH of named bacteria[13].

The pH measurements in this study were performed in the interproximal space between the first and the second primary molars of the upper jaw; however, it is impossible to distinguish between plaque covering the adjacent tooth, i.e., mesial and distal proximal surfaces. Plaque pH measurements should be seen as representing the interproximal space and should be analysed in relation to the status, sound or decayed, of two surfaces. The main result of this study was that the acidogenic potential of the interdental plaque was gradually enhanced from two sound, one sound and one carious and two carious surfaces. A theoretical weak point could be the risk for an underestimation of the number of carious surfaces, as all pre-cavitated lesions were included in the 'sound' surfaces. It is likely that the study findings would be more distinct in only truly sound surfaces were included.

In the literature, several reports describe a difference between plaque pH responses in caries active and caries inactive children[2,24,25]; however, in the present paper, only children with two or three dentinal carious lesions were included and the pH evaluations were performed in terms of the presence of a carious lesion in the space of pH measurements. Information on the relationship between plaque pH and caries status is fairly limited. The available scientific evidence suggests that caries development is related to a higher plaque pH-lowering or acidogenic ability of the dental plaque, where mutans streptococci and lactobacilli within the plaque contribute to a great extent, for a review, see Sansone *et al.*[26] and Lingström *et al.*[5]; however, acid production by plaque is not only due to the presence of mutans streptococci or lactobacilli. Several other microbial species are acidogenic, aciduric and able to promote the caries process[27].

This more acidogenic plaque indicates a more caries-promoting ecological environment, where the different biological factors point towards a higher risk of caries lesion development. This is particularly true for interproximal spaces; it has been reported that interdental plaque is more acidogenic than plaque covering smooth surfaces or exposed root surfaces[28,29]. These differences in plaque pH at different tooth sites are probably related to variations in bacterial composition and saliva access. Saliva access in the interproximal space is limited, especially in primary teeth where a wider contact surface is seen. This is different from the contact point that is more often found in permanent teeth. In addition, if a dentinal lesion is present on one or two proximal surfaces, the plaque will be reached by saliva to a smaller extent, resulting in greater pH-lowering potential compared with plaque located in more accessible sites[14]. By a clinical point of view, this condition can lead to a more rapid progression of the present proximal caries lesion and a higher caries risk for the sound adjacent surface. If this situation does not be changed, both proximal surfaces will become affected by caries in a small period of time and a rapid caries progression of the two lesions will occur due to the high acidogenicity of the dental plaque located in that interproximal space. This finding may help clinicians in treatment decision for the management of primary teeth with proximal carious lesions. Following the Minimal Intervention Dentistry recommendations, early intervention has to be considered when a lesion is present. In the mouth of the child, especially if caries is located in the proximal surface of a primary molar, it is crucial to stop the progression of an initial lesion of the adjacent surface or to prevent a sound from developing caries. Early stages of caries can be brought to a halt and even reversed if the caries challenge is modified or even better removed (i.e., improved oral hygiene and reduced cariogenic diet) or if the protective factors are broadened (i.e., fluoride use as the most relevant agent).

What this paper adds

- The presence of a proximal caries lesion produces a wider plaque pH reduction in primary interproximal molar space, after a sucrose rinse.

Why this paper is important for paediatric dentists

- Early and comprehensive caries management based on the concept of Minimal Intervention Dentistry is to be considered in presence of proximal lesions.

References

1 Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA. The role of sucrose in cariogenic dental biofilm formation – new insight. *J Dent Res* 2006; **85**: 878–887.
2 Scheie AA, Fejerskov O, Lingström P, Birkhed D, Manji F. Use of palladium touch microelectrodes under field conditions for in vivo assessment of dental plaque pH in children. *Caries Res* 1992; **26**: 44–52.
3 Lingström P, Imfeld T, Birkhed D. Comparison of three different methods for measurement of plaque pH in humans after consumption of soft bread and potato chips. *J Dent Res* 1993; **72**: 865–870.
4 van Houte J, Lopman J, Kent R. The final pH of bacteria-comprising the predominant flora on sound and carious human root and enamel surfaces. *J Dent Res* 1996; **75**: 1008–1014.
5 Lingström P, van Ruyven FOJ, van Houte J, Kent R. The pH of dental plaque in its relation to early enamel caries and dental plaque flora in human. *J Dent Res* 2000; **79**: 770–777.
6 van Ruyven FOJ, Lingström P, van Houte J, Kent R. Relationship among mutans streptococci, ''low-pH'' bacteria, and iodophilic polysaccharide-producing bacteria in dental plaque and early enamel caries in humans. *J Dent Res* 2000; **79**: 778–784.
7 Ferro R, Besostri A, Olivieri A. Caries prevalence and tooth surface distribution in a group of 5-year-old Italian children. *Eur Arch Paediatr Dent* 2009; **10**: 33–37.
8 Elfrink ME, Veerkamp JS, Kalsbeek H. Caries pattern in primary molars in Dutch 5-year-old children. *Eur Arch Paediatr Dent* 2006; **7**: 236–240.
9 Twetman S, Fontana M. Patient caries risk assessment. *Monogr Oral Sci* 2009; **21**: 91–101.
10 Young DA, Featherstone JD, Roth JR, *et al.* Caries management by risk assessment: implementation guidelines. *J Calif Dent Assoc* 2007; **35**: 799–805.
11 Axelsson S, Söder B, Nordenram G *et al.* Effect of combined caries-preventive methods: a systematic review of controlled clinical trials. *Acta Odontol Scand* 2004; **62**: 163–169.
12 van Houte J. Bacterial specificity in the etiology of dental caries. *Int Dent J* 1980; **30**: 305–326.
13 van Houte J. Role of micro-organisms in caries etiology. *J Dent Res* 1994; **73**: 672–681.
14 Dong YM, Pearce EI, Yue L, Larsen MI, Gao XJ, Wang JD. Plaque pH and associated parameters in relation to caries. *Caries Res* 1999; **33**: 428–436.
15 Stephan RM. Intra-oral hydrogen-ion concentrations associated with dental caries activity. *J Dent Res* 1944; **23**: 257–266.
16 Huang GF, Guo MK. Resting dental plaque pH values after repeated measurements at different sites in the oral cavity. *Proc Natl Sci Counc Repub China B* 2000; **24**: 187–192.
17 Campus G, Cagetti MG, Sacco G, Benedetti G, Strohmenger L, Lingström P. Caries risk profiles in Sardinian schoolchildren using Cariogram. *Acta Odontol Scand* 2009; **67**: 146–152.
18 Pitts NB, Longbottom C. Preventive care advised (PCA)/operative care advised (OCA) – categorising caries by the management option. *Community Dent Oral Epidemiol* 1995; **23**: 55–59.
19 Bolin AK. Children's dental health in Europe. An epidemiological investigation of 5- and 12-year-old children from eight EU countries. *Swed Dent J Suppl* 1997; **122**: 1s–88s.
20 Castiglia P, Campus G, Solinas G, Maida C, Strohmenger L. Children's oral health in Italy: training and clinical calibration of examiners for the National Pathfinder about caries disease. *Oral Health Prev Dent* 2007; **5**: 255–261.
21 Fleiss JL. *The Design and Analysis of Clinical Experiments*. New York: John Wiley & Sons, 1986: 62–63.
22 Larsen MJ, Pearce EI. A computer program for correlating dental plaque pH values, cH+, plaque titration, critical pH, resting pH and the solubility of enamel apatite. *Arch Oral Biol* 1997; **42**: 475–480.
23 Fejerskov O, Scheie AA, Manji F. The effect of sucrose on plaque pH in the primary and permanent dentition of caries-inactive and -active Kenyan children. *J Dent Res* 1992; **71**: 25–31.
24 Englander HR, Carter WJ, Fosdick LS. The formation of lactic acid in dental plaques. III. caries-immune individuals. *J Dent Res* 1956; **35**: 792–799.
25 Kleinberg I, Jenkins GN, Chatterjee R, Andwijeyeweera L. The antimony pH electrode and its role in the assessment and interpretation of dental plaque pH. *J Dent Res* 1982; **61**: 1139–1147.
26 Sansone C, van Houte J, Joshipura K, Kent R, Margolis HC. The association of mutans streptococci and non-mutans streptococci capable of acidogenesis at a low pH with dental caries on enamel and root surfaces. *J Dent Res* 1993; **72**: 508–516.
27 Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. *Community Dent Oral Epidemiol* 2005; **33**: 248–255.
28 Jensen ME, Schachtele CF. Plaque pH measurements by different methods on the buccal and approximal surfaces of human teeth after a sucrose rinse. *J Dent Res* 1983; **62**: 1058–1061.
29 Scheie AA, Luan WM, Dahlen G, Fejerskov O. Plaque pH and microflora of dental plaque on sound and carious root surfaces. *J Dent Res* 1996; **75**: 1901–1908.