Measuring Early Plaque Formation Clinically

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Purpose: To test a system of measuring early plaque formation (EPF) and its subgingival extension as related to the presence or absence of a plaque free zone (PFZ).

Materials and Methods: EPF was measured by three independent examiners following two consecutive 72-hour periods of undisturbed plaque build-up. One of the examiners further measured EPF following a 96-hour period in the presence of chlorhexidine or placebo. The classification system was composed of criterion 0 (plaque-free dental surface), criterion 1 (presence of plaque and PFZ) and criterion 2 (absence of PFZ, subgingival extension of plaque). Intra- and inter-examiner reliability were evaluated by means of the percentage of absolute agreement (c), Kappa (k) and Kendall (kd) coefficients. The third experiment consisted of a double-blind, placebo-controlled, cross-over trial. Plaque build-up in the presence of 0.12% chlorhexidine was assessed by employing the classification system described.

Results: The percentage of absolute intra- and inter-examiner agreement ranged from 85.43% to 75.63% and from 77.31% to 75.35% respectively. Chlorhexidine and placebo rinses showed similar percentages of criterion 1 surfaces, 62.6% and 51.5% respectively (p = 0.343). Of the surfaces, 44.3% showed criterion 2 after the use of placebo, while 3.4% of surfaces showed this criterion with the chlorhexidine (p = 0.007).

Conclusion: The events associated with EPF can be appropriately scored with this classification system. Chlorhexidine rinses inhibit both the plaque colonization of the dental surfaces as well as its subgingival extension.

Key words: dental plaque/diagnosis, dental plaque/prevention and control, early plaque formation, reliability

Oral Health Prev Dent 2006; 4: 273-278. *Submitted for publication: 08.08.05; accepted for publication: 15.02.06.*

The events associated with the early supragingival plaque formation (EPF) have been widely studied, both microscopically and clinically, as well as in situ, on strips and on plastic discs (Brady, 1973; Waerhaug, 1975; Saglie et al, 1975; Newman and Hardy, 1984; Friedman et al, 1992). As a result, different ways of measuring these events have been proposed in the literature (Hillam and Hull, 1977; Nyvad and Fejerskov, 1989; Quirynen and van Steenberghe, 1989; Quirynen et al, 1991; Furuichi et al, 1992).

Recently the presence of a plaque-free zone (PFZ) has been reported associated with the formation of supragingival plaque (Weidlich et al, 2001). This PFZ is usually present with up to 4 days of plaque accumulation. Subgingival plaque extension is associated with gingival oedema, which is observed in response to plaque build-up at the dentogingival area. The initial formation of a supragingival plaque is in fact dependent on a series of factors, which include diet (Carlsson and Egelberg, 1965; Scheinin and Mäkinen, 1971; Rateitschak-Plüss and Guggenheim, 1982), microbiota (Socransky et al, 1977) and gingival inflammation (Hillam and Hull, 1977; Quirynen et al, 1991; Furuichi et al, 1992; Ramberg et al, 1994; Ramberg et al, 1995). The influence of gingival inflammation on

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the formation of supragingival plaque and possibly its subgingival extension suggests that the process may be modulated not only by microbiological factors but also by the local defence response. Periodontitis is believed to be a result of the immune inflammatory response to the presence of subgingival plaque (Kornman et al, 1997). The subgingival downgrowth of bacteria may thus be an initial step in the line of events associated with the onset of destructive periodontal diseases (Listgarten, 1999).

Adequate measurements of the plaque build-up are necessary for a better understanding of the mechanisms involved in its formation and subgingival extension. Plaque indices, based on scales according with the amount of plaque present, bear simplicity and reproducibility (Furuichi et al, 1992; Ramberg et al, 1994; Ramberg et al, 1995). In general, they require a prolonged accumulation period and they are not able to describe the initial events in plaque formation. Another valuable technique includes photography (Nyvad and Fejerskov, 1989; Quirynen and van Steenberghe, 1989), with which both optical and digital planimetric measurements have been used. These methods are useful in discriminating the extension of the bacterial deposits on the dental surface. However, their characteristics may not be suitable to describe the events associated with the subgingival downgrowth of dental plaque.

In a study by Weidlich et al (2001), 'the supragingival extension of the plaque in the subgingival direction appeared to be much less evident than its incisal extension', suggesting possible modulating mechanisms for extension into that area. It is possible that the PFZ may be a clinical indicator of this interaction. A better understanding of the dynamics involved in the formation of supragingival plaque and its subgingival extension is necessary. From this starting point, it should be possible to evaluate the intervening factors in the process of the formation of supra- and sub-gingival plaques.

The aim of the present study was to test a system of measuring EPF and its subgingival extension as related to the presence or absence of a PFZ.

MATERIALS AND METHODS

EPF and the presence of PFZ were measured by three independent examiners following two consecutive 72-hour periods of plaque build-up. In the sequence, one of the examiners repeated the measuring procedure following a 96-hour period of plaque build up in the presence of chlorhexidine or placebo.

Participants

The participants consisted of 10 non-smoking men between the ages of 20 and 33 (average 23.8 years). The criteria for inclusion in the study were: absence of restorations and cervical irregularities in the upper and lower anterior teeth, absence of gingival recession or marginal bleeding as measured by the index of gingival bleeding (Ainamo and Bay, 1975) and absence of antimicrobial therapy during the last 6 months. The study was approved by the Committee on Ethical Affairs of the Lutheran University of Brazil.

Undisturbed plaque build-up period

In order to determine the intra- and inter-examiner reliability of the classification system, three examiners applied the criteria after two 72-hour periods of plaque accumulation in upper and lower canines and incisors. Plaque accumulation started following a thorough dental polishing guaranteeing absence of disclosed deposits. The participants were requested not to brush or floss upper and lower incisors, canines and premolars. After 72 hours, the teeth were washed, dried and isolated with cotton rolls. The bacterial plaque was then disclosed, with basic fuchsin (Replanic T, Iodontec, Brazil) sprayed using a disposable syringe and a needle (Becton Dickinson, Franklin Lakes, NJ), in order to guarantee the access of the fuchsin to the gingival sulcus. Using a marking pen (UNI PIN 02-200 black fine line, Mitsubishi pencil Co. Ltd, Japan), two reference points were placed on the buccal gingiva, 1–2 mm apically from the gingival margin, dividing the buccal surface longitudinally into three equal thirds.

Three examiners (A/B/C) evaluated the mesiobuccal, buccal and distobuccal areas of the test teeth, using a classification system (Fig 1) that describes the process of plaque formation up to the extinction of the PFZ: criterion 0, plaque-free dental surface; criterion 1, presence of disclosed plaque and presence of a PFZ; criterion 2, subgingival extension of the plaque (presence of disclosed plaque and absence of a PFZ). The scoring procedures were performed by each examiner, who kept a sequential order of examinations (A/B/C), as well as the independence of their evaluation. The average examination time for the three examiners was 5.75 minutes. After an interval of 40 minutes, plaque was disclosed again and each examiner repeated the scoring procedures. The participants received coronal polishing and returned to their routine oral hygiene habits for 4 days. Inter-examiner reliability was further tested in a new experiment, similar to the first one, with the difference that only one examination was performed.

Plaque build-up period controlled by chlorhexidine mouthrinses

In order to further analyse the classification system, the participants took part in a double-blind, placebo-controlled, cross-over trial. Following dental polishing until no disclosed deposits could be detected, the participants were instructed to discontinue all mechanical oral hygiene procedures and were randomly divided into two groups: one group rinsed twice daily with 15 ml of 0.12% chlorhexidine digluconate for 1 minute and the other rinsed twice daily with 15 ml of 0.1% quinine sulphate containing mint essence and blue dye for 1 minute. After 72 and 96 hours, plaque was disclosed with basic fuchsin, as described in the reliability study, and the reference examiner (A) performed the scoring procedures according to the classification system described. Following a 10-day washout period, when the participants returned to routine oral hygiene habits, the experiment was repeated in the same way as described above, with the exception that the participants changed the rinses used.

Statistical analysis

Intra-examiner reliability was calculated in the first series of plaque build-up employing the percentage of absolute agreements and Kappa coefficient. The inter-examiner variation was calculated by comparing results of two consecutive plaque build-up periods, with the aid of the percentage of absolute agreements as well as the Kappa and the Kendall coefficients. To compare results of the clinical trial, the Friedman and Wilcoxon tests were used. All comparisons were set at the 5% significance level.

RESULTS

Undisturbed plaque build-up period

The percentage of absolute intra-examiner agreement showed that the three examiners were similar in their evaluations. Examiner A showed 85.43% absolute agreement, while examiners B and C showed 75.63% and 80.39% respectively. The Kappa coefficient was 0.732 for examiner A, and 0.604 and 0.691 for examiners B and C respectively.

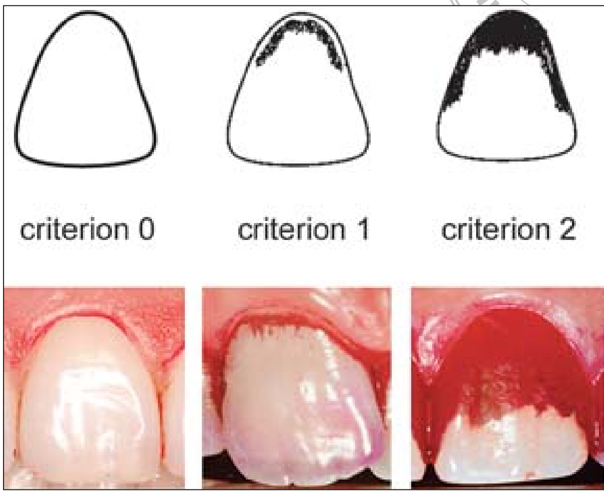

Fig 1 Schematic drawings and clinical photographs illustrating criteria 0, 1 and 2.

Examiner A was chosen as the reference examiner. Inter-examiner reliability, as measured by the Kappa coefficient, was similar when comparing both examiner A with B (0.627) and examiner A with C (0.601). The percentage of absolute agreements was also similar in both comparisons: 77.31% and 75.35% for examiners A/B and A/C respectively. Similar results were obtained in the second series of undisturbed plaque build-up (Table 1). The Kendall coefficient of agreement was 0.831 and 0.745 in the first and second series respectively.

Plaque build-up period controlled by chlorhexidine mouthrinses

The plaque-inhibiting effect exerted by chlorhexidine was clearly demonstrated using this classification system. Fig 2 shows that a significantly lower proportion (10.6%) of the surfaces examined after 72 hours were free of disclosed deposits (criterion 0) after the use of the placebo, when compared with 56.3% of surfaces free of disclosed deposits after using chlorhexidine ($p = 0.018$). The percentage of surfaces exhibiting plaque and PFZ (criterion 1) was higher for placebo (67.8%) than for chlorhexidine (43.4%) ($p = 0.022$). Interestingly, 21.6% of the sites examined presented plaque that extended subgingivally (criterion 2) after the use of placebo, whereas only 0.3% was found in this condition after the use of chlorhexidine ($p = 0.005$). After 96 hours of plaque build-up, the percentage of surfaces without disclosed deposits (criterion 0) reduced

Table 1 Inter-examiner variation determined in two 72-hour plaque build-up periods

Examiners	Period 1		Period 2	
	k	c	k	c
A/B	0.627	(77.31%)	0.615	(77.03%)
A/C	0.601	(75.35%)	0.637	(78.43%)

k, Kappa coefficient
c, absolute agreement

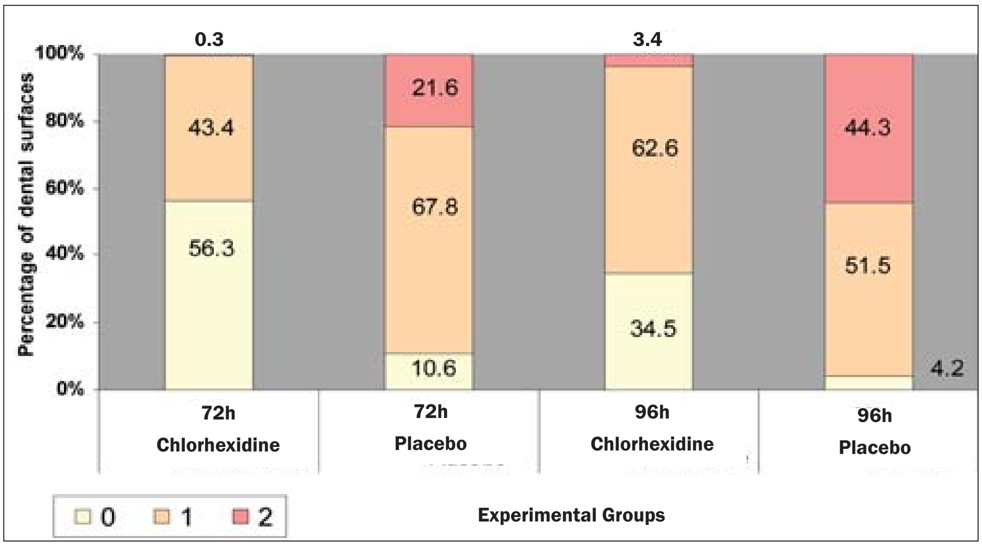

Fig 2 Frequency distribution for plaque-free dental surface (criterion 0), presence of plaque and PFZ (criterion 1) and presence of plaque and subgingival extension of the plaque (criterion 2) 72 and 96 hours after the use of chlorhexidine and placebo (n = 357).

to 4.2% with the use of placebo. whereas 34.5% of surfaces remained in this condition with chlorhexidine (p = 0.005). The percentage of surfaces presenting a PFZ (criterion 1) was similar between chlorhexidine and placebo rinses, 62.6% and 51.5% respectively (p = 0.343). On the other hand, 44.3% of the surfaces showed that the presence of plaque extended subgingivally (criterion 2) after the use of placebo while only 3.4% of surfaces showed this criterion associated with the use of chlorhexidine (p = 0.007). The results observed in the mesiobuccal, distobuccal and buccal areas of the teeth were compared and no significant differences were observed.

DISCUSSION

The present study showed that it is possible to measure the events associated with EPF using the classification system proposed. This observation was made by applying the pre-established criteria in periods of plaque formation up to 96 hours. These periods were repeated twice and under different time intervals with plaque formation evaluated by three independent examiners.

The duration of the observation periods is supported by studies that demonstrated that the main topographical alterations in the formation of plaque occur within the first 96 hours (Bergström, 1981; Quirynen, 1989; Furuichi et al, 1992). Furthermore, Weidlich et al (2001) showed that the transformation of surfaces with a PFZ into surfaces with the subgingival extension of the plaque occurs mainly between 48 and 72 hours.

The variability within the examiners, shown in Table 1, can be considered at satisfactory levels compared with the results from other studies of a similar nature. Spolsky and Gornbein (1996) compared two records reported by Silness and Löe (1964) of plaque index with an interval of 20–30 minutes between each record, and they found 51.2% for absolute agreement (c), 0.4 for unadjusted kappa (k) and 0.57 for adjusted kappa. Kawamura et al (2000), analysing a diagnostic test that evaluated plaque, calculus and gingivitis, calculated values of c = 76.7%, k = 0.67 and c = 71.2%, k = 0.61 for two examiners respectively. Matthijs et al (2001) examined the intra-examiner variation using the Visible Plaque Index (VPI) and the Modified Navy Plaque Index (MPI). The results for the buccal and lingual VPI were c = 78%, k = 0.70 and c = 75%, k = 0.75 respectively. The inter-examiner variations for MPI on buccal surfaces were c = 88%, k = 0.81, and on lingual surfaces c = 73%, k = 0.62.

A similar situation could be observed in the present study considering inter-examiner agreement. The inter-examiner agreement ranged from 75.35% to 78.43%. In the study of Kawamura et al (2000), the percentage of inter-examiner reliability was c = 59% and k = 0.449. Levikind et al (1999), examining occlusal plaque with the same index used by Addy et al (1998), observed adjusted kappa varying between 0.657 and 0.777. The kappa levels observed in this study are considered good according to Landis and Koch (1977) and are reiforced by the Kendall coefficient levels. It is important to observe that the formation of plaque varied between participants. Inter-individual variations in the pattern of plaque formation have already been reported in the literature (Hillam and Hull, 1977; Bergström, 1981; Ramberg et al, 1992; Ramberg et al, 1994; Ramberg et al, 1995).

Chlorhexidine rinses significantly inhibited plaque downgrowth subgingivally. Only 0.3% of the surfaces at 72 hours and 34% at 96 hours exhibited plaque downgrowth subgingivally in association with chlorhexidine rinses, whereas with placebo these figures were 21.6% and 44.3% respectively. The presence of plaque with PFZ after 72 hours was 43.4% with chlorhexidine and 67.8% with placebo. These differences were not observed after 96 hours, when 62.6% with chlorhexidine and 51.5% with placebo showed the presence of plaque and PFZ. These results indicate that chlorhexidine has a more pronounced inhibiting effect towards the apical downgrowth of plaque than the spreading of the plaque on the supragingival area of the tooth. Some studies have used quinine sulphate as a placebo to chlorhexidine due to similar taste (Hansen et al, 1975; Langebaek and Bay, 1976). One study failed to show any antibacterial activity or any effect on bacterial growth for quinine sulphate in vitro (Wolf et al, 2002).

In contrast to existing plaque indices, which measure plaque amount or distribution supragingivally, the plaque measurement tested in the present study evaluated plaque downgrowth towards the gingival crevice. The results of the present study support the observation made by Weidlich et al (2001) concerning a faster spread of the supragingival plaque in the incisal direction than towards the crevice, in spite of the fact that initial colonization occurs very close to the sulcular area. At present there is no clear explanation for this observation. It is known that gingival inflammation speeds up plaque extension on the surface of the teeth. In the present study, as well as in the study developed by Weidlich et al (2001), the participants did not present clinical signs of gingivitis.

The results of the present study showed that chlorhexidine rinses inhibited plaque formation on the dental surfaces, a fact already known, as well as inhibiting the subgingival extension of this plaque. This may represent that the subgingival bacterial challenge to the peridontium may be retarded by this procedure.

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