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A comparison of two chlorhexidine gel brushing regimens and a conventional toothpaste brushing regimen for the development of tooth staining over a 6-week period

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Abstract: *Aims:* A single-centre, randomized single-blind parallel study was undertaken to compare staining seen with three brushing regimens and to determine subject perception of side effects such as staining and effects on taste. *Methods:* This 6-week parallel study used 157 volunteers who were randomized into one of three treatment groups: (i) brushing in the morning and evening with a normal dose of a 1% chlorhexidine gel, (ii) brushing with a low dose of chlorhexidine gel in the evening and a whitening dentifrice in the morning, and (iii) brushing with a standard fluoride paste in the morning and evening. Following home usage of their allocated products, the study volunteers returned after 3 and 6 weeks to record the amount of stain present. After the 6-week period, subject perception of taste and stain acceptability was determined using a questionnaire. *Results:* After 6 weeks of use of the low-dose chlorhexidine gel and whitening dentifrice, significantly more stain was seen compared with the use of a standard dentifrice ($P < 0.0001$). Similarly, significantly more stain was seen with use of the normal-dose chlorhexidine gel compared with the low-dose gel and whitening dentifrice ($P = 0.0007$). Approximately 30% of individuals on the low-dose chlorhexidine gel regimen found the amount of stain unacceptable and 10% noted an effect on their taste perception. *Conclusions:* The use of low dose of chlorhexidine gel at night and a whitening paste in the morning produced a significant amount of stain that 30% of subjects considered unacceptable.

Key words: chlorhexidine gel; clinical trial; staining; whitening dentifrice

Introduction

One of the major limiting factors which preclude the long-term use of chlorhexidine products is extrinsic staining. This staining is seen whether the antiseptic is employed as a mouthrinse or gel, and is specifically cited as the reason for exclusion of long-term use (1). There is a lack of well-conducted studies specifically aimed at measuring staining following the use of chlorhexidine gel. Much of the information has been derived from studies conducted to measure the effects on plaque and gingivitis, with reported incidence of staining being of secondary importance (2–6). For chlorhexidine mouthrinse, some attempt has been made to reduce its staining tendency by reducing its concentration (7, 8) or by adding stain-reducing chemicals such as polyvinyl pyrrolidone (PVP) (9, 10). Unfortunately for the latter, reduction in efficacy can occur whilst lower concentrations of chlorhexidine used in larger volumes can maintain the therapeutic effects for the antiseptic with a possibility of reducing staining (7, 8). Similar approaches to limit chlorhexidine-induced staining with the use of chlorhexidine gel have yet to be documented. Thus, it is not known whether reducing the concentration or the daily dosage of gel results in significant reductions in staining whilst maintaining the therapeutic effects of the antiseptic. Perhaps surprisingly, there appears to be some variation in the regimen recommended for the use of chlorhexidine gel. Thus chlorhexidine gel has been used at 0.5% or 1% w/w concentration, with application via toothbrushing either once or twice a day. The use of the gel, particularly once a day just in the evening, would have one major advantage (4–6). Such timing would be expected to limit the amount of staining which may occur. Certainly evidence for reduced staining with evening use of chlorhexidine mouthrinse compared with morning use is documented but not for use of a gel (11). In terms of gel dosage, it is also possible that the use of smaller amounts brushed at evenings only and the concomitant use of a whitening dentifrice in the morning could effectively limit the amount of staining. The present study was designed to measure the value of such an approach at limiting chlorhexidine-induced staining by reducing the dose of gel and by using a commercial whitening dentifrice. Previous experience with the same PVP dentifrice had shown efficacy in inhibiting chlorhexidine-induced staining following multiple rinses with chlorhexidine mouthrinse and tea (12). Although not the remit of this study, it is possible that using this regimen could result in positive therapeutic effects other than inhibiting plaque formation, such as reducing early morning oral malodour (13, 14) or reducing problems of oral ulceration (15). The primary aim of the present pragmatic study was to compare two regimens of use of a chlorhexidine gel with conventional tooth brushing with toothpaste on the development of extrinsic tooth staining. A secondary aim was to determine the acceptability to the subjects of the staining which developed by the end of the 6-week study period.

Materials and methods

This 6-week, single-blind, randomized parallel study was designed to evaluate the effects on extrinsic staining of three treatment modalities:

1 Normal dose of a 1% chlorhexidine gel (CORSODYL®; GlaxoSmithKline, Weybridge, Surrey, UK) brushed once in the morning and once in the evening. The gel was applied by each subject to provide a full toothbrush head coverage of the brush (MACLEANS® brand toothbrush; GlaxoSmithKline) with the gel (conventional chlorhexidine gel regimen).

2 Normal dose (full toothbrush head) of a commercial fluoride dentifrice (AQUAFRESH® brand toothpaste; mild & minty flavour; GlaxoSmithKline) again brushed once in the morning and once in the evening (conventional tooth brushing with toothpaste regimen).

3 Reduced dose of a 1% chlorhexidine gel brushed in the evening and a normal dose (full toothbrush head) of whitening dentifrice [AQUAFRESH® brand toothpaste (whitening); GlaxoSmithKline] brushed in the morning. The reduced dose of the chlorhexidine gel was applied by each subject extruding a small pea size amount of gel onto the toothbrush instead of the normal full head coverage (test gel regimen). An approximation of the amount of gel applied to the brush was determined by a clinical assistant prior to the study by weighing a brush with or without the applied gel.

Prior to the study, approval was given by the local ethics committee and the study carried out according to good clinical practice (GCP). A total of 164 volunteers were screened prior to the start of the study on the basis of at least 150 (50 per group) volunteers would complete the 6-week period. Subjects were accepted onto the study provided they fulfilled the conditions of the inclusion and exclusion criteria. These included the following: subjects had a minimum of 20 scorable anterior teeth without the presence of tooth-coloured restorations which could stain permanently and had no active oral pathology. Exclusion criteria included idiosyncrasies to chlorhexidine products, and the presence of medical conditions requiring oral antibiotic prophylaxis. Subjects then received a pre-trial toothbrush and standard fluoride dentifrice, and were told to brush at home according to their normal tooth brushing habits. Approximately 2 weeks later, at the baseline visit, each subject

received a dental prophylactic treatment by a hygienist. Any residual extrinsic staining on the buccal surfaces of the upper and lower anterior teeth (premolars, canines and incisors) was scored by an experienced clinician using the modified Lobene Index (16). Thus, the intensity of stain on the gingival crescent and body of the tooth on the buccal surfaces of each assessable incisor, canine and premolar and lingual surfaces of all incisors and canines were observationally scored using the four-point scale (below): 0, no stain; 1, light stain; 2, moderate stain; 3, heavy stain.

Similarly, the area of stain on the gingival crescent and body of the tooth on the buccal surfaces of each assessable incisor, canine and premolar was observationally scored using the four-point scale (below): 0, no stain detected only tooth colour; 1, stain covering up to one-third of the tooth surface; 2, stain covering between one-third and two-thirds of the tooth surface; 3, stain covering more than two-thirds of the tooth.

Without the knowledge of the assessing clinician (NC), each subject was allocated to one of the three treatment groups using a randomization schedule which was stratified to include smokers. Basically, the clinical study site was provided with study products which were labelled with unique subject randomization numbers. Subjects were assigned a unique randomization number according to their smoking status. Subjects who smoked were given sequential numbers from the top of the randomization list, non-smokers from the bottom of the list. At the baseline visit, each volunteer was instructed in the use of each product. This was carried out in another room not in the presence of the assessing clinician. Subjects were not instructed to use any specific tooth brushing technique, but to brush with the allocated product for 1 min, using their usual tooth brushing habit. Subjects were told to refrain from the use of floss, chewing gum and any other mouth rinses. Moreover at this baseline visit, all subjects were provided with a diary to record when brushing with the allocated products was undertaken. After 3 and 6 weeks of using the allocated products, each volunteer returned for inspection by the assessing clinician who was experienced and calibrated in previous stain assessment studies (9, 12). The presence of extrinsic staining was again measured using the modified Lobene Index for stain intensity and area on the buccal aspects of the test teeth. On completion of each 6-week period, each volunteer received a questionnaire from a clinical assistant to determine (1) the effect of the treatment on taste (2), whether any stain produced was considered acceptable or unacceptable. This questionnaire was filled out at the last stain assessment visit so as to ensure a maximum return. At this point all subjects received a scale and polish to remove any residual extrinsic stain.

Statistical analyses

Prior to the study, an estimation of the number of subjects needed for the study was determined, and as such it was planned that 50 evaluable subjects per treatment group would complete the study. It was estimated that with these number of subjects, it would be possible to detect a mean difference of 0.20 (SD = 0.35) in week 6 Lobene Stain Index (intensity × area) between treatments with more than 80% power. The estimate of SD was obtained from a stain removal study (data on file).

The primary efficacy variables reported were the intensity × area product score, intensity score and area score averaged over the body and gingival areas of the buccal surfaces. Differences between treatment groups were analysed using the analysis of covariance with a factor for treatment group, smoking status and gender as co-variates. All statistical testing was performed at the 5% significance level and *P*-values and 95% confidence intervals determined for the following two treatment comparisons:

- Low-dose chlorhexidine gel + whitening dentifrice versus standard dentifrice.
- Low-dose chlorhexidine gel + whitening dentifrice versus normal-dose chlorhexidine gel.

The effects of the three treatment groups on taste perception, both absolute and extent were also analysed from the questionnaire using the same statistical methodology.

Results

The mean weight of a pea-sized amount of gel applied to a brush by a clinical assistant was 0.43 g (SD = 0.09, $n = 10$). This compares with 0.97 g (SD = 0.13, $n = 10$) when a normal dose of gel was applied.

Weight determination of returned unused toothpastes and gel signified satisfactory compliance by all the volunteers of the study. Similarly, examination of the diary cards signified satisfactory compliance. Except for the presence of staining and one occurrence of oral ulceration, no untoward side effects were noted with the use of any of the products.

Of the 164 subjects (56 males, 108 females, mean age 29.2 years) enrolled in the study, 157 completed the 6-week study period with complete data sets. Subsequent analysis (intention-to-treat population) was based on these 157 subjects.

The levels of stain for the body and gingival areas of the buccal surfaces of the teeth increased over the study duration for all treatments (Table 1). Subjects brushing with standard dentifrice developed the least stain and those brushing with normal-dose chlorhexidine gel experienced the most staining. Subjects brushing with low-dose chlorhexidine gel and whitening dentifrice developed staining at levels in between that seen with the other two treatments.

As measured by the intensity × area product score, after 6 weeks of brushing with low-dose chlorhexidine gel at night and whitening dentifrice in the morning, significantly less staining was seen than brushing with normal-dose chlorhexidine gel twice daily ($P = 0.0007$) (Table 2). Similarly, brushing with low-dose chlorhexidine gel at night and whitening paste in the morning produced significantly more staining than brushing with standard dentifrice twice daily at both 3 and 6 weeks (intensity × area product score, $P = <0.0001$) (Table 2). Analysis of the questionnaire found that 30% of the subjects on the low-dose chlorhexidine gel found the level of stain produced unacceptable compared with 57% on the normal-dose chlorhexidine and 6% on the standard dentifrice; 10% of the subjects on the low-dose chlorhexidine reported an effect on their taste, compared with 55% on the normal dose and only 2% of those on the standard dentifrice. The difference in proportion affected between the three treatment groups was significant when comparing the low-dose gel and the normal-dose gel ($P < 0.0001$). For those who reported an effect on taste, there was no significant difference in the extent to which taste was affected when comparing the low-dose gel with either the standard dentifrice or normal-dose chlorhexidine gel ($P > 0.05$).

Table 1. **Summary of intensity × area, intensity and area stain scores following 3 and 6 weeks of use of allocated products**

Assessment	Summary Statistics	Treatment A	Treatment B	Treatment C
Intensity × area				
Week 3	Mean (SD)	0.71 (0.55)	1.48 (1.04)	1.65 (1.16)
Week 6	Mean (SD)	0.82 (0.54)	1.92 (1.28)	2.82 (1.70)
Intensity				
Week 3	Mean (SD)	0.50 (0.28)	0.87 (0.49)	0.95 (0.48)
Week 6	Mean (SD)	0.58 (0.28)	1.09 (0.57)	1.39 (0.64)
Area				
Week 3	Mean (SD)	0.66 (0.48)	1.07 (0.61)	1.21 (0.60)
Week 6	Mean (SD)	0.76 (0.46)	1.20 (0.63)	1.63 (0.67)

A, standard dentifrice; B, low-dose chlorhexidine gel + whitening dentifrice; C, normal-dose chlorhexidine gel; SD, standard deviation.
Treatment A included nine smokers, treatment B ten smokers and treatment C eight smokers.

Discussion

This study was not designed to be exploratory or explanatory in design: numerous paired treatments would have been required. The design simply investigated whether combining a low dose of chlorhexidine gel with a whitening toothpaste would reduce staining compared with the conventional regimen of gel usage and to a level similar to that seen with conventional toothbrushing with toothpaste. No attempt was made to analyse the diet of the subjects or control the dietary intake of chromogens, although all subjects were expected to refrain from eating or drinking following toothbrushing in the evening.

It is well established that products and preparations containing chlorhexidine may be of value not only in reducing plaque and gingivitis but may also be useful in helping to reduce other oral problems such as ulceration and oral malodour (1, 13, 17–19). For chlorhexidine in a gel form, evidence for efficacy in reducing both plaque and gingivitis is equivocal (2–5), whilst some benefit at least in reducing the symptoms of oral ulceration has been noted (15). Irrespective of which type of vehicle used, be it mouthrinse or gel, the

Table 2. **Comparative analysis of mean stain scores for intensity × area, intensity and area following 3 and 6 weeks of use of allocated products**

Assessment	Treatment B versus A	Treatment B versus C
Intensity × area		
Week 3		
Difference	0.84	−0.16
95% CI	0.473 to 1.202	−0.525 to 0.206
P-value	<0.0001	0.3906 (NS)
Week 6		
Difference	1.22	−0.86
95% CI	0.730 to 1.709	−1.353 to −0.371
P-value	<0.0001	0.0007
Intensity		
Week 3		
Difference	0.40	−0.07
95% CI	0.240 to 0.564	−0.237 to 0.089
P-value	<0.0001	0.3740 (NS)
Week 6		
Difference	0.56	−0.28
95% CI	0.363 to 0.765	−0.485 to −0.082
P-value	<0.0001	0.0062
Area		
Week 3		
Difference	0.43	−0.13
95% CI	0.215 to 0.653	−0.353 to 0.086
P-value	0.0001	0.2322 (NS)
Week 6		
Difference	0.49	−0.42
95% CI	0.255 to 0.718	−0.654 to −0.189
P-value	<0.0001	0.0005

A, standard dentifrice; B, low-dose chlorhexidine gel + whitening dentifrice; C, normal-dose chlorhexidine gel; NS, no significance; CI, confidence interval.

side effect of extrinsic staining remains problematic. To reduce this tendency, a number of strategies could be suggested: (i) reduce the overall oral dosage of the gel; (ii) restrict intake of dietary chromogens; (iii) use the product just before retiring to bed; and (iv) use a whitening dentifrice. This study was primarily designed to determine the amount of stain produced when brushing with a low dose of chlorhexidine gel at night and in the morning with a whitening dentifrice, compared with that seen when brushing twice daily with a standard toothpaste or normal dose of the chlorhexidine gel. The findings of the study once more highlighted the significant problem of staining seen with the use of chlorhexidine products, even when used at reduced dosage last thing before bedtime and when used in conjunction with the whitening dentifrice. Moreover, 30% of the volunteers found the degree of staining with the reduced chlorhexidine dose unacceptable, whilst 10% noted an effect on their taste. The use of the whitening paste had previously been shown to reduce chlorhexidine-induced staining and may be expected to have a beneficial effect (12). However, the design of the study would not be capable of demonstrating any benefit by the whitening dentifrice alone but any reduction in staining could equally be due to the low dose of chlorhexidine gel used. Equally, it is acknowledged that the benefits of the whitening paste maybe evident only with its use twice daily, as would be the normal practice at home.

Although staining with chlorhexidine products may be an unwelcome side effect, lack of staining with chlorhexidine products would suggest lack of clinical activity. Thus, previous studies on chlorhexidine mouth rinses, which were claimed not to produce staining, were subsequently shown to lack clinical activity (20). In the present study, staining was seen with the reduced dose of chlorhexidine and as such it would not be surprising that its use would have oral therapeutic effects. This is in spite of its use at a much reduced dose than that normally recommended, say to inhibit plaque and gingivitis. Although it would have been interesting to have obtained study data also on plaque and gingivitis, the reasoning behind the study was that using reduced doses of chlorhexidine gel may be of benefit in the management of oral malodour. Indeed the findings of a study on the effects of the reduced dose on oral malodour showed some benefit at reducing this problem when assessed using a hedonic panel (data on file).

In summary, this study has demonstrated that even using low doses of chlorhexidine gel and a whitening dentifrice, significant amounts of staining is still produced. If staining could have been shown to be not significantly different to that seen with the standard dentifrice, then the use of a low dose of 1% chlorhexidine gel and a whitening dentifrice could have been a recommended strategy to limit this troublesome side effect. The findings of the present study would not appear to support the use of such a regimen to limit chlorhexidine-induced stain.

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