Evaluation of Plaque Removal Efficacy of Dental Floss with/without Chlorhexidine Gel Coating in Patients with Gingivitis - A Clinical and Microbiological Study

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Abstract

BACKGROUND: Chlorhexidine has shown anti-plaque and antimicrobial effects when used as a mouthwash and appears to be effective when used as a topical antiseptic agent.

AIM: The present study aimed to compare the efficacy of chlorhexidine gel coated dental floss with that of uncoated dental floss.

METHODS: This parallel, single-blinded, randomized controlled clinical trial included 30 patients with moderate to severe gingivitis. The total population was randomly divided into three groups, with ten patients in each group. Group A received dental floss with 1% chlorhexidine gel, and Group B received only dental floss, while in Group C no dental floss was provided. All thirty volunteers were provided with standard toothpaste and toothbrush. Clinical parameters such as gingival index, plaque index, and bleeding index were recorded along with supragingival plaque sample collection for microbiological culture. Subjects were recalled after 15 days and clinical and microbiological analysis was performed. All parameters were re-assessed at the follow up visit after two weeks.

RESULTS: All the groups showed a significant reduction in values of plaque index, gingival index, and bleeding index, as well as the microbial counts post-enrollment in the study. A significant reduction in the bleeding indices was noticed in Group A in comparison to C (p < 0.05).

CONCLUSIONS: The use of dental floss coated with 1% chlorhexidine gluconate gel was effective as an interproximal aid for patients with moderate to severe gingivitis.

Introduction

It is an eminent fact that plaque induces inflammatory changes in the periodontium. Plaque is the main cause of gingivitis and periodontitis and needs to be restrained or eliminated on a quotidiant [1, 2]. Plaque removal is crucial for a robust gingiva and dental flossing acts as an adjunct along with a toothbrush. Most of the individuals perform tooth brushing once or diurnally. Dental floss adds on to tooth brushing in the removal of plaque than in comparison to tooth brushing alone [3], particularly in interproximal areas [4]. There are in vivo studies comparing the plaque removal efficacy of dental floss [5]. Perhaps, it is of utmost importance to provide the individuals with modalities for plaque removal from the interproximal surfaces of the teeth as well [6].

Periodontal diseases generally start and are more noticeable in interproximal niches because they are ideal places for biofilm to grow and difficult to eliminate completely. To provide adequate plaque removal from the interproximal areas, several interproximal cleaning devices such as interdental brushes and dental floss are advised. The morphology of gingival embrasures should be considered for selecting the most pertinent interdental device for each individual [7]. Less bleeding from the gums is seen in patients without periodontal attachment loss who brush and floss their teeth regularly in comparison to those who use a toothbrush only [8], thus suggesting that the use of dental floss might be advantageous for these patients.

To maintain good hygiene in the interproximal areas of the teeth, depending on the embrasure area dental floss/untufted or multitufted brushes are advised. It has already been the standard of care [9]. Along with mechanical control of dental plaque, there is a possibility of the chemical mode of prevention for which chlorhexidine has long been gold standard [10]. Chlorhexidine acts by lysis of the bacteria and it also has a good substantivity. Although chlorhexidine mouthwash has certain drawbacks such as staining of the dentition and tongue and oral mucosal desquamation [11], the study showed no side effects of
chlorhexidine when used in the local application in gel form [12]. Mechanical plaque control like tooth brushing should be accompanied with interproximal flossing in every patient. Additionally the effect of chlorhexidine would improve the overall effect of flossing the interproximal surface [13]. Thus, there is a need for a clinical study that addresses the efficacy of dental floss as a mechanical aid with an added benefit of chemical plaque control agent chlorhexidine included during the interproximal flossing.

Considering these facts, the present study sets the goal to evaluate the plaque removal efficiency of the dental floss coated with chlorhexidine. The study would fulfill the following objectives: (A) Compare the plaque removal efficiency between the patients using dental floss with or without the chlorhexidine gel, (B) Compare the microbial colony forming units (CFU) between the patients using dental floss with or without chlorhexidine, and (C) Evaluate the gingival and bleeding indices between the control and the test groups.

Materials and Methods

The study was designed as parallel arm, single blind, and single-centered, open-labeled, non-experimental clinical study. Subjects visiting the Department of Periodontology with moderate to severe gingivitis, aged between 29 to 33 years, were enrolled in the study. The study commenced only after obtaining the approval of the Institutional Ethics Committee. A total of thirty subjects were included in the study. After obtaining written informed consent, the subjects were randomly distributed, by lottery method, into three groups with ten volunteers in each group. All the subjects had a minimum of twenty teeth, with class 1 gingival embrasure. Individuals with carious teeth, severe periodontal disease, were either undergoing orthodontic treatment or using removable partial dentures or having history of use of chlorhexidine or dental floss in the previous six months were excluded from the study. Subjects who were either systemically unhealthy, pregnant or smoked tobacco or were generally found not willing to comply with instructions were excluded.

All the subjects participating in the study were given a demonstration of a standard brushing technique [14] (Modified Bass method). They were also demonstrated the ADA described method of using the dental floss, i.e., wrapping the floss of 18 inches length around the middle finger followed by use of the index finger along with the thumb to guide the floss in up and down movements between the teeth. They were asked to perform it in front of the examiner and were provided with a video of the same procedure. Each subject received a standard soft-bristled toothbrush (Colgate Colgate Plus, Colgate-Palmolive, India) and a standard dentifrice (Colgate Colgate plus, Colgate-Palmolive, India). Furthermore, the volunteers were requested to refrain from the use of any antimicrobial mouthwash throughout the study period. All the participants were instructed to brush twice daily and floss their teeth as instructed every night before bed. A reminder message was sent to the participants of Group A and B to floss after brushing every evening and to reply back once they have completed it. On the 15th day, subjects were recalled for the follow-up. After recording the clinical indices, the plaque sample was collected for microbiological analysis (Figure 1).

Clinical evaluation

An investigator performed supragingival scaling and polishing, to make the dentition free of plaque and calculus. At first microbiological sample was collected from interdental spaces using unwaxed dental floss piece. Second, Loe and Silness gingival index [15] was utilized to mark the gingival condition followed by the recording of interproximal bleeding on probing [16] using Williams probe. Finally, plaque indices were recorded as per Rustogi modified navy plaque index (RMNPI) using a disclosing agent [17]. Subjects were recalled again after 15 days for follow-up and the gingival, papillary bleeding, and plaque indices were recorded and supragingival plaque sample for microbial analysis was collected at this second visit. Other parameters recorded during the follow-up were difficulty experienced by the subjects while flossing their teeth. These parameters were applicable only for Group A and B. Difficulty score was purely a subjective rating on the scale of 1–5 as marked by the participants.

Microbiological evaluation

Dental floss was used to collect the supragingival interdental plaque sample. The plaque containing floss were inoculated aseptically in Robertson's cooked meat (RCM) broth and were transported immediately for microbiological analysis. The RCM bottles were incubated at 37°C for 48 h in the anaerobic chamber (Whitley A35 Anaerobic workstation, Don Whitley Scientific, Shipley, UK). Each sample was vortexed and quantitative cultures were performed in different dilutions (1:10, 1:100, 1:1000 and 1:10,000 dilutions). 10 µl from each dilutions were inoculated on 5% sheep blood agar. The plates were incubated in anaerobic workstation at 37°C for 72 h, following this the colony forming units (CFU/mL) were counted for each specimen. Bacterial colonies were counted and colony-forming units were calculated. The viable organisms were expressed as the total colony-forming units per ml.

The collected data were analyzed using the statistical package, SPSS version 16.0. A student t-test
Total of 42 volunteers were screened for the study with moderate to severe gingivitis.

Excluded due to:
- Orthodontic treatment (6)
- Did not agree to participate (4)
- Wide embrasures (2)

Total of 30 volunteers with moderate to severe gingivitis divided into three groups:

- **Group A (n=10)**: Chlorhexidine with dental floss
- **Group B (n=10)**: Dental floss
- **Group C (n=10)**: No floss

Baseline:
1. Recording clinical indices
2. Collection of plaque sample for microbiological culture

Follow-Up (15 Days):
1. Recording clinical indices
2. Collection of plaque sample for microbiological culture
3. Recording difficulty index experienced by patients

**Figure 1: Consort flowchart of the study design**

was performed for intragroup analysis. p < 0.05 were considered statistically significant.

**Results**

The mean age of the subjects ranged between 29.2 and 32.7 years (Table 1). There was an equal number of males and females in all the groups.

**Table 1: Mean age in each group**

| Parameters | Group A | Group B | Group C |
|------------|---------|---------|---------|
| Number of subjects | 10 | 10 | 10 |
| Mean age | 32.7 | 26.8 | 29.2 |

The mean of the difference in the plaque index and the microbial colony count are summarized in Table 2. The dental floss using chlorhexidine showed significantly better results in plaque index compared to the use of floss alone or the group that did not use floss but only brushed (p < 0.05). Although very similar values of mean reduction (0.68) were noted for the differences in the plaque scores for all three groups (Figure 2), it is important to observe the overall significant reduction in the values post-enrollment in the study. The maximum reduction in microbial colony counts (CFU) ($10^7$) observed for the subjects in the colony-forming units was seen with Group A (108.7±30.6) followed by Group B and least with Group C (Figure 3).

**Table 2: Differences in the means of the plaque index and the microbial count for the three groups with a standard deviation**

| Parameters      | Group A   | Group B   | Group C   | p-value |
|-----------------|-----------|-----------|-----------|---------|
| Plaque score±SD | 0.684 ± 0.16 | 0.685 ± 0.17 | 0.686 ± 0.08 | <0.05*  |
| Microbial counts±SD | 108.7 ± 30.6 | 84.2 ± 16.71 | 88.0 ± 21.57 | <0.05*  |

*p < 0.05 considered statistically significant.

The mean difference between the gingival and bleeding scores was significantly different with p < 0.05 (Table 3). Maximum improvement in gingival scores was seen in the individuals of Group A (0.67±0.19) followed by Group B (0.73±0.17) and the least with Group C (0.56±0.18) (Figure 4). Mean reduction in bleeding score values was highly favorable for Group A followed by Group B and the least for Group C.

**Table 3: Difference in the means of the gingival and the bleeding scores**

| Indices         | Group A    | Group B   | Group C   | p-value |
|-----------------|------------|-----------|-----------|---------|
| Gingival scoresSD | 0.67 ± 0.19 | 0.73 ± 0.17 | 0.56 ± 0.18 | <0.05*  |
| Bleeding scoresSD | 0.85 ± 0.33 | 0.56 ± 0.35 | 0.43 ± 0.21 | <0.05*  |

*p < 0.05 considered statistically significant.
The level of difficulty represented by the Likert scale with 5 as very difficult and 1 as very easy as shown in Figure 5. Of the 20 patients, 2 of them found out the flossing to be difficult (one in each group). About 55% of the total participants mentioned it to be average (7 in Group A and 4 in Group B).

Discussion

The RMNPI was chosen for this study as it allowed for recording minor changes in supragingival plaque, especially with the areas of floss contact to the tooth. This study assessed the efficacy of dental floss coated with chlorhexidine gluconate gel 1% on the reduction of the interproximal plaque in subjects with moderate to severe gingivitis having no interdental papillary loss. Biofilm was disclosed with the solution and then the plaque scores were assessed. Its use along with recording the indices allows good comparisons between the novel and old oral hygiene products [18].

The groups using dental floss, with/without chlorhexidine gel, presented decreased levels of plaque and microbes when compared to groups where no interproximal cleaning was done. These results are consistent with the classic study by Gjermo and Flostra [19], which acclaimed the use of floss for controlling plaque mechanically. A 40% reduction was noticed in the dental biofilm in the interproximal areas for the group, which used the floss for 15 days period. Another study [20], using four types of dental floss, i.e. woven, shred-resistant, un-waxed, and powered flosser, with tooth brushing, demonstrated greater reduction in levels of dental biofilm than when only a toothbrush was used.

The results of the present study showed that dental floss coated with chlorhexidine gluconate gel contributed to a relatively greater decrease in clinical indices and anaerobic microbial load than the other two groups. Chlorhexidine is a di-cationic substance that has both bactericidal and bacteriostatic characteristics, and it causes lysis of bacterial membrane [21]. The chemical action of chlorhexidine united with the mechanical action of flossing achieved more acceptable results in lowering the interproximal plaque. These results substantiate the results of another study.
[22], where the use of an essential oil mouthwash with dental floss daily led to a 13% reduction in the biofilm in the group using dental floss, and 55% reduction in the group using essential oil mouthwash with floss. These studies infer that the combination of mechanical and chemical modalities of controlling dental biofilm are more effective than the individual strategies alone.

Microbiological samples pre- and post-use of floss with and without chlorhexidine influenced the difference in microbial load in the test group as well as between positive and negative controls. This further validates the antimicrobial activity of chlorhexidine-coated floss though limited by the fact that the types of bacteria present were not analyzed. There is a possibility of harboring anaerobic bacteria differently in comparison to aerobic bacteria. In further studies, more specific types of aerobic or anaerobic bacteria could provide detailed results. The results of our study have shown substantially lower CFU counts in the blood agar plates for all the three groups.

In this study, the difficulty level experienced by the subjects while using the floss was evaluated. Around thirteen of the total subjects experienced flossing to be average to difficult to perform. This was mainly because of handling a gel coated floss and performing flossing for the posterior teeth was difficult. Hence there is a need for commercial availability of dental floss with chlorhexidine incorporated in it. Chlorhexidine has been used in the form of toothpaste also, but the suggested use of it as toothpaste is for the short-term to prevent staining. However, we did not observe staining in subjects who reported for follow-up after using dental floss with chlorhexidine gel.

More laboratory phase investigations are required to evaluate the action of 1% chlorhexidine when impregnated into dental floss. Evaluation of dental staining effects with long term use of Chlorhexidine incorporated dental floss in larger population groups is warranted. However, impregnation of the floss with chlorhexidine has been attempted in a study [23], but no antimicrobial activity was assessed. Future studies could also focus on the retention of chlorhexidine in the target site.

Conclusion

Dental floss coated with 1% chlorhexidine gluconate gel, was found to be beneficial in removing the interproximal dental plaque in patients with moderate to severe gingivitis when combined with the regular tooth brushing.

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