Original Research Article

Analogous assay between green tea mouthwash, listerine mouthwash and chlorhexidine mouthwash in plaque reduction, on orthodontic patients: a randomized cross-over study

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ABSTRACT

Background: The aim of the study was to compare the efficiency of green tea mouthwash, Listerine mouthwash and Chlorhexidine mouthwash in plaque reduction among orthodontic patients.

Methods: The study employed a double blinded, simple randomized, cross over design with a control group consisting of 30 orthodontic patients undergoing fixed appliance therapy. All the subjects were divided into group 1 (Green tea), group 2 (Listerine) and group 3 (Chlorhexidine) as 10 subjects per group. Gingival status was assessed using Sulcus Bleeding Index and plaque accumulation was assessed using Turesky-Gilmore-Glickman modification of Quigley Hein Index. After a relapse period of 15 days, group 1 and 2 were crossed over, however, group 3 remained the same. Indices were again recorded at baseline and 15th day.

Results: The mean gingival and plaque score was reduced in all the three groups. However, green tea mouthwash was estimated to have the highest mean difference from 2.17 ± 0.610 at baseline to 1.48 ± 0.474 on the 15th day.

Conclusions: Effective use of mouthwashes as supplements for tooth brushing has proved to be beneficial in oral hygiene and maintenance. The findings of this study provide useful insights on the effectiveness of different compositions of mouthwashes.

Keywords: Chlorhexidine, Green tea, Listerine, Fixed appliance, Plaque, Relapse period

INTRODUCTION

Everyone appreciates the importance of a winning smile. It makes a pleasing appearance, promotes self-esteem and is valuable for social and career success. Creating beautiful smiles is a one of the main objectives in the field of orthodontics. Orthodontic treatment has gained much popularity in the recent years as people want to have the best possible smile. Although there are orthodontic treatment has several positive outcomes, it is imperative for patients undergoing treatment to be extra cautious of their oral hygiene and maintenance.

Dental plaque, a bio film, which usually adheres over the tooth surface, is the common cause of periodontal diseases. The onset or progression of periodontal disease can be controlled by regular plaque control practices. Now a days, patients adopt both mechanical and chemical oral hygiene aids in day to day practice. Although, the efficiency of mechanical methods depends on one's skill and technique, the efficiency of chemical agents in
mouthwash depends on the components of the agents. Recently many mouthwashes have come into market. However, among them chlorhexidine remains gold standard among all. It has broad antibacterial activity, with very low toxicity and strong affinity for epithelial tissue and mucous membranes. Besides its anti-plaque effect, chlorhexidine is substantive; thus reducing levels of microorganisms in saliva up to 90% for several hours. However, Chlorhexidine has certain drawbacks such as staining, altered taste, and mucosal erosion.

An increasing number of people all around the world are turning towards use of natural extracts such as herbal products for both prophylaxis and treatment of different diseases. Plants are the source of more than 25% of prescription and over-the-counter preparations and the potential of natural agents for oral prophylaxis should therefore be considered. Green tea is made solely with the leaves of *Camellia sinensis* that have undergone minimal oxidation during processing. The most abundant components in green tea are polyphenols, in particular flavonoids such as the catechins, catechin gallates (Cg) and proanthocyanidins. Many of the biological properties of green tea have been ascribed to the catechin fraction, which constitutes up to 30% of the dry leaf weight. These potent antioxidants comprise free catechins such as (+) catechin, (+) gallocatechin, (-) epicatechin and (-) epigallocatechin and the galloyl catechins such as (-) epicatechin gallate (ECg), (-) epigallocatechin gallate (EGCg), (-) Cg and (-) gallocatechin gallate.

Green tea also contains carotenoids, tocopherols, ascorbic acid, minerals such as Cr, Mn, Se or Zn and certain phytochemical compounds. Various reported therapeutic and biological activities of catechin are lower incidences of various pathological conditions, including cardiovascular disease, strokes, obesity and cancer. These effects have been attributed, in part, to the antioxidative and free radical scavenging activities of the polyphenolic components of green tea. Studies conducted in the past have shown that the green tea poly phenolic catechins, in particular-EGCg and -ECg, can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. Evidence is emerging that these molecules may be useful in the control of common oral infections, such as dental caries and periodontal disease.

Listerine is another alcohol based mouthwash which contains essential oils like menthol, thymol, eucalyptol and methyl salicylate. It is best known for its anti-inflammatory and anti-bacterial property. Although there are various studies that have explored the effectiveness of acid-based mouthwashes, the literature on comparison of the natural green tea mouthwashes with chemical based counterparts are scarce. Hence this study was conducted with an aim to compare and analyse the anti-plaque efficiency of green tea mouthwash, Chlorhexidine and Listerine mouthwash on orthodontic patients.

**METHODS**

A simple randomized, double blinded, cross over study with a control group was conducted at the Department of Orthodontics and Dentofacial Orthopedics in Priyadarshini Dental College and Hospital. The study was conducted for a period of 45 days between July 2014 and September 2014. The study was approved by the institutional review board of Priyadarshini Dental College and Hospital and the permission to conduct it was obtained from institutional Ethical Committee of Priyadarshini Dental College and Hospital. The study had designated inclusion and exclusion criteria to minimize sampling bias.

**Inclusion criteria**

Subjects aged between 15 and 30 years with fixed orthodontic appliance such as straight wire, stage 2 and metal braces were included in the study. In terms of oral hygiene, the sample included only those subjects who brushed their teeth at least once daily with no history of mouthwashes usage and those who had mild to moderate gingivitis.

**Exclusion criteria**

Subjects suffering from systemic illness, history of smoking, alcohol consumption and antibiotic therapy in the past 3 months were excluded. In addition, those who had oral prophylaxis in the last three months prior to the study were also excluded from the study.

**Study population and randomization**

A total of 70 patients gave consent to participate in the survey for a period of 45 days from July 2014 to September 2014. All the patients undergoing fixed appliance therapy visiting the out-patient of orthodontics department were screened. Considering the criteria for selection, a total of 70 patients were selected. Out of 70, only 30 patients were eligible to participate in the study, and an informed consent was obtained from them.

The study population was randomly divided into three groups of group1, group 2 and group 3 respectively (Figure 1). Subjects under Group 1 were given Green Tea mouthwash, group 2 was given Listerine mouthwash and Chlorhexidine was given to group 3 (Figure 1). All the subjects were given adequate oral hygiene instructions like brushing at least twice daily and mouthwash should be used daily. All the subjects were advised to take 10ml of mouthwash with same amount of water dilution.

**Clinical examination**

Two indices such as sulcus bleeding index and turesky-gilmore-glickman modification of quigley hein index were recorded for each patient. A single examiner examined all the subjects throughout the study to avoid
observer bias. The indices were recorded at 2 intervals such as at baseline and after 15 days. Then a relapse period of 15 days was given to all patients. On 31st day the subjects reported back again to the department. Subsequently, group 1 and group 2 were crossed-over, that is group 1 was given Listerine and Green tea mouthwash was given to subjects under group 2. Group 3 remained same. Indices were subsequently recorded and the subjects were asked to report on 45th for a review (Figure 1).

![Sample population and randomisation](image)

**Figure 1: Sample population and randomisation.**

**Data analysis**

Paired t-test was used to assess the significance of changes within each group at baseline and review. Critical p-values of significance were set at 0.05 and a confidence of 95%.

**RESULTS**

All the 30 subjects recruited for the study were assessed without any dropouts. No side effects were reported by any of the subjects throughout the study period. The results are presented in the following figures.

Figure 2 illustrates the mean gingival score of group 1, group 2 and group 3 at baseline and on 15th day. Group 1 has a significant drop in the mean gingival score between baseline and 15th day, and there was a comparable difference between baseline and 15th day mean gingival score for group 2 and group 3 also. Figure 3 shows the difference in the mean plaque score for group 1, group 2 and group 3 both at baseline and at 15th day. Like gingival score, a comparable drop in the mean values for group 2 and group 3. In addition, a very significant difference was seen for group 1 subjects.

For group 1 (green tea mouthwash), the mean gingival score at baseline was 1.2±0.164 and on 15th day it was
0.69±0.133. The mean gingival score for group 2 (Listerine mouthwash) at baseline was 1.01±0.294 and on 15th day it was 0.73±0.165. The mean plaque score for Group 1 (Green tea) at baseline was 2.17±0.610 and on 15th day it was 1.48±0.474. For group 2 (Listerine mouthwash) the mean plaque score at baseline was 1.74±0.482 and on 15th day it was 1.48±0.474. By comparing the mean values of the two mouthwashes, subjects under group 1 (green tea) showed a better difference in the mean value than group 2 (Listerine mouthwash). The p value at baseline was 0.100 i.e (p<0.100) (t=1.734) for gingival index, (p<0.100) (t=1.735) was for plaque index at baseline. On 15th day gingival index had p-value of 0.599 (p<0.599) (t=0.535), for plaque index (p< 0.746) (t= 0.329). Although, the p-values were not statistically significant, clinical reduction in the plaque accumulation and improvement of the gingival health was seen.

After 15 days, all the subjects from group 1, group 2 and group 3 were given a relapse period of 15 days. Then crossover of subjects belonging to group 1 and group 2 was done. And group 3 remained the same. Figure 4 and 5 describes the mean gingival and plaque score for group 1, group 2 and group 3 after the relapse period. Figure 4 show the mean gingival score of group 1, group 2 and group3 at baseline and after 15th day. Subjects under group 2 had a significant reduction in values when compared to the other two groups. Therefore, group 1 and group 2 had a very less reduction in the mean gingival scores. In Figure 5, the mean plaque score for group 1, group 2 and group 3 at Baseline and at 15th day is shown. Here group 1 and group 3 had a low difference in its mean value, whereas group 2 had a better mean value.

After relapse period of 15 days, subjects under Group 1 and 2 were crossed over. The mean gingival score for group 1 (Listerine mouthwash) was 1.26±0.332 at baseline and 1.03±0.375 on 15th day. For group 2 (Green tea), the mean gingival score was 1.16±0.348 at baseline and 0.71±0.320 on 15th day. For group 1(Listerine mouthwash) 2.07±0.436 was the mean plaque score at baseline and on 15th day it was 1.79±0.385. 1.97±0.3510 was the mean plaque score, for group 2 (Green tea mouthwash), at baseline and the mean plaque score for 15th day was 1.48±0.344. By comparing the mean values of the two mouthwashes, group 2 (green tea) showed a better difference in the mean value than group 1 (Listerine mouthwash). The p value at baseline was 0.528.
i.e \((p<0.528)\ (t=0.643)\) for gingival index, \((p<0.568)\ (t=0.582)\) was for plaque index at baseline. On 15th day gingival index had p-value \((p<0.055)\ (t=2.056)\), for plaque index \((p<0.076)\ (t=1.884)\).

For group 3 (Chlorhexidine mouthwash), the mean gingival score at baseline was 1.16±0.079 and on 15th day it was 0.95±0.082. The mean plaque score at baseline was 2.31±0.308 and on 15th day it was 2.08±0.30. The p-value was found to be statistically significant \((p<0.000)\ (t=6.001)\) for gingival index. However, p-value for plaque index remained non-significant \(p<0.119\ (t=1.637)\). After relapse period of 15 days, the mean gingival score was 1.05±0.063 at baseline and 0.85±0.072 on 15th day. 2.15±0.311 was the mean plaque score at baseline and the mean plaque score for 15th day was 1.95±0.306. The p value was found to be statistically significant \((p<0.000)\ (t=6.332)\) for gingival index but not for plaque index \(p<0.165\, (t=1.447)\).

**DISCUSSION**

The study was carried out to assess and compare the antiplaque effectiveness of Green Tea mouthwash, Listerine mouthwash and Chlorhexidine mouthwash on orthodontic patients. This was a double blinded study where the investigator and the study subjects were not aware to which group the subjects belonged to. No side effects or miss-happenings were seen during the study period.

Comparisons with other studies could not be carried out as the materials used were different as well as the study population was also different. The study period and the time interval also differed from other studies. Popular indices such as the gingival index and plaque index were used for investigating the efficiency of oral hygiene products similar to other studies.5-11

**Green tea mouthwash**

Originating from China, Green tea has gained the world’s taste in the past 2000 years.\(^1\)\(^2\) The economic and social interest of Green tea is clear and its consumption is part of many people daily routine, as an everyday drink and as a therapeutic aid in many illnesses.\(^1\)\(^2\) The first clue to the oral health benefits of tea came from studies in the 1940 s to 50 s showing fluoride to be the active component.\(^1\)\(^3\) Reports suggested not only fluoride but also tannins contributed to the inhibitory effect.\(^1\)\(^4\)-17

In the present study, subjects under Green tea had the maximum desired effect when compared to Listerine and Chlorhexidine. The gingival level scores declined from 1.2 to 0.6 for Green tea mouthwash. Though, not much difference was observed in subjects with Listerine and chlorhexidine. The oral hygiene status improved from poor to good. Green Tea group had upper hand in terms of gingival status, as the response was very good and quick when compared to Listerine or chlorhexidine. One of the reasons for significant reduction in the gingival scores would be attributed to levels of the catechins, tannins, and astringent present in the tea. This finding is consistent with other studies which have reported similar results with that of tea tree oil.\(^1\)\(^8\),\(^1\)\(^9\)

Various other mechanisms have been explained for the effect of tea on gingival health. Green tea catechin has been shown to be bactericidal against Porphyromonas gingivalis and Prevotella species in vitro.\(^2\)\(^2\) Green Tea catechins containing the galloyl radicals possess the ability to inhibit both eukaryotic and prokaryotic cell-derived collagenase, an enzyme that plays an important role in the disruption of the collagen component in the gingival tissues of patients with periodontal disease.\(^2\)\(^0\),\(^2\)\(^1\) Catechin derivatives have been reported to inhibit certain proteases of *P. Gingivalis* and may reduce periodontal breakdown.\(^2\)\(^2\) Green tea catechins have also been shown to inhibit protein tyrosine phosphatase in Prevotella intermedia.\(^2\)\(^3\) EGCg has been reported to inhibit production of toxic metabolites of *P. gingivalis* have shown that purified Green tea polyphenols inhibited in vitro growth and H\(_2\)S production of *P. gingivalis* and Fusobacterium nucleatum associated with human halitosis.\(^2\)\(^4\)

**Listerine mouthwash**

Listerine, an alcohol based mouthwash has proved to have anti-inflammatory and antibacterial property and antiseptic property.\(^7\) In the study, the mean gingival score for Listerine reduced from 1 to 0.7, the mean plaque score reduced from 1.7 to 1.4. After cross over, the mean gingival and plaque score reduced from 1.2 to 1 and 2 to 1.7 respectively. Listerine mouthwash has menthol of 0.042\%, thymol 0.064\%, methyl salicylate of 0.06% and eucalyptol 0.092\% in combination, all has anti-inflammatory effect but methyl salicylate is thought to have anti-inflammatory effects.\(^7\) Ethanol which is toxic to bacteria at concentration of 40\% is present in concentration of 21.6\% in the mouthwash used for the current study.

**Chlorhexidine mouthwash**

Chlorhexidine digluconate, to date is the most thoroughly studied and the most effective anti-plaque and anti-gingivitis agent. However, several side-effects associated with its use have stimulated the search for alternative agents. For this reason, only it is taken as a benchmark control for various mouthwashes. The most commonly prescribed concentration is 0.2\% hence; this was considered in the study.

As expected the mean plaques scores reduced from baseline to 15th day. The lowest plaque was recorded after the first rinse. The drop was found to be clinically significant. Same goes with gingival scores, were clinically significant reduction of gingivitis was seen from score 1.1 at baseline to 1 at the end of 15th day. Oral hygiene which was poor at baseline for subjects, after use...
of mouthwash oral hygiene improved to mild. Similar positive results have been reported in other studies.25-27

In regards to the strengths and limitations of the study, this study was one of the few surveys that compared the natural Green Tea mouthwash with chemical based counterparts among orthodontic patients. The randomized control trial, which is the strongest of the study design was employed to have control of the exposure and to eliminate various bias.26 However, this study has some limitations worth reporting. The limited number of participants in each group may question the validity of the results. Further research could be carried out by recruiting more participants and by evaluating the results with this study.

CONCLUSION

Effective use of mouthwashes as supplements for tooth brushing has proved to be beneficial in oral hygiene and maintenance. All the three mouthwashes used in our study were found to be effective against the plaque accumulation and gingivitis. However, when compared across different groups, Green tea mouthwash showed better effectiveness followed by Listerine and then chlorhexidine mouthwash. The findings of this study provide useful insights for dental practitioners and patients on the effectiveness of different compositions of mouthwashes. Considering the fact that most mouth rinses available in market are chemically based especially in India, a cost-effective and easily available herbal extract of Green Tea would be valuable as an adjuvant to oral hygiene maintenance.

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REFERENCES

1. Page RC, Kornman KS. The pathogenesis of human periodontitis: An introduction. Periodontology. 1997;14:9-11.
2. Balagopal S, Arjunkumar R. Chlorhexidine: The gold standard antiplaque agent. J Pharm Sci Res. 2013;5(12):270-4.
3. Santos A. Evidence-based control of plaque and gingivitis. J Clin Periodontal. 2003;30 Suppl 5:13-6.
4. Allaker RP, Douglas CI. Novel anti-microbial therapies for dental plaque-related diseases. Int J Antimicrob Agents. 2009;33(1):8-13.
5. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull. 2005;2:71-81.
6. Kaur H, Jain S, Kaur A. Comparative evaluation of the antiplaque effectiveness of green tea catechin mouthwash with chlorhexidine gluconate. J Indian Society Periodontol. 2014;18(2):178.
7. Farah CS, McIntosh L, McCullough MJ. Mouthwashes. Australian Prescriber. 2009;32(6):162-4.
8. Khaleesi AM, Pack AR, Thomson WM, Tompkins GR. An in vivo study of the plaque control efficacy of Persica: A commercially available herbal mouthwash containing extracts of Salvadorapersica. Int Dent J. 2004;54:279-83.
9. Arweiler NB, Donos N, Netuschil L, Reich E, Sculean A. Clinical and antibacterial effect of tea tree oil: A pilot study. Clin Oral Investig. 2000;4:70-3.
10. Haffajee AD, Yaskell T, Socransky SS. Antimicrobial effectiveness of an herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. J Am Dent Assoc. 2008;139:606-11.
11. Brecx M, Macdonald LL, Legary K, Cheang M, Forgay MG. Long-term effects of Meridol and chlorhexidine mouth rinses on plaque, gingivitis, staining, and bacterial vitality. J Dent Res. 1993;72:1194-7.
12. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—a review. J Am Coll Nutr. 2006;25(2):79-99.
13. Gershon-cohen J, Mcc lendon HF. Fluorine in tea and caries in rats. Nature. 1954;173:304-5.
14. Soukoulis S, Hirsch R. The effects of a tea tree oil-containing gel on plaque and chronic gingivitis. Aust Dent J. 2004;49:78-83.
15. Elvin-Lewis M, Steelman R. The anticariogenic effects of tea drinking among Dallas children. J Dent Res. 1968;65:198.
16. Rosen S, Elvin-Lewis M, Beck FM, Beck EX. Anticariogenic effects of tea in rats. J Dent Res. 1984;63:658-60.
17. Touyz LZ, Amsel R. Anticariogenic effects of black tea (Camellia sinensis) in caries prone-rats. Quintessence Int. 2001;32:647-50.
18. Lee MJ, Lambert JD, Prabhu S, Meng X, Lu H, Maliakal P, et al. Delivery of tea polyphenol containing gel on plaque and chronic gingivitis. J Dent Res. 2005;84:365-70.
19. Krahwinkel T, Willershausen B. The effect of sugar-free green tea chew candies on the degree of inflammation of the gingiva. Eur J Med Res. 2000;5:463-7.
20. Osawa K, Matsumoto T, Yasuda H, Kato T, Naito Y, Okuda K. The inhibitory effect of plant extracts on the collagenolytic activity and cytotoxicity of human gingival fibroblasts by Porphyromonasgingivalis crude enzyme. Bull Tokyo Dent Coll. 1991;32:1-7.
21. Demeule M, Brossard M, Pagé M, Gingras D, Béliveau R. Matrix metalloproteinase inhibition by green tea catechins. Biochim Biophys Acta. 2000;1478:51-60.
22. Okamoto M, Sugimoto A, Leung KP, Nakayama K, Kamaguchi A, Maeda N. Inhibitory effect of green tea catechins on cysteine proteinases in Porphyromonas gingivalis. Oral Microbiol Immunol. 2004;19:118-20.
23. Okamoto M, Leung KP, Ansai T, Sugimoto A, Maeda N. Inhibitory effects of green tea catechins on protein tyrosine phosphatase in Prevotella intermedia. Oral Microbiol Immunol 2003;18:192-5.
24. Sakanaka S, Okada Y. Inhibitory effects of green tea polyphenols on the production of a virulence factor of the periodontal-disease-causing anaerobic bacterium Porphyromonas gingivalis. J Agric Food Chem. 2004;52:1688-92.
25. Brecx M, Netuschil L, Reichert B, Schreil G. Efficacy of Listerine, Meridol and chlorhexidine mouthrinses on plaque, gingivitis and plaque bacteria vitality. J Clin Periodontol. 1990;17:292-7.
26. Lang NP, Brecx M. Chlorhexidine digluconate: An agent for chemical plaque control and prevention of gingival inflammation. J Periodont Res. 1986;21:74-89.
27. Löe H, Schiött CR, Karring G, Karring T. Two years oral use of chlorhexidine in man. I. General design and clinical effects. J Periodontal Res. 1976;11:135-44.
28. Webb P, Bain C. Essential epidemiology: an introduction for students and health professionals. Cambridge University Press; 2010.

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