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Efficacy of 0.2% Chlorhexidine Gluconate and 1.5% hydrogen peroxide mouthwash on mature and newly formed plaque: A randomised control trial

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Abstract
Aim & Objective: The purpose of this crossover design, randomised controlled clinical trial was to assess the effect of 0.2% chlorhexidine gluconate and 1.5% hydrogen peroxide mouthwash on mature and newly formed plaque.
Materials and Method: Forty-two patients having gingival index score and plaque index score of ≥1 were included in the study. Selected patients were randomly divided into three groups by using chit method. Gingival index score and plaque index score were recorded at each visit using two-tone plaque disclosing solution and respective mouthwash were given to each group and scaling was done as planned. After a certain period of time crossover was done between the test groups and same procedure was repeated.
Results: On intergroup comparison for gingival and plaque index scores at different time intervals between Group B (0.2 % chlorhexidine gluconate) and Group C (1.5% hydrogen peroxide mouthwash) significant difference was observed on newly formed plaque before crossover and at time intervals after crossover i.e., on both mature plaque and newly formed plaque
Conclusion: Both chlorhexidine and hydrogen peroxide mouthwash showed similar effect on mature plaque and newly formed plaque. Hence, 1.5% hydrogen peroxide mouthwash can be used as an alternative to 0.2 % chlorhexidine gluconate mouthwash.

Keywords: Mature plaque, newly formed plaque, chlorhexidine mouthwash, hydrogen peroxide mouthwash

Introduction

Dental plaque is the major etiologic agent for the initiation of gingivitis [1]. Gingival disease can progress to periodontitis which, if left untreated, may eventually compromise the entire periodontium [2]. Thus, the treatment and prevention of gingivitis is a valuable goal in periodontal therapy [3]. Mechanical plaque control is not always sufficient hence, the adjunctive use of chemical plaque control had shown better efficacy in the control of plaque and gingival inflammation. Chlorhexidine remains the gold standard among the mouth rinses [4]. The efficacy of chlorhexidine in reducing oral bacterial viability, strongly inhibiting plaque regrowth and preventing gingivitis has been demonstrated in many studies [5, 6, 7]. However, chlorhexidine has several side effects, like its repeated use often produces stains and taste disturbances. Hydrogen peroxide has been shown to have a good stain removing capability both *in vitro* and *in vivo* [5]. The use of Hydrogen peroxide as an adjunct to chlorhexidine has been found to be very effective in reducing plaque scores and in preventing the stain development [8]. On literature survey, variable results have been found for both mouthwashes when used together adjunctively or individually. More studies are required to evaluate the efficacy of chlorhexidine and hydrogen peroxide mouthwash on plaque regrowth and formed plaque. The present study, therefore aimed to determine the effect of 0.2% chlorhexidine and 1.5% hydrogen peroxide mouthwash on mature and newly formed plaque.

Materials and Method

The present study was carried out as a randomized control clinical trial using crossover design. Ethical clearance for the study was obtained from the Institutional Ethics and Review Board. Total forty-two subjects were selected for the study, satisfying the eligibility criteria and were randomly divided into Control Group-A (Saline) and Test Group- Group B (0.2 % chlorhexidine gluconate) and Group C (1.5% hydrogen peroxide mouthwash) by using chit method.

Inclusion criteria

1. Subjects having gingival index score of ≥1
2. Subjects having plaque index score of ≥1
3. Systemically healthy patients.

Exclusion criteria

1. Subjects who underwent any periodontal surgery within 3 months.
2. Subjects who had underwent scaling in the last 3 months.
3. Subjects taking antibiotic, steroid or hormonal therapy in the last 3 months.
4. Allergy to ingredients used in the study.
5. Subjects who are uncooperative or not complying with oral hygiene instructions.

After assigning the subject to either group, Plaque index (PI) (Sillness and Loe, 1964) [9] and Gingival index (GI) (Loe and Sillness, 1963) [10] score was recorded using two-tone plaque disclosing solution and respective mouthwash was given for 7 days. No scaling and alteration in brushing method was advocated.
On second visit after 7 days, PI and GI were recorded to evaluate the effect on mature plaque (Fig 1, 2 & 4) and scaling was done. Scaling was done and patient was asked

to continue the respective mouthwash for next 7 days and recalled after 7 days.

On third visit after 7 days, i.e., 14 days, PI and GI were recorded to evaluate the effect on plaque regrowth and scaling was done and a washout period of 7 days was given (Fig. 3 & 5).

On fourth visit i.e., after 21 days, PI and GI were recorded and group B and group C was interchanged for a crossover and the same regime was followed (Fig. 6 & 7). All the procedures and clinical assessment were performed by different examiners who were blinded.

Fig 1: Control group A (Saline) after application of disclosing solution

Fig 2: Test group B (CHX) after application of disclosing solution on Day 7

Fig 3: Test group B (CHX) after application of disclosing solution on Day 14

Fig 4: Test group C (H2O2) after application of disclosing solution on Day 7

Fig 5: Test group C (H2O2) after application of disclosing solution on Day 14

Fig 6: Test group B (CHX) after application of disclosing solution after crossover

Fig 7: Test group C (H2O2) after application of disclosing solution after crossover

Results

Effect of mouthwash on mature plaque and newly formed plaque was assessed using gingival index score and plaque index score on 1st, 2nd and 3rd visit. After a crossover period of 7 days test groups are interchanged and the same regime was followed. All the participants completed the study and collected data was statistically analyzed.

On intragroup comparison of both gingival and plaque index scores of test Group B (CHX) and test Group C (H2O2) before crossover showed significant progressive reduction from baseline (1st visit) to 7 days (2nd visit) and 14 days (3rd visit) respectively (Table 2, 3, 5 & 6). On comparing the mean value scores after crossover both groups, group B (CHX) and C (H2O2) results were statistically significant at all time intervals.

On intergroup comparison for gingival and plaque index scores at different time intervals before and after crossover between Group A (Saline) and Group B (CHX) and Group A (Saline) and Group C (H2O2) showed significant difference at the 3rd visit i.e., on newly formed plaque (Table 7 & 8).

Table 1: Control Group A (Saline) intragroup comparison of gingival index scores at different time intervals

Visit	N	Mean difference ± Std. Error	P-value
1st visit	14	2.50 ± 0.139	0.000 S
2nd visit	14	2.29 ± 0.194	0.000 S
3rd visit	14	2.07 ± 0.267	0.000 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant

Table 2: Intragroup comparison of gingival index scores of test Group B (CHX) before and after crossover at different time intervals

Visit	N	Before crossover		After crossover	
		Mean difference ± Std. Error	P-value	Mean difference ± Std. Error	P-value
1st visit	14	2.21 ± 0.214	0.000 S	1.43 ± 0.137	0.000 S
2nd visit	14	2.14 ± 0.231	0.000 S	1.71 ± 0.194	0.000 S
3rd visit	14	0.36 ± 0.133	0.019 S	0.29 ± 0.125	0.040 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant

Table 3: Intragroup comparison of gingival index scores of test Group C (H2O2) before and after crossover at different time intervals

Visit	N	Before crossover		After crossover	
		Mean difference ± Std. Error	P-value	Mean difference ± Std. Error	P-value
1st visit	14	2.21± 0.214	0.000 S	1.36 ± 0.169	0.000 S
2nd visit	14	2.14 ± 0.254	0.000 S	1.93 ± 0.229	0.000 S
3rd visit	14	0.57 ± 0.137	0.001 S	0.64 ± 0.133	0.000 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant

Table 4: Control Group A (Saline) intragroup comparison of plaque index scores at different time intervals

Visit	N	Mean difference ± Std. Error	P-value
1st visit	14	2.45 ± 0.159	0.000 S
2nd visit	14	2.21 ± 0.174	0.001 S
3rd visit	14	2.01 ± 0.389	0.002 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant

Table 5: Intragroup comparison of plaque index scores of test Group B (CHX) before and after crossover at different time intervals

Visit	N	Before crossover		After crossover	
		Mean difference ± Std. Error	P-value	Mean difference ± Std. Error	P-value
1st visit	14	2.27 ± 0.214	0.000 S	1.49 ± 0.137	0.000 S
2nd visit	14	2.18 ± 0.231	0.000 S	1.75 ± 0.190	0.000 S
3rd visit	14	0.39 ± 0.133	0.011 S	0.22 ± 0.135	0.035 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant

Table 6: Intragroup comparison of plaque index scores of test Group C (H2O2) before and after crossover at different time intervals

Visit	N	Before crossover		After crossover	
		Mean difference ± Std. Error	P-value	Mean difference ± Std. Error	P-value
1st visit	14	2.23± 0.214	0.000 S	1.41 ± 0.169	0.000S
2nd visit	14	2.12 ± 0.254	0.000 S	1.95 ± 0.229	0.000 S
3rd visit	14	0.58 ± 0.137	0.001 S	0.67 ± 0.133	0.000 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant

Table 7: Intergroup difference for gingival index scores at different time intervals before and after crossover

Groups	Before crossover			After crossover		
	Mean difference ± Std. Error			Mean difference ± Std. Error		
	1st visit	2nd visit	3rd visit	1st visit	2nd visit	3rd visit
Group A and B	0.286 ± 0.825	0.143 ± 1.027	1.714 ± 0.914	1.071 ± 0.730	0.571 ± 1.158	1.786 ± 1.251
p-value	0.218 N.S	0.612 N.S	0.000 S	0.000 S	0.088 N.S	0.000 S
Group A and C	0.286 ± .611	0.143 ± 1.099	1.50 ± 1.160	1.143 ± 0.663	0.357 ± 0.842	1.429 ± 1.016
p-value	0.104 N.S	0.635 N.S	0.000 S	0.000 S	0.136 N.S	0.000 S
Group B and C	0.857 ± 1.127	0.214 ± 0.579	-0.286 ± 0.469	0.786 ± 0.802	0.429 ± 0.756	0.286 ± 0.469
p-value	0.008 S	0.189 N.S	0.040 S	0.003 S	0.054 S	0.040 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant, N.S.- non-significant

Table 8: Intergroup difference for plaque index scores at different time intervals before and after crossover

Groups	Before crossover			After crossover		
	Mean difference ± Std. Error			Mean difference ± Std. Error		
	1st visit	2nd visit	3rd visit	1st visit	2nd visit	3rd visit
Group A and B	0.281 ± 0.829	0.148 ± 1.022	1.716 ± 0.909	1.074 ± 0.732	0.561 ± 1.134	1.749 ± 1.241
p-value	0.215 N.S	0.602 N.S	0.000 S	0.000 S	0.086	0.000 S
Group A and C	0.281 ± .621	0.143 ± 1.097	1.50 ± 1.162	1.145 ± 0.643	0.367 ± 0.845	1.424 ± 1.019
p-value	0.101 N.S	0.637 N.S	0.000 S	0.000 S	0.133 N.S	0.000 S
Group B and C	0.807 ± 1.137	0.212 ± 0.573	-0.286 ± 0.469	0.784 ± 0.807	0.429 ± 0.756	0.283 ± 0.469
p-value	0.009 S	0.188 N.S	0.040 S	0.004 S	0.051 S	0.045 S

* The mean difference is significant at the 0.05 level. (paired t-test)
N- Sample size, Std.- Standard, S- Significant, N.S.- non-significant

Discussion

Dental plaque is a polymicrobial biofilm, defined as a community of microbial cells embedded in an extracellular matrix, that grows on an interface between two phases of

matter, for example, the solid tooth surface and liquid saliva or GCF [11]. Dental plaque becomes mature in vitro after 24-72 hours while *in vivo* generally after 72 hours [12]. Mature plaque is dominated by pathogens linked to periodontal disease and dental caries.

On the 1st visit subjects were given respective mouthwashes and no alteration in brushing method was advocated to study its effect on already formed (mature) plaque. On the subsequent visit scaling was done and at 3rd visit effect on newly formed plaque was studied. On intragroup comparison of both gingival and plaque index scores of test Group B (CHX) before crossover showed significant progressive reduction from baseline (1st visit) to 7 days (2nd visit) and 14 days (3rd visit) (Table 2 & 5). Reduction in mean value scores from baseline to 7 days shows the effect of chlorhexidine on mature plaque and from 7th day to 14th day shows its positive effect on plaque regrowth. On comparing the mean value scores chlorhexidine showed better effect on newly formed plaque or plaque regrowth as compared to mature plaque. Similarly, on intragroup comparison of gingival and plaque index scores of test Group C (H2O2) before crossover showed significant progressive reduction from baseline (1st visit) to 7 days and 14 days (Table 3 & 6). On comparing the mean value scores after crossover both groups, group B (CHX) and C (H2O2) showed no inhibitory effect on already formed (mature) plaque. However, the results were statistically significant at all time interval before and after crossover for both the test group.

On intergroup comparison for gingival and plaque index scores at different time intervals before and after crossover between Group A (Saline) and Group B (CHX) and Group A (Saline) and Group C (H2O2) showed significant difference at the 3rd visit i.e., on newly formed plaque (Table 7 & 8).

On intergroup comparison for gingival and plaque index scores at different time intervals between Group B (CHX) and Group C (H2O2) significant difference was observed at the 3rd visit i.e., on newly formed plaque before crossover and at all time intervals after crossover i.e., on mature plaque and newly formed plaque (Table 7 & 8).

Both chlorhexidine and hydrogen peroxide mouthwash showed similar effect on mature plaque and newly formed plaque. However, on comparing mean value scores chlorhexidine showed slightly better effect than hydrogen peroxide on newly formed plaque (Table 2, 3, 5, 6).

Chlorhexidine is a cationic bisbiguanide and antimicrobial agent that has broad spectrum antibacterial activity, whereas hydrogen peroxide is an oxygenating agent. The positive inhibitory effect of 0.2% chlorhexidine and 1.5% hydrogen peroxide on mature plaque and newly formed plaque in the present study can be substantiated to its mechanism of action. The antiplaque action of chlorhexidine is purely on the surface. In 1992, Seymour and Heasman [13] stated that killing of bacterial cells is initially dependent on the drug having access to cell walls. Hydrogen peroxide acts therapeutically by releasing oxygen that immediately kills the obligate anaerobes present in the oral infections [14] which might explain its role in dental plaque reduction.

The efficacy of chlorhexidine in reducing oral bacterial viability [15-17] strongly inhibiting plaque regrowth and preventing gingivitis [6] has been demonstrated in many studies[7]. Our result is in accordance to a study by Rosin M et al [16]. who also found inhibitory effect of chlorhexidine on plaque regrowth i.e., newly formed plaque.

Though chlorhexidine is considered as the best agent for plaque control and gingivitis, many patients find its initial bitter taste unpleasant and repeated use often produces stains and taste disturbances. Because of this, daily rinsing with chlorhexidine is not promoted. The absence of stain formation could improve patient compliance and reduce the time necessary to clean the dentition during recall.

Oxygenating agents have been employed for supragingival plaque control and in the treatment of acute ulcerative gingivitis without any harmful side effects on the tissues [18]. 1.5% Hydrogen peroxide has been shown to have a good stain removing capability both in vitro and in vivo. The use of Hydrogen peroxide as an adjunct to chlorhexidine has been found to be very effective in reducing plaque scores and in preventing the stain development [8]. In the present study both 0.2% chlorhexidine and 1.5% hydrogen peroxide when used alone equally reduced formation of mature plaque and newly formed plaque.

In accordance to our study, Gusberti et al. [19] found that the subjects rinsing with 0.12% chlorhexidine showed 95% reduction in gingivitis incidence, 100% reduction in bleeding sites, and 80% reduction in plaque scores compared to the subjects rinsing with placebo. Conversely, the group using 1% hydrogen peroxide showed a marginal reduction in gingivitis incidence of 15% and a 28% reduction in bleeding sites compared to the placebo group, but no significant reduction in plaque scores.

In contrast to our study, Hossainian et al [20]. concluded that mouthwashes containing hydrogen peroxide do not consistently prevent plaque accumulation when used as a short-term monotherapy.

A two-tone dye containing disclosing agent was used in the present study, to make plaque visible and differentiate between mature and newly formed plaque thus making it easier to score on a numerical scale. New plaque stains red while old plaque stains blue.

Though many studies have evaluated effects of 0.2% chlorhexidine and 1.5% hydrogen peroxide on dental plaque reduction, in our knowledge this is the 1st study to compare effect of 0.2% chlorhexidine and 1.5% hydrogen peroxide on mature plaque and newly formed plaque. We found that there is similar significant progressive reduction in formation of mature plaque and newly formed plaque for both chlorhexidine and hydrogen peroxide mouthwash.

Source of support: Nil
Conflict of interest: Nil
Ethical clearance: Obtained

Conclusion

Within the limitation of the present study, authors would like to conclude that 1.5% hydrogen peroxide mouthwash can be used as an alternative to 0.2 % chlorhexidine gluconate mouthwash. However, 0.2% chlorhexidine mouthwash remains the gold standard.

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