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Efficacy of Dental Floss Impregnated with Commercially Available Mouthwashes on Reduction of Supragingival Biofilm: A Randomized Single Blind Study

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the efficacy of dental floss impregnated with commercially available Chlorhexidine gluconate, Cetylpyredinium Chloride and Chlorine dioxide mouthwash on reduction of Supragingival Biofilm.
Materials and Methods: Sixty dental students were randomly divided into four groups (n=15 Control group (group 1-distilled water) included floss impregnated in distilled water. Test groups were (group 2- Rexidine® mouthwash) (group 3- Colgate Plax® mouthwash) (group 4- Freshclor® mouthwash) All the patients used same toothbrush and a unwaxed dental floss impregnated with

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mouthwash twice a day. The presence of the dental biofilm and bleeding on probing was evaluated only on four surfaces, resulting in four scores for each tooth (mesiobuccal, distobuccal, mesiolingual and distolingual). The four surfaces were assessed according to the Quigley–Hein Index , modified by Turesky et al. and papillary bleeding index.

Results: After 15 days, the mean plaque score using Turesky Gilmore Plaque Index of all teeth examined was 0.8473333±0.2868076, for control group, 0.969444 ±0.4494448 for group 2, 0.608333 ± 0.2082143 for group 3, 0.608333 ± 0.2082143 for group 4.

Mean papillary bleeding index scores were 1.60 ± 0.632 for control group, 0.33 ± 0.488 for group 2, 1.07 ± 0.704 for group 3, 1.13± 0.640 for group 4.

Group 3 showed statistically significant levels in reduction of plaque scores with Turesky Gilmore Plaque Index.

Conclusion: The present study concluded that the CHX impregnated floss was more effective in reducing the interproximal plaque levels. And cetylpyridinium impregnated floss was better in reducing the biofilm level when assesed using Turesky Gilmore modification of Quigley Hein plaque index.

Keywords: Biofilm; interproximal areas; dental floss; indices.

1. INTRODUCTION

Main aetiological factor for periodontal disease (PD) are the Dental biofilms [1]. The use of a toothbrush is the best mechanical way for control of the biofilm to prevent PD [2]. However, there is need of additional cleaning devices to supplement the use of toothbrushes due to their reduced efficiency and efficacy in removing the dental biofilm in the inter-proximal [3]. In addition, PD does not necessarily affect all tooth areas with equal severity [4]. Inter-proximal surfaces have larger concentrations of dental biofilm accumulation than on smooth surfaces, suggesting the importance of use of interdental cleaning in these areas [5].

Several inter-proximal cleaning devices are regularly available, including single tufted brushes, toothpicks and dental floss [6]. Size and morphology of the inter-dental spaces should be considered before choosing the appropriate inter dental aid. Evaluation of the skill and ability of the patient to use these devices is also important [7]. Only a small portion of the population uses dental floss on a daily basis, and its consumption is greater in individuals with higher socioeconomic levels, according to Zimmer et al. [8]. The efficiency of flossing increases when its usage is increasingly encouraged. [9], but a large group of dental professionals do not routinely recommend this to the population concerned. All of these difficulties make the habit of flossing less acceptable.

There is a lot of literature establishing, that flossing is effective, but it depends on the patient's situation whether it should be carefully recommended by a dental professional [6,10]. Despite these evidences, one systematic review established that tooth brushing and dental flossing provide no benefit, when compared to tooth brushing alone, on removing plaque and reducing gingivitis [5]. Also, another systematic review states that there is weak and very unreliable evidence suggesting that flossing plus tooth brushing may be associated with a small reduction in plaque [11].

Use of various antimicrobial agents have been suggested as an adjunct to enhance the efficacy of mechanical plaque control. Chlorhexidine is the gold standard for this purpose. It is a dicationic compound that is able to join anionic compounds, such as phosphate and carboxyl radicals from the tooth surface and salivary glycoproteins. It damages the cytoplasmic membrane, leading to bacterial cell lysis [12]. Additionally, retention capacity of chlorhexidine helps it to remains in oral tissues for a prolonged time, exhibiting a high substantivity [13].

On the other hand, Colgate Plax® is an cetylpyredinium chloride containing mouth rinse that has similar antiplaque and antigingivitis effects as chlorhexidine but does not have the unwanted side effects of chlorhexidine [14]. Stabilised chlorine dioxide has marked bactericidal effects against oral bacteria associated with gingivitis and periodontitis [15].

This study aimed to verify the efficacy of the use of dental floss impregnated with commercially available mouthwashes on the reduction of the supragingival dental biofilm.

2. MATERIALS AND METHODS

Ethical aspects: The study was approved by the Ethical Committee of our institution and informed consent was obtained from every participants.

2.1 Study Type

This study followed the CONSORT statement, and it was designed as a single-centre, parallel, single-blind, controlled and randomized clinical trial.

A total of 60 female dental students (aged 18 to 24 years) from our institution were, examined for this clinical trial. Those volunteers who fulfilled the following inclusion criteria were included. As per inclusion criteria, all volunteers had to be in a good general health (free from any major systemic disease) Secondly, they had to display interproximal spaces without any diastema or gingival recession.

Patients with any kind of dental prosthesis, caries or any other plaque retentive factor, with exception of dental calculus, were excluded from this study. Those who used systemic antibiotics within 3 months prior to the start of the study and those who possessed systemic conditions that exerted an effect on periodontal health, such as diabetes, were also excluded.

2.2 Experimental Design

Sixty dental students were equally divided into four groups (n=15). Control group (group 1- distilled water) included floss impregnated in distilled water. Test groups were (group 2- Rexidine® mouthwash) (group 3- Colgate Plax® mouthwash) (group 4- Freshclor® mouthwash) All the patients used same toothbrush and unwaxed dental floss impregnated with mouthwash twice a day.

All the patients were examined at visit one and supragingival scaling and root planning was done with the purpose of making the dentition 100% free of plaque, calculus and extrinsic stains. For present study, only the anterior teeth were selected for evaluation, which includes total twelve inter-proximal spaces on the upper arch and twelve on the lower arch [16].

Except first visit, daily oral hygiene procedures were not supervised. The patients were motivated to perform this procedure twice a day in 12-hours intervals.

To achieve standard conditions, each volunteer received a kit that contained a new toothbrush with soft bristles and fluoride containing dentifrice. They were instructed to follow modified bass method of tooth brushing and demonstration was given for the same at the first visit only. Furthermore, the volunteers were requested not to use any kind of antiseptic mouthwash or chewing gum during the period of study. After 1week they were recalled as Second Visit. The recordings of indices were done which were considered as Baseline readings. Same time they were introduced floss and method of using the same. The intervention started at same visit. On day 15 of the experimental period, subjects attended another appointment as Visit 3 to evaluate clinical parameters again.

The laboratory phase used is an adaptation of the protocol developed by Oppermann et al. [17] and Muniz et al. [16]. Initially, 30-cm lengths of waxed dental floss (Colgate Sensitive Dental Floss) were cut with the help of a ruler. In an autoclave, these floss segments were sterilized and then placed in previously sterilized test tubes with the mouthwashes and distilled water.

During 24 hr., these falcon tubes remained in a incubator at a constant temperature of 37℃ as a tentative to impregnate the solution on the floss. After that time, the floss passed through a drying stage in the same incubator for the same period of time and was then subsequently placed in appropriate packages.

On third Visit, the presence of the dental biofilm and bleeding on probing was evaluated only on four surfaces, resulting in four scores for each tooth (mesiobuccal, distobuccal, mesiolingual and distolingual) [16]. The four surfaces were assessed according to the Quigley–Hein Index [18], modified by Turesky et al. [19] and papillary bleeding index (Muhlemann [20]).

Sample size was estimated using Cohen's d formula for minimum difference expected. Through, it was estimated a sample size of eight volunteers per group taking into consideration a power of study (1-β) 80% and an alpha value of 5%. However, a dropout rate of approximately 20% was added to the sample size which gives 60 volunteers.

From the 82 volunteers evaluated to participate in the study, 60 were randomized to the experimental groups. All volunteers completed this clinical trial without any complications or side effects, such as dental or tongue staining and loss of taste. The subject characteristics are

shown in Fig. 1. All the volunteers were adults with ages between 18 and 24 years. According to ANOVA test, there are no age differences between the four groups (P = 0.994) (Fig. 1).

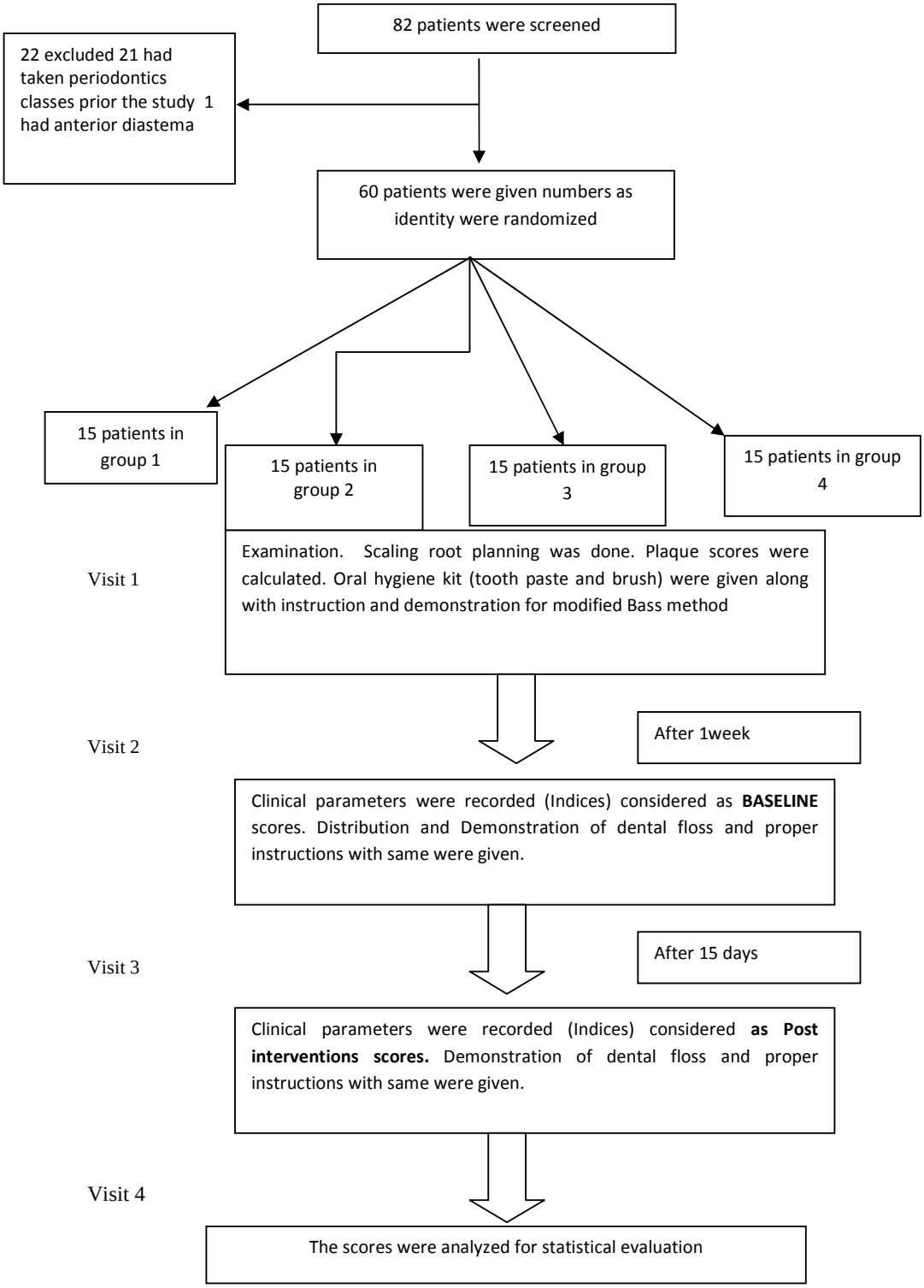

Fig. 1. Study design

3. RESULTS

All the scores were tabulated and entered in to Microsoft Excel 2007. Statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 17. The four groups were compared by ANOVA (Analysis of Variance) followed by intergroup comparison was done by Tukey's post hoc test. Before and after scores (2^{nd} visit /Baseline Scores) and (after 15 days / Post Interventional scores) were compared by paired't' test. p value was set at 0.05 and scores were considered statistically significant only when they are less than 0.05.

The mean plaque score using Turesky Gilmore Modification of Quigley Hein Plaque Index in Group 1(n=15) was 1.566667± 0.1687371 at baseline with minimum score 1.1667 and maximum score 1.7917 while in post intervention mean score was 0.8473333±.2868076 with minimum score 0 and maximum score 1.00.

The mean plaque score using Turesky Gilmore Modification of Quigley Hein Plaque Index in Group 2(n=15) was 1.958333± 0. .3886408 at baseline with minimum score 1.2083 and maximum score 2.5417 while in post intervention mean score was 0 .969444 ±0. 4494448 with minimum score 0 and maximum score 2.0833.

The mean plaque score using Turesky Gilmore Modification of Quigley Hein Plaque Index in Group 3 (n=15) was 1.608333 ± 0. .2518779 at baseline with minimum score 1.2917 and maximum score 2.2500 while in post intervention mean score was 0.608333 ± 0.2082143 with minimum score o.3333 and maximum score 1.0417.

The mean plaque score using Turesky Gilmore Modification of Quigley Hein Plaque Index in Group 4 (n=15) was 1.797222 ± 0. .4151361 at baseline with minimum score 1.0417 and maximum score 2.5833 while in post intervention mean score was 0.608333 ± 0.2082143 with minimum score 0.7083 and maximum score 2.2083.

The GBI score using Papillary Bleeding Index in Group 1(n=15) was 2.27 ± 0.961 at baseline with minimum score 1.00 and maximum score 4.00while in post intervention mean score was 1.60 ± 0.632 with minimum score 1 and maximum score 3.00.

The GBI score using Papillary Bleeding Index in Group 2(n=15) was 2.33± 0.724 at baseline with minimum score 0.00 and maximum score 4.00while in post intervention mean score was 0.33 ± 0.488with minimum score 0 and maximum score 1.00.

The GBI score using Papillary Bleeding Index in Group 3(n=15) was 2.80± 0.862 at baseline with minimum score 0.00 and maximum score 2.00 while in post intervention mean score was 1.07 ± 0.704 with minimum score 0 and maximum score 2.00.

The GBI score using Papillary Bleeding Index in Group 4 (n=15) was 2.57 ± 0.938 at baseline with minimum score 1.00 and maximum score 4.00 while in post intervention mean score was 1.13± 0.640 with minimum score 0 and maximum score 2.00.

More effective supragingival biofilm control was seen with patients using dental floss impregnated with cetylpyredinium chloride mouthwash when assessed with help of Turesky Gilmore Modification of Quigley Hein Plaque Index.

Dental floss impregnated with Chlorhexidine gluconate Mouthwash was more efficient in cleaning interdental areas when assessed using Papillary Bleeding Index.

4. DISCUSSION

Disclosed biofilm was used in this study, to assess plaque scores. Although the use of disclosing solutions in the management of biofilm control can be somehow discouraging for some patients, the use of disclosing solutions combined with index scales enables comparisons between new and existing oral hygiene products [17]. When the mechanical action of flossing was combined with the chemical action of chlorhexidine, it was possible to achieve more satisfactory results in reducing the interproximal biofilms on these surfaces.

This study found out that the dental floss groups, whether or not the floss was impregnated with mouthwash, exhibited lower levels of biofilms as when compared to the baseline levels. This result is supported by the classic study of Gjermo and Fl€otra [21], which established the use of dental floss for the mechanical control of dental biofilms. There was a 40% reduction in the amount of biofilm in the interproximal areas for the group that used dental floss for over 15 days. In a study by Terezhalmy et al. [22], various types of dental floss, such as unwaxed, woven, shred-resistant and powered flosser, in

association with the use of a toothbrush, similarly reduced the level of dental biofilms to a significantly greater extent than the use of a toothbrush alone.

In contrast with these results, a systematic review by Berchier in 2008, concluded that routine instruction to use dental floss is not supported by the literature, and the use of the floss should be analysed carefully by dental professionals so that patient should benefit the most from this type of interproximal cleaning device [6]. Additionally, another clinical trial did not report any additional benefits of the use of dental floss when it was compared to the use of a toothbrush alone [23]. The study found that after 8 weeks, the group that used only a toothbrush and toothpaste showed a reduction of interproximal biofilms which was quite similar to the group that used dental floss as well. Authors also indicated the need for an accurate manual dexterity to obtain the proper efficacy of dental floss. In the present study, at baseline, all patients were instructed on the proper way to use the floss.

This study results showed that dental floss impregnated with cetylpyredinium chloride mouthwash provided statistically significant lower supragingival biofilm assessed using Turesky Gilmore modification of Quigley Hein Plaque Index than those of the other three groups. Colgate plax ® has been studied in many controlled clinical trials which showed plaque reductions ranging from 22-36% and gingivitis reductions ranging from 23-36% with the longest study being 9 months [13]. And patients who used floss impregnated with chlorhexidine mouthwash showed reduced scores of deposits in the interdental areas assessed by Papillary Bleeding Index. Stabilized chlorine dioxide mouthwash exerts its bactetricidal effect by fixing cellular membrane proteins. It is used primarily in reduction of volatile sulphur compounds and hence potent agent for treatment of halitosis. Stabilized chlorine dioxide has marked bactericidal effects against oral bacteria associated with gingivitis and periodontitis [14].

Results of this study corroborate with the results of other studies that combined mechanical and chemical methods to control dental biofilms. In addition, a study by Gisselsson et al. [24] showed that after 3 years, the number of caries lesions was lower in children that used dental floss combined with chlorhexidine 1% gel than in children that used dental floss and a placebo gel.

Addition advantage of the impreganation of dental floss with mouthwash could be that it serves the purpose of both the chemical as well as mechanical plaque control.

However more investigations of the laboratory phase should be done, mainly, to assess how the impregnation with mouthwash happens. Further clinical trials with longer experimental periods should be performed to confirm or reject the absence of side effects, especially in nonprofessional individuals with a larger sample.

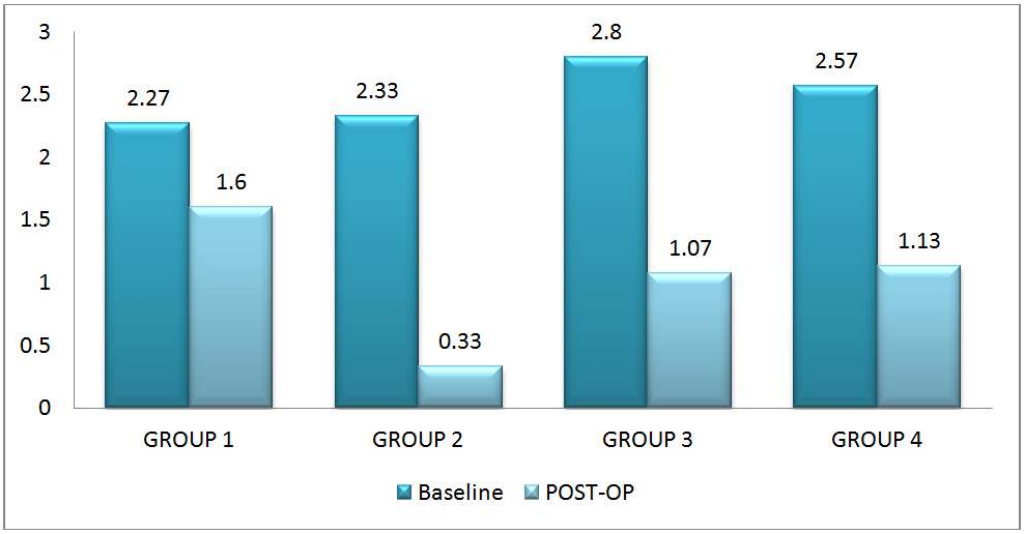

Fig. 2. Papillary bleeding index

Table 1. Descriptive statistics for TGG plaque score among all four groups at baseline and after intervention

Descriptive statistics							
Group	**Pre/post**		**N**	**Minimum**	**Maximum**	**Mean**	**Std. deviation**
Group 1	Baseline	Average	15	1.1667	1.7917	1.566667	.1687371
	Post Intervention	Average	15	.0000	1.0000	0.8473333	.2868076
Group 2	Baseline	Average	15	1.2083	2.5417	1.958333	.3886408
	Post Intervention	Average	15	.0000	2.0833	.969444	.4494448
Group 3	Baseline	Average	15	1.2917	2.2500	1.608333	.2518779
	Post Intervention	Average	15	.3333	1.0417	.608333	.2082143
Group 4	Baseline	Average	15	1.0417	2.5833	1.797222	.4151361
	Post Intervention	Average	15	.7083	2.2083	1.350000	.4100063

Table 2. Comparison of group for Turesky Gilmore Modification of Quigley Hein Plaque Index scores within the group (by pair t Test) as well as intergroup comparison by ANOVA

	Group 1	**Group 2**	**Group 3**	**Group 4**	**ANOVA p value**
Baseline	1.566667 ± 0.1687371	1.958333 ± 0.3886408	1.608333 ± 0.2518779	1.797222 ± 0.4151361	0.056
Post intervention	0. 8473333 ± 0.2868076	0.969444 ± 0.4494448	0.608333 ± 0.2082143	1.350000 ± 0.4100063	**<0.0001**
Mean difference	0.152778	0.6186667	0.8306667	0.4580000	------
Paired t test p value	0.214	**<0.0001**	**<0.0001**	**<0.0001**	--------

Intergroup comparison

Group Vs Group	**Baseline**	**Post intervention**
Group 1 Vs Group 2	0.497	0.0587
Group 1 Vs Group 3	0.078	0.0046
Group 1 Vs Group 4	0.468	**<0.0001**
Group 2 Vs Group 3	0.475	0.0034
Group 2 Vs Group 4	0.884	0.0524
Group 3 Vs Group 4	0.697	**<0.0001**

Table 3. Descriptive statistics for Papillary Bleeding Index score among all four groups at baseline and after intervention

Descriptive statistics						
Group		**N**	**Minimum**	**Maximum**	**Mean**	**Std. deviation**
Group 1	Baseline	15	1	4	2.27	.961
	Post Intervention	15	1	3	1.60	.632
Group 2	Baseline	15	1	3	2.33	.724
	Post Intervention	15	0	1	.33	.488
Group 3	Baseline	15	2	4	2.80	.862
	Post Intervention	15	0	2	1.07	.704
Group 4	Baseline	14	1	4	2.57	.938
	Post Intervention	15	0	2	1.13	.640

Table 4. Comparison of group for Papillary Bleeding Index score within the group (by pair t Test) as well as intergroup comparison by ANOVA

	Group 1	**Group 2**	**Group 3**	**Group 4**	**ANOVA p value**
Baseline	2.27 ± 0.961	2.33 ± 0 .724	2.80 ± 0.862	2.57 ± 0 .938	**<0.0001**
Post intervention	1.60 ± 0.632	0.33 ± 0. 488	1.07 ± 0 .704	1.13 ± 0.640	0.336
Mean difference	0.667	2.000	1.733	1.500	----------
Paired t test p value	**0.045**	**<0.0001**	**<0.0001**	**<0.0001**	_____

Intergroup comparison by Tukey's post hoc test

Group Vs Group	**Baseline (p value)**	**Post intervention (p value)**
Group 1 Vs Group 2	0.349	0.099
Group 1 Vs Group 3	0.997	**<0.001**
Group 1 Vs Group 4	0.785	0.180
Group 2 Vs Group 3	0.468	**0.011**
Group 2 Vs Group 4	0.895	0.991
Group 3 Vs Group 4	0.884	**0.005**

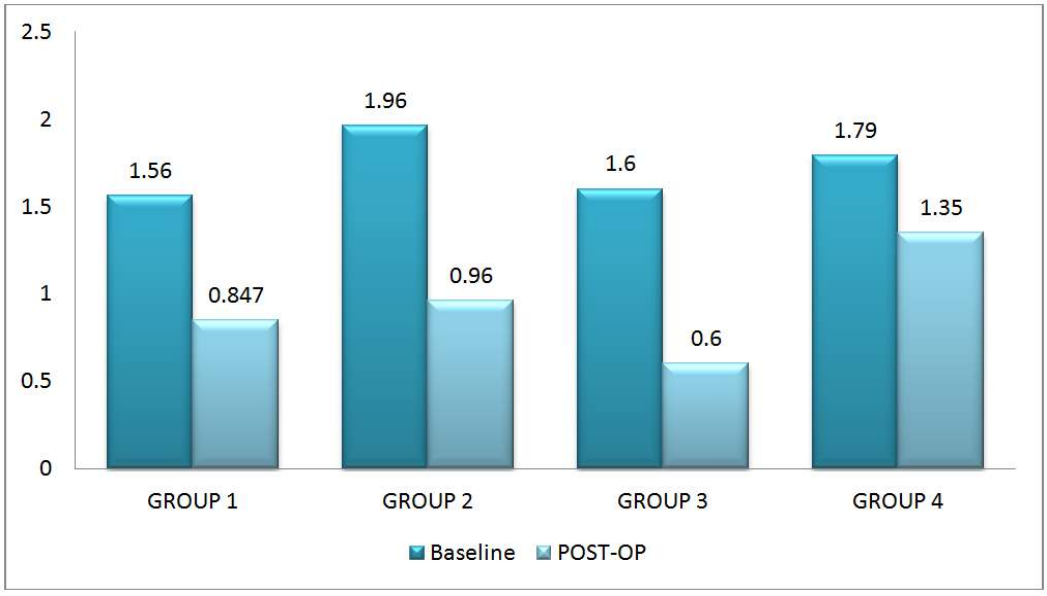

Fig. 3. Turesky Gilmore modification of Quigley Hein Plaque Index

5. CONCLUSION

The present study concluded that the CHX impregnated floss was more effective in reducing the interproximal plaque levels. Cetylpyridinium impregnated floss was better in reducing the biofilm level when assesed using Turesky Gilmore modification of Quigley Hein plaque index.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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