

Comparative Evaluation of Two Different One-stage Full-mouth Disinfection Protocols using BANA Assay: A Randomized Clinical Study

Arjumand Farooqui¹, Vineet V Kini², Ashvini M Padhye³

ABSTRACT

Aim: The aim of this study was to evaluate and compare two different one-stage full-mouth disinfection protocols in the treatment of chronic periodontitis by assessing dental plaque and tongue coat using BANA assay.

Materials and methods: The present study was a prospective randomized clinical parallel arm study design including 40 healthy subjects randomly allocated into two groups, i.e., group A (Quirynen's protocol of one-stage full-mouth disinfection) and group B (Bollen's protocol of one-stage full-mouth disinfection). Subjects were assessed at baseline and six weeks using plaque index, gingival index, and sulcus bleeding index. Probing depth and relative clinical attachment level were also recorded at six weeks. Winkel tongue coat index and BANA were recorded at 8 weeks using subgingival plaque and tongue coat sample.

Results: Both group A and group B demonstrated statistically significant reduction in plaque index, gingival index, sulcus bleeding index, Winkel tongue coat index, reduction in probing depth, and gain in relative clinical attachment level on intragroup comparison. There was no significant difference in BANA assay score of subgingival plaque and tongue coat samples in between group A and group B.

Conclusion: From the findings of this study, both Quirynen's protocol and Bollen's protocol of one-stage full-mouth disinfection are effective in plaque reduction and tongue coat reduction and achieve comparable clinical healing outcomes.

Clinical significance: The difference in duration and mode of use of chlorhexidine as a chemical plaque control agent in the two treatment interventions of Quirynen's and Bollen's protocol of one-stage full-mouth disinfection did not demonstrate statistical significance in reducing sulcus bleeding index scores, reducing probing depths, and gain in relative clinical attachment levels.

Keywords: *n*-Benzoyl-DL-arginine-2-naphthylamide, Dental plaque, One-stage full-mouth disinfection, Red complex, Tongue coat.

The Journal of Contemporary Dental Practice (2019): 10.5005/jp-journals-10024-2623

INTRODUCTION

Periodontitis is a dental plaque-induced host-mediated disease, which leads to the loss of tooth-supporting structures.¹ Bacterial species within the plaque biofilm in chronic periodontitis comprise *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), and *Treponema denticola* (Td) which constitute the Socransky's red complex of dental plaque.² Although an increase in specific microorganisms within the plaque biofilm is associated with periodontitis, it is the cumulative effect of all microorganisms within the plaque biofilm contributing toward periodontal inflammation, leading to loss of tooth support. On the basis of physical and morphological criteria, the oral cavity can be divided into six major ecologic niches: supragingival hard surfaces, subgingival regions adjacent to a hard surface comprising the gingival sulcus and periodontal pocket, lining mucosa of the cheek, palate, and floor of the mouth, dorsum of the tongue, tonsils, and the saliva.³

Scaling and root planing removes the plaque biofilm, resulting in a partly depleted ecologic niche within the periodontal pocket. Bacterial regrowth and recolonization of the periodontal pocket occur within 3–7 days following scaling and root planing, restoring dental plaque biofilms to almost pretreatment levels.⁴ Studies have demonstrated that trans-ecothelial migration of periodontal pathogens occur from several sites including the neighboring periodontal pockets, the dorsum of the tongue, buccal lining mucosa, and saliva. Therefore, it would be of great clinical benefit, if the risk of recolonization of bacteria from adjacent eco-niche be reduced by controlling periodontal pathogens both

^{1–3}Department of Periodontics, Mahatma Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra, India

Corresponding Author: Arjumand Farooqui, Department of Periodontics, Mahatma Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra, India, Phone: +91 9987382635, e-mail: arjumand.farooqui@gmail.com

How to cite this article: Farooqui A, Kini VV, *et al.* Comparative Evaluation of Two Different One-stage Full-mouth Disinfection Protocols using BANA Assay: A Randomized Clinical Study. *J Contemp Dent Pract* 2019;20(8):963–969.

Source of support: Nil

Conflict of interest: None

in subgingival econiche and various other intra-oral econiches colonized by plaque bacteria simultaneously.

One-stage full-mouth disinfection (OSFMD), an approach suggested for the treatment of plaque-induced periodontal disease by Quirynen *et al.*,⁵ completes the full-mouth scaling and root planing in one stage within 24 hours along with tongue surface debridement. One-stage full-mouth disinfection protocols prevent bacterial recolonization of gingival sulci from the translocation of bacteria from dorsum of the tongue and limit the number of appointments in the treatment of chronic periodontitis. Different full-mouth disinfection protocols have been described by Bollen *et al.*,⁶ Mongardini *et al.*,⁷ and Apatzidou *et al.*,⁸ having demonstrated clinical benefits over quadrant-wise scaling and root planing.

Qualitative and semi-quantitative assessment of Socransky's red complex bacteria can be made through chairside diagnostic test of *n*-benzoyl-DL-arginine-2-naphthylamide (BANA). This diagnostic technique detects the presence of arginine hydrolase, an enzyme produced by Pg, Tf, and Td, three anaerobic species consistently associated with periodontal disease. The presence of at least 10^4 cells of Pg, Tf, and Td results in a colorimetric change indicative of presence and quantity of the colony-forming units of aforementioned bacteria in the plaque sample.⁹

The aim of the study was to compare and evaluate two different one-stage full-mouth disinfection protocols in the treatment of chronic periodontitis by assessing dental plaque and tongue coat using *n*-benzoyl-DL-arginine-2-naphthylamide assay.

MATERIALS AND METHODS

The present study was a prospective randomized clinical parallel arm study design. Ethical clearance was obtained from the Institutional Ethical Committee. The study population comprised 40 healthy subjects satisfying inclusion criteria recruited by convenience sampling randomly allocated by computer-generated random numbering sequence method into two groups of 20 subjects each, i.e., group A (Quiryren's protocol of OSFMD) and group B (Bollen's protocol of OSFMD).

The inclusion criteria were:

- Subjects aged 18–60 years rendering informed consent.
- Presence of minimum 20 natural scorable teeth excluding third molars.
- Subjects diagnosed with chronic periodontitis based on American Academy of Periodontology (AAP) classification 1999.¹⁰
- Presence of at least two teeth per quadrant with a probing depth of >3 mm.
- Subjects having good level of oral hygiene (a mean plaque index (PI) score¹¹ <1).

Exclusion criteria were:

- Subjects who have received any form of periodontal therapy, surgical or nonsurgical within past 6 months.
- Subjects who have received antibiotic and/or anti-inflammatory therapy within the past 3 months.
- Subjects wearing orthodontic appliances.
- Subjects suffering from any systemic disease.
- Subjects who give present or past history of drug abuse.
- Subjects who give present or past history of smoking and tobacco consumption.
- Pregnant women and lactating mothers.
- Physically debilitated subjects.

A detailed case history was recorded to include the subjects according to the inclusion criteria. Informed consent was taken from all subjects fulfilling the inclusion criteria. Dental impressions were made and customized acrylic occlusal stents were fabricated. All subjects underwent full-mouth periodontal examination by a single trained calibrated examiner with customized acrylic occlusal stents (excluding third molars) for recording measurements of fixed reference point (FRP) to gingival margin (GM), i.e., sg (Fig. 1A) and fixed reference point (FRP) to base of sulcus (BOS), i.e., ss (Fig. 1B). Plaque index (PI),¹¹ gingival index (GI) by Loe and Silness,¹² sulcus bleeding index (Mühlemann and Son),¹³ and Winkels tongue coat index¹⁴ were also recorded.

The patients were given careful instructions in self-performed plaque control measures:

- Tooth brushing twice daily in the morning and night using the modified Bass brushing technique with a new medium bristle toothbrush and a fluoridated toothpaste.
- Once-daily interdental cleaning at night using Dental floss and/or interdental brushes without any dentifrice.
- Brushing of the tongue dorsum twice a day with a medium bristle toothbrush without any dentifrice.
- Instructions on the use of allocated treatment group mouthrinse protocol as prescribed by respective protocol.
- Oral hygiene control and re-instruction are completed at each visit.

Randomization was done using computer-generated numbering sequence method to allocate the subjects into group A and group B.

In subjects allotted in group A, scaling and root planing was performed in two sessions within 24 hours. Scaling and root planing was done for mandibular arch in first session than later for maxillary arch in second session by same trained operator using ultrasonic scaler and Gracey's curettes. Time taken for scaling and root planing of one quadrant was approximately 1 hour. Both the sessions were followed by:

- Brushing the dorsum of tongue for 1 minute with 1% chlorhexidine gluconate gel with a toothbrush.
- Rinsing twice with 10 mL of undiluted 0.2% aqueous chlorhexidine gluconate solution for one minute during last 10 seconds of which subjects are supposed to gargle.
- Subgingival irrigation of all the pockets thrice in a span of 10 minutes with 1% chlorhexidine gluconate gel using a syringe and irrigation cannula tip.

Additionally, the subjects of this group were instructed as per Quiryren's protocol of OSFMD to rinse twice daily for 1 minute with a undiluted 0.2% aqueous chlorhexidine gluconate solution half an hour after tooth brushing for 2 weeks.⁵

In subjects allotted in group B, scaling and root planing was performed in two sessions within 24 hours. Scaling and root planing was done for mandibular arch in first session than later for maxillary arch in second session by same trained operator using ultrasonic scaler and Gracey's curettes. Time taken for scaling and root planing of one quadrant was approximately 1 hour. Both the sessions were followed by:

- Brushing the dorsum of tongue for 1 minute with 1% chlorhexidine gluconate gel with toothbrush.
- Rinsing twice with undiluted 0.2% aqueous chlorhexidine gluconate solution for one minute during last 10 seconds subjects are supposed to gargle.
- Spraying the tonsil with undiluted 0.2% aqueous chlorhexidine gluconate spray.
- Subgingival irrigation of all the pockets thrice in a span of 10 minutes with 1% chlorhexidine gluconate gel using a syringe and irrigation cannula tip.

Additionally, the subjects of this test group were instructed as per Bollen's protocol of OSFMD to rinse twice daily for one minute with undiluted 0.2% chlorhexidine gluconate solution half an hour after tooth brushing and spray the tonsils twice daily with 0.2% chlorhexidine gluconate spray for 8 weeks.⁶



Figs 1A and B: (A) Measurement from FRP to free gingival margin (sg); (B) FRP to base of sulcus (ss)

Subgingival irrigation with 1% chlorhexidine gluconate gel was done for both groups A and B after 8 days. PI and WTCI were recorded for both the groups at two weeks. Clinical parameters namely PI, GI, and SBI were assessed for both the treatment groups at six weeks.

Measurements of sg, i.e., fixed reference point (FRP) to gingival margin (GM), and ss, i.e., fixed reference point (FRP) to base of sulcus (BOS) were also recorded at six weeks. From these measurements, the following measurements of the clinical parameters were derived:

- Probing depth (PD) = ss – sg.
- Relative attachment level = ss.
- Gingival recession = sg at 6 weeks – sg at baseline.

At 8 weeks, WTCI was recorded. Tongue coat sample (Fig. 2) and subgingival plaque sample (Fig. 3) were collected. BANA assay was performed and color change was noted (Fig. 4).

RESULTS

The data were tabulated and submitted to blinded statistician and analyzed using Statistical Product and Service Solutions (SPSS) software version 17.00. The test of normalcy of data in group A and group B, respectively, was performed by the Kolmogorov–Smirnov test. The analytical statistics for intra-group comparison of both group A and group B were analyzed using repeated measures ANOVA test with *post hoc* Bonferroni correction and paired *t* test.

Intergroup comparison for change in evaluation parameters between group A and group B was performed using unpaired *t* test and Mann–Whitney U test.

Both group A and group B demonstrated statistically significant reduction in PI, GI, SBI, reduction in PD, and gain in RCAL on intragroup comparison, respectively, at 6-week follow-up from baseline.

Group A demonstrated statistically significant reduction in PI scores as compared to group B on intergroup comparison at 2-week follow-up, respectively, and at 6-week follow-up from baseline. ($p < 0.05$) group B demonstrated statistically significant reduction in GI scores as compared to group A on intergroup comparison at 6-week follow-up from baseline ($p < 0.05$). There was no statistically significant reduction in SBI ($p = 0.312$) (Table 1) (Fig. 5).

There was no statistically significant increase in GR ($p = 0.619$), reduction in PD ($p = 0.682$), or gain in RCAL ($p = 0.094$) on intergroup comparison between group A and group B at 6-week follow-up from baseline ($p > 0.05$) (Table 2) (Fig. 6).

There was no statistically significant reduction in WTCI on intergroup comparison between group A and group B at 2 weeks and 8-week follow-up from baseline ($p > 0.05$) (Table 3) (Fig. 7).

There was no significant difference in qualitative aspect of subgingival plaque samples (BANA P) and tongue coat plaque samples (BANA T) assessed through BANA assay scores in between group A and group B at 8-week follow-up from baseline ($p > 0.05$) (Table 4) (Fig. 8).



Fig. 2: Collection of tongue coat sample



Fig. 3: Collection of subgingival plaque sample

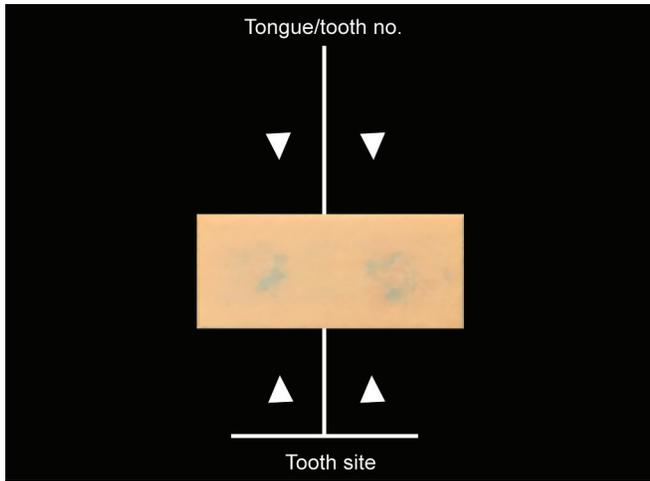


Fig. 4: Upper reagent strip of BANA assay examined for the presence of blue color with reference color indicator

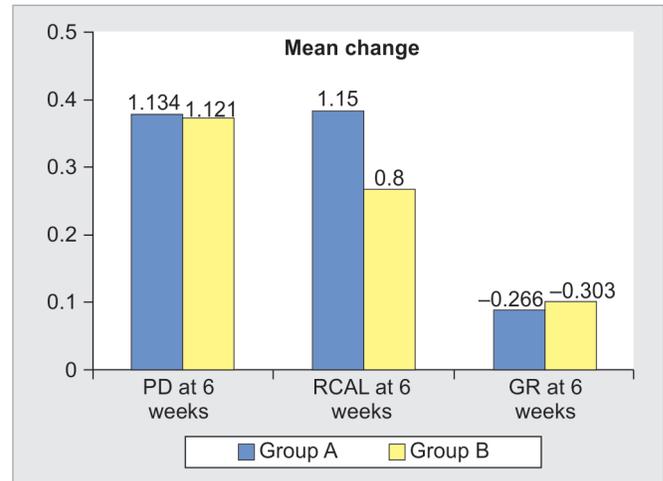


Fig. 6: Mean change in PD, RCAL, and GR for group A and group B

Table 1: Mean reduction in PI, GI, and SBI for group A and group B

Mean reduction	Group A (mean ± SD)	Group B (mean ± SD)	p value (unpaired t test)
PI at 2-week follow-up	0.350 ± 0.237	0.124 ± 0.007	<0.001*
PI at 6-week follow-up	0.276 ± 0.124	0.153 ± 0.007	0.001*
GI at 6-week follow-up	0.015 ± 0.007	0.027 ± 0.012	0.001*
SBI at 6-week follow-up	0.024 ± 0.018	0.020 ± 0.004	0.312

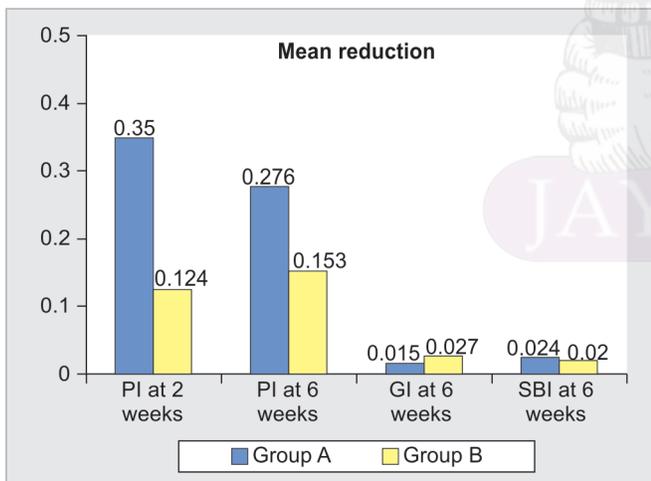


Fig. 5: Mean reduction in PI, GI, and SBI for group A and group B

Table 2: Mean change in PD, RCAL and GR for group A and group B

Mean change	Group A (mean ± SD)	Group B (mean ± SD)	p value (unpaired t test)
PD at 6-week follow-up	1.134 ± 0.650	1.121 ± 0.562	0.682
RCAL at 6-week follow-up	1.150 ± 0.670	0.800 ± 0.615	0.094
GR at 6-week follow-up	-0.266 ± 0.228	-0.303 ± 0.243	0.619

DISCUSSION

Periodontitis is a dental plaque-induced host-mediated disease, which leads to the loss of tooth support from remodeling of tooth-supporting tissues.¹ Traditionally, scaling and root planing has been performed in a quadrant-by-quadrant manner with an interlude of one to two weeks between subsequent treatment appointments.¹⁵ Bacterial regrowth and recolonization of the periodontal pocket occurs within 3–7 days following scaling and root planing, restoring dental plaque biofilms to almost pretreatment levels.⁴ In 1995, Quirynen et al. introduced the concept of OSFMD and concluded that OSFMD demonstrated significant clinical and microbiological advantages over quadrant-wise scaling and root planing.⁵ The difference between the OSFMD protocols as described by Quirynen et al. and Bollen et al. is in the duration and method of use of undiluted 0.2% aqueous chlorhexidine gluconate mouthrinses being limited to two weeks for Quirynen’s protocol and extending to 8 weeks in that of Bollen’s protocol. In addition, Bollen’s protocol involves use of undiluted 0.2% aqueous chlorhexidine gluconate oral sprays over the tonsils for a period of 8 weeks simultaneous with mouthrinsing.

The chairside BANA assay used in this study screens subgingival plaque and tongue coat samples for trypsin-like proteolytic activity that is common to only few known periodontal pathogens, such as Pg, Tf, and Td. The scores of the chairside BANA assay are interpreted as score 0—negative result (no blue color); score 1—weak-positive result (faint blue color) and score 2—strong-positive result (definite blue color). The scores also semi-quantitatively depict the number of colony-forming units (CFUs) of the Pg, Tf, and Td bacterial species cumulatively within the collected plaque sample corresponding to scores in which weak enzymatic reaction (score 1) corresponded to approximately 10⁴–10⁵ colony-forming units whereas strong enzymatic reaction (score 2) would correspond to about 10⁶ or over 10⁶ colony-forming units.⁹

Intragroup comparison of change in PI and WTCL scores at 2-week follow-up as compared to baseline for both group A and group B demonstrated significant reduction. This indicated that the combined brushing and use of chemical mouthrinse of the oral hygiene regimen were adhered to in both the groups. Intragroup comparison of reduction in PI, GI, and SBI scores at 6 weeks demonstrated significant reduction for both group A and group B. This is in agreement with the studies of Quirynen et al.,⁵

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Table 3: WTCL score at baseline, 2 weeks, and 8 weeks for group A and group B

Winkel tongue coat index	Baseline			At 2 weeks			At 8 weeks		
	Group A (N = 20)	Group B (N = 20)	p value (Mann-Whitney U test)	Group A (N = 20)	Group B (N = 20)	p value (Mann-Whitney U test)	Group A (N = 20)	Group B (N = 20)	p value (Mann-Whitney U test)
Score 4	0	0	0.157	0	5	0.063	10	14	0.253
Score 5	2	0		10	8		9	6	
Score 6	9	3		6	7		1	0	
Score 7	6	13		4	0		0	0	
Score 8	1	3		0	0		0	0	
Score 9	2	1		0	0		0	0	
Mode	6	7		5	5		4	4	

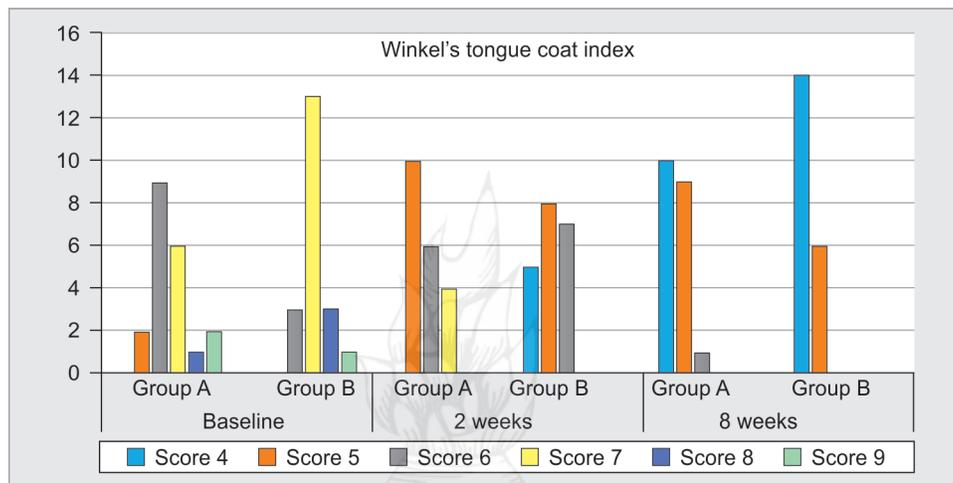


Fig. 7: WTCL score at baseline, 2 weeks, and 8 weeks for group A and group B

Table 4: BANA scores for subgingival plaque sample and tongue coat sample for group A and group B

BANA test score at 8 weeks	BANA P			BANA T		
	Group A (N = 20)	Group B (N = 20)	p value (Mann-Whitney U test)	Group A (N = 20)	Group B (N = 20)	p value (Mann-Whitney U test)
Score 0	13	15	0.59	4	4	0.84
Score 1	7	5		11	10	
Score 2	0	0		5	6	
Mode	0	0		1	1	

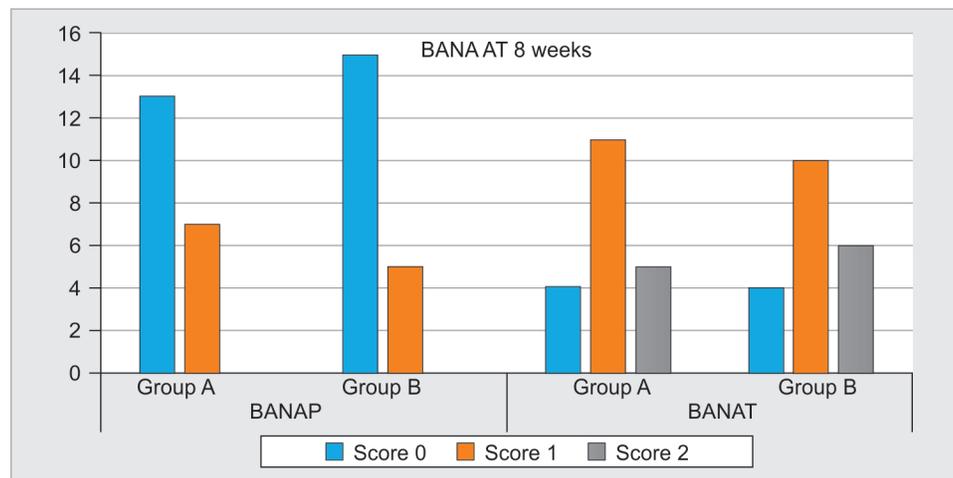


Fig. 8: BANA scores for subgingival plaque sample and tongue coat sample for group A and group B

Vandekerckhove et al.,¹⁶ and Sweirkot et al.,¹⁷ in which significant improvements in PI scores were observed at the follow-up visits following Quirynen's protocol of OSFMD. Also, in the studies of Bollen et al.⁶ and Mongardini et al.,⁷ significant improvements in PI scores were observed at the follow-up visits following Bollen's protocol of OSFMD. Intragroup comparison of reduction in PD and RCAL at 6-week follow-up as compared to baseline demonstrated significant reduction for both group A and group B.

Intergroup comparison for reduction in PI scores at 2 weeks and 6 weeks from baseline depicts significant reduction in PI scores in favor of group A as compared to group B. Intergroup comparison for reduction in GI scores at 6-week follow-up from baseline demonstrates significant reduction in GI scores in favor of group B as compared to group A at 6-week follow-up from baseline. This could be attributed to increased duration of chlorhexidine mouthrinse in group B as compared to that of group A at 6-week follow-up from baseline. At the point of conducting this study, there are no studies to the best of our knowledge that have compared the two protocols.

Intergroup comparison for WTCI scores at 2 weeks demonstrated no significant difference in between WTCI scores for group A (mode 5) and group B (mode 5) at 2-week follow-up ($p = 0.063$). It was observed that intergroup comparison for WTCI scores at 8 weeks demonstrated no significant difference in between WTCI scores for group A (mode 4) and group B (mode 4) at 8-week follow-up ($p = 0.253$). These observations would imply that quantitatively the tongue coat thickness was comparable for both group at 2-week follow-up and at 8-week follow-up. The reduction in the mode values within each group from baseline and at 2-week follow-up and 8-week follow-up indicates that the mechanical tongue brushing protocol was adhered to by the subjects and was equally efficacious in tongue coat removal for both groups A and B. It also indicated the either the discontinuation of chemical plaque control at 2 weeks for group A or use of chemical plaque control beyond 2 weeks in group B did not statistically influence the surface area or tongue coat thickness throughout the study period for both groups A and B, indicating that mechanical tongue brushing proved more instrumental in reducing tongue coat. This in agreement with studies of Soares et al. which show reduction in tongue coat after one-stage full-mouth disinfection as assessed by WTCI.¹⁸ However, Quirynen et al. did not observe a clear tendency in reduction toward tongue coat following one-stage full-mouth disinfection.¹⁹

On intergroup comparison for reduction in PD, gain in clinical attachment level and gingival recession at 6-week follow-up from baseline demonstrated no significant reduction in probing depths between group A compared to group B at 6-week follow-up from baseline. The observation made was that reduction in PD, gain in RCAL, and the GR were comparable in both groups A and B at 6-week follow-up from baseline. It may be permissible to infer that the clinical healing outcomes were comparable for both treatment interventions group A and B at 6-week follow-up from baseline. A cochrane review by Eberhard et al. on OSFMD showed that full-mouth disinfection protocols improved the clinical outcomes of reduction in PD to a greater extent in moderate pockets (4–6 mm) as compared to deep pockets (>7 mm).²⁰

Intergroup comparison of the BANA P scores between group A and group B at 8-week follow-up did not demonstrate any statistically significant difference. Similarly, intergroup comparison of BANA T scores between group A and group B at 8-week follow-up did not demonstrate any statistically significant difference. This would indicate that the difference in chemical plaque control

regimen duration and mode of application of 0.2% aqueous chlorhexidine gluconate did not have an effect on the BANA P and BANA T scores at 8-week follow-up; therefore, the qualitative nature of plaque at 8-week follow-up in both groups A and group B is comparable and similar when assessed by the chairside BANA assay. At the point of conducting this study, there are no studies to our knowledge that have compared the two protocols of one-stage full-mouth disinfection based on the BANA scores. However, in the study of Quirynen et al., it was found that there was eradication of Pg from subgingival pocket, tongue mucosa following full-mouth disinfection.²¹ In a study of Faveri et al., it was found higher frequency of BANA-negative sites after full-mouth scaling along with use of chlorhexidine mouthrinse.²²

CONCLUSION

From the findings of this study, both Quirynen's protocol and Bollen's protocol of OSFMD are effective in plaque reduction, tongue coat reduction, and achieve comparable clinical healing outcomes with no difference in the qualitative aspect of the subgingival plaque and tongue coat in the treatment of chronic periodontitis.

CLINICAL SIGNIFICANCE

The difference in duration and mode of use of chlorhexidine as a chemical plaque control agent in the two treatment interventions of Quirynen's and Bollen's protocol of one-stage full-mouth disinfection did not demonstrate statistical significance in reducing sulcus bleeding index scores, reducing probing depths, and gain in relative clinical attachment levels.

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