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REDUCTIONS IN CLINICAL GINGIVITIS, DENTAL PLAQUE BACTERIA AND MICROBIOME ANALYSIS AFTER USE OF A CHX MOUTH RINSE IN COMPARISON TO A CONTROL TREATMENT.

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ABSTRACT

Background: A recognition of microbial influences in the initiation and progression of common oral conditions has resulted in a significant emphasis on avenues to augment oral hygiene. **Aims:** Analyze clinical outcomes along with evaluation of anaerobic dental plaque bacteria by microbial culture and high throughput 16S rRNA sequencing after rinsing with a 0.12% chlorhexidine or a fluoride mouthwash. **Settings and Design:** The study protocol was approved by the Institutional Ethics Committee of the SDM College of Dental Sciences and Hospital and the population were from the local area who provided voluntary written informed consent. **Materials and Methods:** Adults aged 20-55 years with gingival index scores of 1.0 or more underwent examinations for dental plaque and gingivitis followed by collection of dental plaque Post-treatment clinical assessments and microbial culture were conducted after one and two weeks use of treatments with microbial 16S rRNA gene (V3-V4) sequencing of dental plaque. **Results:** Chlorhexidine group demonstrated a 66% and 76% reduction respectively for plaque bacteria evaluated by anaerobic microbial culture at the one- and two-week evaluations compared to the fluoride group. 16S rRNA analysis of dental plaque identified 290 OTU's representing 18 phyla and 133 genera. 57.2% of these OTU's were observed in all evaluations with principal component analyses. α-diversity (chao 1) assessments demonstrated significant reductions in microbial density after Chlorhexidine treatment. **Conclusions:** Significant changes in microbial genera including Veillonella, Haemophilus, Treponema and Aggregatibacter were observed after Chlorhexidine treatment. Results align with the clinical assessments for dental plaque and gingivitis.

KEYWORDS: Chlorhexidine, Dental Plaque, Gingivitis, Polymorphonuclear leukocytes.

INTRODUCTION

Endogenous microorganisms of the human mouth representing complex microbial ecosystems are readily found in the dental plaque, saliva, mucosal surfaces and the exposed surfaces of the tongue, cheek, gums and palate.[1-3] Microbial proliferation in these regions is facilitated by diet, environmental influences, regional differences in oral anatomy along with physiological factors.[2-3] The by-products of microbial proliferation have antigenic and immunologic features.[3] Contemporary practices in dentistry identify self-care representing optimal routine cleansing of the teeth and oral surfaces.[5-6] as an important means to attenuate the influences of microbial colonization and proliferation.[7] Associated with the lack of effective optimal hygiene are common oral conditions such as gingivitis representing an inflammation of gums[2,3,6,7] with some populations at greater risk for complications due to pre-existing or systemic conditions.[4,8] Additionally, microbial influences are reported in the progression of gingivitis to periodontitis with clinical observations reporting loss of bone supporting the tooth and may lead to tooth loss.[9]

A recognition of microbial influences in the initiation and progression of common oral conditions has resulted in a significant emphasis on avenues to augment oral hygiene.[2,4] An approach to control these organisms is the use of oral hygiene formulations with antimicrobial ingredients.[9] Available widely in the literature are investigations of formulations with herbal extracts and ingredients such as chlorhexidine gluconate [CHX] with proven antimicrobial features. CHX, a guanide has a significant history of use for oral and medical applications based on its established safety profile and efficacy parameters.[10] The US FDA has approved CHX as an ingredient in mouthwash and for other applications.[11] In clinical studies, CHX demonstrates substantivity to oral surfaces with clinical effects that

include reductions in scores of dental plaque and gingivitis.[9,12]

Distinct regions of the human body are dominated by heterogeneous microbial communities.[13] A recognition of the interactions and influences of these microbial communities with the human host has led to strategies for their analyses.[13-15] Microbial 16S rRNA sequencing is widely reported to profile endogenous communities to examine their differences in health and disease[16], the skin microflora[17] along with analysis of dental plaque and oral samples.[1, 18] Available readily are several approaches for high-throughput microbiome analysis[15] including those that can be accessed as a service provided by laboratories. To the best of our knowledge, there are few studies examining the effects of CHX on the dental plaque microbiome reported from randomized clinical trials. A recent clinical study reported salivary microbiome and biomarker evaluations from a single-blind, non-randomized design with sequential assignment of water followed by the CHX mouthwash with each treatment lasting one-week.[19] Accordingly, the present parallel design clinical investigation with randomized treatment assignment evaluated the effects of daily oral hygiene after use of either a CHX or a fluoride mouthwash on dental plaque bacteria by anaerobic culture in conjunction with analysis of dental plaque microbial communities by high throughput Illumina HiSeq 2500 sequencing of 16S rRNA (V3-V4) gene amplicons. Included were clinical assessments of dental plaque and gingivitis as widely established oral health measures, microbiological analysis of anaerobic dental plaque organisms by culture. Also included were an analysis of effects on oral polymorphonuclear leukocytes [PMN] representing immune effector cells and commonly referred to as the first-responders[20] with a recent report demonstrating the effects of CHX on PMN.[21] Together, these efforts were designed to provide a comprehensive analysis of these interventions.

MATERIALS AND METHODS

Clinical Study population and Experimental Design

This was designed as a double-blind, parallel design single center study to evaluate outcomes over the two-week treatment period with treatments assigned randomly. The study protocol was approved by the Institutional Ethics Committee of the SDM College of Dental Sciences and Hospital with all study related steps conducted in accordance with widely accepted procedures for clinical studies. The study population were from the local area who provided voluntary written informed consent prior to study enrollment. Subjects expressing a willingness to participate in the study were scheduled for a screening visit at the dental clinic to evaluate their eligibility for study enrollment.

Criteria for Subject Enrollment

Prospective subjects of either gender (age range 18-70 years) in good health from the local area were scheduled for the initial screening visit. Screening visits were scheduled in the morning and included a whole mouth exam by a dentist at the dental operatory with examinations conducted under constant lighting conditions. Enrolled subjects presented with 20 natural teeth and no removable of fixed dental prostheses. Those presenting a whole mouth dental plaque score of 1.5 by the Turesky modification of the Quigley-Hein Index[22] and a minimum gingivitis index score of 1.0 by the Loe-Silness Index[23] were enrolled.

Subject exclusion criteria

Subjects requiring dental care or reporting pregnancy, or an anticipated pregnancy were excluded. Those reporting chronic or serious conditions including diabetes mellitus, heart, renal or liver disease along with infectious conditions were also excluded. Additionally, subjects under the care of a physician, taking prescription medications or anticipating medical or dental procedures did not meet study inclusion criteria. During the screening clinical exam those presenting oral conditions and requiring dental care such as ulcers, abscess, carious lesions or restorations along with reported allergy to oral hygiene formulations were excluded. Additionally, subjects who reported clinical study participation in the preceding months or in an ongoing study were not eligible for study enrollment. Enrolled subjects were scheduled for baseline clinical evaluations described in section below.

Clinical Study Procedures

Baseline examinations and test product assignment

Subjects enrolled in the study were assigned a commercially available fluoride toothpaste and a soft bristled toothbrush for use during the one-week washout phase. Following study enrollment, subjects were instructed to refrain from using all other oral hygiene formulations for the study duration.

Following the washout phase, subjects arrived at the dental clinic for baseline evaluations with these and all subsequent procedures conducted between 7-9 AM. Prior to each visit, subjects refrained from oral hygiene for 12 hours or food prior to clinical evaluations for plaque and gingival indices by a calibrated dental examiner. After the baseline assessments and sampling described below, subjects were randomly assigned to either a fluoride mouthwash (control) or a commercially available CHX mouthrinse (test) with all products overwrapped to mask all identifying features and assigned a unique code to blind the subjects and examiners of treatment assignments. Subjects were also provided a commercially available fluoride toothpaste and a soft-bristled toothbrush for oral hygiene and instructed to brush their entire mouth for 2 minutes followed by rinsing with 15 ml of assigned mouthwash for 30 seconds as described previously (Sreenivasan & Prasad 2020). To facilitate compliance, all subjects completed the first use of assigned treatment at the dental clinic. Subjects were instructed to maintain their normal dietary habits and refrain from using any other oral

hygiene formulations or share provided test articles with anyone.

Post-treatment evaluations

Post-treatment evaluations were conducted weekly over the two-week study period with these procedures similar to those described for baseline. During each post-treatment visit, subjects were interviewed by study personnel for adverse events and the products weighed to evaluate compliance. Additionally, subjects were periodically contacted over the study period to reinforce study procedures. All issued treatments were collected from subjects at the conclusion of the study for compliance evaluations.

Clinical measurements:

Subjects underwent an oral examination during each visit to the dental clinic and were interviewed for adverse events. Whole mouth clinical measurements for dental plaque and gingivitis were conducted on all scorable maxillary and mandibular teeth at each visit. A calibrated dentist conducted these examinations using a dental light and mirror with scoring procedures excluding third molars or teeth with cervical restorations or crowns. Treatment efficacy was based on average scores from the whole mouth for gingivitis and dental plaque. The Loe-Silness gingival index is described widely in the literature and represented a primary clinical outcome[23]. In brief, the Loe-Silness utilizes a 3 point scale on 6 surfaces per tooth: (1) mesio-facial; (2) mid-facial; (3) disto-facial; (4) mesio-lingual; (5) mid-lingual; and (6) disto-lingual. A whole mouth score for gingival index for each subject is determined by adding the values provided by the dental examiner to each scorable surface and dividing that by the number of surfaces scored. A maximum tooth score of 18 is possible by the Loe-Silness index and clinical criteria for scoring is as follows.

0 Absence of inflammation
1 Mild inflammation-slight change in color and little change in texture
2 Moderate inflammation-moderate glazing, redness, edema and hypertrophy. Tendency to bleed upon probing.
3 Severe inflammation-marked redness and hypertrophy. Tendency to bleed spontaneously.

The whole mouth dental plaque examination based on the Turesky Modified Quigley-Hein plaque index represented an additional clinical assessment.[22] A disclosing agent was used to stain the dental plaque of all scorable maxillary and mandibular teeth. Scores from 0 to 5 was evaluated by a dental examiner using a dental light and mirror and each tooth was scored on six surfaces: (1) mesio-facial; (2) mid-facial; (3) disto-facial; (4) mesio-lingual; (5) mid-lingual; and (6) disto-lingual. Scores were not recorded from third molars or teeth with crowns or restorations. Average whole mouth scores for each subject at each examination were determined by adding all dental plaque scores assigned by the dental examiner and dividing by the number of scored surfaces. The Turesky Modification of the Quigley-Hein Plaque Index is as follows.

0 No Plaque
1 Separate flecks of plaque at the cervical margin of the tooth.
2 A thin continuous band of plaque (upto 1 mm) at the cervical margin of the tooth.
3 A band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth.
4 Plaque covering at least one-third but less than two-thirds of the crown of the tooth.
5 Plaque covering two-thirds of more of the crown of the tooth.

Laboratory Procedures

Analysis of dental plaque for anaerobic organisms: Dental plaque samples were evaluated for anaerobic organisms by culture in accordance with previously described procedures.[24] In brief, collected plaque samples were transported to the laboratory immediately after collection. Samples were sonicated and rapidly diluted prior to plating in duplicate on enriched agar and incubated under anaerobic conditions at 37°C. Media were scored for viable organisms and reported as colony forming units per ml of sample (CFU/ml). Statistical analysis of viable organisms (CFU/ml) were conducted with Log_{10} transformed results as described previously.[24]

Dental Plaque Sample collection for metagenomics

Dental plaque samples were collected from each subject in tubes with 1.5 ml phosphate buffered saline sterile and coded with label information for clarity and unambiguous sample registration. Baseline and post-treatment samples at the conclusion of the two-week treatment phase were collected and stored at -80°C. Samples were shipped on dry ice to the central laboratory for microbial sequencing. Microbial DNA was extracted from samples using QIAamp BIOStic kit from Qiagen and DNA quantified using Qubit dsDNA HS Assay kit (ThermoFisher Scientific). Genomic DNA (20-100 ng) was amplified using 16S specific primers for the V3 and V4 regions. Amplicons were used for library preparation using Kapa DNA Hyper kit (Kapa Biosystems, KK8504) and Truseq Nano HT (Illumina, #FC-121-4003) where the amplicons were ligated with indexed Illumina adapters followed by PCR to amplify the ligated molecules. Libraries from each sample were sequenced on a HiSeq 2500 sequencer and tracked using a sequence barcode. Bioinformatics included a quality report of sequences to establish base quality score distribution, average base content per read and GC distribution. Based on assigned barcode fastq files were de-multiplexed and consensus sequences generated for each sample. Microbial taxonomy was assigned based on consensus sequence and compared across different samples. Bacterial diversity with each sample were conducted using Shannon index and rarefaction plots and diversity across samples evaluated by principal

component analysis and UPGMA tree. Results from sequencing analyses are reported as group 1 and 3 from the baseline assessments of control and test groups respectively and from groups 2 and 4 for the control and test groups at the two-week post-treatment evaluations.

Statistical Methods

Sample size outcomes for this study were estimated to enroll approximately 15 subjects in each treatment group to detect a difference of approximately 0.2 units between the treatment groups for gingival index scores. Calculations were based on standard deviation values of 0.2 units with α of 0.05 and statistical power of 80%.

Statistical Analysis

A chi-square analysis and an analysis of variance (ANOVA) compared the gender and ages of enrolled subjects between treatment groups with descriptive statistics presented as mean ± SD over the study period. Microbiological outcomes for anaerobic organisms by culture were transformed (log_{10}) for statistical analysis. Clinical outcomes for dental plaque and gingival index measures within treatment groups from baseline to each post-treatment examination were evaluated by t-tests. Differences between treatment groups for clinical and from anaerobic microbiological culture were evaluated by an analysis of covariance (ANCOVA) with the corresponding baseline scores serving as the covariable. Treatment differences were evaluated by a Tukey-HSD with all statistically significant results reported at p values less than 0.05.

RESULTS

Fifty adults who provided their voluntary informed consent were scheduled for a screening visit at the dental clinic conducted by a dentist to determine study eligibility. Nineteen subjects were excluded during the screening visit for not meeting study criteria. Thirty-one subjects met study criteria and were enrolled in the study and completed the one-week washout phase during which they used the provided commercially available fluoride toothpaste for oral hygiene. Subjects were instructed to refrain from using any other oral formulations after study enrollment. At the conclusion of the washout phase, subjects arrived at the dental clinic for their baseline examinations and sampling. All enrolled subjects completed the two-week study with no drop-outs or adverse events reported by study subjects or the dental examiners. Shown in Table 1 is a summary of demographic characteristics of study subjects. The study population comprised 17 females and 14 males with an average age of 33.13 years and a standard deviation of 9.09. The minimum and maximum age of the population were 20 and 55 years respectively. The average age of subjects in the control and test groups were 33.25 and 33.00 years respectively with no significant differences by ANOVA ($p>0.05$). Similarly, chi-square analysis revealed no differences in gender between treatment groups ($p>0.05$).

Results from the clinical evaluations and anaerobic microbiological assessments are shown in table 2 and presented as a summary of the mean and SD of these evaluations conducted twelve (12) hours after oral hygiene. Average dental plaque scores (Table 2 a) at baseline were 2.26 and 2.42 for the control and test and were not significantly different ($p>0.05$). While the control group demonstrated some reduction in plaque scores over the study period, average plaque scores in the test group were 2.02 and 1.93 at the one-week and two-week evaluations respectively representing significant differences from control ($p<0.05$). Corresponding observations were noted for gingival index scores (Table 2b). While baseline scores were 1.27 for the two treatment groups, the test group demonstrated significantly greater reductions with progressive increases over the two-week study period in comparison to the control ($p<0.05$). Average gingival index scores at the one and two-week post treatment evaluations for the test group were 0.96 and 0.84 respectively representing reductions of 19.3% and 23% versus the control.

Analysis of the anaerobic organisms over the study is presented in Table 2c. Results are presented as numbers of viable organisms (Log CFU/ml) over the study period (Average ± SD). Whereas baseline scores between treatment groups demonstrated no statistically significant differences ($p>0.05$), a sequential reduction in the numbers of viable organisms were observed from the baseline over the two-week evaluation period for the test group. The control group demonstrated slight increases in average numbers of anaerobic organisms at all post-treatment evaluations. In comparison to the control, statistical analyses by ANCOVA indicate significantly lower numbers of anaerobic organisms in the test group at each post-treatment assessment ($p<0.05$). The test group registered a 66% reduction in anerobic organisms at the one-week evaluation that increased to 79% in comparison to the control.

Post-treatment evaluations of groups (3 & 4) revealed significant changes in microbial composition. The major phyla which showed significant changes were *Fusobacteria, Actinobacteria & Spirochaetes.* The genera *Streptococcus, Veillonella, Haemophilus, Leptotrichia, Actinomyces, Lautropia, Treponema & Aggregatibacter* produced significant changes in relative abundances. Samples were evaluated for diversity and richness by measuring alpha diversity as shannon and chao1 metrics respectively (Figures 1 & 2). The data showed test treatment to cause a decrease in overall richness of the samples as observed from the chao1 index. All groups showed similar shanon index distribution. Group 4 distribution, though showed slight reduction were not statistically significant. Results suggested that, though the test treatment reduced the overall microbial load, it did not affect the diversity of the microbiota and resultant microbial dysbiosis.

A weighted unifrac analysis (beta diversity) for each individual between before and after treatment samples provided diversity between two communities based on phylogenetic information. The beta diversity in test group was slightly higher than the control group suggesting test treatment altered microbial composition to some extent than the control but the difference was not statistically significant ($p>0.05$).

Analysis of all OTUs (Figure 4) indicate that 57.2% were found across all the groups and only a few OTUs (0.3% to 8.3%) were group specific. Similarly, the total number of genera did not vary within each group. The results suggest similarities in microbiota across all the groups. The commonality among the groups suggested the absence of dysbiosis due to treatment.

Table 1: Summary of subject demographics who completed the entire study.

Parameter	Number of subjects	Age [Mean]	Age [SD]	Age [Minimum]	Age [Maximum]
Entire population	31 [17 females; 14 males]	33.13	9.09	20	55
	Female [n=17]	35.06	8.92	22	55
	Male [n=14]	30.79	9.07	20	46
Control Mouthwash ǂ	16 [8 females; 8 males]	33.25	8.82	21	46
	Female [n=8]	34.75	7.15	22	45
	Male [n=8]	31.75	10.53	21	46
Test Mouthwashǂ	15 [9 females; 6 males]	33	9.68	20	55
	Female [n=9]	35.33	10.69	23	55
	Male [n=6]	29.50	7.42	20	40

ǂ No significant differences between treatment groups for age or gender by ANOVA and chi-square analysis respectively ($p>0.05$).

Table 2 a: Summary of dental plaque index scores over the study period [Average ± SD].			
Treatment	**Baseline**	**1 week post-treatment**	**2 weeks post-treatment**
Control Mouthwash	2.26±0.41	2.26±0.45	2.14±0.39
Test Mouthwash	2.42±0.34	2.02±0.45*	1.93±0.42*
Percent differences between treatments		16.30%	10.50%
*Test mouthwash significantly different from control mouthwash at all post-treatment evaluations by ANCOVA ($p<0.05$).			
Table 2 b: Summary of gingival index scores over the study period [Average ± SD].			
Treatment	Baseline	1 week post-treatment	2 weeks post-treatment
Control Mouthwash	1.27±0.22	1.19±0.19	1.16±0.0.21
Test Mouthwash	1.27±0.21	0.96±0.26*	0.84±0.32*
Percent differences between treatments		19.30%	23.60%
Table 2 c: Summary of viable anaerobic organisms [Log CFU/ml] over the study period [Average ± SD].			
Treatment	Baseline	1 week post-treatment	2 weeks post-treatment
Control Mouthwash	6.44±0.3	6.54±0.4	6.52±0.38
Test Mouthwash	6.73±0.46	6.27±0.8*	6.10±0.6*
Percent differences between treatments		66.00%	79.00%

*Test mouthwash significantly different from control mouthwash at all post-treatment evaluations by ANCOVA ($p<0.05$).

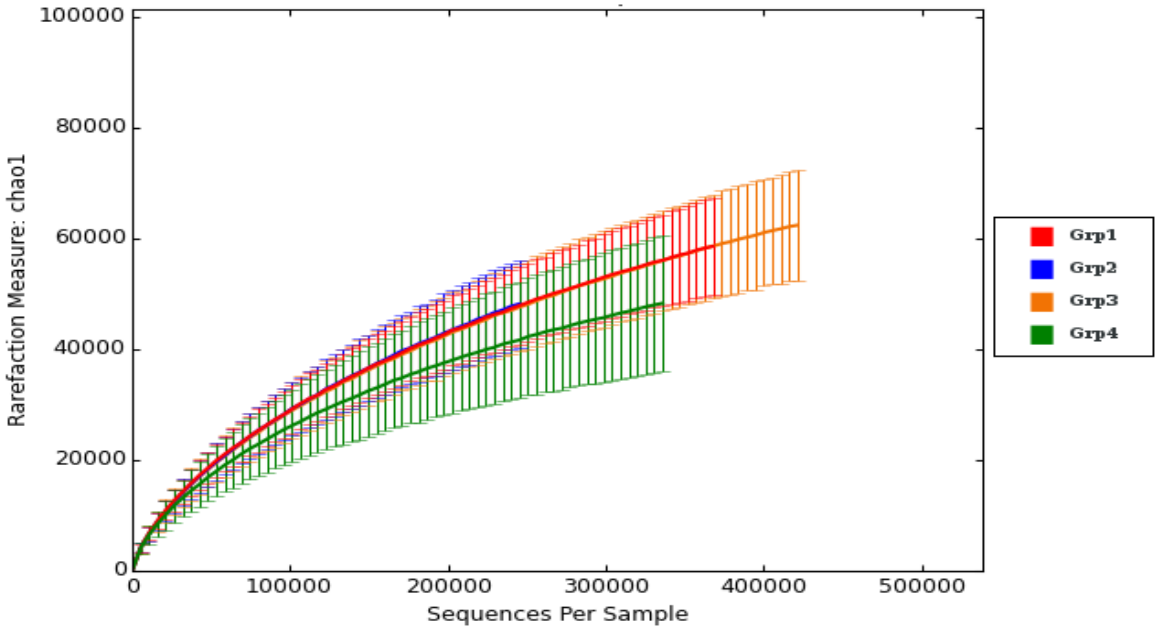

Figure 1: Alpha diversity in treatment groups (chao1 metric).

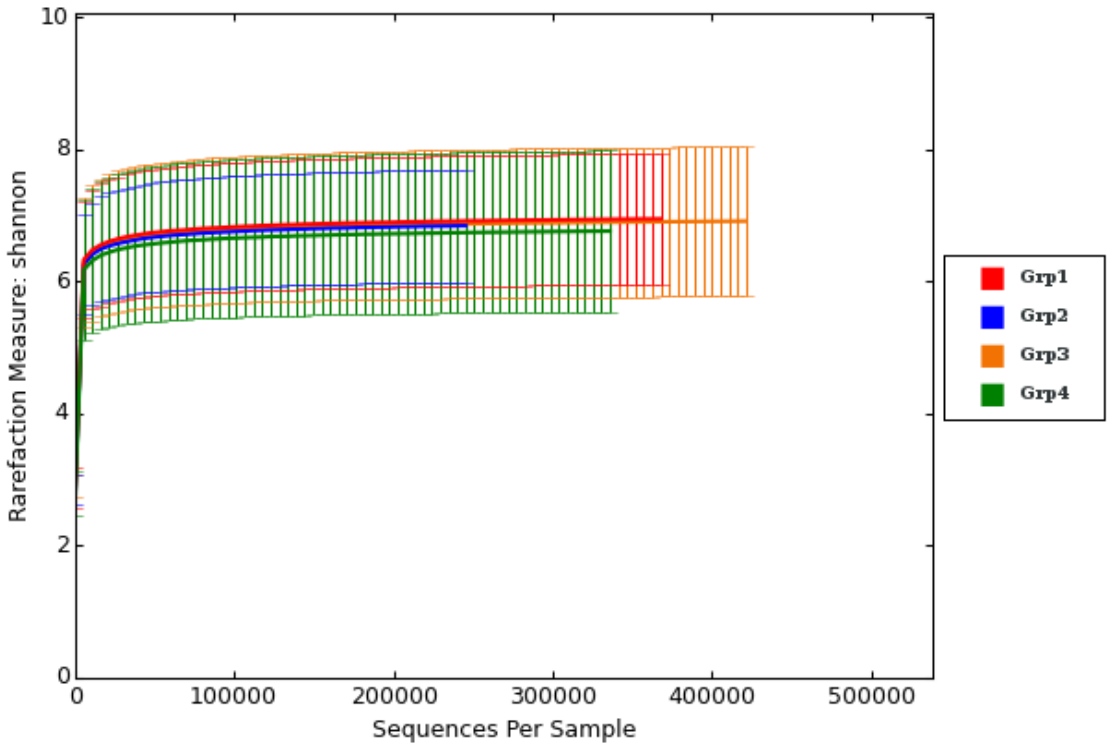

Figure 2: Alpha diversity in treatment groups (Shannon diversity).

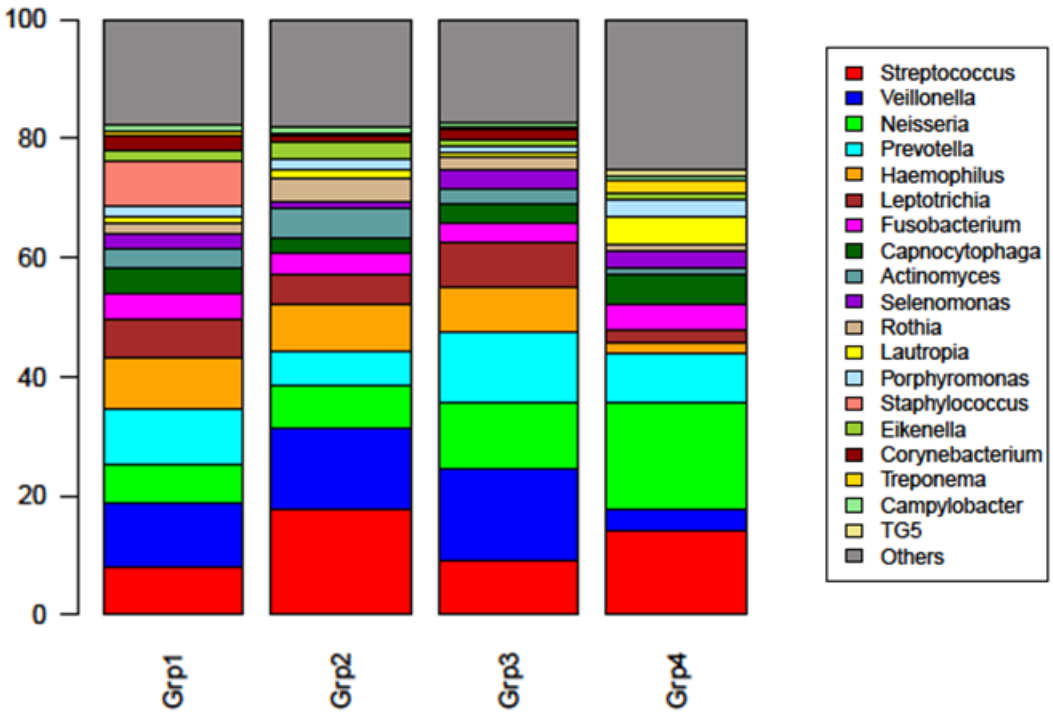

Figure 3: Boxplot presenting phyla level analysis of all treatment groups.

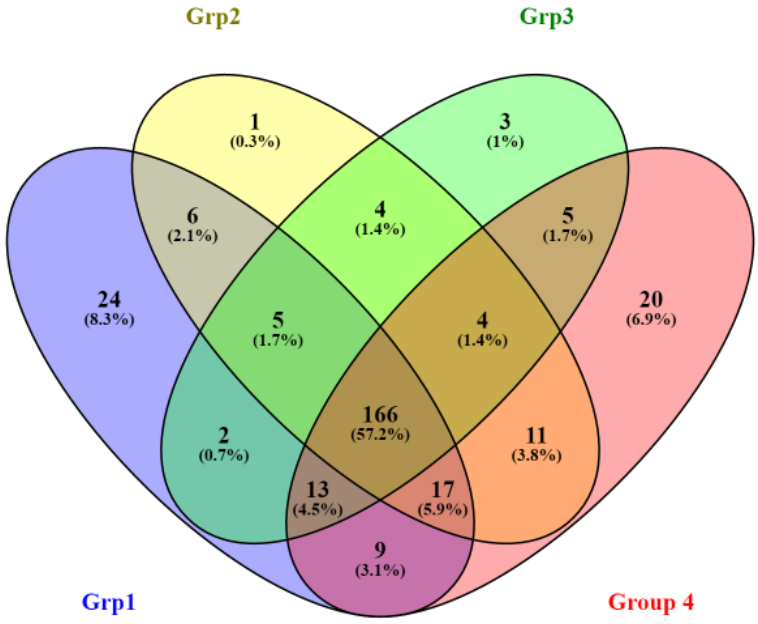

Figure 4: Venn Diagram for OTUs across all the groups.

DISCUSSION

Chronic oral conditions such as gingivitis and periodontal diseases have a global epidemiology.[3,7,25] with microbiological investigations examining the role of endogenous bacteria in their initiation and progression.[2-3] Recent research has placed a substantial focus on emerging technologies to explore the relationships between oral health status and the complex microbial communities of the human mouth.[1] The purpose of this study was to extend the knowledge of dental plaque microbial communities after routine use of mouthrinses formulated with CHX in comparison to a control mouthwash. Included in the study were well established clinical indices of oral health, microbiological outcomes and an assessment of oral polymorphonuclear neutrophils as a measure of ongoing inflammation.

Assessments of oral health status over the study included commonly utilized clinical indices for dental plaque and gingivitis representing widely referenced determinants of

efficacy[22,23,26] and have a significant history. These indices have contributed to the development, validation and acceptance of current therapies including CHX.[9,10,12] CHX represents a "gold-standard" with a substantial literature and history of dental application.[9,12] Commercially available formulations were utilized in this study and enrolled community dwelling subjects who presented with gingivitis but not undergoing any medical or dental care. The study commenced with a washout phase to provide a period of standardized oral hygiene with a commercially available fluoride toothpaste. This washout phase was designed to reduce the influence of previously utilized oral hygiene formulations in accordance with previous study designs.[21]

Several additional aspects of this study require highlight. Subjects drawn from this study represented adults of either gender from the local area who were not seeking any medical or dental care. Enrolled subjects presented gingival index scores greater than 1.0 with the average scores in the treatment groups at baseline at 1.2 representing an average gingival index values reported in previous population surveys.[27,28] During interviews, the subjects in this study indicated no previous participation in clinical studies or routine dental care and were not asked to change their diet or other practices after study enrollment. The study compared the effects of a CHX mouthrinse to a control formulation with these treatments assigned randomly. Treatments in this study are commercial with ingredients, excipients and flavor components appropriate for routine use. These selections enabled similar product use features by the subjects in treatment groups with product compliance evaluated over the study period. Treatments were randomly assigned and subjects enrolled after they provided voluntary informed consent with the study protocol approved by the hospital ethics committee.

The study incorporated clinical screening by a dentist to establish the oral health status of enrolled subjects. Clinical evaluations by a dentist established oral health status of enrolled subjects with well-established clinical indices recoded as outcome measures and contrast procedures from a recent report.[19] These steps included in the present research allows comparison to clinical results in the published literature.[9,12] Clinical indices for dental plaque and gingivitis scores in the treatment groups were similar at baseline with progressive decreases noted from their corresponding baselines over the study. While the changes in the control group were relatively modest over the study period, the test group assigned CHX registered significant reductions from baseline to all post-treatment examinations. Similar observations were noted in the comparisons between the CHX and control groups at each post-treatment visit with these effects increasing from the first to the second recall visit. Microbiological results enumerating dental plaque bacteria by anaerobic culture indicate effects similar to those observed for the clinical indices. While treatment groups demonstrated no differences at baseline, the CHX group registered progressive reductions in anaerobic organisms over the study period with the lowest number of bacteria recorded at the final visit. Outcomes from this analysis reporting treatment assessments by an established microbiology technique corroborates with the clinical observations and overall study results.

Microbial communities dominate the endogenous organisms in distinct regions of the human mouth. The recognition of the multiple microbial interactions in health has led to a substantial increase in high-throughput analyses of these communities in recent years.[14] Approaches in genome sequencing offer several important advantages by revealing the microbial constituents of the dental plaque ecosystem. An important outcome from this investigation is the overall reduction in microbial constituents by CHX with an effect on the microbial load and a more limited effect on microbial diversity. While some differences in microbial composition were noted in the test group, these differences remained non-significant in comparison to the control. Furthermore, no notable differences were observed in the number of genera between the groups. The phyla with the most changes included *Fusobacteria, Actinobacteria & Spirochaetes.* Differences in relative abundance were observed for several genera including *Streptococcus, Veillonella, Haemophilus, Leptotrichia, Actinomyces, Lautropia, Treponema & Aggregatibacter*. It is plausible that reductions in dominant genera such as Streptococcus and Actinomyces can help to explain the broad reductions in microbial load by the test treatment.

The results reported from this investigation is likely one of the initial studies evaluating the effects of CHX on the dental plaque microbiome in conjunction with established clinical indices in a randomized, double-blind study. Results from this study indicates that twice-daily use of CHX mouthwash demonstrated a significant improvement in clinical measures of oral hygiene reducing both the gingival and dental plaque indices. Concurrently, the CHX treatment also demonstrated significant reductions of dental plaque anaerobic organisms assessed by microbial culture. These outcomes are widely aligned with established efficacy features of CHX reported in the literature. Notable findings from the microbiome investigation include broad reductions in the dental plaque organisms with no changes in the microbial diversity of the samples. These represent critical outcomes with no microbial dysbiosis in the time-frame of the present study. Although not presented, the CHX group also demonstrated reductions in polymorphonuclear leukocytes collected in oral rinse samples in comparison to the control. PMN are critical effector cells and are referred to as first-responders to inflammation.[20] Reductions in oral PMN noted in the CHX group corroborate previously reported findings.[21] In summary, this investigation reports a comprehensive multiplexed assessment of oral hygiene treatment

utilizing clinical, microbiological, microbial community and immunological outcomes.

CONCLUSION

Significant changes in microbial genera including Veillonella, Haemophilus, Treponema and Aggregatibacter were observed after Chlorhexidine treatment and align with the clinical assessments for dental plaque and gingivitis.

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