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Comparison of Effects of Mouthwash Containing Chlorhexidine and Chlorine Dioxide on Salivary Bacteria-A Randomized Control Study

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Authors' contributions

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

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ABSTRACT

Aims: The present study aimed to compare the effectiveness of Chlorhexidine (CHX) and Chlorine dioxide (ClO_2) mouthwashes in reducing the microbial load in saliva.
Place and Duration of Study: Department of Periodontics, Government Dental College and Hospital, Afzalgunj, Hyderabad, between January and March 2020.
Methods and Materials: 60 Patients with gingivitis were included in the study. Ultrasonic scaling was done and saliva samples of the participants were collected and transferred onto blood agar plates. These plates were sent for the microbial count. Later they were given the mouthwash (Chlorhexidine or Chlorine dioxide or Distilled water by random selection) which they used for four weeks. Each group of participants was instructed to rinse
• 10 ml of 0.2% CHX for one minute twice per day. (Group A)
• 10 ml of ClO_2 (Freshclor) for one minute twice per day. (Group B)
• 10 ml of Distilled water for one minute twice per day. (Group C)
After four weeks of usage of prescribed mouthwashes, the participants were recalled and salivary samples were again collected and sent for the microbial count.

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Results: The intergroup comparison of CFU between the groups after four weeks showed significant reduction of CFU Groups A and B when compared to Group C. When compared to Group A (CHX), Group B (ClO_2) witnessed statistically significant reduction of CFU in with a mean difference of 0.26±0.09 ($p<0.001$).
Conclusion: The present study demonstrated that ClO_2 mouth rinse was effective in reducing microbial load after four weeks of usage than CHX.

Keywords: Chlorine dioxide; chlorhexidine; colony forming units (CFUs).

1. INTRODUCTION

Microorganisms found in saliva are derived from various surfaces of the oral cavity including gingival crevices and pockets. Anaerobic bacteria species make up a significant proportion (25-65%) of the subgingival microflora which is involved in the etiology of different forms of periodontal disease [1]. So, saliva which harbors these microorganisms can go about as a delegate example for a general perspective on the oral microbiota.

Microorganisms colonize the oral cavity a couple of hours after birth. Colonization of the gingival crevice happens at first by bacterial collaborations with the tooth and later by interbacterial associations prompting the development of a coordinated harmonious network, called biofilm. Momentum proof shows that gum disease and periodontitis are polymicrobial contaminations brought about by the biofilm-related bacteria [2]. To forestall periodontal sickness, disposal of dental plaque is important by mechanical and chemical techniques. The utilization of antimicrobial oral washes assume a significant part in keeping up oral cleanliness, fundamentally by lessening the number of dental plaque microorganisms. Among the accessible mouthwashes, CHX (Chlorhexidine) is compelling in the decrease of dental plaque and pathogenic microorganisms [3]. CHX connects with outer cell segments and the cytoplasmic membrane, inciting the leakage of intracellular segments. Harm to the external cell layers alone is insufficient to initiate cell death [4]. Though powerful, CHX has certain results like staining of the teeth, oral mucosal disintegration, and unpleasant taste [5]. Therefore research for new and alternative mouthwashes with fewer side effects continues to obtain desirable results. Chlorine dioxide (ClO_2) mouthwash has been tested in recent times. The chlorite anion (ClO_2^-) present in ClO_2 is considered to be bactericidal to microorganisms [6]. The current study proposes that using a ClO2 mouthwash will reduce periodontal bacteria in saliva (in vivo). The goal of this study was to examine the inhibitory effects of a mouthwash containing ClO2 and CHX on salivary bacteria during a four-week period.

2. MATERIALS AND METHODS

The present study was conducted in the department of Periodontics. Prior approval was obtained from the institutional ethical committee. Information regarding the study was explained to the subjects before the sample collection and written informed consent was taken.

The eligible subjects were selected based on the following clinical parameters: 1) Subjects of age group 25-35 years of age, 2) Clinically presenting with bleeding on probing and gingival erythema. 3) Absence of clinical attachment loss.

Exclusion from the study was based on the following criteria: 1) Presence of any systemic illness, 2) Smokers 3) Pregnant and lactating women, 4) Patients undergoing orthodontic treatment. 5) Patients with removable and fixed prosthodontic appliances 5) Patients under antibiotic treatment within the last three months, 6) Subjects allergic to any of the ingredients of mouthwashes used in this study.

The 60 patients were arbitrarily chosen into three groups, Group A (CHX group) and Group B (ClO2 group) and Group C (Distilled water) (Fig. 1). Ultrasonic scaling was performed for all participants before carrying out the study for standardization. Each participant's whole saliva sample was collected. Each participant received a sterile 50-mL wide-mouth test tube and was instructed to collect unstimulated saliva throughout a 20-minute period. The saliva sample, which was at least 10 mL in volume, was immediately chilled and examined the same day. Samples after serial dilution were transferred onto blood agar plates. These plates were sent for the microbial count. Later they were given the mouthwash by random selection which they used for four weeks. Each group of participants was instructed to rinse:

- 10 ml of 0.2% CHX for one minute twice per day. (Group A)
- 10 ml of ClO_2 (Freshclor) for one minute twice per day. (Group B)
- 10 ml of Distilled water for one minute twice per day. (Group C)

After the usage of prescribed mouthwashes, the participants were recalled and salivary samples were collected as described earlier and sent for the microbial count.

Bacterial count (colony forming units [CFUs]) in each sample was determined by culture and microscopy at the Department of Medical Microbiology, Hyderabad. The collected saliva samples were inoculated on agar plates. Inoculated agar plates were incubated at 37°C for 24 hours (Fig. 2). The developed colonies on blood agar were counted against standard inoculum utilized. The Semi-quantitative method was used and the microbiologist was kept oblivious to dodge the bias.

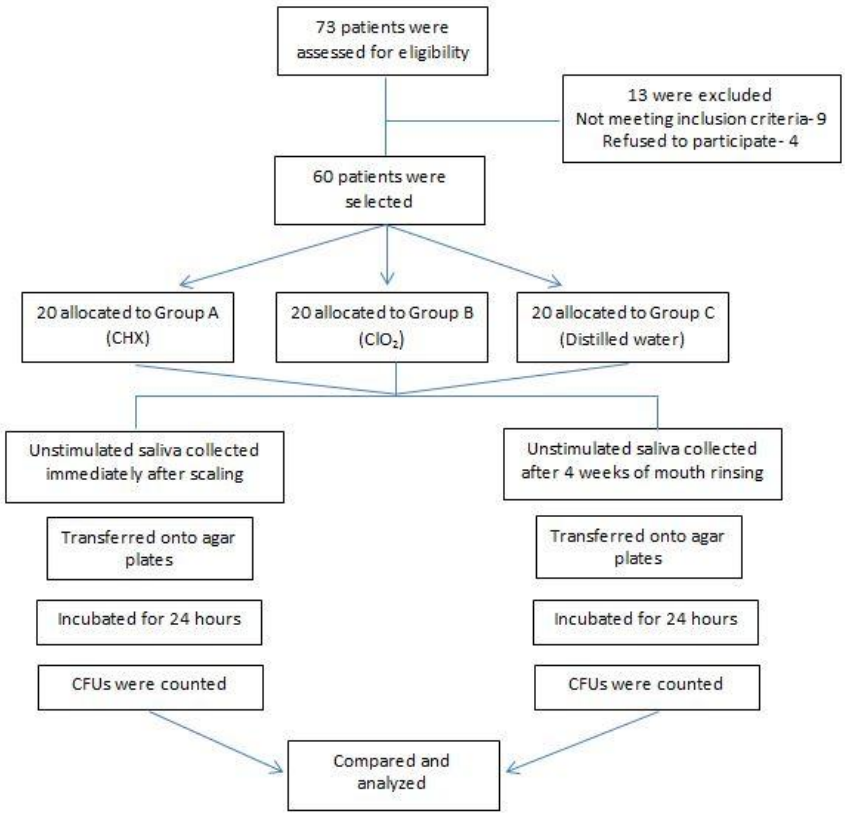

Fig. 1. Study design

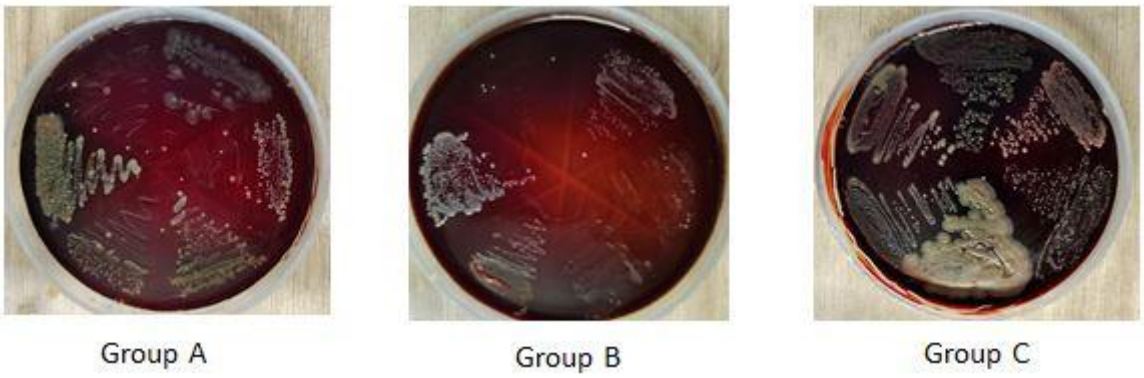

Fig. 2. Comparison of CFUs of three groups after four weeks

3. RESULTS

Table 1 demonstrates the intragroup comparison of CHX group (Group-A) at baseline and four weeks. CHX shows a statistically significant difference with a mean difference of 0.85±0.08 (p< .001). The intragroup comparison of ClO_2 group (Group-B) at baseline and four weeks is presented in Table 2. This group also shows a statistically significant reduction in CFU with a mean difference of 1.05±0.15 (p<.001). Table 3 shows the intragroup comparison of Distilled water group (Group-C) with a mean difference of 0.12±0.01. Wilcoxon Signed Ranks Test was used in all cases. Table 4 witnesses the intergroup comparison of CFU between Group A and Group B after four weeks. Statistically significant reduction of CFU was seen in Group B with a mean difference of 0.26±0.09 (p<.001). Table 5 demonstrates the intergroup comparison of Group A and Group C with a mean difference of 0.88±0.04 and statistically significant P value (p < .001). Table 6 witnesses the intergroup comparison of CFU between Group B and Group C with a mean difference of 1.14±0.13 (p<.001).

Table 1. Intra-group comparison of Group A

Group A (Chlorhexidine)							
Variables	**Min**	**Max**	**Mean**	**SD**	**Difference Mean±SD**	**% of Mean change**	**P value**
CFU/ml immediately after scaling	1.10	1.90	1.53	0.28	0.85±0.08	-55.74	<0.001 Significant
CFU/ml four weeks after mouthwash	0.30	0.90	0.68	0.21			

CFU- Colony Forming Units Min- Minimum Max- Maximum SD- Standard Deviation

Statistical Analysis: Wilcoxon Signed Ranks test. Statistically significant if P<0.05

Table 2. Intra-group comparison of Group B

Group B (Chlorinedioxide)							
Variables	**Min**	**Max**	**Mean**	**SD**	**Difference Mean±SD**	**% of Mean change**	**P value**
CFU/ml immediately after scaling	1.10	1.90	1.47	0.27	1.05±0.15	-71.33	<0.001 Significant
CFU/ml four weeks after mouthwash	0.20	0.60	0.42	0.12			

CFU- Colony Forming Units Min- Minimum Max- Maximum SD- Standard Deviation

Statistical Analysis: Wilcoxon Signed Ranks test. Statistically significant if P<0.05

Table 3. Intra-group comparison of Group C

Group C (Distilled water)							
Variables	**Min**	**Max**	**Mean**	**SD**	**Difference Mean±SD**	**% of Mean change**	**P value**
CFU/ml immediately after scaling	1.10	1.90	1.44	0.26	0.12±0.01	8.33	<0.05 Significant
CFU/ml four weeks after mouthwash	1.10	2.00	1.56	0.25			

CFU- Colony Forming Units Min- Minimum Max- Maximum SD- Standard Deviation

Statistical Analysis: Wilcoxon Signed Ranks test. Statistically significant if P<0.05

Table 4. Inter-group comparison between Group A and Group B

GROUP A Vs Group B						
CFU/ml four weeks after mouthwash	Min	Max	Mean	SD	Difference Mean±SD	P value
Group A	0.30	0.90	0.68	0.21	0.26±0.09	<0.001
Group B	0.20	0.60	0.42	0.12		Significant

CFU- Colony Forming Units Min- Minimum Max- Maximum SD- Standard Deviation

Statistical Analysis: Mann-Whitney U test. Statistically significant if P<0.05

Table 5. Inter-group comparison between Group A and Group C

GROUP A Vs Group C						
CFU/ml four weeks after mouthwash	Min	Max	Mean	SD	Difference Mean±SD	P value
Group A	0.30	0.90	0.68	0.21	0.88±0.04	<0.001
Group C	1.10	2.00	1.56	0.25		Significant

CFU- Colony Forming Units Min- Minimum Max- Maximum SD- Standard Deviation

Statistical Analysis: Mann-Whitney U test. Statistically significant if P<0.05

Table 6. Inter-group comparison between Group B and Group C

GROUP B Vs Group C						
CFU/ml four weeks after mouthwash	Min	Max	Mean	SD	Difference Mean±SD	P value
Group B	0.20	0.60	0.42	0.12	1.14±0.13	<0.001
Group C	1.10	2.00	1.56	0.25		Significant

CFU- Colony Forming Units Min- Minimum Max- Maximum SD- Standard Deviation

Statistical Analysis. Mann-Whitney U test. Statistically significant if P<0.05

4. DISCUSSION

Plaque is a biofilm that grows on oral surfaces and is constantly bathed by saliva and contains layers of microorganisms encased in a matrix [7]. Antimicrobial oral rinses aids in maintaining oral hygiene because they reduce the microbial load of dental plaque. Chlorhexidine (CHX) appears to be the most effective chemical agent in both short- and long-term use [8]. Although CHX has low toxicity after oral administration, it is not spared from side effects [9]. Several disadvantages, such as an unpleasant taste, tooth discoloration, burning sensation, soreness were reported which limit its long-term use and urge the adoption of alternatives. Attributing to the persistently proved efficacy of CHX, other chemical agents should be assessed for their potency as an alternative. Hence, the present study was conducted to evaluate the efficacy of ClO2 compared to CHX in reducing salivary bacteria. The results of this study show that rinsing with a mouthwash containing ClO2, over a four-week time frame, was viable in diminishing CFU in saliva samples when contrasted with CHX mouth rinse. Chlorhexidine mouth rinse was used twice daily in the study since its persistence in the oral cavity and its ability to decrease bacterial count lasts for 12 hours [10]. There was a significant reduction in CFU after four weeks ($p< 0.001$). This was as per the investigation led by Herrera et al [11].

Chlorine dioxide has been widely used in various fields because of its strong antibacterial properties [12]. Research also shows that it is a proven bactericidal agent against bacterial pathogens causing periodontitis such as *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, and Prevotella intermedia* [13]. The fundamental favorable circumstances of ClO2 are, it is non-staining, alcohol-free, and non-irritating and it does not cause taste alteration [14]. After using ClO_2 mouth rinse the microbial load (CFU) significantly reduced from baseline. ($p<0.001$). This was in accordance with the study by Shinada et al who suggested that rinsing with a 0.1% chlorine dioxide mouth rinse effectively reduces the number of Gram-positive and Gram-negative anaerobic bacteria in the oral cavity [12]. This was also supported by another study which states that chlorine dioxide mouth rinse can kill up to 90% of oral pathogens in <30 min [15]. The intergroup comparison showed that ClO_2 had a greater reduction in CFU count than CHX after four weeks of usage. Chlorine dioxide infiltrates the bacterial cell wall and binds to the imperative amino acids (cysteine, methionine, tyrosine, and tryptophan) that are fundamental for microorganisms in the cell wall and bacterial cytoplasm [16, 17]. It destabilizes the permeability of the cell membrane and the cell wall ruptures [18]. The proliferation of anaerobic bacteria through oxygenation is also limited by chlorine dioxide [11]. ClO_2 is not carcinogenic or allergenic as it does not form chlorinated hydrocarbons with organic compounds. Invitro studies also show that ClO_2 is less toxic to gingival cells than CHX [19]. All these properties

of ClO_2 might provide an additional benefit to the participants when compared to CHX. Based on these findings, ClO2 mouth rinse can be considered a viable alternative to CHX due to the drawbacks of the latter.

5. CONCLUSION

The present study demonstrated that ClO_2 mouth rinse was effective in reducing microbial load after four weeks of usage than CHX. However further investigations with a huge sample size should be led to affirm the drawn-out impacts of ClO2 mouthwash.

CONSENT

Informed consent was obtained from participants after explaining the study.

ETHICAL APPROVAL

As per university standard guideline, ethical approval have been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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