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ORIGINAL ARTICLE

Efficacy of ozonated water over chlorhexidine mouth rinse in chronic gingivitis patients – A comparative clinical study

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KEYWORDS

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Abstract *Aim:* This study aimed to compare the efficacy of ozonated water mouth rinse over chlorhexidine mouth rinse against the accumulation of plaque in chronic gingivitis patients.

Materials and methods: A total of 30 patients with chronic generalized gingivitis were choosen for the study. The duration of this study was 15 days and was divided into three groups. Group 1 patients were advised to rinse with ozonated water¸ group 2 with a chlorhexidine mouth rinse and group 3 with water for 15 days. The clinical parameters like plaque index, gingival index, and bleeding index were recorded at baseline, 7th, and 15th day respectively. Saliva samples were collected for microbial culture at baseline that is before mouth rinse and on the 15th day that is after using mouth rinse from each patient.

Results: The statistical analysis was performed by using ANOVA and Bonferroni's Post Hoc test on 30 patients at the end of the 15thday. There was a significant decrease in mean plaque, gingival, and bleeding scores in group 2 patients that are chlorhexidine mouth rinse. There was also a decrease in the microbial culture at the end of the 15th day in patients with chlorhexidine mouth rinse compared to ozonated water.

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Conclusion: The results of this study demonstrate that ozonated water was less effective compared to chlorhexidine mouth rinse in reducing plaque in gingivitis patients. But ozonated water can be used as an alternative to CHX.

1. Introduction

Dental plaque is a living organized community of microorganisms that are a main etiological factor for gingival and periodontal diseases. Plaque control is a critical component in the long-term success of periodontal health. A large variety of chemical agents in the form of mouthwashes have been tested for their ability to reduce plaque accumulation (Marcotte and Lavoie, 1998).

Several mechanical aids and local and systemic antimicrobials play a principal role in plaque control and further prevent periodontal diseases. The primary concern of periodontal therapy is the resolution of soft tissue inflammation by reducing the microbial load and restoration of lost alveolar support. Mouth rinses have been popularly used as a supplementary oral hygiene aid (Krayer et al., 2010). Among them, bisbiguanide chlorhexidine has been studied extensively for over 20 years and is currently the most potent chemotherapeutic and antiplaque agent. CHX is bacteriostatic at low concentrations and bactericidal at high concentrations. It has a broad antimicrobial spectrum thus making it effective against many oral bacterial species. But the long-term use of CHX has a few disadvantages such as teeth staining, altered taste sensation, supragingival calculus formation, and desquamation of oral mucosa which restricted its usage in the pediatric age group (Anumula et al., 2017).

One such effective and safe alternative to CHX mouthwash is the use of ozone water. In various kinds of literature, ozone and its uses have been discussed in dentistry as a possible alternative antiseptic agent. It is a powerful non-antibiotic biocide that is effective as a gas and can be dissolved in water. Its antimicrobial spectrum includes a wide variety of bacteria, viruses, and fungi. Its mechanism of action is by inducing oxidation of the cell wall and cytoplasmic membranes that leads to the lysis of the microorganism. Even though ozonated water is a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses, less attention has been paid to its antimicrobial activity in bacterial biofilms (Gupta and Mansi, 2012).

To know the potency of Ozone as an effective antimicrobial agent against the microbial complexes in oral biofilm, a study was conducted in our department.

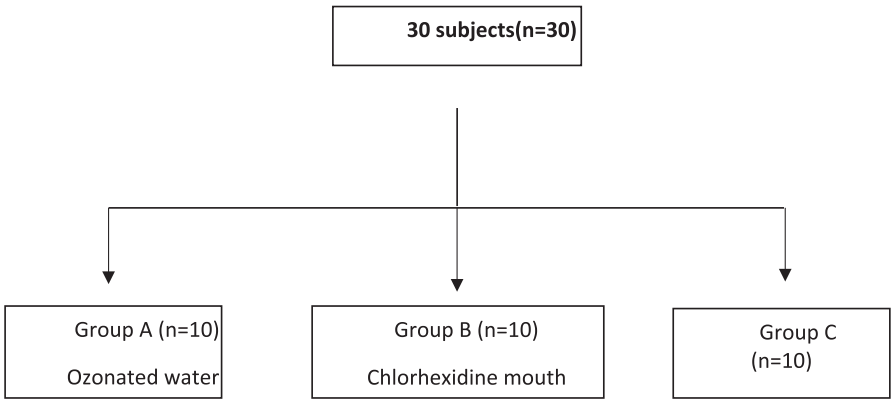
This present study aimed to compare and evaluate the antimicrobial effect of ozonated water and chlorhexidine mouth rinse against the accumulation of plaque in chronic gingivitis patients.

2. Materials & method

After taking the ethical approval form the institutional review board GDCH-IEC/PG/1940 the current randomized controlled trial was conducted in the Department of Periodontics, Govt dental college, Hyderabad.

2.1. Sample size

A study population including 30 students with chronic generalized gingivitis was selected. The duration of this study was 15 days and was divided into three groups. Group A patients were advised to rinse their mouth with ozonated water, group B with a chlorhexidine mouth rinse and group C with water. For the next 15 days of the study, the subjects were instructed to rinse their mouth with 10 ml of mouthwash twice a day (after breakfast and after dinner) for 1 min. The subjects were told to refrain from all other oral hygiene measures such as dental floss, chewing gum except the directed mouth rinses, and routine tooth brushing habits and to avoid eating or drinking for at least 30 min after rinsing the mouth. The clinical parameters like plaque index, gingival index, and bleeding index were recorded at baseline, 7th, and 15th day respectively. Saliva samples were collected for microbial culture at baseline that is before mouth rinse and on the 15th day that is after using mouth rinse from each patient. The culture media used for this study is blood agar which is cost-effective and most used for culturing bacterial colonies in laboratories.



2.2. Inclusion criteria

- Dental students (females) aged 19–22 years
- Students with mild gingivitis
- DMFT/deft score ≤3

2.3. Exclusion criteria

- Students with systemic diseases
- Students with chronic periodontitis
- Students with history of antibiotics usage in past 1 month
- Students undergoing orthodontic treatment

3. Results

A total of thirty subjects participated in the study, with 10 subjects in each group. All the subjects turned up for follow-up with no attrition. All the subjects included in the study were females with a mean age of 19–22 years.

A Repeated measures ANOVA and Bonferroni Post Hoc test for multiple comparisons of plaque (Table 1) and gingival scores (Table 1) showed a statistically significant difference ($P < 0.05$) between three different intervals of time.

3.1. Plaque Index (PI) (Löe, 1967)

All the groups including the water group showed a reduction in plaque index score after 15 days. These values slightly increased after follow-up for another 15 days without using mouth rinses but were significantly lesser than the baseline scores. All groups showed maximum reduction in plaque score during the use of mouth rinses. From this study, it was clear that even normal rinsing with water can remove plaque. Ozonated water was comparatively better in reducing plaque after 15 days of usage of mouth rinses (Figs. 1 and 2).

3.2. Gingival index (GI) (Löe, 1967)

Chlorhexidine mouth rinse and ozonated water group showed a decrease in GI compared to water. Ozonated water was equally effective as CHX in reducing gingival index after 15 days of usage of mouth rinses (Figs. 1 and 2).

3.3. Bleeding Index (BI) (Ainamo and Bay, 1975)

Similar to GI, CHX and ozonated water showed a reduction in the bleeding index compared to water. Ozonated water was equally effective as CHX in reducing bleeding index after 15 days of usage of mouth rinses (Figs. 1 and 2).

3.4. Total microbial colony count

There was a reduction in microbial colony count in the two groups other than the water group after the usage of mouthrinses for 15 days (Table 2). CHX indirectly affects the enzymatic function of dehydrogenase and adenosine triphosphatase present in the cell wall of bacteria resulting in the disruption of the cell membrane (Mon et al., 2019). The oxidation potential of ozonated water destructs the cell wall and cytoplasmic membranes of bacteria and fungi. Ozone, when dissociated into oxygen, creates an oxygen-rich environment, thus disturbing the normal ecosystem of the plaque. The enzymatic control system of the cell is blocked as ozone inhibits glycoproteins, glycolipids, and other amino acids the outcome of which is the functional cessation and death of the microorganism (Anumula et al., 2017). Ozonated water has also been proposed to promote remineralization of incipient carious lesions by causing decarboxylation of the pyruvic acid produced by acidogenic bacteria to acetic acid during cariogenesis (Ainamo and Bay, 1975). Though CHX was found to be superior to ozonated water one cannot rule out that it has shortcomings such as mucosal desquamation, impaired wound healing, and fibroblast attachment to the tooth surfaces (Anumula et al., 2017).

4. Discussion

Oral health is an integral part of general health and a proper oral hygiene routine helps in maintaining a healthy oral environment. Poor oral hygiene practices can lead to plaque accumulation and calculus formation. Dental plaque has been proven to be a significant factor in the initiation and progression of gingival and oral diseases (Nazir, 2017). Hence, dentists often recommend chemical adjuncts in addition to tooth brushing and flossing for routine home care. Mouth rinses can deliver therapeutic ingredients and benefits to all accessible surfaces in the mouth including interproximal surfaces. They

Table 1 Multiple comparisons for mean plaque scores between groups.

Groups	PI			GI		
	Mean difference	P value	95 % Confidence Interval	Mean difference	P value	95 % Confidence Interval
Ozonated water versus chlorhexidine	0.06	1.000 NS	−0.144 to 0.250	0.12	0.006 S	0.0375–0.207
Ozonated water versus water	0.10	1.000 NS	−0.376 to 0.184	−0.05	0.606 NS	−0.169 to 0.0610
Chlorhexidine versus water	−0.16	0.020 S	−0.275 to −0.0234	−0.17	0.008 S	−0.302 to −0.0496

Statistical Analysis: Repeated measures ANOVA and Bonferroni's Post Hoc test for multiple comparisons. Statistically significant if $P < 0.05$.

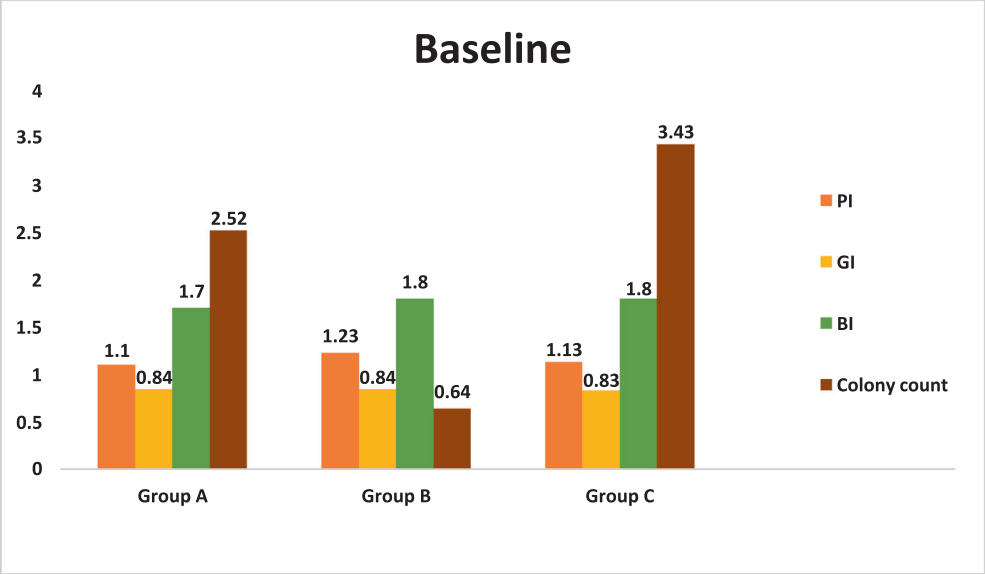


Fig. 1 Plaque index, gingival index, Bleeding index and colony count scores at baseline.

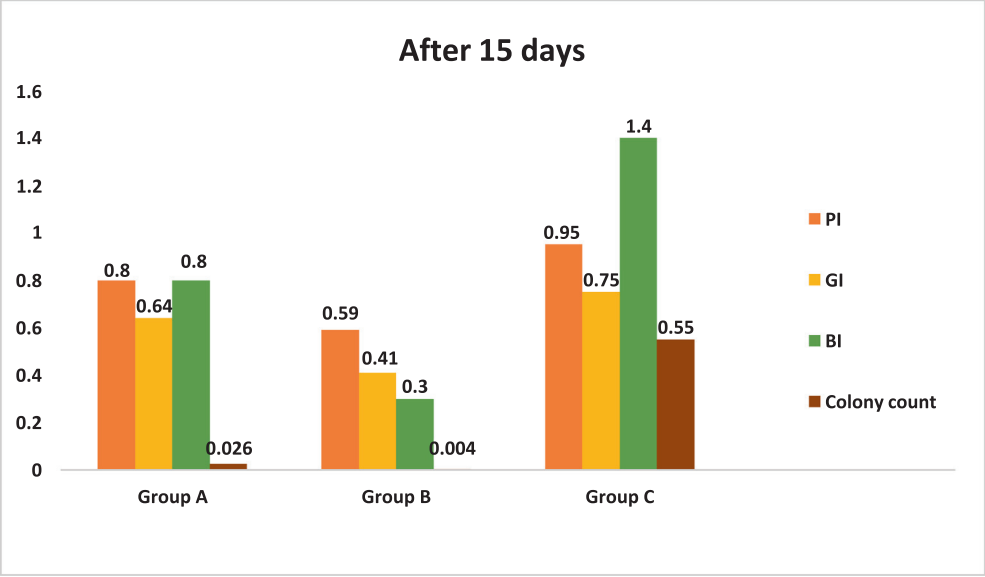


Fig. 2 Plaque index, gingival index, Bleeding index and colony count scores after 15 days of treatment.

Table 2 Multiple comparisons for mean Colonies count between groups.

Groups	Mean difference	P value	95 % Confidence Interval
Ozonated water versus chlorhexidine	0.951	0.516 NS	−0.9302 to 2.833
Ozonated water versus water	−0.717	1.000 NS	−3.3971 to 1.964
Chlorhexidine versus water	−1.668	0.202 NS	−4.022 to to 0.686

Statistical Analysis: Repeated measures ANOVA and Bonferroni's Post Hoc test for multiple comparisons. Statistically significant if $P < 0.05$.

also remain effective for an extended period depending on their substantivity (Kour et al., 2019). Ozonated water is cost-effective as the ozone gas is a one-time investment in buying the ozone generator. Purifying water with a lower power generator at cheaper rates is also available in the market. The participants who used ozonated water were more willing to use it as its appearance did not differ much from the normal water other than in slight odor and taste (Azarpazhooh and Limeback, 2008). The only shortcoming is that the O3 molecule is unstable and ozonated water should be prepared immediately before its use. Its oxidizing property gradually decreases with time (Anumula et al., 2017).

Commercially available mouthwashes containing synthetic and/or semi-synthetic active agents have several disadvantages like staining on the teeth, more alcohol content, and irritation

during their use. One such example is CHX which is to date considered to be the most effective anti-plaque agent, but with certain limitations (Kaur et al., 2014).

Ozonated water could be a good alternative as a mouth rinse which is very effective in inhibiting bacterial growth in biofilms. Mere thirty seconds rinse of ozonated water can reduce the dental plaque bacterial load to almost half. Its biocompatibility with oral epithelial cells has also been established. The cost-effectiveness and ease of preparation of ozonated water make it a potential adjunct to tooth brushing and flossing. A higher significance (p-value < 0.001) was seen in the ozonated water group than in the CHX group indicating a better antibacterial property. (Anumula et al., 2017).

Hence, this study aimed to evaluate the antibacterial effect of ozonated water and chlorhexidine mouth rinse against the accumulation of plaque in gingivitis patients. The study was a randomized controlled trial conducted over 15 days.

Ozone is currently being discussed in dentistry as a possible alternative antiseptic agent. Both gaseous, as well as aqueous forms of ozone, are equally effective as antimicrobial agents against oral pathogens (Lubojanski et al., 2021). It was first suggested as a disinfectant for drinking water in terms of its powerful ability to inactivate microorganisms (against bacteria, fungi, protozoa, and viruses). In our study, we used aqueous ozone which was freshly prepared each day by ozonation of water for 20 min by using an ozone generator (Martinelli et al., 2017).

Ozonated water is effective in killing gram-positive, gram-negative bacteria and oral Candida albicans causing periodontal disease. The aqueous form of ozone, as a potential antiseptic agent, showed less toxicity than volatilized gas or established antimicrobials like chlorhexidine digluconate, sodium hypochlorite, or hydrogen peroxide under most conditions. Therefore, aqueous ozone fulfills optimal cell biological characteristics in terms of biocompatibility for oral application (Tiwari et al., 2017).

Summary: The present randomized controlled trial was conducted at the Department of Periodontology and Implantology, Government dental college and hospital, Hyderabad. The study was conducted among undergraduates in GDCH, Hyderabad to determine plaque index, gingival index, bleeding index, and microbial colony count. Thirty students aged 19–22 years with DMFT/dmft ≤ 3 were included in the study. They were randomly divided into 3 equal groups ($n = 10$): chlorhexidine mouth rinse, ozonated water, and water. They were instructed to use their respective mouthrinses for 15 days. PI, GI, BI, and microbial colony count were estimated at baseline, after 7 days, and after 15 days. Saliva samples were used to evaluate CFU counts that were seen in blood agar media. Results were tabulated and analyzed statistically.

5. Conclusion

Chlorhexidine mouth rinse and ozonated water showed a decrease in PI, GI, BI, and microbial colony count after 7 days and 15 days. Ozonated water showed maximum reduction in overall PI, GI, and BI score after 15 days but it is less effective compared to chlorhexidine in reducing plaque and gingivitis. From this study, ozonated water may also be used as an alternative to CHX.

Limitations of the study:

- There is a need to study the long-term effects of the mouth rinses on oral health.
- The role of these mouth rinses needs to be assessed with emphasis on individuals with poor oral hygiene.
- Further studies are required to find the optimum ozonation time and concentration as required for mouthrinse (Parkar et al., 2017).

Ethical statement

This material is the authors' own original work, which has not been previously published elsewhere. All sources used are properly disclosed (correct citation). Since this is a review an Ethical Approval was not obtained.

Conflict of Interest Statement

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Roja Reddy Talasani: Conceptualization. **Santhi Priya Potharaju:** Conceptualization, Formal analysis, Writing – original draft. **B. Vijaya Lakshmi:** Data curation, Formal analysis, Writing – review & editing. **Y. Durga Bai:** Data curation, Formal analysis, Resources, Writing – review & editing. **Ravi Kanth Chintala:** Formal analysis. **Pradeep koppolu:** Conceptualization, Software, Validation. **Abdul Rahman Saeed AlGhamdi:** Formal analysis, Funding acquisition.

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