Original Research

Comparison of the Antimicrobial Effect of Hydrogen Water and Chlorhexidine Mouth rinse in Toothbrush Disinfection Among Dental Students

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Submitted: 25-Oct-2024
Revised: 18-Nov-2024
Accepted: 18-Nov-2024
Published: 28-Feb-2025

ABSTRACT

Background: Toothbrushes are essential for eliminating dental biofilm and preventing caries and periodontal disease. Regular disinfecting is necessary to maintain a clean toothbrush. Chlorhexidine is the gold standard, but it may be resistant to periodontal pathogens. Hydrogen-rich water, due to its antioxidant properties, may be beneficial against periodontal infections. **Method:** A study comparing hydrogen water and chlorhexidine as toothbrush disinfectants was conducted. Thirty dental students were divided into two groups: A and B. Group A students used hydrogen water and Group B used chlorhexidine mouth rinse as a disinfecting solution. They were instructed to brush twice a day for seven days and disinfect their toothbrushes with the disinfection solution for 10 minutes. Bristles from the collected toothbrush were sectioned and incubated for 24 hrs and colony-forming units (CFU) were counted. **Result:** The results showed that hydrogen water had significantly less colony-forming units in brushes disinfected with hydrogen water compared to chlorhexidine. **Conclusion:** Hydrogen water has better antimicrobial properties than chlorhexidine mouthwash. Therefore, hydrogen water is recommended as a regular disinfectant for toothbrushes.

Keywords: *Chlorhexidine, disinfection, hydrogen water, toothbrush*

Introduction

Toothbrushes can prevent dental caries and periodontal disease by removing plaque biofilm.[1] However, they can be contaminated by enteric bacteria, such as *Lactobacilli*, *Enterococcus*, *Streptococcus*, and *Staphylococcus*, which can survive on used brushes for up to a week.[2] Regular use of the same container among family members can also spread pathogens. Therefore, minimizing toothbrush contamination may be a useful strategy to prevent diseases and transmission of infections.[3] Chlorhexidine is considered the gold standard, but periodontal pathogens are resistant.[4] Hydrogen water has shown antibacterial activity against *Actinobacillus actinomycetemcomitans*, *P.gingivalis*, and *P.intermedia*, making it a more suitable alternative.[5] This study evaluates the effectiveness of hydrogen water as a toothbrush disinfectant and compares its results with the industry-standard, chlorhexidine solution.

Materials and Methods

A clinical comparative study evaluating hydrogen water and chlorhexidine solution for toothbrush disinfection was conducted on 30 dental students at a dental college for women. The study, in compliance with the 1975 Declaration of Helsinki, involved hostel residents, aged 18–25, with at least 24 caries-free teeth. Participants were excluded from having any oral or

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How to cite this article: Vaishnavi C, Elangovan GP, Thirumal M, Prashanth SV, Deepika D, Pragathi TG. Comparison of the antimicrobial effect of hydrogen water and chlorhexidine mouth rinse in toothbrush disinfection among dental students. J Pharm Bioall Sci 2025;17:S528-30.

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jpbs

DOI: 10.4103/jpbs.jpbs_1565_24

systemic diseases, taking medications, using antibiotics or antiseptic mouthwashes, undergoing orthodontic treatment, having an intraoral prosthesis, or using herbal or chemical plaque control agents as mouthwash.

Thirty dental students were randomly divided into two groups: Group A and Group B. Each group received a new toothbrush and a disinfecting solution. Group A received hydrogen water daily, prepared using commercially available bottles with electrodes. Group B received a chlorhexidine mouth rinse. For seven days, participants were instructed to brush twice a day and disinfect their toothbrushes after each use. They were also instructed to store their toothbrushes in a container after cleaning.

Following a seven-day period, all toothbrushes were collected and transferred them to a laboratory for microbial culture and colony counting units assessment. Surgical spirit was used to disinfect the handles. Two-thirds of the bristles were submerged in a saline solution, and following a serial dilution of 10^{-8}, then samples were distributed on a nutritive agar plate. The plates were incubated at 37 degrees Celsius for 24 hours, and colony-forming units (CFU) were counted using a digital colony counter.

Statistical analysis

The data were analyzed using SPSS version 26, revealing significant variation in samples. The data were converted into a log form and analyzed using Student's *t*-test to compare colony-forming units in hydrogen water and chlorhexidine groups. A *P* value of less than 0.05 was considered statistically significant.

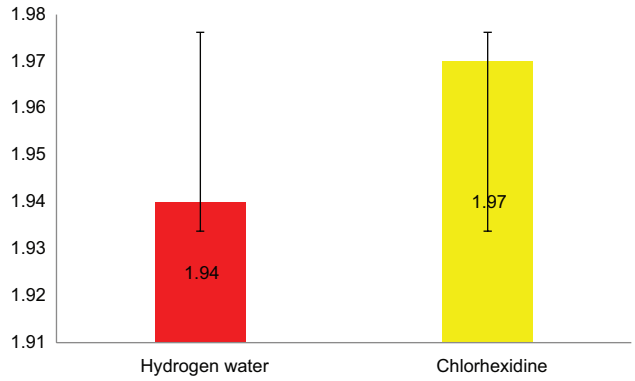
Result

The study analyzed toothbrushes from both groups for microbiological analysis, determining the number of colony-forming units (CFUs). The results showed significant differences between the groups, with hydrogen water having a lower number of CFUs than chlorhexidine [Table 1]. Graph 1 shows the mean CFUs/ml differences between the two groups. Group A showed superior disinfection properties compared to Group B.

Table 1: Mean number of colony-forming units/ml between the two groups

Group	*n*	Mean	Std. deviation	Std. error mean	*P*
CFU					
Hydrogen water	15	89.2000	7.15342	1.84701	0.029*
Chlorhexidine	15	95.5333	7.94505	2.05140	
TRCFU					
Hydrogen water	15	1.9491	.03475	0.00897	0.032*
Chlorhexidine	15	1.9787	.03720	0.00960	

**P* value is statistically significant



Graph 1: The graphical representation of mean colony-forming units/ml in each group

Discussion

Toothbrushes are a crucial mechanical plaque control device, but contaminated ones can cause recurrent oral infections.[6] The ADA recommends changing toothbrushes every three months for healthy individuals and every three days for chemotherapy patients.[7,8] Regular tap water rinses can lead to contamination and biofilm development. Chlorhexidine is recommended as the gold standard disinfection agent, but some periodontal pathogens are resistant to it.[9] Disinfection techniques must be simple, economical, nontoxic, and effective. Most suggested methods, such as tetrasodium EDTA, UV sanitization, and chlorhexidine gluconate, are expensive and difficult to use.[10] Therefore, hydrogen water is a natural agent with antimicrobial action against periodontal pathogens that can be used regularly to clean toothbrushes.

The study used hydrogen water and chlorhexidine to disinfect toothbrushes due to their antibacterial properties. The participants, all dental interns, were familiar with proper toothbrushing and storage protocols. Since all were residents from the college hostel, it was easier for the examiner to provide freshly prepared hydrogen water every day and make sure the toothbrush was properly stored and disinfected.

The seven-day toothbrush collecting period is comparable to previous research by Grewal and Swaranjit.[11] Chlorhexidine was discovered by Komiyama *et al*. and Hamal *et al*.,[12,13] to be a more efficient toothbrush disinfection. However, this study found hydrogen water to be more effective in disinfecting toothbrushes. This suggests that hydrogen water can be used regularly as a disinfection solution and is an ideal substitute for chlorhexidine in cleaning toothbrushes.

Hydrogen water, produced when an electric current passes through the water between metal electrodes, minimizes oxidative stress and improves antibacterial activity.[14] It impacts oral hygiene by bactericidal action, biofilm removal, and preventing bacterial development. Hydrogen water has antibacterial properties against oral microorganisms linked to oral disorders, such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Streptococcus mutans*.[15] It is a rapidly effective, cost-effective, and nontoxic agent for disinfecting toothbrushes.

Conclusion

It can be concluded that hydrogen water is more effective than chlorhexidine for dental brush disinfection, suggesting its regular use. The public should adopt proper storage procedures and daily brush disinfection practices to prevent further disease spread.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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