Assessment of antibacterial effect of hydrogen water on plaque from patients with chronic periodontitis

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INTRODUCTION

Dental plaque is a highly complex biofilm comprising various species and strains of microbiota, adhering to tooth surfaces, other oral surfaces, and restorations in the oral cavity. Plaque associated with a tooth surface is characterized by Gram-positive rods and cocci including Streptococcus mitis, Streptococcus sanguis, Actinomyces viscosus, Actinomyces naeslundii, and Eubacterium species. Tissue-associated plaque shows a predominance of Streptococcus oralis, Streptococcus intermedius, Peptostreptococcus micros, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Fusobacterium nucleatum.

Periodontitis is an inflammatory disease of the supporting tissues of teeth initiated by microorganisms, resulting in progressive destruction of the tissues surrounding a tooth, leading to the eventual loss of the tooth. Hence, overall reduction of the bacteria present in the dental plaque may help prevent periodontal disease and limit its progression.

Various agents are used to combat the microbial load in the oral cavity. The use of chemical agents for this purpose is proven to be effective, but the duration of action of these agents is short lived and they cannot be consumed lifelong. Therefore, the use of natural agents, which can be consumed on a daily basis and also have antibacterial effects on oral bacteria, is desirable. One such agent is “hydrogen water.”

Hydrogen water has shown antibacterial activities on standard strains of Actinobacillus actinomycetemcomitans, F. nucleatum, P. gingivalis, P. intermedia, and Treponema denticola. It has also been proven to be an antifungal agent by showing its activity against Candida albicans biofilm.

How to cite this article: Nayak A, Bhatt A, Bhat K, Nayak R, Hooli A, Naik S. Assessment of antibacterial effect of hydrogen water on plaque from patients with chronic periodontitis. J Indian Soc Periodontol 2021;25:193-6.
Studies conducted have confirmed its efficacy in suppressing the progression of periodontitis by reducing gingival oxidative stress, due to its antioxidative activity.6

Taking into account the above applications and advantages of hydrogen water, we conducted a study on plaque samples obtained from patients with chronic periodontitis. The bacteria present in the plaque were exposed to hydrogen water at various intervals of time, and the antibacterial effect of hydrogen water was assessed by direct contact test.

**MATERIALS AND METHODS**

**Production of hydrogen water**

Generation of hydrogen water was done using a commercially available hydrogen water bottle. The base of the bottle has electrodes, which helps in the production of hydrogen water. Distilled water was infused in the hydrogen water bottle. The process of electrolysis is initiated in 3 s after the power of the bottle is in an ON mode. The electrolysis of water generated hydrogen (H₂) at the cathode, thus producing hydrogen water in the bottle at the end of 5 min.

**Sample collection and procedure**

Subjects for the study were selected from patients visiting the Department of Periodontology, in the Institute. Plaque samples were collected from 20 patients with chronic periodontitis aged 30–50 years after obtaining approval from the institutional ethical committee. Written informed consent was obtained from all the 20 participants. Patients with the presence of bleeding on probing, probing depth (PD) ≥3 mm, or clinical attachment loss (CAL) ≥5 mm were included in the study. Pregnant and lactating women, patients who received any medications 3 months before sampling, patients with cancerous or precancerous lesion, patients who underwent periodontal therapy in the last 6 months, patients with medically compromised conditions, and patients using mouth rinses and tobacco habits were excluded from the study. The clinical examination included Löe and Silness gingival index, PD, and CAL using the Williams probe.

The plaque samples were collected in reduced transport fluid (RTF) and transported to the laboratory for analysis. It was vortexed for 30 s to allow equal dispersion of the plaque in the RTF.

A pilot study was conducted earlier to standardize the dilution of the plaque sample, and it was finalized that the plaque sample would be diluted with thiglycollate broth with hemin and Vitamin K in the ratio of 1:25.

The diluted sample was then centrifuged at 1000 rpm for 2 min. Postcentrifugation, the supernatant was discarded. For the growth of bacteria, blood agar media with hemin and Vitamin K was used. The culture plate was divided into four quadrants. The diluted sample was inoculated in one quadrant to facilitate readings at baseline (negative control) in two different culture plates of blood agar using streak method of culturing. 1 ml of hydrogen water was then added to the pellet, and further, inoculation was done at 1, 2.5, and 5 min, respectively, in the culture plates.

One plate with the above culture was studied under aerobic conditions, which is in 5% CO₂ and incubated for 24 h at 37°C in an incubator. The second plate was placed in an anaerobic jar with modified gas pack and incubated for 72 h at 37°C. After the respective incubation time period, the colony forming units and total aerobic and anaerobic bacterial count of both the plates were recorded using a digital colony counter.

The same procedure and interpretation were followed for all the 20 samples with hydrogen water (test group) and chlorhexidine (positive control).

**Statistical procedure**

Data collected were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS v 21.0, IBM, Armonk, NY). Normality of numerical data was checked using Shapiro–Wilk test. It was observed that the data did not follow a normal curve; hence, nonparametric tests have been used for comparisons. Intragroup comparison was done by Friedman’s test while pair-wise comparison was done using Wilcoxon signed-rank test. A P < 0.05 was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

**RESULTS**

The effect of hydrogen water on the microbial count in the plaque obtained from patients with chronic periodontitis is presented in the following results.

Intragroup comparison was done using Friedman’s test, for both aerobic and anaerobic cultures [Tables 1 and 2, respectively]. A statistically significant difference (P < 0.01) was seen for the values between the time intervals. This was followed by pair-wise comparison to determine the antibacterial activity of hydrogen water at exposure of 1, 2.5, and 5 min on the bacteria present in the plaque in patients with chronic periodontitis.

Table 3 shows the pair-wise comparison of the hydrogen water group using Wilcoxon sign-ranked test for aerobic culture. There was a statistically significant difference (P < 0.01) for the values between the time intervals baseline–1 min, baseline–2.5 min, and 1–2.5 min, indicating that there were a lesser number of colony forming units at 1 and 2.5 min when compared to baseline and at 2.5 min when compared 1 min. However, statistically insignificant difference was seen for the values between baseline–5 min (P = 0.067) and 1–5 min (P = 0.756).

Pair-wise comparison test of the hydrogen water group for anaerobic culture is depicted in Table 4. A statistically significant difference (P < 0.01) was seen, suggesting that the number of colony forming units was reduced at 1, 2.5, and 5 min when compared to baseline. However, there was no statistically significant difference seen for the values between the time intervals 1–2.5 min (P = 0.179), 1–5 min (P = 0.327), and 2.5–5 min (P = 0.231).

The above results indicate that hydrogen water does act as an antibacterial agent against pathogens associated with chronic periodontitis.
**DISCUSSION**

Mechanical plaque control is an effective strategy for preventing the progression of oral diseases. A daily use of an effective mouth rinse as well as can be considered as an adjuvant strategy to tooth brushing. Chlorhexidine, as a mouth rinse, has been considered as a gold standard for many decades now, but long-term use of chlorhexidine is known to cause side effects. Hence, discovery of potential natural agents exhibiting antibacterial effect on oral bacteria is desirable. Hydrogen water is one such natural agent with an antibacterial potential.

In a study carried out by Lee et al., antibacterial effect of hydrogen water was checked in vitro and in vivo. When compared to the effect of tap water, samples treated with hydrogen water showed a significant reduction in the growth of all periodontopathogens (standard strains) in culture, toothbrushes, and saliva. The results of our study were similar to the above study. In our present study, there was a statistically significant reduction in the colony forming units of aerobic and anaerobic bacteria when they were treated with hydrogen water as compared to baseline.

Kim et al.[7] studied the effect of hydrogen water on streptococcal biofilm formation. The results showed that there was a significant decrease in *in vitro* biofilm formation. Furthermore, oral rinse with hydrogen water for 1 week led to significantly fewer salivary streptococci than did that with control tap water. Hence, the authors concluded that oral rinse with hydrogen water would be helpful in treating dental biofilm-dependent diseases with ease and efficiency. In our present study, we found that the aerobic and anaerobic bacteria which constitute the biofilm were significantly reduced by hydrogen water.

The results of the present study were also in accordance with the study done by Baek et al.,[8] wherein the antibacterial activity of hydrogen-rich water was tested against bacteria associated with oral disease. Bacterial strains of *Streptococcus mutans, F. nucleatum, P. gingivalis*, and *Tannerella forsythia* were cultured and treated with hydrogen water and tap water, and the number of bacterial colonies was measured. Hydrogen water showed antibacterial effect against all four pathogens in comparison to tap water.

Zeng et al.,[9] through their research on the antibacterial mechanism of electrolysed water on *Escherichia coli* and *Staphylococcus aureus*, suggested that the products obtained after electrolysis of water increased the permeability of the bacterial cell membrane and led to the leakage of cellular components, thus killing the bacteria. However, the exact mechanism by which the hydrogen water reacts with bacterial membrane is beyond the scope of this study.

Studies on rats by Yoneda et al.[10] and Kasuyama et al.[11] have shown that consumption of hydrogen-rich water was beneficial in suppressing periodontitis progression and limiting the alveolar bone resorption. The results of our present study suggest that hydrogen water has an antibacterial effect on aerobic and anaerobic bacteria in the plaque obtained from patients with chronic periodontitis. Considering that the plaque microbiota is the initiating etiological agent for chronic

**Table 1: Intra group comparison of hydrogen water group for aerobic culture**

| Time pairs | Z    | P     |
|------------|------|-------|
| Baseline 0 min-1 min | -2.696 | 0.007** |
| Baseline 0 min-2.5 min | -3.099 | 0.002** |
| Baseline 0 min-5 min | -1.831 | 0.067*  |
| 1 min-2.5 min | -2.418 | 0.016*  |
| 1 min-5 min | -0.310 | 0.756   |

*P<0.05 statistically significant difference, **P<0.01 Statistically highly significant difference, #P>0.05 nonsignificant difference, P – Level/test of significance, Z – Number of standard deviations from mean

**Table 2: Intragroup comparison of hydrogen water group for anaerobic culture**

| Time pairs | Z    | P     |
|------------|------|-------|
| Baseline 0 min-1 min | -3.324 | 0.001** |
| Baseline 0 min-2.5 min | -3.025 | 0.002** |
| Baseline 0 min-5 min | -2.744 | 0.006** |
| 1 min-2.5 min | -1.945 | 0.179   |
| 1 min-5 min | -0.980 | 0.327   |
| 2.5 min-5 min | -1.198 | 0.231   |

*P<0.01 Statistically highly significant difference, **P<0.05 nonsignificant difference, P – Level/test of significance, Z – Number of standard deviations from mean

**Table 3: Pairwise comparison using Wilcoxon sign-ranked test of hydrogen water group for aerobic culture**

**Table 4: Pairwise comparison using Wilcoxon sign-ranked test of the hydrogen water group for anaerobic culture**
periodontitis, we can assume that the antibacterial potential of hydrogen water will help halt the disease progression.

Azuma et al.[12] studied the effects of drinking hydrogen water on nonsurgical periodontal treatment in 13 patients. The participants in the hydrogen water group showed greater improvements in probing pocket depth and clinical attachment level as compared to the control group at 2, 4, and 8 weeks.

To the best of our knowledge, no studies have reported any unwanted/adverse effects of consumption of hydrogen water.

The results of our in vitro study have been very encouraging to say the least. They indicate an antibacterial potential for hydrogen water against clinical plaque samples in patients with chronic periodontitis. The adjunctive use of hydrogen water as an irrigant, mouth rinse, especially in patients with chronic periodontitis, needs to be further explored.

CONCLUSION

Chronic periodontitis is a multifactorial disease although bacterial onslaught through accumulation of plaque is responsible for initiating and increasing the severity of disease.

The analysis of data from the present study is suggestive that hydrogen water has an antibacterial activity against aerobic and anaerobic bacteria present in the plaque obtained from the patients with chronic periodontitis. This remarkable finding of something as natural as water, but having an antibacterial potential, has certainly opened our minds to further research on hydrogen water and its applications.

Presence of bacterial biofilm causes a low-grade inflammation, which leads to persistent activation of inflammatory cells, resulting in a high degree of reactive oxidative species (ROS) release. Over-produced ROS deplete the endogenous antioxidants thereby leading to increased oxidative damage. Recent studies also indicate that treatment with hydrogen water has an antioxidant effect on cells, tissues, and organs as well as a beneficial role in the prevention against oxidative stress-related disorders.

In continuation to this study, we are now inspired to take this forward and test hydrogen water with respect to its antioxidant potential in patients with chronic periodontitis.

Acknowledgements
For statistical support, Dr. Dheeraj Kalra (Professor and Head, Department of Public Health Dentistry, YMT Dental College and Research Centre, Kharghar, Navi Mumbai) is acknowledged.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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