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ORIGINAL ARTICLE

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Management of gingival inflammation in orthodontic patients with ozonated water irrigation – a pilot study

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Abstract: *Objectives*: Ozonated water irrigation has recently been tried for its antimicrobial and anti-inflammatory effects in treatment of periodontitis. During orthodontic treatment, gingival inflammation occurs along with increased lactate dehydrogenase (LDH) enzyme levels in gingival crevicular fluid (GCF). Thus, the aim of this pilot study was to evaluate the clinical effects of a single subgingival irrigation with ozonated water on gingival inflammation in orthodontic patients and also to correlate the clinical effects with LDH enzyme activity in GCF. *Methods*: Fifteen systemically healthy orthodontic patients (seven men and eight women, mean age 17.3 years) with full-mouth brackets were included in this prospective, cross-sectional, clinical and laboratory investigation. Clinical parameters, LDH enzyme activity and GCF volume were measured at baseline (0 day) followed by subgingival irrigation with 0.01 mg l^{-1} ozonated water. These parameters were again assessed on 14th and 28th day. *Results*: There was significant ($P < 0.05$) reduction in values of clinical parameters, GCF LDH activity and GCF volume after subgingival irrigation with ozonated water. Also, a significant correlation ($r = 0.50$, $P = 0.01$) was observed only between the post-treatment changes of plaque index and LDH values, among the clinical parameters assessed. *Conclusions*: A single subgingival irrigation of 0.01 mg l^{-1} ozonated water can effectively reduce the gingival inflammation in orthodontic patients, which is also reflected in the reduction of LDH enzyme levels. However, further randomized controlled trials are required to validate the use of ozone irrigation in orthodontic patients for plaque control measures.

Key words: gingival crevicular fluid; gingivitis; irrigation; orthodontics; ozone

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Introduction

Adequate levels of plaque control are difficult to achieve in patients with fixed orthodontic appliances. As a result, orthodontic patients often develop gingivitis, with the response characterized by gingival inflammation and hyperplasia (1). Powered toothbrushes (2) and various chemical agents, such as chlorhexidine (1, 3), sanguinarine (3) and saline (3) in the form of subgingival irrigation, have been shown as beneficial adjuncts to normal manual toothbrushing for plaque removal in orthodontic patients.

An alternative approach to conventional antimicrobial or antiseptic agents in the suppression of subgingival bacteria is to inhibit their growth by changing the subgingival environment, which has been shown to be highly anaerobic with a prevailing low oxygen tension (4). First advocated by Dunlop in 1913, various agents have been applied, such as molecular oxygen (5), hyperbaric oxygenation (6) and hydrogen peroxide (7). It has been shown that repeated subgingival oxygen irrigation in previously untreated deep periodontal pockets resulted in a significant clinical improvement in the periodontal baseline conditions (8).

Recently, the use of ozone (O_3), which is an allotrope of oxygen, has gained momentum in dentistry. In the field of orthodontics, ozone gas has been tested for its anticaries effect (9) and also for its effect on shear bond strength of orthodontic brackets to enamel (10).

With regards to application of ozone in the field of periodontics, very few *in vitro* and *in vivo* studies have been attempted. In an *in vitro* study, Nagayoshi *et al.* (11) observed that gram-negative bacteria such as *Porphyromonas endodontalis* and *Porphyromonas gingivalis* were substantially more sensitive to ozonated water (0.5–4 mg l^{-1}) than gram-positive oral *Streptococci* and *Candida albicans* in pure culture. Huth *et al.* (12) in their *in vitro* study found that the aqueous form of ozone (1.25–20 μg ml^{-1}), as a potential antiseptic agent, showed less cytotoxicity than gaseous ozone (4×10^6 μg m^{-3}) or established antimicrobials (2%, 0.2% chlorhexidine digluconate; 5.25%, 2.25% sodium hypochlorite and 3% hydrogen peroxide) and thus concluded that aqueous ozone fulfils optimal cell biological characteristics in terms of biocompatibility for oral application. In another *in vitro* study, Huth *et al.* (13) showed that nuclear factor-kappa B (NF-*k*B) activity in oral cells in periodontal ligament tissue from root surfaces of periodontally damaged teeth was inhibited following incubation with aqueous ozone (20 μg ml^{-1}), suggesting that it has an anti-inflammatory capacity. A recent systematic review also concluded that there is good evidence of ozone biocompatibility with human oral epithelial cells, gingival fibroblast and periodontal cells in *in vitro* conditions (14).

In an *in vivo* study, Ramzy *et al.* (15) attempted the management of aggressive periodontitis patients using ozonized water and found highly significant improvements in pocket depth, plaque index (PI), gingival index (GI) and bacterial counts in relation to quadrants treated by scaling and root planing together with ozone application compared to quadrants treated by scaling and root planing alone. Also, Kshitish and Laxman (16) reported higher percentage of PI, GI and bleeding index reduction using 0.1 mg l^{-1} ozone irrigation as compared to chlorhexidine irrigation in periodontitis patients.

On the other hand, lactate dehydrogenase is an enzyme that is normally limited to the cytoplasm of cells, and it is only released extracellularly after cell death. Previous studies have demonstrated that the activity of lactate dehydrogenase (LDH) in gingival crevicular fluid (GCF) is significantly correlated with gingival inflammation (17–19) and tissue destruction consequent to periodontitis in humans (19, 20). Also, it has been demonstrated that GCF LDH activity increases during the course of orthodontic treatment (21) and that its levels are significantly greater in dental sites undergoing compression stress (22). Moreover, this enzyme activity may increase in teeth wearing orthodontic appliances even if they do not undergo orthodontic movement, potentially as a consequence of gingival inflammation produced by the presence of the plaque-retentive appliances (22).

The anti-inflammatory effects of ozone seen in the periodontal field need to apply in the control of gingival inflammation and accompanying increased GCF LDH activity in orthodontic patients. Hence, the purpose of this pilot study was to evaluate the clinical effects of a single subgingival irrigation with ozonated water on gingivitis in patients with fixed orthodontic appliances and also to correlate the clinical effects with the LDH activity in GCF.

Study population and methodology

Subjects

This single-centre, single-blind clinical study included individuals currently in orthodontic treatment with full-mouth brackets (bands or bonds) and archwires. The subjects were recruited from Department of Orthodontics and Orofacial Orthopedics, College of Dental Sciences, Davangere, Karnataka, India. From the fifty orthodontic patients examined, 15 subjects (seven men and eight women, mean age 17.3 years) met the inclusion criteria and agreed to participate in the study.

Subjects were enrolled if they had a medical history showing good general health, at least 24 natural teeth in the mouth excluding the third molars, and also full-mouth fixed orthodontic appliances in place for a minimum of 3 months. At the screening examination, each subject needed to have at least 50% bleeding sites and 50% dichotomous plaque score. Oral and written information was given to each enrolled subject. Both the parent and subject signed the consent form. The study protocol, consent form and instructions were approved by the Ethical Committee, Rajiv Gandhi University of Health Sciences, Karnataka, India, and the study was conducted based on the principles outlined in the Declaration of Helsinki of 1975, as revised in 2008, on experimentation involving human subjects.

Subjects were excluded from the study if they had any of the following: (i) medical history of any liver, heart, kidney and muscle diseases that are known to affect LDH levels, (ii) medical history of heart murmur, rheumatic heart disease, rheumatic fever, mitral valve prolapse or history of any condition that might put them at risk if a bacteremia were to occur, (iii) pregnant or planning a pregnancy within the next 3 months, or if they were taking antibiotics, (iv) current history of medications likely to affect gingival health, (v) advanced periodontitis or rampant dental caries based on a non-invasive examination, (vi) removable oral prostheses or removable orthodontic appliances, (vii) requirement of premedication with antibiotics for dental appointments, (viii) who had received any surgical or non-surgical therapy 6 months prior to

the start of the study, (ix) who had received any chemotherapeutic mouth rinses and oral irrigation during the past 6 months, and (x) who were smokers.

Treatment procedure

The study was carried out in Department of Periodontics, College of Dental Sciences, Davangere, Karnataka, India. The study period of 28 days was divided into three time intervals, i.e. baseline (0 day), 14th day and 28th day.

At baseline, the clinical parameters, viz. PI (23), GI (24), gingival bleeding index (GBI) (25), were recorded at distofacial, facial, mesiofacial and entire lingual gingival marginal surfaces of all the teeth present. Probing pocket depth (PPD) was accomplished for the same four surfaces of all the teeth with a William's graduated periodontal probe and was recorded to the nearest millimetre demarcation. All the recordings of clinical parameters were made by the same calibrated examiner (dental hygienist), who was blinded as to the treatment condition and who did not review the data sheets from previous visits while recording his measurements.

Later, the patients were subjected to full-mouth irrigation with ozonated water (along with the use of suction device) that was released from an irrigation device, 'Kent ozone Dental Jet TY-820' (Pure Water House, Bangalore, India). The device released a single pulsating stream of ozonated water from the nozzle that could be adjusted for different speeds and pressures ranging from 66 to 130 kPa (kilo pascals) and an ozone output of 0.1 mg l^{-1} (0.1 ppm), at a noise output of <70 dB (decibels) and water outflow of 280–420 ml. To facilitate subgingival ozone irrigation, a 20-gauge blunt needle was bent and attached to the tip of the nozzle of the ozone dental jet holder. A stop clock was used to set the irrigation time to 15 s at each site, after which the irrigation was stopped. A total time of 5–10 min was spent for irrigation for each patient.

After irrigation, the patients were instructed to perform regular oral hygiene habits, i.e. twice daily brushing for a minimum of 2 min, using a standard tooth brush (Colgate Super Flexible with medium consistency bristles) and tooth paste (Colgate dental cream) provided to them. The patients were dispersed and instructed to report on the subsequent 14th day. The procedure of assessment of clinical indices and PPD was then repeated on the 14th and 28th day.

GCF sampling procedure

The determination of LDH enzyme activity was carried out at baseline before ozone irrigation, and then, subsequent analysis was carried out at 14th and 28th day along with the clinical parameters.

Maxillary teeth were selected for sampling to reduce the possibility of contamination with saliva. The mesial aspect of most inflamed site in the maxillary arch was selected for sampling of GCF, which was then analysed for LDH enzyme activity. Each crevicular site included in this study was isolated with cotton rolls, gently air dried and the supragingival plaque carefully removed. The GCF was collected using #30 standardized sterile paper point inserted 1 mm into the gingival crevice and left *in situ* for 30 s (21). In cases of visible contamination with blood, the points were discarded and other sites showing clinical signs of inflammation were sampled.

Gingival crevicular fluid volume determination was carried out at all the three time intervals of the study. The GCF volume in each paper point was calculated as follows: each single paper point was weighed on an analytical balance before the sampling procedure, and then this was repeated immediately after the sampling; by considering the relative density of GCF as being equal to 1.0, the difference in weight showed the adsorbed GCF volume (23). Immediately after GCF volume determination, the paper points were transferred to plastic vials containing 100 μl phosphate-buffered saline with pH 7.4. The points were then allowed to elute for 1 h at 25°C. Thereafter, the points were discarded, and the vials with the GCF samples were taken to the Central laboratory of Biochemistry at Bapuji Hospital, Davangere, Karnataka, India, for the procedures of chemical analysis.

Lactate dehydrogenase activity determination

All the vials containing GCF samples were put in the spectrophotometric automatic apparatus (COBAS INTEGRA 400 *plus*; Roche Diagnostics, Mannheim, Germany) to be automatically analysed without any manual procedures. The COBAS INTEGRA 400 *plus* is a flexible system for both classical and special clinical chemistries. It has an on-board capacity of 36 tests and throughput of up to 400 photometric tests per hour for analysis of serum, plasma, urine, cerebrospinal fluid, hemolysate and whole blood. The system has preloaded reagent cassettes for various enzyme activity determinations with automatic sample dilution and concentration and later automatic cassette reconstitution when required. The minimum sample requirement for the system is 2–10 μl (microlitre), and the results for the LDH enzyme volume activity (International units per litre [IU l^{-1}]) are given in approximately 7 min.

By means of this apparatus, the total volume of GCF was expressed in μl, and the LDH activity was calculated as total LDH unit activity (milli unit per sample [mU per Sample]) by using the formula (18): GCF volume (μl) × LDH volume activity (IU l^{-1}) × $l/10^6$.

Statistical analysis

Results were expressed as mean ± SD and proportions as percentages. Wilcoxon signed rank test was used to compare post-treatment changes (at 14th and 28th day) with pretreatment values (at baseline). Spearman's ranked correlation coefficient was used to assess the relation between post-treatment changes in total LDH enzyme activity and clinical parameters. Non-parametric methods were used for statistical analysis because heterogeneous data were observed. For all the tests, a *P*-value of 0.05 or less was considered for statistical significance.

Results

In case of effect of ozone irrigation on clinical parameters, a significant reduction was observed for all the clinical parameters, viz. mean PI, mean GI, mean GBI % and mean PPD (Table 1). Plaque index was significantly reduced from 1.93 ± 0.22 (P = 0.0086) at baseline to 1.57 ± 0.23 (P = 0.005) at 14th day and to 0.89 ± 0.24 (P = 0.002) at 28th day. GI showed a significant reduction from 1.78 ± 0.14 (P = 0.002) at baseline to 1.59 ± 0.13 (P = 0.001) at 14th day and to 1.07 ± 0.12 (P = 0.001) at 28th day. GBI was significantly reduced from 87.1 ± 6.2% (P < 0.001) at baseline to 75.4 ± 6.4% (P < 0.001) at 14th day and to 30.9 ± 4.5% (P < 0.001) at 28th day. PPD (mm) was also significantly reduced from 3.10 ± 0.12 (P = 0.016) at baseline to 3.09 ± 0.13 (P = 0.001) at 14th day and to 2.92 ± 0.10 (P = 0.001) at 28th day.

Besides clinical parameters, ozonated water irrigation also caused significant reduction in GCF volume and GCF LDH enzyme activity from baseline to 14th day, from 14th day to 28th day and also from baseline to 28th day (Table 2). GCF volume (μl) was significantly reduced from 0.30 ± 0.03 (P = 0.002) at baseline to 0.29 ± 0.03 (P = 0.001) at 14th day to 0.23 ± 0.03 (P = 0.001) at 28th day. GCF LDH enzyme activity (mU per sample) was also significantly reduced from 102.7 ± 59.2 (P = 0.001) at baseline to 64.8 ± 35.0 (P = 0.001) at 14th day to 18.9 ± 5.9 (P = 0.001) at 28th day.

Table 3 summarizes the data concerning the concurrent changes in clinical parameters and total LDH enzyme unit activity after ozone irrigation, between baseline and 28th day. Results showed a statistically significant correlation (r = 0.50, P = 0.01) between the concurrent changes of PI and LDH values from baseline to 28th day. Rest of the clinical parameters showed a non-significant correlation with concurrent changes in the LDH enzyme activity values from baseline to 28th day, after ozone irrigation.

Discussion

We designed a short-term, single-centre, single-blind prospective clinical study to evaluate the clinical effects of a single subgingival irrigation with 0.01 mg l^{-1} ozonated water on gingivitis in patients with fixed orthodontic appliances and also to correlate the clinical parameters, viz. PI, GI, GBI and PPD, with the LDH activity in GCF.

Results of the present study showed that subgingival irrigation with 0.01 mg l^{-1} ozonated water caused significant reduction for all the clinical parameters, viz. mean PI, mean GI, mean GBI % and mean PPD (mm), from baseline to 14th day,

Table 1. **Mean and standard deviations of clinical parameters at three time intervals during study period (n = 15)**

	Plaque index		Gingival index		Gingival bleeding index (%)		Probing pocket depth (mm)	
Time interval	Mean ± SD	P-value*	Mean ± SD	P-value*	Mean ± SD	P-value*	Mean ± SD	P-value*
Baseline (0 day)	1.93 ± 0.22	–	1.78 ± 0.14	–	87.1 ± 6.2	–	3.10 ± 0.12	–
14th day	1.57 ± 0.23	–	1.59 ± 0.13	–	75.4 ± 6.4	–	3.09 ± 0.13	–
28th day	0.89 ± 0.24	–	1.07 ± 0.12	–	30.9 ± 4.5	–	2.92 ± 0.10	–
0–14th day	0.36 ± 0.07	0.0086	0.19 ± 0.6	0.002	11.7 ± 1.5	<0.001	0.01 ± 0.01	0.016
14–28th day	0.68 ± 0.15	0.005	0.52 ± 0.11	0.001	44.5 ± 5.0	<0.001	0.17 ± 0.05	0.001
0–28th day	1.04 ± 0.14	0.002	0.71 ± 0.14	0.001	56.2 ± 5.2	<0.001	0.18 ± 0.04	0.001

*Wilcoxon signed rank test.
P < 0.05 – statistically significant.
SD, standard deviation.

Table 2. **Mean and standard deviations of gingival crevicular fluid (GCF) volume and total GCF lactate dehydrogenase (LDH) activity at three time intervals during study period (n = 15)**

	GCF (μl)			LDH (mU per sample)		
Time interval	Mean ± SD	Median (Range)	P-value*	Mean ± SD	Median (Range)	P-value*
Baseline (0 day)	0.30 ± 0.03	0.31 (0.24–0.35)	–	102.7 ± 59.2	89.2 (37.5–258.1)	–
14th day	0.29 ± 0.03	0.27 (0.23–0.3)	–	64.8 ± 35.0	51.0 (27.5–147.6)	–
28th day	0.23 ± 0.03	0.23 (0.17–0.28)	–	18.9 ± 5.9	18.5 (8.1–27.6)	–
0–14th day	0.01 ± 0.01	–	0.002	37.9 ± 27.7	–	0.001
14–28th day	0.06 ± 0.01	–	0.001	45.9 ± 30.8	–	0.001
0–28th day	0.07 ± 0.02	–	0.001	83.8 ± 55.4	–	0.001

*Wilcoxon signed rank test.
P < 0.05 – statistically significant.
SD, standard deviation.

Table 3. **Descriptive statistics for post-treatment changes (baseline – 28th day) in clinical parameters along with Spearman's correlation (*r*) with lactate dehydrogenase (LDH) changes (*n* = 15)**

Reduction in parameters	Mean reduction ± SD	Percent of reduction (%)	Correlation with LDH changes
LDH	83.8 ± 55.4	82.0	–
Plaque index	1.03 ± 0.14	53.0	$r = 0.50$*, NS
Gingival index	0.71 ± 0.14	40.0	$r = 0.15$, NS
Gingival bleeding index (%)	56.2 ± 5.2	65.0	$r = 0.21$, NS
Probing pocket depth (mm)	0.19 ± 0.04	6.0	$r = 0.15$, NS

*P-value = 0.01 (significant).
S, significant; NS, non-significant; SD, standard deviation.

from 14th day to 28th day and also from baseline to 28th day. The results followed an expected pattern in accordance with the reduction in PI, GI and GBI seen in the study by Kshitish and Laxman (16), which evaluated the effect of oral irrigation with 0.01 mg l^{-1} ozonated water and 0.2% chlorhexidine in moderately deep periodontal pockets on the clinical parameters of chronic and aggressive periodontitis patients.

The possible mechanism for reduction in the PI and gingival inflammation associated with subgingival ozone irrigation may be because of the antibacterial effect of ozone on the plaque microorganisms (11) or by a disruption of subgingival plaque rather than an instant killing of microorganisms (26). The anti-inflammatory effects of ozonated water (aqueous ozone) observed in the present clinical study are also supported by an *in vitro* study by Huth *et al.* (13) which showed that NF-*k*B activity in oral cells in periodontal ligament tissue from root surfaces of periodontally damaged teeth was inhibited following incubation with aqueous ozone (20 μg ml^{-1}), suggesting that it has an anti-inflammatory capacity.

There was a significant reduction in GBI % scores in this study at both 14th and 28th day. It cannot be ruled out that even though this reduction was maintained throughout the study duration of 28 days, GBI scores might not have been maintained if the duration of the study was longer. A prolonged observation period will allow a better estimation of extinction of the effect.

Pseudopockets were found at most of the sites. A slight (6%) but significant reduction in probing depths seen at 14th and 28th day could have resulted from reduction in gingival inflammation.

In addition to the effects of subgingival ozonated water irrigation, the improvement seen in clinical parameters may also be attributed to the subject's improved oral hygiene as a response to the required twice daily brushing for 2 min and anticipation of forthcoming oral examination during study intervals, i.e. Hawthorne effect (27).

Along with measuring changes in clinical parameters for reduction in gingival inflammation, this study also included measurement of changes in total enzyme unit activity of LDH along with changes in GCF volume. In this study, the GCF collection for LDH enzyme activity estimation was carried out using #30 standardized sterile paper point inserted 1 mm into the gingival crevice for 30 s, similar to methodology of Serra *et al.* (21). However, the LDH enzyme estimation in the present study was carried out by a fully automated analyser, which can be considered as a better method of analysing LDH enzyme activity than using manual spectrophotometer used in previous studies (21, 22).

There was significant reduction observed after ozone irrigation in GCF volume from baseline to 14th day, from 14th day to 28th day and also from baseline to 28th day. This significant reduction is in accordance with the known reduction in GCF volume with periodontal treatment as seen in many studies (19, 28–30).

In the present study, there was a significant reduction after ozone irrigation in total LDH enzyme activity from baseline to 14th day, from 14th day to 28th day and also from baseline to 28th day. The presence of high total LDH enzyme unit activity at baseline (i.e. sites with full-mouth fixed orthodontic appliances in place for a minimum of 3 months showed a mean total LDH unit activity of 102.7 ± 59.2 mU per sample) in our study is in accordance with the results of Serra *et al.* (21) who attribute this increased GCF LDH levels to the tissue resorption in both the compressed and tensional sites, or even secondary to a possible cell necrosis in the periodontal ligament during the orthodontic treatment. Also, this enzyme activity may increase in teeth wearing orthodontic appliances even if they do not undergo orthodontic movement, potentially as a consequence of gingival inflammation produced by the presence of the plaque-retentive appliances (22).

The GCF LDH levels in our study reduced significantly by 82% from 102.7 ± 59.2 mU per sample at baseline to 18.9 ± 5.9 mU per sample at 28th day, after ozone irrigation. These GCF LDH values at baseline and 28th day are comparable to the mean GCF LDH values for diseased sites (0.129 ± 0.406 IU per site) and healthy sites (0.029 ± 0.035 IU per site), respectively, as observed in a cross-sectional study by Wolff *et al.* (19), thus indicating the return of diseased sites to healthy sites following ozone irrigation. This post-treatment significant reduction in GCF LDH values can also be correlated to studies showing significant reduction in GCF LDH activity levels after successful periodontal treatment (19, 20).

The concurrent changes between the changes in GCF LDH values and the clinical parameters, viz. PI, GI, GBI and PPD between baseline and 28th day, were also seen using Spearman's correlation coefficient, which revealed a significant correlation only between changes in GCF LDH levels and PI. This significant correlation is similar to study by Wolff *et al.* (19) and can again be attributed to effect of ozone on the plaque because of the antibacterial effect of ozone on the plaque

microorganisms (11) or by a disruption of subgingival plaque rather than an instant killing of microorganisms (26). The absence of significant correlation between changes in GCF LDH levels and GI is similar to study by Wolff *et al.* (19). For deriving a significant relationship between concurrent changes in GCF LDH levels and GI, GBI and PPD, a possible further subgingival ozone irrigation over a prolonged period is proposed along with the use of more number of GCF sampling sites to correlate better with the clinical parameters recorded.

The Medline search does not reveal any comparative study for assessing the effects of subgingival irrigation on clinical parameters and LDH enzyme activity in gingival inflammation in orthodontic patients. Previous studies (21, 22) have shown an increased LDH enzyme activity in orthodontic patients, which needs to be controlled along with gingival inflammation to facilitate orthodontic tooth movement. The results of this pilot study showed that a single subgingival irrigation of 0.01 mg l^{-1} ozonated water can effectively reduce the gingival inflammation in orthodontic patients, which is also reflected in the reduction of total LDH unit activity levels. The effect of reduced inflammation is maintained over a 28-day (4-week) period, which frequently coincides with scheduled orthodontic appointments during active treatment. This would conveniently allow for repeated treatments at specific interproximal sites that are difficult for plaque control and are most severely affected by inflammation in orthodontic patients. Also, the Kent ozone Dental Jet used in our study can be used as a home care appliance by using the device directly for irrigation (supragingival) near the orthodontic brackets, and also for professional irrigation (both supra- and subgingival) in dental office, for effective plaque control in orthodontic patients. In addition, no adverse effects were observed with the use of subgingival irrigation with 0.01 mg l^{-1} ozonated water during the 1-month study trial period.

In comparison with classical periodontal treatment modalities, such as systemic and local antimicrobials, ozone therapy is quite inexpensive, and according to many case reports and scientific studies, it is very promising. However, further research is needed to standardize the indications and treatment procedures of ozone therapy. To the best of our knowledge, the present clinical trial is the first report to determine the beneficial clinical effects of a single subgingival irrigation of 0.01 mg l^{-1} ozonated water on gingival inflammation and increased GCF LDH activity in orthodontic patients. An obvious shortcoming of the present study was the absence of a control group, as in this pilot study, orthodontic patients were subjected to only one intervention with outcomes evaluated at 2 and 4 weeks. Further randomized control trials with larger sample size comparing the efficacy of subgingival ozonated water irrigation with positive control as 0.2% chlorhexidine and negative control as saline are being undertaken in our department.

Conclusion

At the end of one month, a single subgingival irrigation of 0.01 mg l^{-1} ozonated water could effectively reduce the gingival inflammation in orthodontic patients. Thus, subgingival ozone irrigation can be an effective method that can be performed during monthly visits on orthodontic patients to reduce the gingival inflammation because of plaque-retentive orthodontic appliances. However, further randomized controlled trials are required to validate the use of ozone irrigation in orthodontic patients for plaque control measures.

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