Assessment of the effect of ozonated water irrigation on gingival inflammation in patients undergoing fixed orthodontic treatment

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Abstract:
Background: A split-mouth longitudinal study was designed here to evaluate the effect of ozonated water delivered subgingivally through a jet device (Kent Ozone dental TY 820 jet) in controlling gingival inflammation of patients undergoing fixed orthodontic therapy. Materials and Methods: Twenty-eight patients undergoing fixed orthodontic treatment following thorough oral prophylaxis were recalled after 2 weeks, and baseline gingival inflammation was estimated. This study was conducted as split-mouth technique with the upper left quadrant (Control side) was irrigated by jetting saline into sulcus by air–water syringe with gauge 22 needle tip and upper right quadrant (Test side) irrigated with ozonated water by a jet device using similar delivery tip. The patients were recalled at the end of 1st, 2nd, 3rd, and 4th month and the irrigations as well as gingival inflammation estimation were repeated. A biochemical assessment of gingival inflammation (estimation of aspartate aminotransferase [AST] in gingival crevicular fluid [GCF]) and clinical assessment (gingival index scoring) was done at each visit. Results: At the test side, the AST level in the GCF was found to be maintained almost the same value as baseline till the end of 4th month, indicating not much of change in inflammatory status. Statistically significant difference in GCF-AST and gingival index score between test and control sites were found at the end of 3rd and 4th month with a significance of P < 0.05. Conclusion: The patient undergoing fixed orthodontic treatment showed a consistent improvement in gingival inflammation on frequent irrigation with ozone jet irrigation as compared with saline irrigation delivered with same jetting force.

Key words:
Fixed orthodontic therapy, gingival index, gingival inflammation, ozone irrigation

INTRODUCTION

O one of the major drawbacks of fixed orthodontic treatment lies in its need to fix complex appliances on teeth for considerably long time, which hinder the plaque control regimen. Chemical plaque control agents become a mandate in such conditions. Chlorhexidine mouthrinse is found to be the most effective agent among the commonly used chemical plaque control agents, but its long-term usage results in several side effects. However, its long-term use is associated with increased staining and temporary taste disturbances.[1]

The undisputed disinfection power of ozone over other antiseptics makes the use of ozone in dentistry a very good alternative or an additional disinfectant to standard antiseptics. Ozone therapy can activate the antioxidant system, helps in proper oxygen metabolism, induce a friendly ecologic environment, increase circulation, and also can modulate immune system. Ozonated water is not only proven to be an adjunct to scaling and root planning procedure[2,3] but also irrigation with ozonated water in gingival sulcus is shown to reduce microbial load on teeth even before the scaling and root planning (SRP) procedure.[4]

The current study is to evaluate the clinical effects of subgingival irrigation with ozonated water on gingival inflammation in patients undergoing fixed orthodontic treatment, where mechanical plaque control means are partially hindered. The literature review on this kind of studies showed no long-term studies that evaluated the progress at frequent intervals.[2,3] Split-mouth studies done previously compared two chemical agents...
which could have resulted in overlapping of the results.\[3\]

Thus, a study with a rationale to overcome these limitations was required to elucidate the effect of ozonated water irritant.

**Aim**

This study aims to evaluate the effect of ozonated water as a subgingival irritant in controlling gingival inflammation in patients undergoing fixed orthodontic therapy.

**The objectives of study**

1. To ascertain whether ozonated water jet irrigation alone without mechanical plaque control measures can cause reduction in gingival inflammation
2. To compare ozone therapy with a control group of normal saline irrigation in controlling gingivitis during fixed orthodontic treatment.

**MATERIALS AND METHODS**

A longitudinal study was performed on 28 fixed orthodontic treatment undergoing patients. Sample size was calculated by Winpepi software which is having 5% of significance level and 90% of power.\[3\] The patient selection was limited to people who had completed 1 year of treatment and within an age group 13–22 years. The cases were selected from the patients who sought treatment at the Department of Orthodontics at the study-conducted institution. Written informed consent was taken from each patient under study and ethical clearance for the study was received from the Institutional Ethical committee.

**Inclusion criteria:**

1. Patients who had completed 1 year of fixed orthodontic treatment
2. Patients with baseline, mild-to-moderate gingival index\[6\]
3. Systemically and physically healthy individuals
4. Patients who have not received any antimicrobial therapy for the past 3 months

**Exclusion criteria:**

1. Patients with clinical attachment loss with severe gingival index scores were excluded\[9\]
2. Patients with any kind of enamel anomalies
3. Smokers, pregnant women, and patients with systemic disorders

In the first visit, the procedure was explained to patients and consent to be a part of the study was obtained. A detailed case history including the periodontal status of the patient was assessed using the plaque index,\[7\] gingival index,\[6\] clinical attachment level,\[8\] and gingival bleeding index.\[9\] The periodontal assessment was done by a trained and calibrated dentist. It was a double-blinded study. In the second visit, full-mouth scaling was done for each person. Oral hygiene instructions were given.

After 2 weeks, the patients were recalled, and gingival crevicular fluid (GCF) sample was collected with a microcapillary tube by a trained periododontist and sent for the estimation of baseline aspartate aminotransferase (AST) level in it. AST is an inflammatory biomarker, released from injured and dead cells into extracellular fluid and can be readily assayed in serum, tears, and GCF. After GCF collection, gingival index scoring was performed at both the sides to assess the gingival inflammation by the dentist.

Split-mouth evaluation technique was planned with the upper right quadrant as the test side where ozone irrigation was done and the upper left quadrant as the control side where saline irrigation was done. Initially, the subgingival areas of the left upper quadrant were irrigated by saline through an air–water syringe. The test area was then irrigated with ozonated water through ozone water jet (Kent Ozone dental TY 820). The ozone irrigation was set in a mode 4 so that it equalizes with the air water syringe pressure. A total of 900 ml of ozone water was used to irrigate on test side and same quantity of saline irrigation was used on the control side each time. The overflowing irrigant was evacuated with high-power vacuum suction to prevent it from reaching the control site. Quadrilateral side was isolated with cotton rolls. The method of ozone water and saline irrigation is described in Figures 1 and 2.

At the fourth visit (1 month following first irrigation), the GCF samples were collected from the same areas for estimation. Gingival index scoring and the irrigation were repeated in the same manner. Following this, the patients are recalled at 2nd, 3rd, and 4th month GCF collection; GI examination and irrigation with respective agents were repeated. Figures 3-6 show pre- and post-operative treatment of two cases.

GCF sample of 1 µL was collected from the area around the premolar region so as to standardize the area of sample collection on both test and control site. GCF was collected by a volumetric microcapillary tube placed passively at the entrance of gingival sulcus, without inducing any stress on the tissue. This was immediately transferred to Cryovial containing 99 µL of phosphate-buffered saline stored in the refrigerator. The container was labeled with patient code, stored at 4°C and was transported to the laboratory on the same day. The AST level was estimated biochemically by the International Federation of Clinical Chemistry and Laboratory Medicine method without pyridoxal phosphate using the principle of Kinetic determination of AST activity.\[10\]

The results were subjected to statistical evaluation and were used to intercompare the inflammatory status of gingiva in patients using test and control group. Dependent \( t \)-test was used for paired samples, and correlation was found by Karl Pearson’s technique.

**RESULTS**

Twenty-eight patients comprising 14 females and 14 males, aged between 13 and 22 years (mean 16.8 years) who had completed 1 year of their fixed orthodontic therapy were enrolled in this study. Three of them could only undergo examination till the third visit; however, their obtained data till the time they attended, were included in the analysis.

GCF-AST was estimated at definite intervals from baseline at 1st, 2nd, 3rd, and 4th month in both the test and control sites [Tables 1 and 2]. At the test side, there was a gradual reduction in GCF-AST levels at various intervals. However, at the control side, there was gradual progression in GCF-AST levels.
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Table 1: Gingival crevicular fluid-aspartate aminotransferase level (µUL) of baseline, 30th, 60th, 90th, and 120th day at test side and control side

| GCF-AST    | n   | Test side Mean | SD  | Control side Mean | SD  |
|------------|-----|----------------|-----|-------------------|-----|
| Baseline   | 28  | 2926.57        | 1277.801 | 2967.04           | 1314.592 |
| 30th day   | 27  | 2521.52        | 1002.744 | 2951.93           | 1368.664 |
| 60th day   | 25  | 2712.08        | 1168.159 | 3265.48           | 1666.828 |
| 90th day   | 25  | 2496.32        | 1182.367 | 3371.52           | 1918.265 |
| 120th day  | 25  | 2256.44        | 1090.297 | 3459.20           | 1687.605 |

n – Number of samples; GCF – Gingival crevicular fluid; AST – Aspartate aminotransferase; SD – Standard deviation

A statistically significant difference ($P < 0.05$) was observed in the GCF-AST level between the test and control sides at 90th day and 120th day.

Gingival index was found to gradually reduce at test side consistently throughout the study, whereas at the control side, it did not exhibit much of a difference after the 30th day. There was statistically significant ($P < 0.05$) difference between the gingival index of test and control side at each intervals of 30, 60, 90, and 120 days [Tables 3 and 4].

Pearson’s correlation technique was used to test the direction and strength of the relationship between GCF-AST and gingival index [Table 5]. On the 30th day, though the test side exhibited a positive correlation between GCF-AST and
gingival index, the control side showed a negative correlation at this point. However, there was a significant ($P < 0.05$) and positive correlation observed on 90th and 120th day between the parameters at control side, a negative correlation was found at 60th, 90th, and 120th day at the test side.

**DISCUSSION**

The action of ozone water irrigation was found to be comparable with several current techniques available in controlling plaque accumulation and gingival inflammation. Following 30 days of scaling and root planning and irrigation

Table 2: Paired samples test of gingival crevicular fluid-aspartate aminotransferase level ($\mu$UL) at test and control side

| GCF-AST | Paired difference between test AST levels and control AST levels |   | $t$ | df | $P$ ($P<0.05$) |
|---------|---------------------------------------------------------------|---|-----|----|----------------|
| Baseline | 40.46 | 1396.69 | 0.153 | 27 | 0.879 |
| 30th day | 430.40 | 1438.01 | 1.555 | 26 | 0.132 |
| 60th day | 553.40 | 1816.55 | 1.523 | 24 | 0.141 |
| 90th day | 875.20 | 1397.02 | 1.523 | 24 | 0.033 |
| 120th day | 1202.76 | 1454.78 | 4.134 | 24 | 0.000 |

SD – Standard deviation; $t$ – Paired difference test value; df – Degree of freedom; $P$ – Probability value; GCF – Gingival crevicular fluid; AST – Aspartate aminotransferase

Table 3: Gingival index of initial visit, baseline, 30th day, 60th day, 90th day, and 120th day at test side and control side

| Gingival index | n | Test side | Control side |
|---------------|---|-----------|--------------|
|              | Mean | SD | Mean | SD |
| Initial visit | 28 | 1.495 | 0.198 | 1.522 | 0.188 |
| Baseline     | 28 | 1.419 | 0.181 | 1.452 | 0.183 |
| 30th day     | 27 | 1.262 | 0.128 | 1.378 | 0.144 |
| 60th day     | 25 | 1.226 | 0.116 | 1.440 | 0.151 |
| 90th day     | 25 | 1.198 | 0.096 | 1.427 | 0.165 |
| 120th day    | 25 | 1.147 | 0.114 | 1.420 | 0.146 |

$n$ – Number of samples; SD – Standard deviation

Table 4: Paired samples test of gingival index at test and control side

| Gingival index | Paired difference between test gingival index and control gingival index |   | $t$ | df | $P$ ($P<0.05$) |
|---------------|-------------------------------------------------------------------|---|-----|----|----------------|
| Initial visit | 0.03 | 0.19 | 0.749 | 27 | 0.460 |
| Baseline     | 0.03 | 0.19 | 0.901 | 27 | 0.376 |
| 30 days      | 0.12 | 0.11 | 5.518 | 26 | 0.000 |
| 60 days      | 0.21 | 0.14 | 7.674 | 24 | 0.000 |
| 90 days      | 0.23 | 0.17 | 6.668 | 24 | 0.000 |
| 120 days     | 0.27 | 0.13 | 10.384 | 24 | 0.000 |

$t$ – Paired difference test value; df – Degree of freedom; $P$ – Probability value; SD – Standard deviation

Table 5: Correlation of gingival crevicular fluid-aspartate aminotransferase with gingival index of test and control side

| Correlation of GCF-AST with gingival index | 30th day | 60th day | 90th day | 120th day |
|------------------------------------------|---------|---------|---------|---------|
| Test side | Control side | Test side | Control side | Test side | Control side | Test side | Control side |
| Pearson correlation | $P$ | 0.342 | -0.195 | -0.094 | -0.059 | -0.398 | 0.824 | -0.152 | 0.769 |

$P<0.05$ is considered significant. $P$ – Probability value; GCF – Gingival crevicular fluid; AST – Aspartate aminotransferase
affected the outcome of the study. The studies done on similar equipment that delivers test agent through a jet was compared with control group that was delivered through irrigation syringes that did not possess jet. In the present study, both control and test group were delivered through jet technique. In addition to the clinical parameters, the study also substantiates its clinical effects with an inflammatory biomarker, AST through its enzyme activity in GCF of the irrigated areas.

Thus, this study has been successful in reducing most of the errors that would have affected its result. However, this research work can be continued with presently available biomarkers in GCF for better evaluation of the treatment outcome. Also by evaluation of changes in pocket depth, conducting a longitudinal study from the very beginning of orthodontic treatment and the changes that occur after discontinuing the ozone therapy are few regions that require further research.

CONCLUSION

The result of the study supports the usage of ozonated water through jet irrigation in effectively reducing gingival inflammation even without the aid of SRP in patients wearing fixed orthodontic appliances. To conclude, the observations of this study consider this device as a valuable tool in controlling gingival inflammation in cases of patients undergoing fixed orthodontic treatment and recommending it as a professional irrigation device that could be used for irrigating subgingival areas in patients wearing fixed orthodontic appliances.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mathew MJ, Jithesh J, Peter SS, Vijayakumar B, Sudeep CB, Reshmi J. Antimicrobial efficacy of ozonated water and chlorhexidine mouth rinse on Porphyromonas gingivalis: An in vitro study. J Odontol Res 2013;1:11-6.
2. Dhingra K, Vandana KL. Management of gingival inflammation in orthodontic patients with ozonated water irrigation – A pilot study. Int J Dent Hyg 2011;9:296-302.
3. Kshetish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. Indian J Dent Res 2010;21:341-8.
4. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T, et al. Efficacy of ozone on survival and permeability of oral microorganisms. Oral Microbiol Immunol 2004;19:240-6.
5. Ramzy MI, Gomaa HE, Mostafa MJ, Zaki BM. Management of aggressive periodontitis using ozonized water. Egypt Med J N R C 2005;6:229-45.
6. Loehlin H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
7. Silness J, Loehlin H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.
8. Ramford SP. The periodontal disease index (PDI). J Periodontol 1967;38:Suppl. 602-10.
9. Muhlemann HR, Mazor ZS. Gingivitis in Zurich school children. Helv Odontol Acta 1958;2:3-12.
10. Murray RL. Aspartate aminotransferase. In: Kaplan LA, Pesce AJ, editors. Clinical Chemistry: Theory, Analysis and Correlation. United states: C.V. Mosby Company; 1984. p. 1105-8.
11. Dodwad V, Gupta S, Kumar K, Sethi M, Masamatti S. Changing paradigm in pocket therapy–ozone versus conventional irrigation. Int J Public Health Dent 2011;2:7-12.
12. Patel PV, Patel A, Kumar S, Holmes JC. Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: A randomized, controlled, double blind, clinical and microbiological study. Minerva Stomatol 2012;61:381-98.
13. Hayakumo S, Arakawa S, Maeno Y, Izumi Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. Clin Oral Investig 2013;17:379-88.
14. Katti SS, Chava VK. Effect of ozonised water on chronic periodontitis – A clinical study. J Int Oral Health 2013;5:79-84.
15. Yilmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T, et al. Evaluation of the clinical and antimicrobial effects of the er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. Photomed Laser Surg 2013;31:293-8.
16. Shoukheba MY, Ali SA. The effects of subgingival application of ozonated olive oil gel in patient with localized aggressive periodontitis: A clinical and bacteriological study. Tanta Dent J 2014;11:63-73.
17. Baysan A, Lynch E, Ellwood R, Davies R, Petersson L, Borsboom P, et al. Reversal of primary root caries using dentifrices containing 5,000 and 1,100 ppm fluoride. Caries Res 2001;35:41-6.
18. Wolff LF, Smith QT, Snyder WK, Bedrick JA, Liljemark WF, Aeppli DA, et al. Relationship between lactate dehydrogenase and myeloperoxidase levels in human gingival crevicular fluid and clinical and microbial measurements. J Clin Periodontol 1988;15:110-5.