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ORIGINAL ARTICLE

Antibacterial efficacy of prophylactic ozone treatment on patients with fixed orthodontic appliances

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Abstract

Objective. This study investigated the efficacy of ozone on microflora of patients with fixed orthodontic appliances. ***Materials and methods.*** Ten subjects undergoing fixed appliance therapy were enrolled in the study. Before any application, two elastic ligatures were removed with the aid of a sterile probe from the premolar brackets on the upper right and lower left jaws for baseline bacteriological counts. Then ozone gas was applied for 180 s by running the device on prophylaxis mode with included mouth tray according to the manufacturer's recommendations on all brackets. After the ozone application, two other elastic ligatures were removed from the opposite sides of the mouth of the patients. One week after the application two more elastic samples, which were not changed in the previous session, were collected from the upper right and lower left premolar brackets. The elastic ligatures were immersed in transfer solutions and cultured to determine the *Streptococcus mutans* and *Lactobacillus acidophilus.* Statistical analysis of the data was obtained by paired Student's *t*-tests. ***Results.*** The ozone treatment reduced the *S. mutans* and *L. acidophilus* immediately after the application and these reductions were found to be statistically significant ($p = 0.038$ and $p = 0.020$). Both the *S. mutans* and *L. acidophilus* values increased by the 1-week period and the *S. mutans* values were significantly higher than the baseline values ($p = 0.01$). ***Conclusion.*** It can be concluded that the ozone treatment may have an instantaneous lethal effect on *S. mutans* and *L. acidophilus;* however, within the limitations of this study, a long-term preventive effect could not be observed.

Key Words: *orthodontic appliances, oral microbiology, ozone*

Introduction

Fixed orthodontic appliances increase the risk of plaque accumulation and bacterial colonization, which leads to enamel decalcification and white spot lesion formation. Due to the increased retentive sites, which lead to retention of the biofilm and bacterial accumulation, orthodontic treatment might be a factor that increases the caries risk of the patient [1–4].

To avoid enamel decalcification and formation of the white spot lesions in high-risk patients, numerous preventive strategies are used and recommended in the literature [5–7]. These strategies are mainly focused on the elimination of the cariogenic microflora or the mechanical removal of the plaque [8].

Ozone therapy is amongst these strategies and based on the concept of complete elimination of acidophilic bacteria, fungi and viruses, thus, creating a sterile environment for re-mineralization to take place [9]. Nowadays the ozone treatment is gaining wider acceptance in dentistry. Many *in vitro* studies suggest a promising potential of ozone, but no clinical evidence for application of ozone in dentistry is existing [10,11]. Ozone is one of nature's most powerful oxidants and has been shown to kill *mutans streptococci* efficiently; however, the antibacterial efficacy of ozone is still discussed and has to be investigated [10–12].

A recent review of dental literature reported conflicting evidence for the *in vitro* application of ozone in varying fields. Namely these are; endodontics, oral and maxillofacial surgery and treatment of oral mucosa diseases and disinfection during implant applications. On the other hand, the same review concluded that the *in vitro* application of ozone is a useful prophylactic

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antimicrobial treatment prior to etching and placement of dental sealants and restorations [13].

In the literature there are some reports about ozone application in orthodontically-treated patients or the effect of ozone application on bond strength of orthodontic brackets [12,14]. The preventive effect of ozone on the development of white spot lesions was evaluated in a study by Kronenberg et al. [12]. They concluded that the caries protective effect of Cervitec/Fluor Protector during fixed appliances therapy was superior to ozone. Ozone treatment prior to bracket bonding has also been investigated and it is concluded that the ozone application does not affect the shear bond strength of orthodontic brackets [14]. The clinical effects of a single subgingival irrigation with ozonated water on gingival inflammation in orthodontic patients has also been investigated and it was found that ozonated water can effectively reduce the gingival inflammation in orthodontic patients [15]. To our knowledge, up to date there is no study that evaluated antibacterial efficacy of ozone in patients who were undergoing fixed orthodontic appliance treatment. The hypothesis tested was that the application of gaseous ozone would suppress the level of *S. mutans* and *L. acidophilus* levels in patients with fixed orthodontic appliances. Therefore, the aim of this study was to investigate the effectiveness of ozone on *S. mutans* and *L. acidophilus* in patients with fixed orthodontic treatment.

Materials and methods

Study sample

A total of 10 right-handed subjects (four male, six female) aged between 21–23 were enrolled in the study. The participants had no relevant medical history and had not taken antibiotics nor used antibacterial mouth rinse within the last month. The inclusion criterion was being orthodontically treated for a minimum of a 3-month period. The participants were informed about the aim of this trial well in advance and informed consent was obtained. The Ethical Committee of the University of Ege approved the study protocol (12-6/7).

The same operator performed the routine orthodontic controls of the individuals 1 week before the samples were obtained in order to place standard elastic ligatures around the brackets. The patients were informed and encouraged to use fluoride toothpaste (Colgate, 1450 ppm F, Colgate-Palmolive Company, Herstal, Belgium) twice a day during the 1-week period. Patients were advised not to consume any type of food and not to brush their teeth in the morning of the sample collection sessions.

Clinical procedure

The analysis of saliva included the stimulated salivary flow rate, buffer capacity and saliva pH. Participants chewed paraffin wax for 30 s for saliva stimulation based on the manufacturer's instructions (GC Corporation, Tokyo, Japan) before before saliva collection. Salivary flow rate was recorded after a 5-min collection period (values <3.5 ml were regarded as very low, 3.5–5 ml as low and >5 ml was regarded as normal). Buffering capacity was also recorded by using a saliva check buffer (GC Corporation). The measurement of saliva pH was carried out with a pH test strip (GC Corporation) placed into the saliva sample for 10 s. The change in the color of the strip was compared with the testing chart in the package. The manufacturer grouped buffering capacity according to the change in color of test pads supplied (0–5 points: very low, 6–9 points: low and 10–12 points: normal/high).

The application sequence of the method is summarized in Figure 1 and as seen in the figure at baseline, two elastic ligatures were removed from the premolar brackets on the upper right and lower left quadrants (14 and 35) with the aid of a sterile probe and immersed into a transfer solution (10 mL of 0.85% sterile saline solution). An available size of full mouth tray (FMT) was chosen for each patient and ozone gas (Ozonytron OZ, MIO international Ozonytron GmbH, Munich, Germany) was applied for 180 s by running the device on prophylaxis mode according to the manufacturer's recommendations. After the ozone application, two other elastic ligatures were removed from the premolar brackets of teeth 24 and 45. The elastic ligatures were then again immersed into transfer solutions.

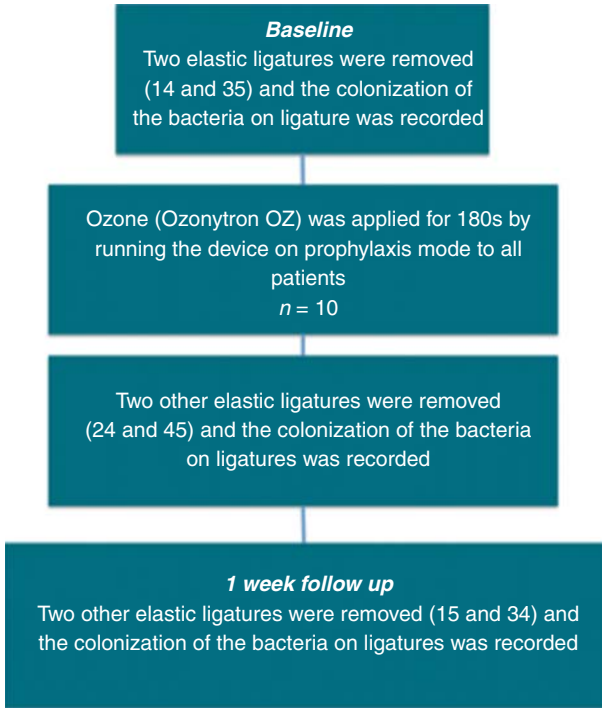

Figure 1. The flow chart of the study.

Table I. Test of resting and stimulated saliva samples.

Salivary flow rate			Buffering capacity			Salivary pH		
Very low	Low	Normal	Very low	Low	Normal/high	Highly acidic	Moderately acidic	Healthy saliva
0	2	8	0	10	0	0	4	6

The expelled ligatures were replaced by new elastic ligatures. Patients were recalled 1 week later with the same conditions mentioned above in the morning. Two more samples, which were not changed in the previous session, were then collected from the upper right and lower left premolar brackets (15 and 34) and the ligatures were then immersed into transfer solution. The missing elastic ligatures were replaced again as in the previous session.

Microbiological analysis

In all sessions the transfer solutions were delivered to the Microbiology Laboratory immediately after collecting the sample and cultured to determine the *S. mutans* and *L. acidophilus*.

The tubes containing the transfer solutions were vortex mixed and the homogenized solutions were then diluted. MRS Agar (Tween-80 supplemented) and Mitis Salivarius Agar (20% sucrose and 0.2 U/mL bacitracin supplemented) plates were used to determine the number of bacteria surviving on the elastic ligatures. The colonization of the bacteria on ligatures was recorded as Colony-Forming Units/ligatures (CFU/ligatures).

Statistical analyses

The data were transferred to SPSS and normality test was applied. The data were analyzed using SPSS 15.0 Software. Microbiological counts from baseline and test samples were transformed as logarithmic values (colony forming units + 1) prior to statistical analyses in order to normalize their distributions. Statistical analysis of the data was obtained by paired Student's *t*-tests to determine significant differences. The level of significance was set at $\alpha = 0.05$.

Results

Because of the fact that the data for *S. mutans* were not normally distributed, the logarithms were used for statistical analysis. The log-transformed data were normally distributed for both *S. mutans* and *L. acidophilus*.

The results of the chair-side tests applied to saliva samples are presented in Table I and the values of *S. mutans* and *L. acidophilus* are shown in Table II for all measurements (baseline, direct after ozone application and after 1 week). The ozone treatment reduced the *S. mutans* and *L. acidophilus* immediately after the application and these reductions were found to be statistically significant ($p = 0.038$ and $p = 0.020$, respectively).

After the 1-week period the treatment with ozone did not exert any significant effect for both *S. mutans* and *L. acidophilus*, even the evaluated *S. mutans* and *L. acidophilus* values increased after the 1-week period and these increases were found to be statistically significant ($p = 0.03$ and $p = 0.01$, respectively). Both the *S. mutans* and *L. acidophilus* values increased by time and the *S. mutans* values were significantly higher than the baseline results ($p = 0.01$).

Discussion

The effect of fixed orthodontic appliances on microbial flora and periodontal status has been evaluated previously [16]. It is well known that fixed orthodontic appliances create retention areas for *mutans streptoccocci* and *lactobacilli*, which increases the risk of white spot lesions and formation of caries in orthodontic patients [17]. These challenges directed clinicians to search for preventive measures for orthodontic patients such as fluoride, chlorhexidine or CPP-ACP containing preventive agents in order to overcome this problem [6,12,18].

Ozone, in the gaseous or aqueous phase, has been shown to be a powerful and reliable antibacterial agent against bacteria [13] and it is used also as a caries preventive agent in patients who have fixed orthodontic appliances [12,19]. Therefore, this study investigated the antibacterial effectiveness of a newly-designed ozone appliance in patients who has fixed orthodontic appliances. The ozone system used in this study can be used in many conditions such as prophylaxis, bleaching or desensitizing the teeth. This system is manufactured with a novel application technology, which allows the disinfection of all teeth and periodontal ligament with a full-mouth-tray. It can be used in patients with fixed

Table II. Bacterial counts (mean values ± SD $\log_{10}$ cfu) before and after ozone treatment.

	Time periods (mean values ± SD $\log_{10}$ cfu)		
Microorganisms	Baseline	Immediately after the ozone treatment	After 1 week
S. mutans	5.27 ± 0.63	4.83 ± 0.49	5.53 ± 0.61
Lactobacillus	5.55 ± 0.41[a]	4.92 ± 0.31	5.69 ± 0.21[a]

The groups with comparisons at each time point measurement that are not significantly different are marked with the same letters.

orthodontic appliances with the aid of the FMT as prophylaxis in one session, which is more applicable compared to the others that have been used, which can be applied only tooth-by-tooth.

Elastomeric rings were used in this study to collect the biofilm and to assess it microbiologically because there has been interest in investigating the role of materials used for ligation of orthodontic arch wires on the increase of bacterial contamination in the areas adjacent to the brackets [20,21].

The ozone treatment reduced the *S. mutans* and *L. acidophilus* immediately after the application of ozone, but both the *S. mutans* and *L. acidophilus* values increased by the 1-week period. The reduction immediately after the application might be explained with the killing effect of ozone. Previous *in vitro* studies have also showed the antibacterial and killing effect of ozone on cariogenic bacterial species [10,11,22]. It is reported that the tested cariogenic species (*Actinomyces naeslundii*, *Lactobacilli casei* and *Streptococcus mutans*) are affected to various degrees by ozone gas, with almost 100% killing after only 60 s of ozone application species [10]. In our study with the aid of the FMT we applied 180 s of ozone in total and 85–90% of the bacteria were still alive immediately after ozone treatment. Although ozone seems to have an antibacterial effect on bacteria under *in vitro* conditions, there is no consensus of how efficiently ozone kills bacteria under *in vivo* conditions in the presence of saliva and the dental biofilm, a barrier well-known to be highly resistant to various types of antibacterial treatments [23,24]. The low reduction found in the present study might be related to the fact that in the present study the bacteria were organized in biofilm and so only a lower number of bacteria will be reached by the ozone.

Baysan and Lynch [25] and Holmes [26] reported the caries inhibiting effects of ozone, but there are no conclusions about the bacteria in the lesions. Our results also showed that ozone treatment has an instantaneous lethal effect on *S. mutans* and *L. acidophilus*, but no long-term inhibitory effect of ozone was found.

The increase in *S. mutans* and *L. acidophilus* 1 week after the application when compared with the baseline results might be explained by the aging of the elastomeric ligatures. The elastomeric ligatures were changed before the application of ozone and by time and it is possible that, because of an elastomeric ligature, deformation due to the orthodontic forces might lead to a retention area, which causes an increase in the number of bacteria. In a study of Magno et al. [20], who used Super Slick elastomeric rings which are developed for controlling bacterial colony forming units, thus reducing the risk of iatrogenic enamel demineralization during orthodontic therapy, it had been reported, after a 15 day intra-oral period, these elastomeric rings had significantly greater contamination by *S. mutans* than conventional elastomeric rings and also related their findings due to the formation of fissures on the rings when they are tied to the orthodontic brackets [20].

Since it was reported previously that *mutans streptococci* can be suppressed effectively for a prolonged period of time following the application of highly concentrated chlorhexidine varnishes [27,28], it may be anticipated that chlorhexidine treatment combined with an ozone therapy may be implemented as a caries prevention strategy, for patients undergoing fixed appliance.

It can be concluded that the ozone treatment may have an instantaneous lethal effect on *S.mutans* and *L. acidophilus*; however, within the limitations of this study, a long-term preventive effect could not be observed and the efficacy of combined use of ozone and other preventive strategies should be a question for further research.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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