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IN VIVO STUDY OF THE EFFECTIVENESS OF OZONIZED OLIVE OIL GEL ON INHIBITING ENAMEL DEMINERALIZATION DURING ORTHODONTIC TREATMENT

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ABSTRACT:

The present study assessed the effect of ozonized olive oil gel during orthodontic treatment to inhibit demineralization around orthodontic brackets.

Material and Methods: *Fourteen patient, aged 15-18 years, scheduled to have premolars extraction as a part of orthodontic treatment participated in the present study. The patients were randomly divided into 3 groups; control group (16 upper and lower premolars) and two experimental groups (20 upper and lower premolars for each). In the Control group no treatment was applied on the premolars. In experimental groups, after etching and bonding, T-loops were formed with 0.014 inch stainless steel wire and engaged on the experimental teeth. In experimental group II, ozonized olive oil gel was applied sparingly to the tooth surface with concentration of 20:25 µgm/ml. Participants were instructed to apply the ozonized olive oil gel to the buccal surface of the premolars three times daily for 8 weeks after regular oral hygiene procedures. After 8 weeks, the brackets were deboned and the premolars extracted carefully. The premolars of each group were divided into 2 equal numbers; 28 premolars prepared for elemental analyses (calcium and phosphorus) followed by scanning electron microscope investigation, and 28 premolars examined with polarized light. ANOVA test was applied followed by post hoc LSD test to compare between each 2 groups.*

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Results: *Ca/P ratio of enamel in control group was 3.09. In experimental group I, the Ca/P ratio reduced to 1.75, while in group II, the ratio was 2.33, with statistically significant difference between all groups. Polarized light examination of experimental group I revealed increased depths of demineralized areas, and extending deeper in the enamel which was indicated by positive form birefringence. Experimental group II showed the enamel surface with improvement of demineralized lesions in the form of negative form birefringence resembling normal enamel in the control group. Scanning electron microscopic examination of experimental group I showed comparatively increased depths of demineralized areas with deep wavy perikymata. Enamel surface of experimental group II showed almost normal appearance of perikymata, with new hydroxyapatite crystal deposition.*

Conclusion: *The use of ozonized olive oil gel in addition to the standard oral hygiene regimen was found to be beneficial for orthodontic patients to prevent enamel decalcification during treatment.*

INTRODUCTION

Despite the advances in orthodontic materials and techniques in recent years, the development of decay around brackets during orthodontic treatment continues to be a problem[1]. The presence of orthodontic attachments makes patient's dental hygiene more difficult and the accumulation of plaque easier[2,3]. This environment changes the biological balance of the mouth and increases the patient's risk of caries[4-7]. In addition it may restrict the ability of the tongue to remove food particles from the mouth[8].

After the use of fixed appliances, decalcification marks are more pronounced at the gingival third of the teeth, where higher plaque accumulation usually occur[9].Clinically, they appear as white opaque spot chalky lesions around the brackets. White spot lesions are softer than the surrounding enamel[10]. They are seen as early as 4 weeks after band/ bracket placement[4,5]. The obvious degree of iatrogenic enamel damage during orthodontic treatment suggests the need for preventive programs.

The two methods that have been proposed to date to prevent white spot formation include professional hygiene instructions and enamel surface modification by means of fluoride agents, chlorhexidine, sealants, or Nd: YAG laser and others [11-16].

In the field of conservative dentistry, ozone has gained popularity as a means of preventing dental caries[16,17]. Ozone is a natural oxidant consisting of three atoms that is also known as triatomic oxygen. Ozone treatment is reported to inactivate microorganisms that cause tooth decay, with no negative interactions with the physical properties of enamel and adhesive restorations[18]. The effects of gaseous ozone and aqueous ozone have been investigated in several studies[19-25].

Ozone gas dissolved in plant oils, such as olive oil, is being discussed in dentistry for its excellent antimicrobial property, without the development of drug resistance and facilitation of wound healing[26-29]. In orthodontics, clinicians tend to seek out an effective antimicrobial agent to apply prior to bracket bonding in order to prevent dental caries during fixed appliance treatment [30-34]. Despite the current lack of substantiated evidence for the benefits of its application, ozone may prove a viable alternative to conventional prophylactic treatments in the near future[35]. In light of various findings in conservative dentistry[36], it seems appropriate to speculate that application of ozone gas for 20 seconds around orthodontic brackets during interval visit will prevent or reduce the potential of demineralization and development of white spot lesions without affecting the shear bond strength of the bonded brackets[37,38].

Although ozone appears to be an integral part of noninvasive therapy of dental caries, specifically as a disinfectant prior to placing a direct restoration[36,39-41], knowledge of its in-vivo effect on enamel surface around the orthodontic brackets still deficient. The present study aimed to determine quantitatively and qualitatively:

- The amount and extent of demineralization occurring around orthodontic appliance after 2 months in vivo.
- The ability of ozone olive oil gel to inhibit such orthodontically related demineralization.

MATERIALS AND METHODS

Fourteen patients, aged 15- 18 years, scheduled to have premolars extraction as a part of orthodontic treatment participated in the present study. The criteria for inclusion in this study were: intact buccal enamel, absence of any clinical evidence of demineralization lesions, lack of visible structural defects on enamel and no restorations on the surface. The patients were duly informed about the study and signed informed consent forms. The buccal surfaces of the maxillary premolars were polished with fluoride-free pumice and rubber cup for 10 seconds, sprayed with water and dried with compressed oil-free stream. Precise oral hygiene instructions were given to all patients.

The patients were randomly divided into 3 groups; control group and two experimental groups. A total of 56 premolars were involved including 16 in the control, and 40 in the experimental groups, 20 for each (Fig 1).

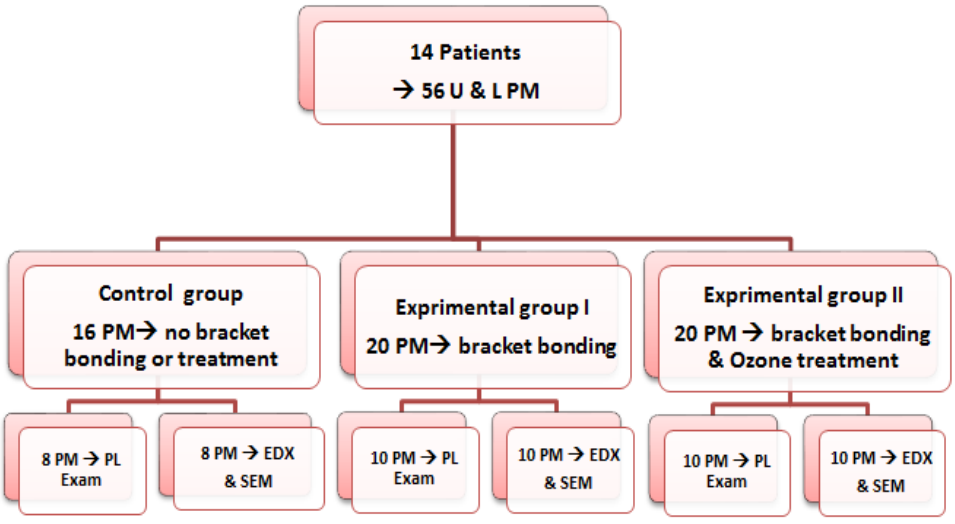


Fig (1): Illustration of study design. U&L PM= upper and lower premolars, PL Exam= polarized light examination, EDX= energy dispersive x-ray analysis, SEM=scanning electron microscope.

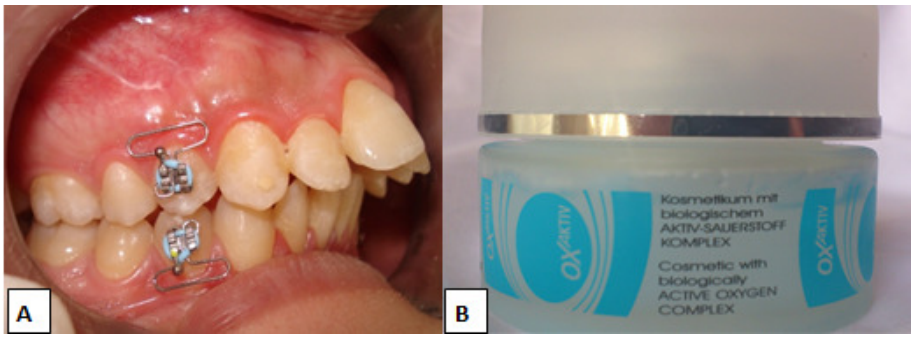


Fig (2) A: Intraoral photograph showing the bonded brackets and T-loops were formed with 0.014 inch stainless steel wire and tied with elastomeric rings. B: The jar of ozonized olive oil gel

Control group:

No treatment was applied on the premolars. The patients in this group instructed to brush daily with the supplied dentifrice.

Experimental group I: Bracket bonding without enamel treatment

A 37% phosphoric acid gel was applied to the enamel surface area corresponding to the bracket base for 30 seconds. The acid gel was washed with water jet, tooth surface dried and the bracket bonded to the enamel surfaces following manufacturer's instructions with a fluoride free self-curing composite (Rely A Bond; Reliance Orthodontic Products, Itasca, II, USA). The brackets (American orthodontics/ mini master series) were positioned on the facial surface at the height of contour mesiodistally, in the middle third occlusogingivally, and parallel to the long axis of the tooth, and pressed on the enamel surface until fully seated. Excessive adhesive around the bracket was removed with a clinical probe. T-loops were formed with 0.014 inch stainless steel wire and engaged on the experimental teeth with elastomeric rings after 1 hour of bonding (Fig 2, A).

Experimental group II: Bracket bonding and treatment of enamel with ozonized olive oil

After etching, bonding, and ligation of T- loops as done in group I, the premolars were kept dried by careful tooth isolation and the enamel

received a single topical application of a small dab of the ozonized olive oil gel with concentration of 20:25 µgm/ml (Pharmoxid Arznei Gmb H & Co.KG, Ifferzheim, Germany, Fig 2,B) sparingly to the tooth surface with the aid of a brush applicator. Participants were instructed to apply the gel to the buccal surfaces of the premolars three times daily for 8 weeks after regular oral hygiene procedures. The subjects were monitored weekly for application reinforcement, and supplied with a list of specific instruction outlining the sequence of brushing and application of ozonized gel. To ensure compliance, the subjects noted the time of brushing and checklist, which the parent or guardian signed daily. After 8 weeks the brackets were deboned and the premolars extracted carefully, scrapped and cleaned of remnants of periodontal ligaments, and fixed in a solution containing 2.5% formaldehyde freshly prepared from Para-formaldehyde. The roots were separated from the crowns with diamond discs under water spray just at the cemento- enamel junction, then each crown was cut on a mesiodistal line from occlusal to cervical and the buccal surfaces were used for the study.

Scanning electron microscope examination:

For scanning electron microscope study, the premolar crowns were washed with distilled water, dehydrated in grades of ethanol baths and dried in air. The specimens were mounted on aluminum stubs. Firstly, they were subjected to x-ray microanalysis, then they were coated with gold in a high vacuum evaporator to thickness of 10 nm. The cervical, middle and occlusal thirds of the buccal surfaces were examined with a scanning electron microscope (JEOL JSM 5410, Tokyo, Japan), at 25KV at the Faculty of Science, Alexandria University.

Energy dispersive X-ray analysis:

Energy dispersive X-ray analysis (elemental microanalysis) cervical to the bracket was performed. A total of 10 analysis were done in each experimental group, and 8 in control group. Thus, elementary chemical analysis for calcium and phosphorus were obtained (mineral components) and the Ca/P ratio for each analysis was calculated for subsequent statistical analysis.

Polarized light examination:

The premolar crowns of each group were sectioned buccolingually adjacent to midline (Fig 3) using water cooled diamond disc and further by hand grinding on polishing boards until a thickness of about 100:120 microns was obtained. The samples were then washed with deionized water, and mounted longitudinally on glass cover slides.

Fig (3): The position of cutting through the crown for polarized light examination.

The teeth in the three groups were then examined under a polarized light microscope (Olympus dual stage, CX 31 Japan) in Geology Department in Faculty of Science Alexandria University and photographed with magnifications X40 (Fig 4:8 A) and X 100 (Fig 4:8 B).

Statistical analysis:

Analytic data were collected and analyzed using SPSS version 18 under windows 7. Statistical analysis was done using one way ANOVA test to compare the Ca, P, and Ca/P ratios of enamel surface in each group, followed by post hoc LSD test to compare between each 2 groups. The value of significance was set at $p<0.001$.

RESULTS

Energy dispersive X-ray analysis:

It was seen that for all the study groups the comparison revealed very highly significant differences among groups (Table 1). Ca/P ratio of

enamel in control group was 3.09. In experimental group I, the Ca/P ratio reduced to 1.75, while in group II, the ratio was 2.33. Post hoc test showed statistical significant difference between all groups.

Table (1): Elemental analysis

Minerals	Mean	±SD	Min.	Max.	ANOVA- F	P value
PKa						
control	21.09	1.26	21.5	23.3	235.12	P<0.001**
group I	33.35	1.56	32.1	35.2		
group II	28.01	2.92	23.9	30.00		
CaKa						
control	68.04	1.68	66.9	69.0	135.77	P<0.001**
group I	58.68	1.78	56.5	61.3		
group II	65.20	1.99	61.30	68.5		
Ca/P ratio						
control	3.09	0.3	3.0	3.90	256.74	P<0.001**
group I	1.75	0.5	1.5	1.90		
group II	2.33	0.19	2.05	2.86		

**Significant at P< 0.001.

Histological findings of the polarized light microscope

The histological results of the polarized light microscopic sections demonstrated variations in the depths and areas of the demineralized lesions among the three groups.

Control Group: The enamel surface of this group appeared to have the least depth of demineralized areas. This was represented by the negative form birefringence in the body of the lesion (translucent zone) (Fig4). Some lesions showed very faint positive form birefringence band at the inner border of the lesion (Fig 5). In all control samples the surface prismless enamel layer was intact.

Experimental Group I: The enamel of this group showed comparatively increased depths of demineralized areas, affecting the

surface of some specimens, body of the lesion and extending deeper in the enamel which were indicated by positive form birefringence (Fig 6 and 7).

Experimental Group II: The enamel of this group showed comparatively improvement of demineralized lesions. This appeared in the form of negative form birefringence (Fig 8).

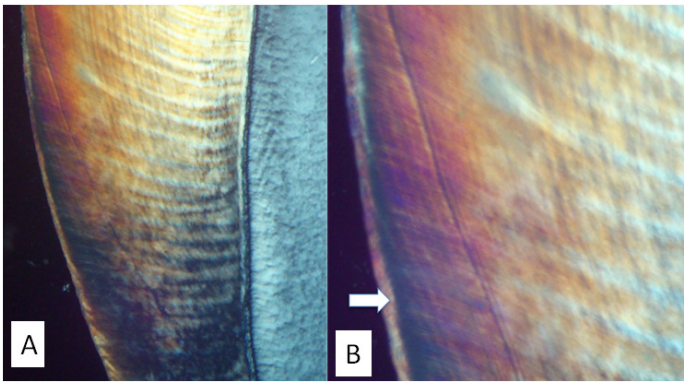


Fig (4): Polarized light micrographs of longitudinal ground sections of control group showing translucent zone with intact outer surface of enamel (arrow).

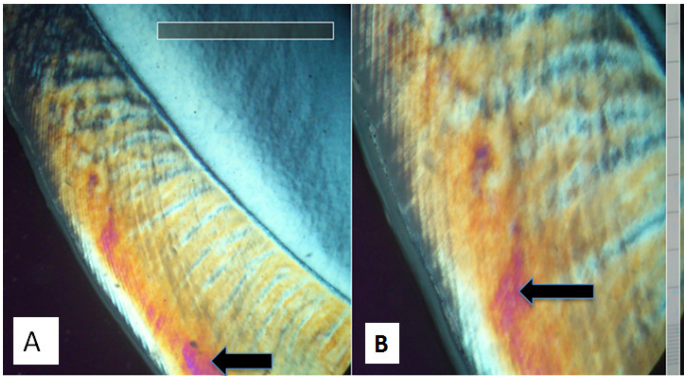


Fig (5): Polarized light micrographs of longitudinal ground sections of control group showing the translucent area and a very faint band (arrows) at the inner border of the lesion the scale on the figure equal to 1 mm.

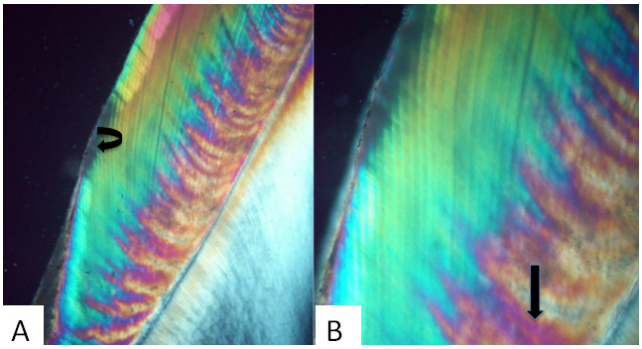


Fig (6): Polarized light micrographs of the longitudinal ground sections of the enamel of experimental group I showing laminated, broad, dark, positive birefringent zone. Note the outer enamel surface is partially demineralized (curved arrow).

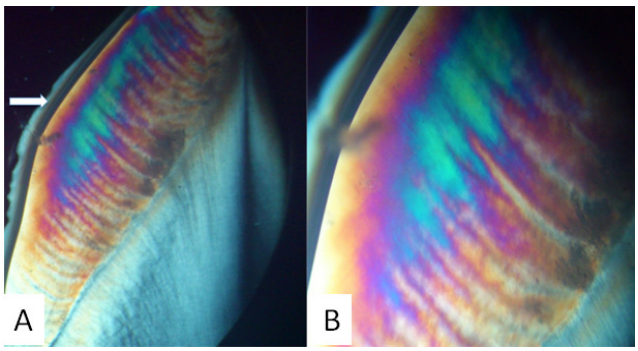


Fig (7): Polarized light micrographs of longitudinal ground sections of the enamel of experimental group I showing larger area of laminated, broad, dark, positive birefringent zone. The outer enamel surface (arrow) is still intact.

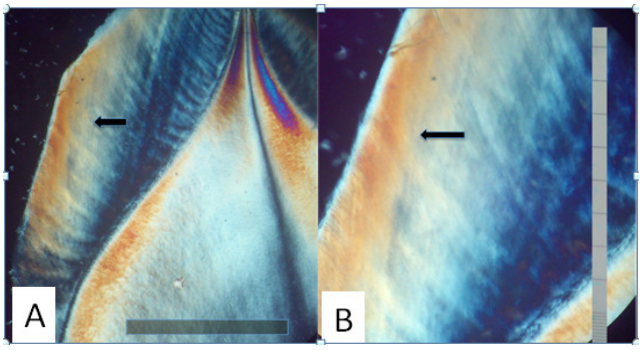


Fig (8): (A & B) Polarized light micrographs of longitudinal ground sections of enamel of experimental group II showing translucent zone with negative form birefringence indicated by arrows.

Scanning electron microscope evaluation:

Scanning electron microscope examination of the enamel surface in control group showed normal appearance of perikymata at cervical third while the middle and occlusal thirds showed smooth surface of enamel with some scratches (Fig 9). In contrast, the enamel surface of experimental group I showed deep wavy perikymata occupies most of the buccal surface with exposure of enamel rod ends in furrows between perikymata (Figs 10& 11).

Experimental group II showed almost normal appearance of perikymata restricted to the cervical third while enamel of middle and occlusalthirds were almost smooth except minimal fissures (Fig 12). Occasionally, rough enamel surface and irregular depressions of variable size that did not expose the enamel rod ends with new hydroxyapatite crystal deposition were detected (Fig 13).

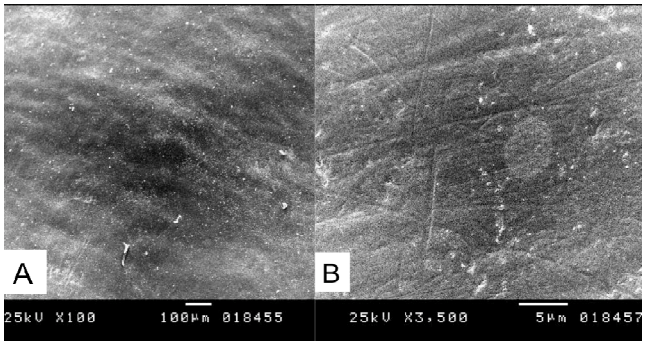


Fig (9): SEM of enamel surface of control group showing normal appearance of perikymata at cervical third (A). The middle and occlusal thirds showing smooth surface of enamel with some scratches (B).

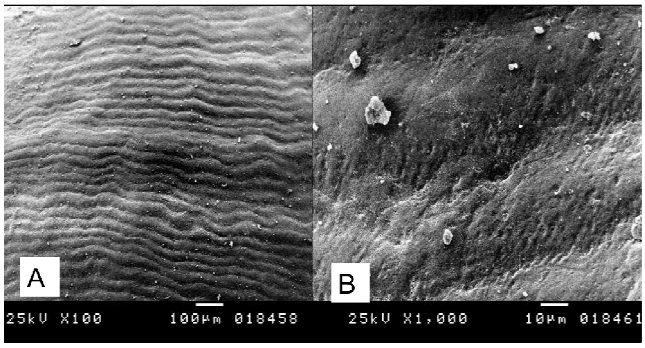
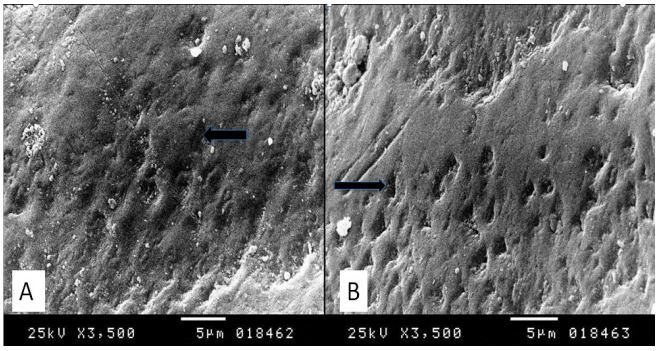


Fig (10): SEM of enamel surface of experimental group I showing deep wavy perikymata occupy most of the buccal surface (A). Higher magnification of the previous figure showing exposed enamel rod ends in the furrows between perikymata (B).



Fig(11): SEM of enamel surface of experimental group I, showing exposed enamel rod ends with characteristic key holes appearance (arrows in A and B).

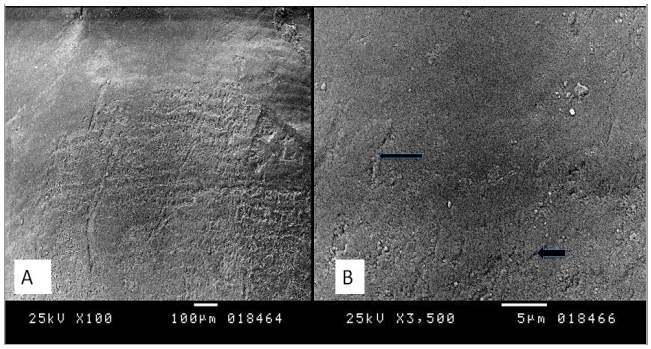


Fig (12): SEM of enamel surface of experimental group II (A) showing almost normal appearance of perikymata restricted to the cervical third the enamel of middle and occlusal thirds with almost smooth surface except minimal fissures (arrows, B).

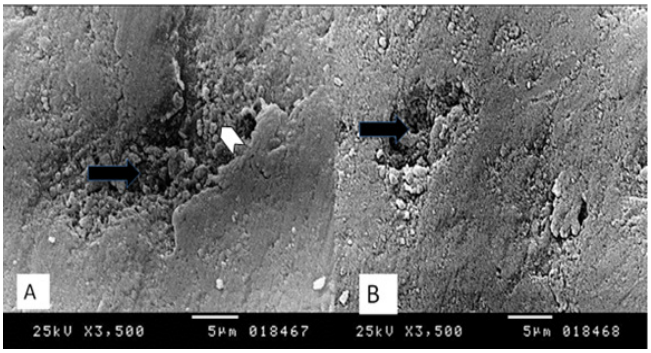


Fig (13): (A & B) SEM of enamel surface of experimental group II showing rough enamel surface and an irregular depression (arrow) that did not expose the enamel rod ends. Note the deposition of new hydroxyapatite crystals (arrow head).

DISCUSSION

The foremost goal of orthodontic treatment is to improve function and aesthetics. To achieve these goals, the orthodontist must do extensive preventive treatment to maintain tooth structure.

In spite of optimal oral hygiene measures, there is a chance of developing white spot lesions during orthodontic treatment with fixed appliances, which may present subsequent aesthetic problems that need to be considered. Assessment of ozone's effect on caries of permanent teeth showed that ozone reverse caries in the surface lesion by shifting the chemical balance promoting remineralization of tooth structure regardless of lesion type or location[22].Ozone gas application around orthodontic brackets was described as a new protocol to reduce enamel demineralization[42]. For that, the present study was conducted to prove the capability of ozone treatment to reduce the development of white spot lesions during fixed orthodontic treatment.

Although the ozonized water is easy to use, the concentration became a half in about 30: 40 minute [43]. It was found that ozone could be kept for a long time by making a virgin olive oil dissolve ozone, under controlled cooling and pressure reaction, and obtained in gel form[44]. In the present study, ozonized olive oil gel was used, and for accurate prophylaxis, it was applied three times daily as the effect of ozone reduced gradually by time[45]. Energy dispersive X-rays analysis has been used for elemental analysis at the ultra-structural level. It is one of the latest micro-analytical techniques that are used in conjunction with scanning electron microscope as an efficient way to quantitatively assess the changes in mineral content[46].

The results of the current study showed that ozonized olive oil gel have a substantial ability to preserve the Ca and P contents in enamel by its protective effect against decalcification of teeth although the Ca/P ratios (2.33) did not reach the values of sound enamel (3.09). Since the Ca and P are the main components of hydroxyapatite crystals[47], it might be said that ozonized olive oil gel could decrease the potential of decalcification during orthodontic treatment.

In experimental group I, Ca/P ratio was decreased to the extent of 1.75, which denoted reduction in the crystallinity and an increase in the extent of dissolution of the apatite with subsequent enamel destruction[48]. This result was matched with polarized light and scanning electron microscope observations. The results of polarized light examination of group II showed the enamel surface with comparatively improvement of demineralized lesions. This appeared in the form of negative form birefringence resemble the normal enamel in control group. Scanning electron microscope revealed that the surface texture demonstrated almost normal appearance of perikymata with almost smooth surface except minimal fissures. Enamel surface roughness and irregular depressions could be detected with no exposure of the enamel rod ends.

The efficiency of ozone treatment in reducing decalcification around orthodontic brackets was explained by the fact that ozone can significantly reduce the number of Streptococcus mutans in plaque, and effectively penetrate into the lesion and kill the great majority of micro-organism, resulting in a delayed recolonization compared with untreated enamel surface[42]. The re-application of ozone could slow the recolonization pattern and achieve long-term suppression. Ozone enables the shifting of microbial flora from acidogenic and acidouric microorganisms to normal oral commensals, which will allow remineralization to occur[49]. It has the ability to remove protein in carious lesion and to enable Ca and P ions to diffuse through the lesions leading to remineralization[50]. Accordingly, in the current study, new hydroxyappitite crystal depositions could be seen adjacent to the small area of demineralizationon of the surface treated with ozone application. Saliva could be considered as another factor which led to the remineralization as Amaechi and Higham[51] speculated that, saliva with its mineral content, can possess a reparative effect on early erosion which is characterized by softened surface and slight subsurface demineralization in addition to a crater.

Dahnhardt et al[22], Al-shamsi [42] and ALjehani et al[52] support the results of the present study. In contrast, Kronenberget al[38] reported low effects of gaseous ozone on white spot lesions. They evaluated the preventive effect of ozone on the development of white spot lesions

during multibracket appliance therapy. Their patients were selected with very poor oral hygiene, with an average observation period of 26 months. This conflict may be due to using the ozone as a remineralization tool, rather than an antibacterial agent[53].Furthermore, white spot formation or enamel decalcification was evaluated visually and by quantitative light-induced fluorescence using a DIAGNO dent device, which does not seem to be a suitable device for the detection of initial lesions during fixed orthodontic treatment[54-56.]

In experimental group I, the untreated enamel surface showed partial enamel demineralization, affecting the surface of some specimens extending deeper in the enamel which were indicated by positive form birefringence with polarized light. Scanning electron microscope demonstrated more surface changes that revealed exposed enamel rod ends with spreading of focal holes and more accentuation of the perikymata. The numerous so-called focal holes observed along the perikymata are not empty spaces but most likely are filled with organic material, which considered as the initial sites of acid penetration that diffuse easily into the defects and then into the spaces between the crystallites[57, 58]

Decalcification of untreated enamel with orthodontic treatment is to be expected and reported earlier, as tooth brushing and flossing become more challenging in the presence of the orthodontic appliances[59,60]. Several studies have revealed that Streptococcus mutants colonization in plaque was higher in teeth with fixed orthodontic appliances compared with control teeth without appliances [61, 62]. Plaque acts as a physical barrier for the diffusion of acid away from the enamel surface and it can also prevent remineralization by calcium and phosphate ions from the saliva. Moreover it is a source of acid production in the presence of fermentable substrate attaching directly to enamel[8]. Also, during orthodontic treatment acid etching and composite resin adhesives increase enamel decalcification[63]. Acid etching dematerializes the enamel surface, exposes the enamel rods and prisms, and removes the protective acquired pellicle[63,64]. The exposed enamel rods provide a way for the bacteria and cariogenic by-products. So it is essential for the orthodontist to be proactive in oral hygiene control during treatment and in monitoring all patients closely.

CONCLUSION

- There was significantly less decalcification of teeth that were protected with ozonized olive oil gel than do teeth not protected.
- The uses of ozonized olive oil in addition to the regular oral hygiene regimen were found to be beneficial for orthodontic patients in preserving the enamel surface.

RECOMMENDATIONS

No study in the literature so far shows complete prevention of enamel decalcification[65], so good oral hygiene and proper diets are still more effective than any adjunctive agent. It is important to continue the development of a preventive agent that can be effectively delivered in the orthodontic patient who lacks compliance with oral hygiene.

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