SYNERGISTIC CARIES INHIBITORY EFFECT OF A REMATERIALIZING AGENT AND CO₂ LASER ON HUMAN ENAMEL AND ROOT DENTIN

Maha Ahmed Niazy(1) and Ehab Saeed Abdul-Hamid(2)

1. Associate Professor of Restorative Dentistry, Faculty of Dentistry- AlAzhar University (Girls).
2. Associate Professor of Oral Pathology- Faculty of Dentistry, Ain Shams University.

**ABSTRACT**

Objectives: Aiming to determine the potential of combined enamel cream/ laser treatment on enamel and root dentin to inhibit caries. Methods: A total of 36 premolars were split into two halves; the buccal half received the treatment; enamel cream (Casein phosphor-peptide-Amorphous calcium phosphate CPP-ACP, laser or combination while the lingual halves served as controls. Results: Enamel cream produced an insignificant increase in surface roughness of the experimental surface as compared to the control surface, whereas laser and combined treatment produced significant increase in surface roughness. Dentin treatment in all groups did not produce any significant difference as far as surface roughness is concerned. Polarized light microscopy revealed areas of remineralization varying from focal areas of different densities with enamel cream to wide translucent areas and loss of incremental lines for CO₂ laser and combined treatment. In root dentin, the remineralization extended deep into the dentinal structure filling almost all the demineralization zones for the combined treatment. Conclusions: Enamel cream and laser were effective in inhibiting caries. Significant synergistic effect exists for combined enamel cream and laser on inhibition of caries in root dentin. Enamel cream and laser individually and in combination created surface and subsurface alterations that could provide protection against caries though more evident in root dentin. The combined therapy seems to be promising in controlling root caries.

**INTRODUCTION**

Dental caries is a bacterially based disease that progresses when the acid produced by bacterial action on dietary fermentable carbohydrates diffuses into the tooth and dissolves the minerals, that is demineralization. Although the prevalence of dental caries has declined markedly over the last 20 years in most countries in the western world, the disease is still a serious threat to the oral health for both adults and children. Considerable advances in caries prevention have been realized to search for the most cost-effective therapy. Protective factors which include salivary calcium, phosphate and proteins,
salivary flow and fluorides in the saliva can balance, prevent or reverse dental caries. Several researchers have evidenced that white spot lesions could be reversed to sound enamel rather than proceeding inevitably to cavitations by remineralizing solutions containing high calcium concentrations\textsuperscript{1-3}. Moreover, it was suggested that freshly prepared highly supersaturated calcifying solutions can be used to enhance mineral appositions in accidental etch pits\textsuperscript{4}.

These results have led to the emerging of new products that can release supersaturated levels of calcium and phosphate ions in proportions favorable for apatite formation. Dairy products including milk, milk concentrates and cheese have long been known to exhibit anti-caries activity where the responsible components being identified as casein, calcium and phosphate. The repair of early tooth enamel lesions has been recently demonstrated by tryptic phospho-peptides derived from milk caseins that associate with amorphous calcium phosphate forming stable complexes. Casein phospho-peptides amorphous calcium phosphate (CPP-ACP) nano-complexes have shown to have anticariogenic potential in lab, animal and human in-situ experiments\textsuperscript{5-10}. The twice daily use of a 1.0 % W/V CPP-ACP solution produced a 51\% reduction in enamel mineral loss caused by frequent sugar-solution exposure. The CPP-ACP, unlike fluoride, can be added to sugar containing foods and therefore have commercial potential as an additive to foods as well as tooth pastes and mouth washes for control of caries\textsuperscript{11}. The addition of CPP-ACP to either sorbitol or xylitol – based gum resulted in a dose-related increase in enamel remineralization with 0.19, 10, 18.8 and 56.4 mg of CPP-ACP produced an increase in enamel remineralization of 9, 63, 102, and 152\% respectively, relative to the control gum, independent of gum weight or type\textsuperscript{5}. Moreover, Mazzaoui et al, in 2003,\textsuperscript{12} reported an enhanced release of calcium, phosphate and fluoride ions at neutral and acidic pH by incorporating CPP-ACP into self-cured glass ionomer cement.

An enamel improving cream containing casein phosphoprotein-calcium phosphate complex (Topical C-5) was introduced to the market. Researches proved that the depth of enamel demineralization was greatly reduced compared with teeth not covered with Topical C-5\textsuperscript{13}.

On the other hand, laser technology has launched among dentists after being approved by FDA and the inhibitory effects of laser irradiation on enamel demineralization have been clearly evidenced in the past two decades\textsuperscript{14-17}. The CO\textsubscript{2} laser irradiation by specific wavelengths resulted in a greater than 98% reduction in mineral loss and resistance to acid challenge to a depth about 54 um\textsuperscript{16}. Moreover, recent studies proved some synergistic benefits from combining of fluorides and laser treatment\textsuperscript{19-29}. The best results were obtained when the fluoride was used before irradiation at an energy density of 1.5 J/ cm\textsuperscript{2} per pulse \textsuperscript{30}.

The aim of the present study is to emphasize the synergistic effects of enamel improving cream (CPP-ACP) and laser treatment on human enamel and root dentin for inhibition of artificial caries. The ultra structure was also studied using polarized light microscope.

\section*{MATERIALS AND METHODS}

\subsection*{Tooth selection and grouping}

A total of 36 freshly extracted non-carious permanent human premolars scheduled for extraction for orthodontic reasons were selected for this study. Teeth were brushed with non-fluoridated tooth paste and soft brush, then washed and stored in saline solution at room temperature (26°C) to be used within the duration of one month. The solution was changed on weekly basis until the teeth were used. Teeth were then randomly divided into three main groups of 12 each according to the type of the treatment used. In the first group, the samples received CO\textsubscript{2} laser treatment; the second group of samples was subjected to combined treatment of laser and Topical C-5\textsuperscript{TM} enamel improving cream whereas the third group samples were treated with Topical C-5\textsuperscript{TM} alone.
Sample preparation

The samples were splitted into 2 halves mesio-distally, the buccal halves received the treatment while the lingual halves served as matched controls. The exposed pulpal sides were sealed with wax then coated with nail polish then samples were painted exposing only the buccal surface and part of the root surface.

CO₂ Laser irradiation treatment

The buccal surfaces were subjected to CO₂ irradiation by means of Uni-laser device (450P- ASAH Medico A/S Valseholmen 11 – 13 DK – 2650 Hvidovre Denmark). The irradiation parameters were set according to the study by Hsu et al; 2000 as follows: 2 watts, 5 millisecond per pulse, 20 Hz repetition rate, wave length = 10.6 um. The tip of laser unit was clamped to prevent movement, and the buccal surface was irradiated starting at the center then moving systematically to the peripheries. The amount of time for each spot was one second and this was repeated so that each spot in the buccal surface received four times. The samples were then rinsed with distilled water for 10 minutes. Thereafter, all teeth halves were submitted to demineralizing solution.

Topical C-5™ application

A sufficient amount (one small scoop 0.5 gm covered two buccal surfaces) of Topical C-5™ enamel improving cream* was dispensed to coat the buccal surface and the root dentin of the samples. After 4-min treatment, the cream was wiped off with tissue paper. The process was repeated 4 times then the samples were washed using distilled water then dried with a stream of air.

In the combination group, the tooth surfaces were irradiated after being treated with Topical C-5™ then washed and dried.

PH cycling process

The samples were then subjected to a four-day pH cycling scheme of 18 hours in demineralizing solution at a pH 4.6 then 6 hours in remineralizing solution at a pH 7.0 to form artificial caries-like lesions.

The demineralizing solution contained 0.05 M acetic acid, 2.2 M calcium and 2.2 M phosphate ions. The remineralizing solution contained 0.15 M potassium chloride, 1.5 M calcium and 0.9 M phosphate ions. The solutions were stirred constantly at a speed of 132 rpm. The pH cycling process started with the demineralizing phase then 5 min wash in the distilled water between demineralizing and remineralizing phases and at the end of the process. The solutions were not changed during the process and the samples were stored at plastic containers at 100 % humidity.

Roughness calculations

Surface roughness measurements were performed using profilometer (Portable surface roughness tester TR100) conforming to traceable standards. The large LCD display shows roughness parameter Ra, range 0.05 μm to 10.0 μm. The TR 100 tester operates on various surfaces, not only flat but also outer cylinder, outer cone, grooves, diamond tracer tip (radius 10 μm ± 10 μm) with tracing length 6 mm and tracing speed 1.0 mm/second. The experimental surfaces (buccal) and the control surfaces (lingual) (n=10) were adjusted in the horizontal plane parallel to the horizontal machine base. The profiles of different surfaces were determined and plotted. Three readings were recorded for each sample and their mean was calculated. The quantitative assessment was based on automatic calculation of the mean Ra value (central line average). It is an expression of the arithmetic mean for the absolute value of the deviation of the profile curve from the mean line within the length of the reference or its absolute distance from the actual profile to the medium profile.

* Topical C-5™, a thixotropic tooth surface coating cream which contains phoscal™, casein phosphoprotein –calcium phosphate complex NSI Dental Pty Ltd, Hornsby NSW 1630 Australia.
The mean values of each group were then used for statistical analysis. One way analysis of variance (ANOVA) and Post-Hoc test for multiple comparisons (Bonferroni method) were used to compare the mean of different groups. The statistical difference was considered significant when P value <0.05.

**Polarized light microscopy**

Two samples from each group were processed for polarized light microscope. Serial ground sections of 20 microns thickness were prepared from each longitudinal section of the different groups. Sections were mounted on glass slides and Canada balsam was applied as a mounting medium for examination under polarized light microscope (BX 60, Olympus, Japan). The surface and subsurface changes of enamel were observed.

### RESULTS

#### Roughness results

**I- Enamel Treatment**

When comparing the mean Ra values of roughness in different groups after enamel treatment, statistical analysis revealed a significant difference between groups (P = 0.0023) (Table 1). Post Hoc multiple comparison test (Bonferroni method) revealed statistically insignificant increase in Ra roughness values of the experimental group of enamel cream surface treatment as compared to their controls (P=1) (Table 2).

On the other hand, there was statistically significant increase in the roughness Ra values for the experimental groups; laser and combined enamel cream and laser treatment as compared to their controls. (P= 0.0232 and 0.0141 respectively).

**TABLE (1) Analysis of Variance (ANOVA) test for comparison of mean Ra roughness values in different groups for enamel surface treatment (n=10).**

<table>
<thead>
<tr>
<th>Ra roughness</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
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<td>40.2804</td>
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<td>Within Groups</td>
<td>65.6985</td>
<td>12</td>
<td>5.4749</td>
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</tr>
<tr>
<td>Total</td>
<td>267.1005</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE (2) Post Hoc multiple comparisons test (Bonferroni method) for comparison of mean Ra values in different groups for enamel surface treatment (n=10). S= significant, NS= insignificant.**

<table>
<thead>
<tr>
<th></th>
<th>Mean Ra</th>
<th>S D</th>
<th>% of Change</th>
<th>P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enamel Cream</strong></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>control</td>
<td>132.3300</td>
<td>0.0300</td>
<td>2.49</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>experimental</td>
<td>134.2100</td>
<td>0.0800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Laser</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>129.4600</td>
<td>0.0600</td>
<td>4.75</td>
<td>0.0232</td>
<td>S</td>
</tr>
<tr>
<td>experimental</td>
<td>137.2400</td>
<td>0.0400</td>
<td></td>
<td></td>
<td></td>
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<td><strong>Combined</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>128.5167</td>
<td>5.7303</td>
<td>3.69</td>
<td>0.0141</td>
<td>S</td>
</tr>
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<td>0.0400</td>
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</tbody>
</table>
II- Root dentin treatment

When comparing the mean Ra values of roughness in different groups after root dentin treatment, statistical analysis revealed a significant difference between groups (P = 0.0395) (Table 3).

Post Hoc multiple comparison test (Bonferroni method) revealed statistically insignificant increase in Ra roughness values between the control and experimental groups of enamel. Post Hoc multiple comparison test (Bonferroni method) revealed statistically insignificant increase in Ra roughness values between the control and experimental groups of enamel cream, laser or combined surface treatment (P=1 & 1 & 0.976 respectively) (Table 4).

Polarized light microscopy

Examination of the enamel by polarized light microscope performed after treatment with enamel improving cream revealed loss of normal prismatic architecture and replacement with focal translucent areas of different densities. These areas extended from the sub-surface zone till the dentino-enamel junction where the translucent remineralization areas were more evident (Figure 2 a). On the other hand, CO₂ laser surface treatment revealed wide loss of prismatic structure with disappearance of incremental lines of the enamel and replacement by focal translucent areas of remineralization that appeared to fill most of the dark demineralization zones (Figure 2b). Combined surface treatment showed alternative translucent remineralization and dark demineralization zones that extended deep into the dentino-enamel junction. The surface enamel showed loss of incremental lines and presence of a wide band of translucent remineralization area (Figure 2c).

A wide zone of remineralization of dentine was noted with enamel cream. This reaction appeared more obvious in the deeper layers of dentine and alternating bands of translucent and dark areas were also observed near the surface (Figure 3a). The same reaction was also recognized with CO₂ laser surface treatment. However, the remineralization translucent zone was extending into deeper layers of dentine with an intermediate dark zone (Figure 3b). Combined treatment revealed a homogeneous translucent wide zone that extended from the surface into the deeper layers of dentine with no dark demineralization intermediate zone (Figure 3c).

### Table 3

<table>
<thead>
<tr>
<th>Ra Roughness</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<td>45.8626</td>
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<td>0.0395</td>
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<tr>
<td>Within Groups</td>
<td>163.5423</td>
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<td>13.6285</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>392.8554</td>
<td>17</td>
<td></td>
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<td></td>
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</tbody>
</table>
TABLE (4) Post Hoc multiple comparisons test (Bonferroni method) for comparison of mean Ra values in different groups for root dentin (n=10)

<table>
<thead>
<tr>
<th></th>
<th>Mean Ra</th>
<th>S D</th>
<th>% of Change</th>
<th>P Value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel Cream</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>142.023</td>
<td>4.587</td>
<td>2.510</td>
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<td>NS</td>
</tr>
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<td>experimental</td>
<td>144.100</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laser</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>143.357</td>
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<td>4.220</td>
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<td>NS</td>
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<tr>
<td>experimental</td>
<td>146.753</td>
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<tr>
<td>Combined</td>
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</tr>
<tr>
<td>control</td>
<td>135.137</td>
<td>4.124</td>
<td>4.370</td>
<td>0.976</td>
<td>NS</td>
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<tr>
<td>experimental</td>
<td>141.257</td>
<td>3.281</td>
<td></td>
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</tbody>
</table>

Fig. (2) Photomicrograph of surface treatment of enamel (Polarized light x4). (A) Enamel cream showing focal translucent areas of different densities. (B) CO₂ laser showing translucent areas of remineralization that appeared to fill most of the dark demineralization zones. (C) Enamel cream combined with CO₂ laser showing alternative translucent remineralization and dark demineralization zones that extended deep into the dentino-enamel junction.

Fig. (3) Photomicrograph of root dentin surface treatment (Polarized light x4). (A) Enamel cream showing wide zone of remineralization of dentine that appeared more obvious in the deeper layers of dentine. (B) CO₂ showing the remineralization translucent zone that extended into deeper layers of dentine with an intermediate dark zone. (C) Enamel cream combined with CO₂ showing homogeneous translucent wide zone of remineralization that extended from the surface into the deeper layers of dentine.
In this study, enamel surfaces treated with enamel improving cream expressed an insignificant increase in surface roughness as compared to their controls (Fig. 1). By examination of enamel surface by polarized light microscope, focal translucent areas of different densities were observed extending from the subsurface zone till the dentino-enamel-junction where these areas were more evident. These findings support previous studies who confirmed the role of CPP-ACP in depressing enamel demineralization and enhancing remineralization. The enamel improving cream Topacal C-5 contains Phoscal which is unique complex between the casein protein derived from milk, and calcium phosphate that is the principal building block of teeth. Thus it may boost the natural protective and repair functions of saliva.

The proposed anticariogenic mechanism of CPP-ACP is the incorporation of the nano-complexes into the plaque and onto the tooth surface. The localized CPP-ACP nano-complexes purportedly then act to buffer the free calcium and phosphate ion activation, thereby maintaining a state of supersaturation with respect to tooth enamel, preventing enamel demineralization and promoting remineralization. Moreover, the remineralization results demonstrate the importance of CPP in stabilizing ACP and producing a highly soluble calcium phosphate phase thereby facilitating remineralization. The protective effect of the enamel cream may also involve interaction with salivary proteins forming enamel pellicle as the integrity of the tooth enamel is maintained by selective adsorption of salivary proteins that form a protective barrier. The polishing of the specimens at the initiation of this study might have also played a role as the contaminated enamel surface was removed exposing fresh enamel more prone to calcium uptake.

On the other hand, CO₂ laser as well as combined enamel improving cream /laser treatment of enamel exhibited significant increase in surface roughness compared to the controls. Focal translucent areas of remineralization were observed by polarized light microscope in enamel that received laser treatment, while the combined treatment showed alternative translucent remineralization and dark demineralization zone extending deep into the dentino-enamel junction.

High energy laser irradiation at specific wavelength has been shown to cause crystalline transformation in apatite, which may lead to the formation of tri-calcium phosphate with a resulting decrease in acid resistance. The decrease in solubility was attributed to the change in the rate of dissolution kinetics due to the change from the more accessible dissolution site of hydroxyapatite to a less reactive site of heat treated apatite. Another explanation for the resistance of lased enamel to subsurface demineralization is the carbonate loss, which is a soluble mineral from carbonated apatite tooth mineral. This carbonate content being less stable and having poor fit in the crystalline lattice, its loss could decrease demineralization of the substrate. In addition, less soluble tetra calcium diphosphate monoxide was identified as being a component of the melting surface and this layer presented reduced carbonate content. The combination of the reduced enamel permeability together with the reduced solubility with melting, fusion and recrystalization of enamel crystallites thereby sealing the enamel surface, could protect enamel against the subsequent acid challenge.

Meanwhile, the significant increase in the surface roughness of enamel subjected to CO₂ laser in this study could be attributed to the surface changes induced by the action of laser which varied from crazing, cratering and exfoliation of surface enamel. These micro morphological changes could also allow the efficient incorporation of the enamel cream containing calcium nano-complexes into the tooth in the combination group, where the casein phosphoprotein carrying the
stabilized nano-clusters of hydrated calcium phosphate bind onto the intercellular matrix macro-molecules localizing the bioavailable calcium and phosphate ions at the tooth surface.

In such a way, these numerous nano-clusters of calcium deposits on the dental surfaces could act as a reservoir to replenish the soluble calcium and phosphate ions capable of diffusing into the subsurface enamel thereby augmenting the action of laser to inhibit the demineralization.

Of particular interest is that the results of the present study suggested that the remineralization effect of the different treatments used appeared to be more pronounced on the root dentin. Several hypotheses have been proposed concerning the carious lesion inhibition in root dentin by CO₂ laser. The crystallographic changes that occurred due to laser irradiation namely the recrystalization and crystalline growth were observed and the dentin with a low order of crystallinity, structurally changed in such a way as to closely resemble the crystalline structure of the hydroxyapatite of normal enamel. Moreover, the preferential removal of the inherent content of water and proteins, being much higher in dentin than enamel, by laser irradiation may result in decreasing the contribution of the mineral phase and emphasizing the role of water and proteins in the light absorption. Furthermore, having much greater porosity and surface roughness than enamel, the root dentin may have much greater propensity for calcium and phosphate deposits than enamel, with a much higher crystalline density than that of the root. This is in addition to the surface changes observed on the root dentin surfaces by laser irradiation ranging from charring, cratering, poring, fissuring and cracking up to melting. Many small molten and rehardened particles were found on the lased dentin surfaces.

This could also account for the insignificant percentage of change in the root surface roughness for all groups as compared to their controls (Table 4 and figure 1). This was also supported by the polarized light microscopic observations where the treated dentinal surfaces showed wide zones of remineralization extending in deep layers of the dentin structure for both enamel cream and CO₂ laser treatments (figure 3a&b).

It seems that the inherent dentin surface roughness as well as the surface changes produced by the laser effects could enhance the binding of enamel improving cream to the hydroxyapatite in deeper layers thus reducing susceptibility to cariogenic challenge and providing an additive effect compared with the separate remineralization effects of laser or enamel cream. The permeability of dentin surfaces could have also played a role in this respect as it was reported that the amount and depth of fluoride in dentin were several times greater than those in enamel. This holds true, specifically that the polarized light microscopic examination revealed a wide homogenous translucent zone of remineralization extending from the surface to deeper layers of dentin with almost no evidence of demineralization (figure 3c).

CONCLUSIONS

1- Both enamel improving cream and CO₂ laser treatment were effective in inhibiting demineralization and enhancing remineralization.

2- There is a significant synergistic effect of combined enamel improving cream and CO₂ laser treatment on inhibition of subsequent cariogenic attack in root dentin.

3- Enamel improving cream and CO₂ laser treatment individually and in combination created surface and subsurface alterations that could provide protection against cariogenic attack though more evident in root dentin.

4- The combined therapies seem to be a promising approach to prevent or control caries attack.
RECOMMENDATIONS

1- The combination of laser treatment and enamel improving cream is appreciated for inhibition of demineralization of root dentin.

2- Further studies are recommended to investigate the effects of different energy intensities of laser in the combination treatment for caries prevention as well as their effects on the pulp

REFERENCES


