



## Research article

Effect of sodium fluoride plus tricalcium phosphate with and without CO<sub>2</sub> laser on remineralization of white spot lesionsNouran M. Eissa<sup>\*</sup>, Eman M. Elshourbagy, Nahla E. Gomaa

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## ABSTRACT

**Objectives:** The aim of this in vitro study was to evaluate the effect of NaF plus TCP with and without CO<sub>2</sub> laser irradiation on management of demineralized enamel using microhardness test and digital microscopy.**Methods:** Eighty intact extracted human premolar teeth were randomly divided into 4 groups (20/each). Each group was subjected to a demineralizing solution to create white spot lesion. **Group 1** was treated with 3M Vanish™. **Group 2** was irradiated with CO<sub>2</sub> laser. **Group 3** was subjected to CO<sub>2</sub> laser followed by 3M Vanish™. **Group 4** was treated by 3M Vanish™ then CO<sub>2</sub> laser. The teeth were immersed in artificial saliva. Surface microhardness was measured for each tooth before demineralization at base line (M1 as a control), after demineralization (M2) and after management (M3). Comparison of microhardness values between groups was performed using one way ANOVA test with significant level (0.05) followed by multiple comparisons post-hoc Tukey test between groups. Enamel surface was photographed by digital microscope.**Results:** All intervention methods used in the current study significantly increased microhardness values of demineralized enamel ( $P < 0.001$ ). Little improvement of enamel appearance was observed in all groups meanwhile using CO<sub>2</sub> laser on demineralized enamel directly led to signs of white and black dots affecting the appearance of enamel surface.**Significance:** The most effective intervention regarding microhardness was group 2 followed by group 3, group 4 and group 1. Coating the enamel surface with 3M Vanish™ before CO<sub>2</sub> laser irradiation acted as a protective layer from the undesirable effects of laser on the teeth with increasing enamel microhardness values more than using Vanish alone. So the promising intervention method regarding both microhardness and appearance was group 4.

## 1. Introduction

The occurrence of white spot lesions (WSLs) is a concern for orthodontists whose patients are treated with fixed appliances especially in the presence of poor oral hygiene and creates an esthetic problem for the patients [1]. The irregular surfaces of brackets, bands, wires, and other attachments hinder naturally self-cleaning mechanisms and increase the accumulation and retention of bacterial plaque on the labial surface of crowns which results in enamel demineralization [2].

WSLs are manifested clinically as an early enamel caries and its white appearance is caused by an optical phenomenon owing to subsurface tissue loss and is exaggerated by thorough drying. The prevalence of WSLs was reported to vary from 2 to 96%, according to different methods of diagnosis and grading of WSLs. [1, 3, 4, 5].

The management of WSLs can be divided into remineralization techniques, noninvasive infiltration and surface alternation of

the lesion [6]. Remineralization techniques can be categorized into fluoride and non-fluoride remineralization systems, noninvasive infiltration as resin infiltration techniques and surface alternation as hard tissue lasers [6, 7]. Remineralization can be defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to produce net mineral gain by promoting ions deposition into crystal voids in demineralized enamel [8].

Calcium-phosphate-based remineralization delivery systems provides stabilization of bioavailable calcium, phosphate and fluoride ions and the localization of these ions to non-cavitated caries lesions for controlled remineralization [9]. The combination of fluoride and functionalized  $\beta$ -tricalcium phosphate gives stronger, more acid-resistant mineral relative to fluoride or functionalized tricalcium phosphate (fTCP) alone. The functionalization of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) with organic or inorganic molecules provides a barrier that prevents premature

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fluoride-calcium interactions and helps in mineralization when applied via common procedures [10].

3M™ Vanish™ 5% Sodium Fluoride (NaF) White Varnish contains 5% NaF and fTCP, which is sold exclusively through 3M. The varnish is an alcohol-based suspension of modified resin. Vanish White Varnish is sweetened with xylitol. The product is supplied in unit-dose packages containing 0.5 ml of Vanish White Varnish. Each 0.5 ml dose contains 25 mg of NaF, equivalent to 11.3 mg of fluoride ion.

fTCP is a hybrid material produced by a milling technique that fuses  $\beta$ -TCP and sodium lauryl sulfate or fumaric acid. This blending produces a functionalized calcium and a free phosphate, designed to improve the efficacy of fluoride remineralization [11, 12]. The combination of fluoride and fTCP can produce more acid-resistant and stronger mineral relative to fluoride [10].

CO<sub>2</sub> laser (with 9.3, 9.9, 10.3 and 10.6  $\mu$ m wavelengths) is considered a main laser type in inhibition of caries formation because the absorption bands of phosphate, carbonate, and hydroxyl groups of enamel and dentin structures are in the range of 9–11  $\mu$ m. Structural and chemical alternations in enamel occurred after laser absorption. These alternations include decomposition of organic matrix, decrease in carbonate compounds, and fusion and recrystallization of hydroxyapatite crystals that make enamel more resistant to acid solution [13, 14, 15, 16].

The increase in acid resistance is due to the melting and fusion of enamel hydroxyapatite crystals and the sealing of the enamel surface. The melting of the enamel surface was not homogenous and usually occurred in limited areas. Beneath the melted surfaces, there is a significant increase in inter- and intra-crystalline voids. The new chemical products in the melted enamel structure are less resistant to acid attack than is enamel hydroxyapatite [6, 17].

As attention has been focused on the combination of laser radiation and topical fluoride to achieve additional effect on prevention and treatment of enamel demineralization. Therefore, it is important to study the effect of CO<sub>2</sub> laser, 3M™ Vanish™ and their combination on management of demineralized enamel.

## 2. Materials and methods

### 2.1. Sample size calculation

At a confidence level of 95%, a power of 80%, a ratio of one (exposed: nonexposed) {this means equal number of teeth in each group of the study}, and an average of improvement in hardness from the baseline among all the intervention groups of about 40%. The average change in hardness in the baseline measurements was estimated to be 1%. So the least calculated sample size needed in each group was estimated to be  $N = 16$  teeth.

The sample size increased to include  $\{N = 20\}$  teeth in each group to cover the differences of improved hardness within the groups. The total sample size was  $\{N = 80\}$  teeth.

Eighty human premolar teeth that were extracted as a part of orthodontic treatment were included in the current study. The extracted teeth were collected from orthodontic clinics after taking informed consents from patients requiring extraction for orthodontic treatment.

### 2.2. Inclusion criteria of the sample

The teeth included in the current study were fulfilled the following criteria: Intact buccal enamel, no caries or hypoplastic areas, no cracks or gross irregularities of enamel surface, no restorations.

### 2.3. Ethical considerations

The purpose of the present study was explained to the parents and informed consents were obtained from them, in addition to the ascent from the children to use the extracted teeth in the research according to

the guidelines on human research adopted by the Research Ethics Committee, Faculty of Dentistry, Tanta University.

### 2.4. Samples cleaning and storage

The selected teeth were cleaned using toothbrush and scaled with periodontal scaler to remove soft tissue debris. The teeth were stored in de-ionized water at room temperature and used within one month after extraction.

### 2.5. Sample preparation

The crown of each tooth was separated from the root using a high-speed diamond tipped disc.

Each crown was embedded in an acrylic block with the buccal surface faced upward and parallel with the base. The acrylic blocks were randomly divided into 4 groups and color coded: group I taking green color, group II taking violet color, group III taking red color, group IV taking yellow color. The buccal enamel surfaces of the teeth were polished with 600, 800, 1200 grit paper consecutively to remove aprismatic enamel layer.

### 2.6. Creation of WSL

Two layers of acid-resistant nail varnish (former, Milano, Italy) was applied on each tooth, leaving a window of 4 mm  $\times$  4 mm of enamel exposed at the middle third of the tooth representing orthodontic bonding area. These standardized windows were made by using a custom-made square sticker that was pasted on the center of tooth surface. All the samples were immersed into a container containing 40 ml of demineralizing solution for a period of 72 h at 37 °C using an incubator (BTC, Egypt) no stirring [18].

The composition of demineralizing solution used: 8.7 mmol/L CaCl<sub>2</sub>, 8.7 mmol/L KH<sub>2</sub>PO<sub>4</sub>, 0.05 ppm sodium fluoride NaF, 75 mmol acetic acid, the final pH was adjusted to 4 with KOH [18].

The pH value was checked daily using a pH meter. The solution was changed with fresh solution every day. After 72 h, all samples were taken out of the solution, rinsed with running tap water for 5 min, rinsed again with distilled water for 30 s and dried with air to be visualized for their chalky white appearance.

### 2.7. Experimental groups

The teeth were randomly divided into 4 groups of 20/each.

**Group 1:** The demineralized enamel surface was treated with a thin layer of 3M™ Vanish™ according to the manufacturer's instructions. The tooth was rinsed and dried, and the material was applied to the tooth surface and left for 1 min [19].

**Group 2:** The tooth was irradiated with ablative fractional CO<sub>2</sub> laser (DEKA Laser Technologies, Florence, Italy) at a wavelength of 10.6  $\mu$ m, a frequency of 5 Hz, an output power of 0.4 W and an operation time of 0.9 s based on a previous study [20]. The procedure was carried out with a uniform scanning motion from an approximate distance of 5 mm between the enamel surface and the tip of indenter in total time of 20 s to cover the entire sample area and promote homogenous irradiation [20].

**Group 3:** The demineralized enamel was first irradiated with CO<sub>2</sub> laser as explained for Group 2, followed by application of white varnish according to the manufacturer's instructions.

**Group 4:** The demineralized enamel in this group was covered with a thin layer of white varnish according to the manufacturer's instructions, then laser beams with the same parameters described for Group 2 were applied through the paste over a period of 20 s.

- Then all the samples were washed with **de-ionized water** and immersed in the artificial saliva in a separate closed container.

## 2.8. Artificial saliva

Each sample were immersed into a separate container containing 30 ml of artificial saliva for a period of 28 days at 37 °C using an incubator [21] (Figure 1).

The composition of artificial saliva used: 0.33 g  $\text{KH}_2\text{PO}_4$ , 0.34 g  $\text{Na}_2\text{HPO}_4$ , 1.27 g KCl, 0.16 g NaSCN, 0.58 g NaCl, 0.17 g  $\text{CaCl}_2$ , 0.16 g  $\text{NH}_4\text{Cl}$ , 0.2 g urea, 0.03 g glucose, 0.002 g ascorbic acid in 1000 ml distilled water. The final pH was adjusted 7.00 at 37 °C with 85% lactic acid [21, 22]. The pH value was checked daily using a pH meter. The solution was changed with fresh solution every 48 h [21].

All samples were taken out of the solution, rinsed with running tap water for 5 min, then rinsed again with distilled water for 30 s and dried with air.

## 2.9. Microhardness test

The Vickers hardness number (VHN) in all teeth was measured with a Vickers diamond indenter. Surface microhardness was measured for each tooth before demineralization at base line as a control (M1), after demineralization (M2) and after management (M3). A Vickers microhardness tester (INDENTEC, Zwick/Roell, U.K) was used under a 300-g load and a dwell time of 15 s to assess Vickers microhardness and using a 10X objective lens. The indenter was placed on a 4 × 4-mm exposed enamel surface. For each sample three indentations were made on three points of enamel surface and the mean value was recorded as VHN [20]. Indentation result was seen at projector screen in the form of shadow shaping rhomb; the diagonal length was measured with micrometer.

## 2.10. Digital microscopy

The appearance of the enamel surface was photographed by digital microscope after demineralization and after management of tooth. Five samples from each group were evaluated by digital microscope. Specimens were photographed using USB Digital microscope with a built-in camera (Scope Capture Digital Microscope, Guangdong, China) connected with a compatible personal computer using a fixed magnification of 50X and at constant distance between the enamel surface and digital microscope. The images were recorded with a resolution of 1280 × 1024 pixels per image. The appearance of enamel after demineralization and formation of WSL was compared with its appearance after management.



Figure 1. Each sample were immersed into a separate container in the incubator.

## 2.11. Statistical analysis

Descriptive statistics including the mean, standard deviation, maximum and minimum values, standard error and 95% confidence interval were calculated for each group. In addition, the comparison of two dependent quantitative variables was calculated by T paired test while the comparison of quantitative variable among the five independent groups was calculated by ANOVA test along with post hoc analysis (if needed). Significant differences for all statistical tests were predetermined at  $p \leq 0.05$ . All statistical evaluations were made with a software program (SPSS version 23).

## 3. Results

### 3.1. Microhardness test evaluation

The descriptive statistics for microhardness values before demineralization base line (M1), after demineralization (M2) and after management (M3) are presented in Table 1 including the mean, standard deviation, minimum and maximum values, standard error and 95% confidence interval.

The percentage of surface hardness recovery  $(M3 - M2)/(M1 - M2)$  was 41.17% for group 1, 62.42% for group 2, 57.91% for group 3 and 41.91% for group 4.

The results of one way ANOVA test revealed statistically non-significant difference in M1 and M2 values among the group ( $P = 0.568$ ) ( $P = 0.639$ ) respectively and statistically significant difference in M3 values ( $P = 0.013$ ;  $P \leq 0.05$ ) among the groups. Applying multiple comparison post hoc Tukey's test for microhardness values after management (M3) as illustrated in Table 2 revealed statistically significant difference between group 2 and group 4 ( $P = 0.047$ ), whereas no statistically significant difference between the rest of groups was found.

As illustrated in Table 3, mean microhardness values of the management groups were compared using the paired t-test. There were highly significant difference ( $P < 0.001$ ) between M2 and M3 microhardness values of the groups.

The statistical analysis of microhardness values for difference between M3-M2 with one way ANOVA test showed that there was a highly significant difference among the groups  $P < 0.001$  as appeared in Table 4. Group 2 yielded the highest mean of microhardness value (90.8 HV) followed by group 3 (88.05 HV) then group 4 (71.2 HV) and finally group 1 (52.1 HV).

The results of post hoc Tukey's test between the four managed groups as illustrated in Table 4 revealed that there was a highly significant difference ( $p \leq 0.001$ ) between group 1 and group 2 and between group 1 and group 3. A significant difference between group 1 and group 4 ( $P = 0.022$ ) and group 2 and group 4 ( $P = 0.018$ ) was found. No significant difference between group 2 and group 3 ( $P = 0.973$ ) and group 3 and group 4 ( $P = 0.054$ ) was detected.

### 3.2. Digital microscope image observation

Comparing the appearance of enamel surface before and after management of WSLs by White Varnish in group 1 resulted in some improvement in appearance of enamel surface with visible WSL. In group 2 and group 3 showed slight improvement in appearance of enamel surface in addition to signs of white dots representing non-homogenous surface management and carbonization (black dots) affecting the appearance of some teeth in some areas. In group 4 showed some improvement in appearance of enamel surface and slightly visible WSL with no signs of dots and black dots (Table 5).

**Table 1.** Microhardness values.

Group	SD ± Mean	Min	Max	Std.error	95% Confidence Interval		Percentage of surface hardness recovery	
					Lower Bound	Upper Bound		
Group 1	M1	41.206 ± 327.7	226	387	9.214	308.415	346.985	41.17%
	M2	43.035 ± 201.15	128	289	9.623	181.009	221.291	
	M3	40.575 ± 253.25	181	313	9.073	234.26	272.24	
	M3-M2	13.345 ± 52.1	24	74	2.984	45.854	58.346	
Group 2	M1	48.297 ± 338	237	411	10.8	315.396	360.604	62.42%
	M2	41.347 ± 192.45	134	287	9.246	173.099	211.801	
	M3	39.256 ± 283.3	228	378	8.778	264.927	301.673	
	M3-M2	22.553 ± 90.85	51	140	5.043	80.295	101.405	
Group 3	M1	39.434 ± 342.8	259	391	8.818	324.394	361.306	57.91%
	M2	48.26 ± 190.75	100	267	10.791	168.164	213.336	
	M3	33.443 ± 278.8	223	345	7.478	263.148	294.452	
	M3-M2	25.228 ± 88.05	49	142	5.641	76.243	99.857	
Group 4	M1	38.403 ± 349.3	288	409	8.587	331.326	367.274	41.91%
	M2	49.363 ± 179.4	101	251	11.038	156.297	202.503	
	M3	42.173 ± 250.6	180	317	9.43	230.862	270.338	
	M3-M2	19.182 ± 71.2	42	123	4.289	62.222	80.178	

**Table 2.** One way ANOVA test for microhardness values among groups followed by multiple comparison by post hoc Tukey's test.

	Group 1	Group 2	Group 3	Group 4	F	P value
	Mean					
Base line (M1)	327.7 ± 41.2 (226–387)	338 ± 48.29 (237–411)	342.8 ± 39.43 (259–391)	349.3 ± 38.4 (288–409)	0.739	0.568
After demineralization (M2)	201.2 ± 43.03 (128–289)	192.5 ± 41.3 (134–287)	190.8 ± 48.3 (100–267)	179.4 ± 49.4 (101–251)	0.635	0.639
After management (M3)	253.3 ± 40.6 (181–313)	283.3 ± 39.3 (228–378)	278.8 ± 33.4 (223–345)	250.6 ± 42.2 (180–317)	3.77	0.013*
Post Hoc Tukey test						
P1 0.078	(between group 1 and 2)					
P2 0.171	(between group 1 and 3)					
P3 0.996	(between group 1 and 4)					
P4 0.983	(between group 2 and 3)					
P5 0.047*	(BETWEEN GROUP 2 AND 4)					
P6 0.11	(between group 3 and 4)					

**Table 3.** Paired t-test of Microhardness values between (M2) and (M3).

Group	After demineralization (M2)	After management (M3)	T	P Value	
GROUP 1	Mean ± SD	201.15 ± 43.035	253.25 ± 40.575	-17.45	<0.001*
GROUP 2	Mean ± SD	192.45 ± 41.347	283.3 ± 39.256	-18.01	<0.001*
GROUP 3	Mean ± SD	190.75 ± 48.26	278.8 ± 33.443	-15.6	<0.001*
GROUP 4	Mean ± SD	179.4 ± 49.363	250.6 ± 42.173	-16.59	<0.001*

#### 4. Discussion

A lot of studies were conducted to prevent and manage WSLs with different techniques [20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]. The current study evaluated the efficiency of NaF plus TCP, CO<sub>2</sub> laser irradiation and their combination on microhardness of demineralized enamel.

A combination of a laser and fluoride is considered the most promising treatment for caries prevention. This combination improved fluoride uptake, decreased enamel solubility in acid, reduced the progression of caries like lesions, and this treatment was more effective than laser or fluoride alone [37]. CO<sub>2</sub> laser application before fluoride compounds increased the uptake of fluoride and converted hydroxyapatite into fluorapatite in the

presence of fluoride by incorporating fluoride into apatite structure [38, 39].

In the present study, results revealed that microhardness values increased after management with 3M Vanish™ in group 1. This result supports the finding of Ulkur et al [23], Elkassas and Arafa [24], Said et al [25], Memarpour et al [21] and Soltaninehr et al [40] who reported that TCP varnish had a greater effect on the microhardness of enamel. fTCP has the highest potential for remineralization and Clinpro™ White Varnish (5% NaF with fTCP) had a significant remineralization effect on enamel carious lesions when evaluated by surface microhardness.

Microhardness values increased significantly after management by CO<sub>2</sub> laser in group 2. This result is in agreement with Farhadian et al [20], Rafiei et al [41] and Fekrazad et al [42] who showed that the CO<sub>2</sub> laser irradiation increased demineralized enamel microhardness. The





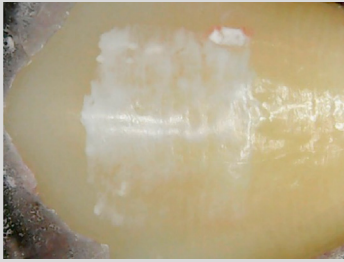



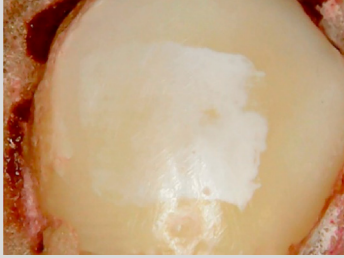

**Table 4.** ANOVA test followed by multiple comparison by post hoc Tukey's test of Microhardness values for difference between M3-M2 among the groups.

M3-M2	Group 1	Group 2	Group 3	Group 4	F	P value
	Mean ± SD (range)					
	52.1 ± 13.3 (24–74)	90.8 ± 22.5 (51–140)	88.05 ± 25.23 (49–142)	71.2 ± 19.2 (42–123)	15.1	<0.001*
post hoc Tukey's test						
P1 <0.001*	(BETWEEN GROUP 1 AND 2)					
P2 <0.001*	(BETWEEN GROUP 1 AND 3)					
P3 0.022*	(BETWEEN GROUP 1 AND 4)					
P4 0.973	(Between group 2 and 3)					
P5 0.018*	(BETWEEN GROUP 2 AND 4)					
P6 0.054	(Between group 3 and 4)					

combination of CO<sub>2</sub> laser and 3M Vanish™ had a significant effect on increasing the microhardness values in group 3 and group 4. These results are in agreement with, Mahmoudzadeh et al [43], Poosti et al [35], Rafiei et al [41], Fekrazad et al [42] and Belcheva et al [44] who used CO<sub>2</sub> combined with others material containing fluoride.

Mahmoudzadeh et al [43] found that combination of fluoride varnish and CO<sub>2</sub> laser irradiation is able to decrease enamel demineralization around orthodontic brackets using SEM in evaluation. Poosti et al [35] revealed that CO<sub>2</sub> laser irradiation before fluoride treatment caused a significant increase in the surface microhardness of the enamel.

**Table 5.** Enamel surface appearance before and after management of WSLs with digital microscope.

	Before management	After management
Group 1		
Group 2		
Group 3		
Group 4		

Rafiei et al [41] showed that the combined application of CO<sub>2</sub> laser with Remin Propaste was suggested for rehardening of WSLs. Fekrazad et al [42] reported that CO<sub>2</sub> laser irradiation after fluoride application increased the microhardness of initially demineralized enamel and Belcheva et al [44] showed that the sub-ablative CO<sub>2</sub> laser irradiation combined with fluoride treatment has a great effect in protecting enamel surface and resisting demineralization than CO<sub>2</sub> laser irradiation or fluoride alone.

On the other hand, Farhadian et al [20] reported that the application of CPP-ACP paste either alone or in combination with CO<sub>2</sub> laser did not increase enamel microhardness significantly compared to the control. Soltanimehr et al [40] showed that no synergistic effect was found between materials and lasers.

There is controversy regarding whether laser irradiation should be performed before or after fluoride application. Tagomori et al [39] showed significantly higher acid resistance when enamel was irradiated before fluoride application. Esteves et al [45] believed that laser application should be performed following fluoride treatment. Poosti et al [35] indicated that the application of a fractional CO<sub>2</sub> laser either before or through the APF was more effective in restoring the appearance of carious enamel and irradiation prior to fluoride application was more effective in increasing surface microhardness but surface cracking and melted areas were not observed when laser was applied after fluoride application.

In the current study, digital microscope was used to detect the appearance of enamel surface before and after management. Image analysis of clinical digital photographs is a method for evaluation of demineralized areas and has attracted wide attention [46]. On the other hand Guotao et al [47] revealed that the prevalence of enamel demineralization is overestimated when detected by digital photographs due to the flashlight from the camera and light reflection from the environment which provides a false WSL on the enamel surface. Benson et al [46] found that the camera flash and the angle of the camera makes a difference to the area measured and images taken at a larger angle than 20° to the perpendicular will record a significantly reduced area of demineralization. This explains partly why the WSLs still evident with little improvement in all management groups and taking into consideration the short duration management.

It was found that in group 1, there is little improvement in appearance of enamel surface with visible WSL. This may be attributed to short duration of the study. Multiple applications of 3M Vanish™ may be recommended for further enhancing its mineralizing effect. As the study period of 28 days may be regarded as short compared with other studies [48, 49]. Salamara et al [48] reported that the 5% NaF with fTCP appeared clinically effective in reversing post-orthodontic WSLs 16 weeks after debonding. Kannan et al [49] showed that Clinpro™ XT varnish significantly restored the colour and lightness of the WSLs significantly at 3 and 6 months.

In group 2 and in group 3, some improvement in appearance of enamel surface was found in addition to signs of white dots representing non-homogenous surface management and black dots affecting the appearance of some areas of managed enamel. This result supports the finding of Poosti et al [35], Hossain et al [50] and Yamada et al [51]. Poosti et al [35] observed surface cracking and melted areas when fractional CO<sub>2</sub> laser was applied before fluoride application, they also used fractional type of CO<sub>2</sub> laser as used in the current study. Hossain et al [50] noted slight carbonization on the enamel and dentin surfaces with the laser irradiation. Yamada et al [51] reported that CO<sub>2</sub> lasers irradiation on the enamel surface showed an opaque white color, but on the dentin showed signs of carbonization (black) affecting the appearance of tooth.

According to the parameters of CO<sub>2</sub> laser used in the current study, the power density was 50 J/cm<sup>2</sup>. Stern et al [52] utilized a pulsed CO<sub>2</sub> laser with energy density range from 13 to 50 J/cm<sup>2</sup> on the structure and appearance of the human enamel surface, they found that the enamel surface became fissured and porous at an energy density of 50 J/cm<sup>2</sup>.

Similarly, previous studies used CO<sub>2</sub> laser at 5 mm distance on the sample area as that applied in the present study [20, 43, 53]. On the other hand Fekrazad et al [42] applied CO<sub>2</sub> laser at 2 cm distance away from the enamel surface. Decreased distance and smaller irradiation point of laser in the present study may have increased the irradiance of the laser on enamel surface with unfavorable mechanical alterations. Accordingly, the distance between the tip of the indenter and the tooth surface may be needed to increase for decreasing the heat generation.

Several studies used the same laser parameters that used in the current study to detect the effect of CO<sub>2</sub> laser on prevention of WSL and the application of laser was on sound enamel [43, 53]. Whereas in the current study, the laser was applied on demineralized enamel. Laser parameters plays a key role in the effect of laser systems. Although high irradiation may better induce remineralization of demineralized enamel, it may cause macrostructural changes in enamel. So more studies are necessary to determine the best laser parameters with good role in remineralization without any harmful effect on teeth [42].

In group 4, slight improvement in appearance of enamel surface and slightly visible WSL with no signs of white and black dots was observed. 3M Vanish™ may provide a protective layer on tooth surface. This finding is in consistency with Poosti et al [35] who reported that surface cracking and melted areas were not observed when fractional CO<sub>2</sub> laser was applied after fluoride application. Although group 2 presented the highest increase in microhardness followed by group 3 then group 4 and finally group 1. Group 4 showed the promising results regarding both microhardness and aesthetic appearance.

Despite of little improvement of enamel appearance, all the management groups showed highly significant difference between M2 and M3 values. Percentage of surface hardness recovery showed that enamel treated with 3M Vanish™ or CO<sub>2</sub> laser or its combination was not as hard as the enamel at the base line. It is not possible to simply expect a complete solution to the problem with this short duration study (one month management period). Further long-term evaluation is required to detect the effect of 3M Vanish™ and CO<sub>2</sub> laser on demineralized enamel to see if the aesthetic of enamel and microhardness are completely restored.

#### 4.1. Limitations of the study

- Short duration of the study, so multiple applications of White Varnish may be recommended for further enhancing its remineralizing effect.
- Prevalence of enamel demineralization is overestimated when detected by digital microscope due to the flashlight from the camera and light reflection from the environment.
- The parameters of CO<sub>2</sub> laser used in the current study provided high energy density affected on the appearance of teeth surface.
- Focusing on Microhardness evaluation and appearance of tooth surface, but it would be better if scanning electron microscope was used to explain what happened micromorphologically.

#### 5. Conclusions

- All intervention methods used in the current study increased significantly enamel microhardness values of demineralized enamel, the most effective intervention was group 2 followed by group 3, group 4 and group 1.
- Some improvement of enamel appearance was observed, meanwhile using CO<sub>2</sub> laser on demineralized enamel directly leads to signs of white and black dots affecting the appearance of some areas of some teeth.
- Coating the enamel surface with 3M Vanish™ before CO<sub>2</sub> laser irradiation acted as a protective layer from the undesirable effects of laser on the teeth with increasing enamel microhardness values more than

using Vanish alone. So the promising intervention method regarding both microhardness and appearance was group 4.

## Declarations

### Author contribution statement

Nouran M. Eissa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Eman M. Elshourbagy, Nahla E. Gomaa: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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