



ORIGINAL ARTICLE

Effect of topical fluoride application and diode laser-irradiation on white spot lesions of human enamel



Mohammad A. Alqahtani^{a,*}, Naif A. Almosa^b, Khalid A. Alsaif^c, Naif M. Alsaif^d,
Yazeed J. Aljaser^e

^a Department of Dentistry, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia

^b Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Riyadh, Saudi Arabia

^c Prosthodontics Department, College of Dentistry, King Saud University, Riyadh, Saudi Arabia

^d King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

^e Orthodontic Resident, College of Dentistry, Taibah University, Madina, Saudi Arabia

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KEYWORD

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Abstract Objectives: This study aims to evaluate the effectiveness of topical fluoride application and diode laser-irradiation on the hardness of demineralized enamel and to evaluate the esthetic improvement of the white spot lesions (WSLs) using a visual analog scale (VAS).

Materials and Methods: Artificial WSLs (3x3 mm) were created on the enamel surface of 45 human third molars. The teeth were randomly assigned into three groups (n = 15): group A, fluoride only; group B, combined therapy of fluoride and diode laser; and group C, control. Vicker's hardness number (VHN) was measured at baseline, after demineralization and after treatment. To evaluate the esthetic improvement after treatment, 14 raters evaluated each group's photographs using a 100-millimeter VAS. A one-way ANOVA or Brown-Forsythe and Games-Howell post hoc procedure were performed for statistical analysis. The level of significance was set at $\alpha = 0.05$ for all tests.

Results: Group A mean VHN was significantly higher than groups B and C, and group B was significantly higher than group C ($P < 0.05$). However, the mean VAS rating for the combined therapy group (B) was significantly higher than that for groups A and C ($P < 0.05$).

* Corresponding author.

E-mail address: malqahtaniub@gmail.com (M.A. Alqahtani).

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Conclusion: Combination therapy significantly improved the esthetic appearance of WSLs compared to the fluoride only group. However, there was less enamel hardness versus treatment with fluoride only.

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1. Introduction

During orthodontic treatment, dental plaque may accumulate around fixed orthodontic appliances in frequent patterns, and when the dynamic equilibrium of minerals exchange between the tooth surface and oral fluids is disturbed, enamel demineralization occurs, leading to a porosity underneath the enamel surface. This enamel porosity is known as white spot lesions (WSLs) of the enamel. WSL has a chalky white appearance as a result of the increased difference in the refractive index of sound enamel apatite and its surrounding environment (Fejerskov, Bente Nyvad et al. 2015). WSL is one of the most common undesirable adverse effects of fixed orthodontic therapy, and it has become an esthetic issue for many patients after removal of orthodontic appliances. The reported prevalence of WSLs after removing the orthodontic fixed appliance varies widely in the literature from 2% to 97% (Mitchell 1992, Boersma, Van der Veen et al. 2005). This variation can be attributed to the variety of the methods that were used to assess and record the WSLs in these studies. Three main methods frequently used in the literature include the following: 1) Evaluating the color changes via a spectrophotometer to determine the delta E values for the WSLs (Knösel, Eckstein et al. 2013). 2) Evaluating the severity of the demineralization via quantitative light fluorescence technique and then calculating delta F (Kim and Kim 2015). 3) Evaluating the esthetic improvement of WSLs via a visual analogue scale (VAS) or customized scoring system (Senestraro, Crowe et al. 2013).

Different methods of treatment have been proposed to improve the WSLs appearance after removing the fixed orthodontic appliances. Some investigators found that post-orthodontic demineralized WSLs can be improved when enamel micro-abrasion was performed using pumice and hydrochloric acid at different concentrations from 6.6% and up to 18% (Murphy, Willmot et al. 2007, Gu, Yang et al. 2019). The low-viscosity resin infiltration technique was also introduced recently as a treatment option to mask the WSLs (Knösel, Eckstein et al. 2013, Senestraro, Crowe et al. 2013). In this technique, 15% hydrochloric acid should be applied first to allow the resin to infiltrate into the porous lesion. Thus, the resin will make the refractive index of the lesion closer to the refractive index of sound enamel. Enamel micro-abrasion and resin infiltration techniques can provide a significant and immediate improvement in the post-orthodontic WSLs. However, it was claimed that these techniques are invasive and eliminate the chance for enamel remineralization and natural color recovery (Nainar 2014).

Topical fluoride varnish contains 5% sodium fluoride has been proven to be effective in remineralizing the post-orthodontic WSLs (Du, Cheng et al. 2012). However, it requires multiple applications over time (up to 6 months) to get a significant improvement. A recent systematic review concluded that topical fluoride application of 5% sodium fluoride

varnish is effective remineralizing therapy when it is applied once a month for 6 months after removing the fixed orthodontic appliance (Fernández-Ferrer, Vicente-Ruiz et al. 2018).

Over the past twenty years, few in vitro studies have investigated the effect of using combined therapy of diode laser irradiation and topical fluoride application on the enamel (Vlacic, Meyers et al. 2007, González-Rodríguez, de Dios López-González et al. 2011, Vitale, Zaffe et al. 2011). These studies showed increased enamel hardness and fluoride uptake for the combined therapy groups versus fluoride-only treatment. Only one study looked at the esthetic effects of diode laser irradiation and topical fluoride treatment, and found that this combination therapy enhanced the hardness and esthetic appearance of WSLs in bovine teeth (Alqahtani, Andreana et al. 2019).

Thus, this in-vitro controlled laboratory study aims to evaluate the effectiveness of topical fluoride application with diode laser-irradiation in enhancing the enamel remineralization of artificially developed WSLs in human teeth and improving the appearance from an esthetic point of view. The after-treatment mean Vicker's hardness number (VHN) and Visual Analog Scale (VAS) ratings are the study's outcome measures. The alternative hypothesis was that WSL experimental groups differ in mean VHN and/or mean VAS after treatment.

2. Materials and methods

2.1. Sample preparation

Forty-five sound human third molars teeth were obtained and cleaned with pumice, and then stored in 10% buffered formalin. The roots of the experimental teeth were sectioned from the crowns using a diamond disc under cooling (IsoMet® Low Speed Saw, Buehler, Lake Bluff, IL, USA). The crowns were then mounted in a chemical-cure acrylic resin and flattened by 280-, 400-, 600-, 800-, 1200-, and 4000-grit silicon carbide grinding papers. Next, the sample was examined under a 4.5X magnifying loupe to ensure no residual resin on the teeth surfaces and to exclude teeth with cracks or defects in the enamel structure. Ethical approval was obtained from the College of Dentistry Research Center at King Saud University (Registration No. FR 0489).

2.2. Preparation of White Spot-Like lesions

The teeth were painted with nail varnish except for a small window (3X3 mm) of enamel left without painting as the experimental area for the surface where artificial WSLs were formed later. The teeth were then stored in an acidic solution containing 0.2 wt% 450 kDa MW polyacrylic acid (Poly-science Inc.USA) and 0.1 M lactic acid (Sigma-Aldrich, USA) solution partially saturated with hydroxyapatite

(BioRad, Hercules, CA, USA) and adjusted to a pH of 5 for 2 weeks (White 1987). The teeth were then cleaned with deionized water, and the varnish was removed using a surgical blade and cotton pellet soaked in 50% acetone solution.

2.3. Treatment of WSLs

The samples were randomly assigned into three groups ($n = 15$) using the lottery method of randomization (Table 1).

Group A (Fluoride only)

In this group, topical fluoride (5% sodium fluoride (NaF) varnish (Enamel Pro® Varnish, Premier Dental Co, USA)) was applied for 4 h as it was recommended by the manufacturer that the varnish should remain on the teeth for 4–6 hr, and then cleaned with deionized water.

Group B (combined therapy of fluoride and laser application)

In this group, laser treatment was performed immediately after applying 5% NaF (Enamel Pro® Varnish, Premier Dental Co, USA) that was left on the enamel for 4 h after receiving the laser irradiation. Laser irradiation was carried out using a 400- μ m fiber with a pulse diode laser at 940 nm wavelength (Epic X, Biolase Inc, USA) with a power of 2 W for 60 s. This diode laser setting was selected based on the most favorable results of a previous study that showed no significant difference between using different durations of time (15 s, 30 s, and 60 s), however, we used the longest duration to optimize the irradiation's benefit, if any exists. (Alqahtani, Andreana et al. 2019). The fiber tip was maintained during the procedure at a distance of 5 mm from the tooth surface using a customized tip that was connected to the laser holder.

Group C (control group)

A group of 15 teeth was assigned to the control group. The teeth did not receive any kind of treatment.

2.4. Evaluation of WSLs

2.4.1. Color evaluation

Three photographs of each tooth were taken at three different stages of treatments: baseline, after creating the WSLs, and

after treatment. A digital camera (Nikon D5300, Nikon, Tokyo) with a macro lens (AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED, Nikon, Tokyo) was used to take the photographs inside a photo booth with daylight exposure (HAVOX®, France). The camera was positioned at a fixed focal distance from the teeth using a copy stand device. The photographs were then presented on a large screen inside dark room to 16 postgraduate orthodontic students at King Saud University to rate the aesthetic improvement of the WSLs using the 100-millimeter visual analog scale (VAS). Three photographs of each specimen at three different stages were presented together in one slide. A group of photographs was shown more than one time to evaluate the intra-examiner reliability of the raters in which 2 raters were excluded from the study because of their poor intra-examiner reliability.

2.4.2. Microhardness assessment

A Micro-Vickers hardness tester (Innovatest Micro Vickers Hardness Testers 240, Netherlands) was used under a load of 200 g and a dwell time of 15 s. To obtain the mean Vicker's hardness number (VHN), five readings were averaged at the three different experiment stages: baseline, after demineralization (WSL), and after treatment.

2.5. Statistical analysis

The sample size was determined using G*Power software and based on the effect size of a previous study by Alqahtani et al (Alqahtani, Andreana et al. 2019). Statistical analysis used SPSS Statistics 24 (IBM SPSS, Armonk, NY). Levene's test for homogeneity was used to assess the equality of variances. A one-way ANOVA was performed to evaluate the influence of the fluoride and laser applications on Vicker's microhardness of enamel and VAS ratings. The Tukey HSD test was used to determine group differences for any significant ANOVA results. Brown-Forsythe and Games-Howell post-hoc procedures were performed when the equal variance assumption was not met. A paired *t*-test was performed on the control group to evaluate whether there were any significant changes in VHN at the WSL and after-treatment stages. The level of significance was set at $\alpha = 0.05$ for all tests.

3. Results

3.1. Micro-hardness assessment

The mean VHN was 392.15 ± 17 for sound enamel at baseline; which dropped to 57.7 ± 28 after making the artificial WSL (Table 2). One-way ANOVA indicated no significant differences in mean VHN by the experiment group at the first two stages: baseline stage ($P = 0.876$) or WSL stage ($P = 0.12$).

A paired *t*-test was performed on the control group to evaluate whether there were any significant changes in VHN values at the WSL and after-treatment stages during the course of the experiment. The results indicated no significant differences in the control group measured at WSL stage or after-treatment stage ($P = 0.144$).

Levene's test for homogeneity of variance indicated that the equal variance assumption for mean VHN in the experimental groups after treatment has not been met ($p < 0.05$). The

Table 1 45 specimens were randomly assigned to three groups ($n = 15$).

	Group A (fluoride only)	Group B (fluoride + laser)	Group C (control)
Sample size	15	15	15
Fluoride*	Yes (4 hr)	Yes (4 hr)	No
Laser application	No	Yes	No
Power (W)	N/A	2	N/A
Duration (s)	N/A	60	N/A
Energy Density (J/mm ²)	N/A	6.6	N/A

* Topical fluoride 5% sodium fluoride (NaF) varnish was applied for 4 h then cleaned with deionized water. In the combined therapy group, diode laser was carried out immediately after placing the fluoride.

Table 2 The means and standard deviations of VHN for each group (n = 15) at three stages.

	Baseline	WSL	Treatment	Δ VHN
Group A (Fluoride only)	393.21 \pm 18.29	69.98 \pm 43.63	103.56 \pm 11.38	33.57 \pm 40.76
Group B (combined therapy)	392.97 \pm 12.53	50.01 \pm 12.96	83.98 \pm 16.87	33.97 \pm 18.91
Group C (control group)	390.26 \pm 20.38	53.33 \pm 15.36	58.74 \pm 23.34	5.41 \pm 13.53
Total	392.15 \pm 17.04	57.77 \pm 28.50	82.09 \pm 25.48	24.32 \pm 29.72

Brown-Forsythe test indicated significant differences in mean VHN by the experiment group after-treatment ($p < 0.05$).

Follow up with multiple comparisons (Games-Howell) revealed a significantly higher mean VHN for the fluoride only group "A" when compared with the combined therapy group "B" and the control group "C" ($P < 0.05$). The combined therapy group "B" mean was significantly higher than the control group C ($P < 0.05$). (Fig. 1).

3.2. Visual analog scale (VAS) ratings

Initially, 16 raters were included in the evaluation but two were excluded for poor intra-examiner reliability after calculating the intra-examiner and inter-examiner reliability using intra-class correlation coefficient (ICC). The remaining 14 raters showed good reliability of 85% or above.

The mean VAS ratings were 21.54 ± 17 for the control group, 56.14 ± 21 for the fluoride group, and 76.42 ± 17 for the combined therapy group "B" (Table 3). One-way ANOVA indicated significant differences in mean VAS by the experimental groups ($p < 0.05$) (Figs. 2-3). Multiple comparisons

indicated a significantly higher mean VAS for the treatment groups ($P < 0.05$) when compared with the control group. However, the mean for group A was significantly higher than group C and significantly lower than the combined therapy group B ($P < 0.05$).

4. Discussion

The effects of topical fluoride application in combination with laser irradiation on dental enamel surfaces were assessed in this in vitro study. Two parameters were considered including the enamel hardness assessment and esthetic improvement evaluation.

The results suggest that mean VHN was 392.15 ± 17 for sound enamel at baseline, which agrees with previously published studies (Kutuk, Ergin et al. 2019). Gutiérrez-Salazar and Reyes-Gasga found that the VHN has a range of 327 to 397 when measured parallel to the occlusal surface. (Gutiérrez-Salazar and Reyes-Gasga 2003). The VHN dropped from 392.15 ± 17 to 57.77 ± 28.5 after creating the WSL, which is higher than the readings obtained by White who pro-

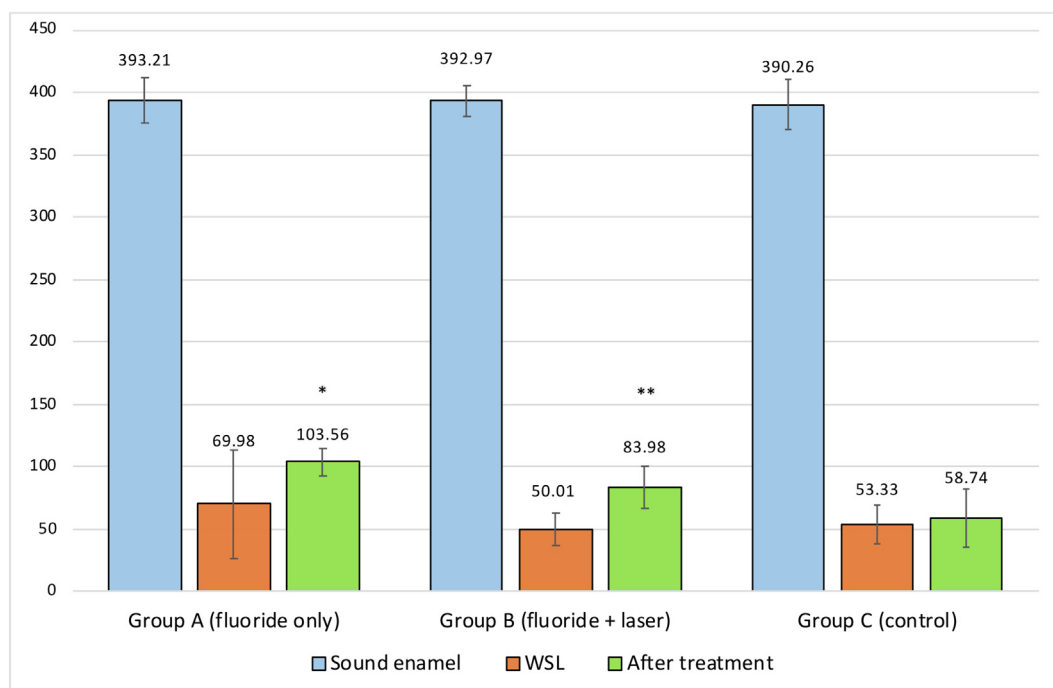


Fig. 1 The mean of VHN for each group at WSL stage. No significant differences were seen in the mean VHN between groups at baseline and WSL stages. * The mean VHN of group A after treatment was significantly higher than for groups B and C ($P < 0.05$). ** The mean VHN of group B after treatment was significantly higher than group C ($P < 0.05$).

Table 3 Averaged mean VAS by treatment groups.

Group	Mean (SD)
Group A (fluoride only)	56.14 (21.72)
Group B (combined therapy)	76.42 (17.05)
Group C (control group)	21.54 (17.78)
Total	51.37 (29.46)

posed using the synthetic high molecular weight polyacrylic acid (Carbopol C907) in the demineralization technique (White 1987). Indeed, White showed that the surface VHN reached 15 ± 4.9 after 6 days of demineralization.

Post-treatment readings showed significant differences in VHN between groups. The fluoride group “A” mean was significantly higher than groups C and B. Different laboratory studies have shown that 5% NaF varnish significantly increases the enamel hardness versus the control group (Cardoso, de Castilho et al. 2014, Mohd Said, Ekambaram et al. 2017). In the fluoride group, the Δ VHN of the after treatment and the WSL readings is 33.57 ± 40 which is very close to that reported by Dehailan et al (Dehailan, Martinez-Mier et al. 2019). Two clinical studies found that topical fluoride varnish can significantly reverse the post-orthodontic WSLs when applied once a month for 6 months (Du, Cheng et al. 2012, He, Li et al. 2016).

Here, topical fluoride application with pulsed laser irradiation showed a significantly higher mean VHN than the control group, and a significantly lower mean VHN than the fluoride group. This finding is in contrast to previously reported values when topical fluoride was applied with continuous laser irradiation (Alqahtani, Andreana et al. 2019). Different materials and protocols were used here relative to these previous studies including the type of commercial fluoride varnish and the mode of laser application. These differences might explain

the differences in the results. However, a recent study showed that using topical fluoride varnish in combination with a diode laser application at a wavelength of 810 nm and a power of 2 W in continuous mode was not more effective than fluoride application alone (Moghadam, Seraj et al. 2018). In the latter study, the investigators first exposed sound enamel from primary teeth to different kinds of treatment and then exposed the teeth to a pH-cycling process. Regardless of whether they support combining topical fluoride application with laser irradiation or not, most reported studies have great differences in the materials and methods used. This makes it very difficult to make firm conclusions (Santaella, Braun et al. 2004, Vlacic, Meyers et al. 2007, González-Rodríguez, de Dios López-González et al. 2011, Vitale, Zaffè et al. 2011). These differences also include the type of teeth used (animal vs human, primary vs permanent), topical fluoride varnish (active ingredient, surface contact duration), mode of the laser application (wavelength, power, duration, continuous vs pulse mode), and method of assessment (enamel hardness, fluoride uptake, lesion depth of the polarized microscopy).

Besides that, the surface microhardness can be used to evaluate the physical aspect of enamel surface remineralization but it does not study the mineral distribution of the subsurface enamel lesion (Lippert and Lynch 2014). The diode laser irradiation might lead to more fluoride uptake and remineralization at the deepest areas of the lesion that resulted in color improvement. However, without examining the mineral distribution of the subsurface enamel lesion, we cannot confirm this hypothesis, so we suggest using the transverse microradiography test (TMR) in future studies.

In this study, we used the VAS rating to evaluate the esthetic improvement of WSL after treatment. This method is the gold standard for esthetic evaluation, but it has some disadvantages primarily related to the rater's reliability (Chu, Trushkowsky et al. 2010). The intra- and inter-examiner reli-

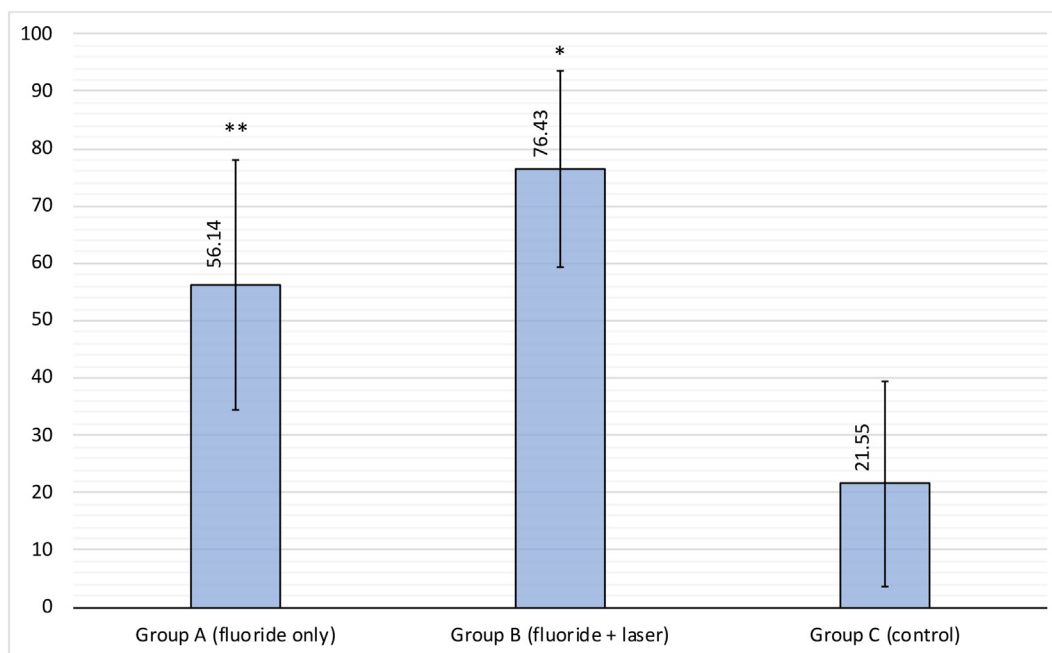


Fig. 2 Averaged VAS ratings for each group. * The mean VAS of group B was significantly higher than groups A and C ($P < 0.05$). ** The mean VAS of group A was significantly higher than group C ($P < 0.05$).

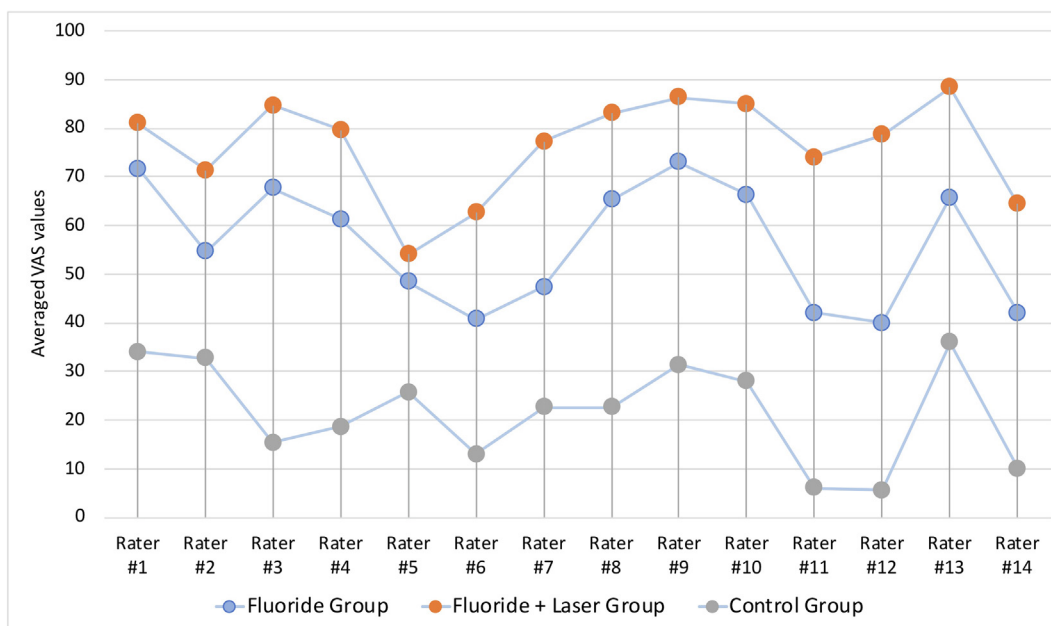


Fig. 3 Averaged VAS values for each rater by experimental groups. All the raters reported mean VAS ratings for the three experimental groups in the same sequence: Combined therapy group “B” is the highest followed by fluoride group “A” and then the control group “C”.

bility were evaluated in this study with a good reliability of 85% or more. The mean VAS ratings for the fluoride application with diode laser irradiation group was significantly higher than that for control and fluoride groups; the mean VAS rating for the fluoride group was significantly higher than that for the control group. Despite presenting the specimens randomly to examiners who are blind to the experimental groups, the mean VAS ratings for the control group was 21.54 ± 17 . This high rating percentage of the control group could be explained as a response bias, specifically a central tendency form of response bias. However, when we look at the mean individual VAS ratings for each rater, all the raters reported mean VAS ratings for the three experimental groups in the same sequence: group B is the highest followed by group A and then the control group (Fig. 3).

This study has some limitations including the use of surface microhardness method solely to evaluate the enamel remineralization. It might be better if we used the transverse microradiography (TMR) method, which is considered the gold standard in studying the mineral distribution of the sub-surface enamel lesion. The surface microhardness assessment could then be added as a complementary test to evaluate the physical aspects of the enamel lesion. (Lippert and Lynch 2014).

5. Conclusion

In this study, topical fluoride application significantly increased the hardness of demineralized human enamel versus a control group and combination therapy of topical fluoride varnish and diode laser-irradiation group. The combination of diode laser-irradiation at 940 nm and 2 W for 60 s with topical fluoride application significantly improved the esthetic appearance of WSL versus control and fluoride-only groups.

CRedit authorship contribution statement

Mohammad A. Alqahtani: Conceptualization, Methodology, Supervision, Validation, Formal analysis, Data curation, Visualization. **Naif A. Almosa:** Methodology, Resources, Data curation, Supervision, Validation, Project administration. **Khalid A. Alsaif:** Methodology, Investigation. **Naif M. Alsaif:** Methodology, Investigation, Writing – original draft. **Yazeed J. Aljaser:** Investigation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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