Effect of Nd:YAG Laser Irradiation on the Number of Open Dentinal Tubules and Their Diameter with and without Smear of Graphite: An in Vitro Study

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Abstract:

Introduction: Dentin hypersensitivity (DH) is characterized by tooth pain arising from exposure of dental roots. In this study the efficiency of neodymium yttrium-aluminum-garnet (Nd:YAG) laser in association with graphite on dentinal surface changes as the alternative to the treatment of DH was evaluated.
Methods: Sixteen noncarious human third molars were collected and sectioned into 5 parts from cementoenamel junction (CEJ) to the furcation area. The prepared samples were randomly assigned into five groups (Gs) of each 16: Control (G1), treated by Nd:YAG laser at 0.5 W (G2), irradiation of Nd:YAG with a 0.25 W output power(G3), smeared with graphite and then using Nd:YAG laser at output powers of 0.5 W (G4) and 0.25 W (G5). For all groups the parameters were 15 Hz, 60 s, at two stages and with a right angle irradiation. The number and diameter of dentinal tubules (DT) were compared and analyzed by SPSS software, One way ANOVA and Post hoc LSD tests.
Results: The number of open dentinal tubules varied significantly between all groups except among G1 with G3 and G2 with G5. Multiple comparison tests also exhibited significant differences regarding the diameter of tubules between the groups two by two except among G2 with G5.
Conclusion: Nd:YAG laser used at 0.25 W and 0.5 W with application of graphite smear was able to reduce the number and diameter of dentinal tubules.
Keywords: dentin hypersensitivity; Nd:YAG lasers; scanning electron microscopy.

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Introduction

Dentin hypersensitivity (DH) refers to an abnormal response to different stimuli- typically chemical, thermal, evaporative, tactile or osmotic- recognized by a sharp located short duration pain from denuded dentin that would not exist under normal conditions in a healthy tooth[1]. This phenomenon is a rather common problem that has been reported usually in 30 to 40 year old patients with a mean prevalence of 4 to 57% in many literatures, depending on the studied individuals. In patients with periodontitis this range rises to 60%-98%[2,3]. There are many varieties of potential causes for dentin sensitivity. The loss of enamel and removal of cementum from the root with exposure of dentin, however, is a major contributing factor[4].

The most common etiologic factors considered for DH are abrasion, abfraction and erosion, as results of the intensive tooth brushing, occlusal forces, acidic environment, and so on[5]. Although various theories have been reported for biological mechanism of DH, nowadays the acceptable one is Brannstrom's hydrodynamic theory which states that the movement of fluid in the dentinal tubules results in displacement of odontoblasts located in the external layers of the pulp or in the internal terminals of tubules[6].

The treatment modalities to reduce DH are based on decreasing dentinal permeability[7]. For this purpose, the occlusion of dentinal tubules has attracted attentions by means of resins, glass ionomers, sodium fluoride gel (the most common tubule occluding agent), desentisizing agents and etc [8-11]. With the advent of laser technology and its growing utilization in dentistry, a therapeutic option was added for minimizing dentinal pain. Miserendino et al. in the investigation of laser interaction with biologic tissues, pointed out that some parameters such as wavelength specificity (which is determined by the inherent optical properties of the irradiated tissue), power density (that affects the type of interaction with the tissue) and irradiation time influence this interaction[12].

The first use of Nd:YAG (neodymium yttrium-aluminum-garnet) laser for the treatment of DH was reported by Matsumoto et al. and its efficacy range was between 5.2% to 100%[13]. Comparing the therapeutic effects of Nd:YAG and Erbium-Doped Yttrium Aluminum Garnet (Er:YAG) lasers for the management of DH, Birang et al. found significant reduction in visual analogue scale (VAS) by using Nd:YAG laser rather than another[14]. Abed et al. compared the sealing ability of Nd:YAG laser application (1 W, 10 Hz, 60 seconds, noncontact mode without cooling) to that of a resin applied to exposed human dentinal tubules in vitro. In comparison with the control group, laser application showed a homogeneous dentinal surface with less exposed tubules and a reduction in the diameter of the exposed tubules of 50% [15]. In an in-vivo study it was shown that Nd:YAG wavelength was highly absorbed by the pigmented tissue, making it a very effective surgical laser for cutting and coagulating dental soft tissues, with good homeostasis[16].

Due to these results, pigmentation of dental surface can result in increasing Nd:YAG laser absorption.

When using Nd:YAG laser and black ink as an absorption amplifier, deep penetration of laser light through enamel and dentine is evaded so that excessive harmful effects on the pulpal tissue can be avoided[17] and superficial sealing effects can be enhanced[18,19]. Based on these findings, in the present study our aim was to evaluate the alterations in dentin surface irradiated with Nd:YAG laser beams (0.25 and .05 W) alone and in combination with graphite smear (as an absorber of the laser wavelength), to evaluate the number of open dentinal tubules and their diameter as well as craters and microcracks to distinguish its ability for treatment of DH.

Methods

Samples

Sixteen caries free adult (18-25 years of age) human teeth collected in the school of dentistry at Islamic Azad University were kept in distilled water and cool environment. Further inclusion criteria for gathering teeth were based on: having adequate mesiodistal width and a long root trunk, absence of restoration or congenital anomaly, absence of calculus or signs of attrition. The long root trunk allowed taking 5 samples from each tooth and then they were divided into 5 groups. Each tooth was vertically sectioned using a precision section device (Hobbymat 2000, Germany) so that five samples were obtained with the dimensions of 2×2×1 mm.

This procedure produces a smear layer on dentin surface that makes it impossible to evaluate its changes. Thus, the smear layer was removed by application of ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite before lasing.

Treatments

As said before, the samples were divided into five groups as below:

Group 1: no treatment, considered as the control group [The remained groups were irradiated by Nd:YAG laser of 1064 nm wavelength with an optic fiber of 300 micro millimeter diameter (Fontana, Fidelis Plus, Ljubljana, Solvenia)]. In group 2 the specimens were lased by Nd:YAG laser with a power of 0.5 W, whereas 0.25 W power was used for group 3. In groups 4 and 5 the teeth were stained with a graphite pencil having a rectangular tip and with 0.5 W and 0.25 W, respectively. In fact we painted two samples obtained of each tooth by a graphite pencil (instead of black ink) for the pigmentation of dentinal surface.

The parameters used for laser treated groups were the following: non-contact mode (the distance between the optical fiber and the irradiated surface was 2 mm), delivered energy densities per second 47.13 and 23.49 J/Cm^2 for the following output power settings: 0.5 W and 0.25 W, respectively.

Specimens were placed on a flat surface and the topical fiber was moved by the operator with a right angle (90°) at two stages with the frequency of 15 HZ for 60 seconds and without cooling. The irradiation distance (2 mm) was standardized by using an orthodontic wire with a flat sectional area that was attached on the laser handpiece and put on the samples in direct contact and perpendicular to the specimen surface so that this distance was maintained during the irradiating procedure.

Scanning electron microscopy (SEM) analysis

First, the specimens were fixed by glutaraldehyde 2.5% for 12 hours, and rinsed in phosphate buffer, then dehydrated in alcohol %25, %70, %95 and %100, dried by freeze dry technique/ critical point dry. Then samples were put on a specific by carron adhesive double side pad, and were gold coated by a gold coating machine (Balzer SCD040/USA). The microscopic fields were observed under 100× magnification to evaluate microcracks and 1500× magnification to assess changes in dentinal tubules (DT), craters and microcracks. The studied factors were analyzed by SEM (LEO 43vp/USA).

Considered variables

1- Number of open dentinal tubules: By numbering them in a field without numeration of the tubules in the margin with undetermined parts.

2- Diameter of dentinal tubules: The largest DTs seen in a field (in similar areas of samples) was measured by AutoCAD software. The mean of studied fields was determined as diameter of DTs using specified scale of the image.

3- Craters: This character was registered just by observing cavities and valley like structures by 1500× magnification.

4- Microcracks: Existence or absence of microcracks determined this option at two magnifications 100× and 1500×.

Statistical analysis

The data obtained were analyzed using SPSS version 20, One way ANOVA (analysis of variance) and LSD (least significance difference) Post hoc test for multiple comparisons. Each value represents the mean ± SD. A significant level of 5% was employed for all analysis.

Results

Number of open dentinal tubules

One way ANOVA revealed a P < 0.001 indicating differences between the subgroups; so LSD test was done to represent the exact comparison among 5 groups two by two. This statistical test showed significant differences (P < 0.001) between the groups except among G1 with G3 (P = 0.62) and G2 with G5 (P = 0.83) (Tables 1 and 2).

Diameter of dentinal tubules

Based on the results obtained from One-way ANOVA

Table 2. P-values obtained by two by two comparisons of different studied groups regarding the number of dentinal tubules

	G1	G2	G3	G4	G5
G1	–	<0.001	0.629	<0.001	<0.001
G2	<0.001	–	<0.001	<0.001	0.83
G3	0.629	<0.001	–	<0.001	<0.001
G4	<0.001	<0.001	<0.001	–	<0.001
G5	<0.001	0.83	<0.001	<0.001	–

Table 1. The mean number of open dentinal tubules and their diameter (µm) in different studied groups

Group	N	min/max	Number	min/max	Diameter
1	16	34 / 70	52 ± 11.2	3.1 / 4.9	3.78 ± 0.61
2	16	23 / 52	33.4 ± 9.6	1.7 / 3.32	49 ± 0.48
3	16	32 / 68	50.3 ± 11.3	2.6 / 4.3	3.32 ± 0.58
4	16	8 / 29	14.9 ± 6.7	1.1 / 2.21	53 ± 0.35
5	16	23 / 51	32.7 ± 9.7	1.6 / 3.1 2	28 ± 0.44
P		< 0.001		< 0.001	

G1: No treatment
G2: Lased by Nd:YAG laser with a power of 0.5 W
G3: Lased by Nd:YAG laser with a power of 0.25 W
G4: Stained with graphite and lased by Nd:YAG laser with a power of 0.5 W
G5: Stained with graphite and lased by Nd:YAG laser with a power of 0.25 W

(P < 0.001), data analysis was followed by a two by two analysis of LSD. There were meaningful differences between all studied groups except between G2 with G5 (P = 0.25) (Tables 1 and 3).

In G2 and G3 (non-smeared groups), no micro crack was observed under 100× magnification, although superficial globules and cracks were seen in some of the specimen in graphite used groups (G4 and G5) (Figures 1-5). For exact examination of this item, micrographs with 1500× magnification were prepared. In groups treated with laser alone, no cracks were detected, while in G4 some cracks were revealed on the dentine surface. On the other hand, in G5 -with higher output power (0.5 W) the cracks were deeper, more in number and associated with rupture of melted dentin. There was not any crater in microscopic fields of all groups (Figures 6-10).

Table 3. P-values obtained by two by two comparisons of different studied groups regarding the diameter of dentinal tubules

	G1	G2	G3	G4	G5
G1	–	<0.001	0.012	<0.001	<0.001
G2	<0.001	–	<0.001	<0.001	0.254
G3	0.012	<0.001	–	<0.001	<0.001
G4	<0.001	<0.001	<0.001	–	<0.001
G5	<0.001	0.254	<0.001	<0.001	–

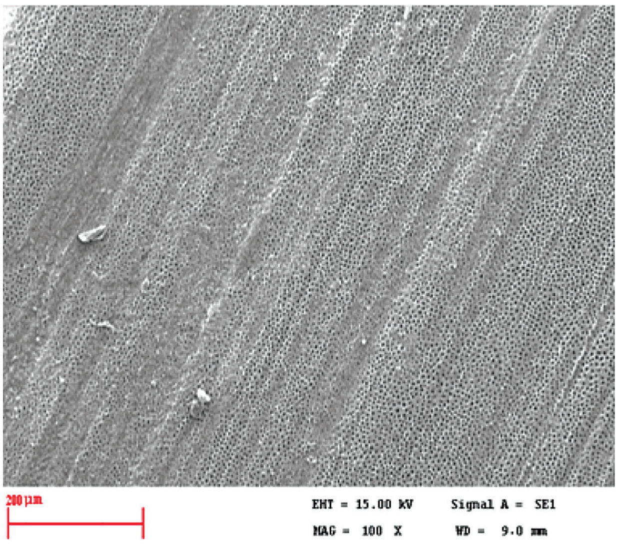

Figure 1. Scanning electron micrograph of specimen in control group (×100)

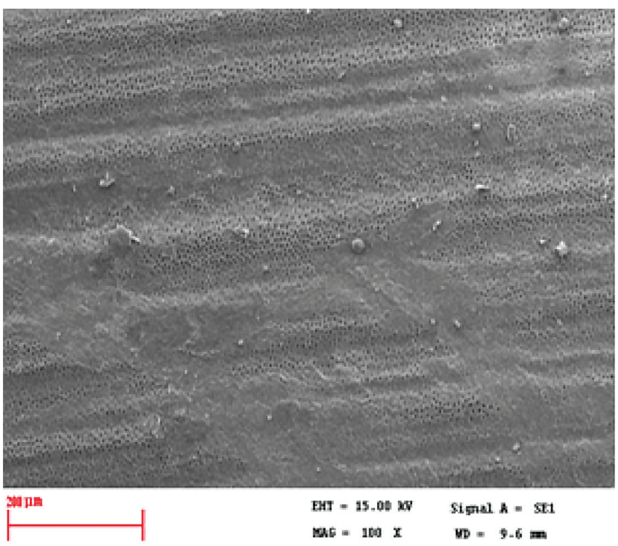

Figure 2. Scanning electron micrograph of specimen using Nd:YAG 0.5 W (×100)

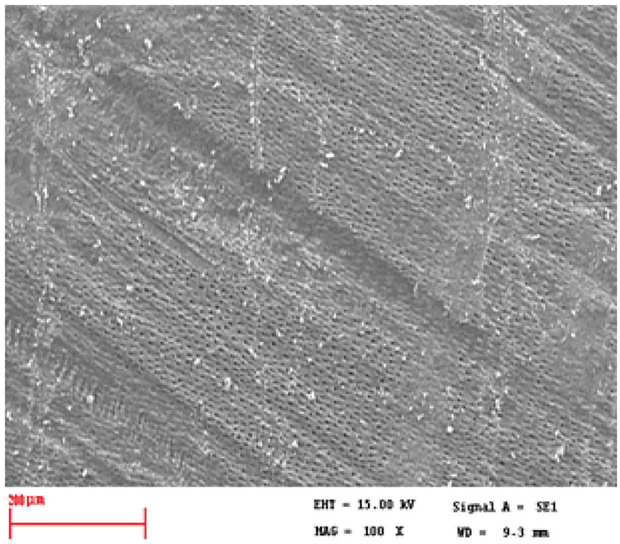

Figure 3. Scanning electron micrograph of specimen using Nd:YAG 0.25 W (×100)

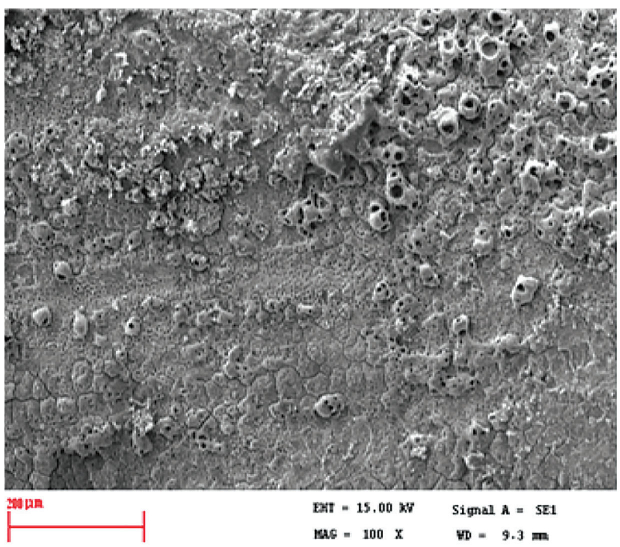

Figure 4. Scanning electron micrograph of specimen using Nd:YAG 0.5 W with smear of graphite (×100)

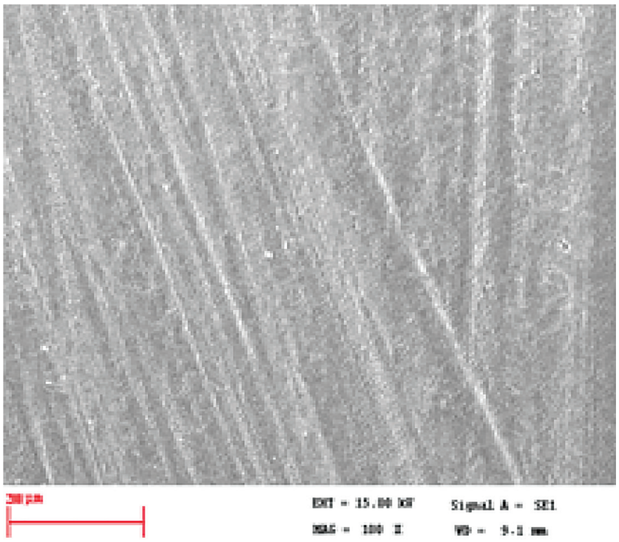

Figure 5. Scanning electron micrograph of specimen using Nd:YAG 0.25 W with smear of graphite (×100)

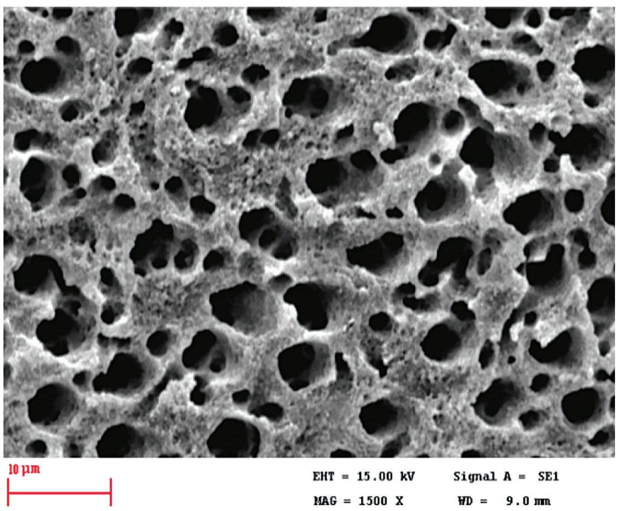

Figure 6. Scanning electron micrograph of specimen in control group (×1500)

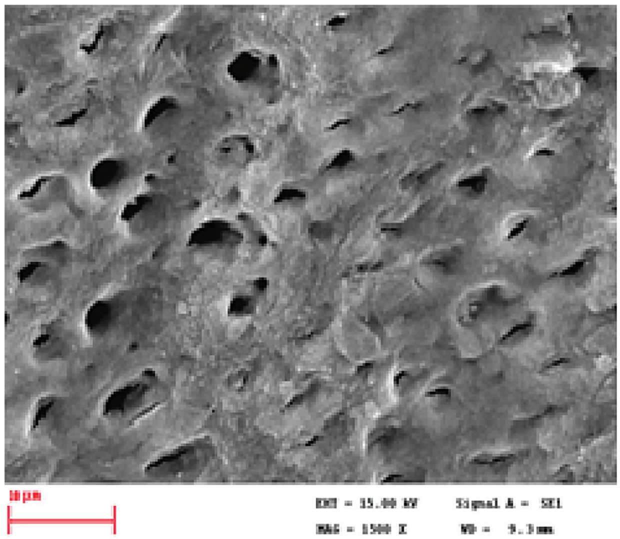

Figure 7. Scanning electron micrograph of specimen using Nd:YAG 0.5 W (×1500)

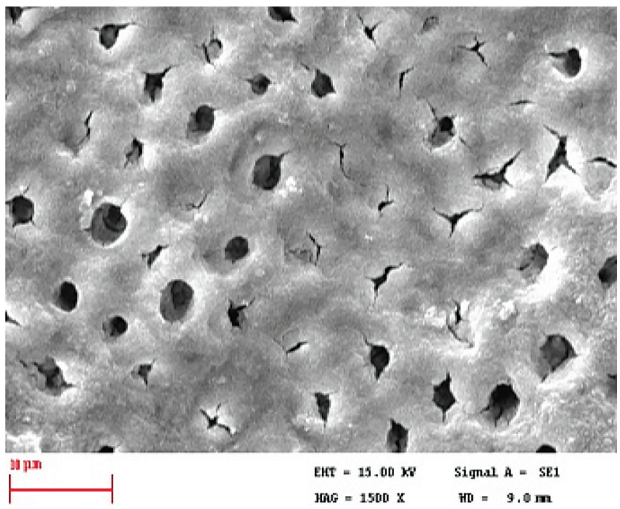

Figure 8. Scanning electron micrograph of specimen using Nd:YAG 0.25 W (×1500)

Discussion

Indeed, efforts to apply lasers in dentistry date back to the introduction of this technology, and its applicability to different procedures has become a subject of great interest for treatment of DH ever since. The prevalence of dental hypersensitivity is on increase due to two reasons: first, people can keep their teeth for longer periods; second, root surface of teeth are mostly exposed as a result of gingival recession and periodontal surgery[20]. There is a significantly high correlation between the morphology and the number of open dentinal tubules and DH[21,22].

The first laser used for the treatment of dentin hypersensitivity was the Nd:YAG laser. With the use of the Nd:YAG laser, the treatment effectiveness ranged from 5.2 to 100%. When using this type of laser, the use of black ink as an absorption enhancer is recommended in order to prevent Nd:YAG laser beam's deep penetration through the enamel and dentin, and excessive effects in the pulp. Although pulpal disruption did occur in laser-treated specimens with remaining dentin thickness of less than 1 mm, it did not happen when the dentin thickness exceeded to 1 mm. The mechanism of Nd:YAG laser effects on DH is thought to be a laser-induced occlusion or a narrowing of dentinal tubules, as well as direct nerve analgesia[23,24]. The output power when using Nd:YAG laser usually varies from 0.3 to 2 W[17,25], so in the present study the powers of 0.25 W and 0.5 W were used which

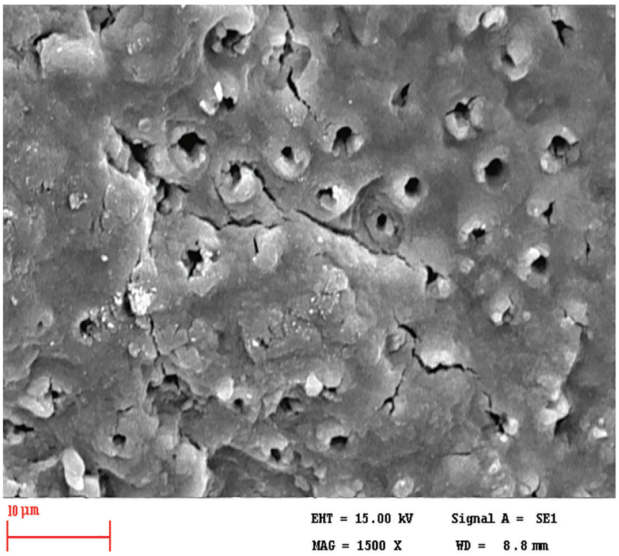

Figure 9. Scanning electron micrograph of specimen using Nd:YAG 0.5 W with smear of graphite (×1500)

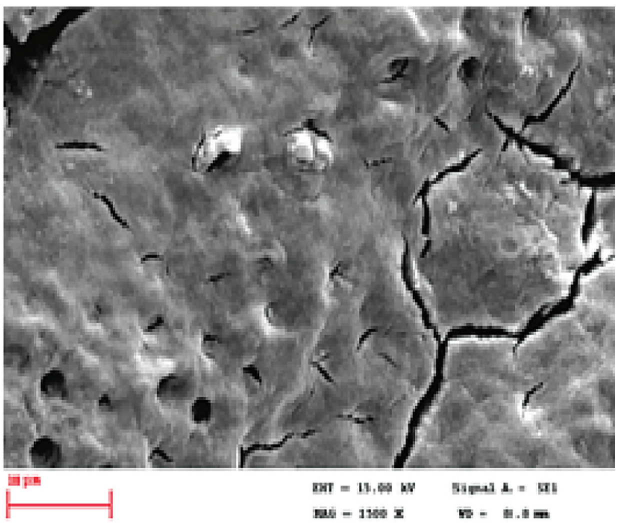

Figure 10. Scanning electron micrograph of specimen using Nd:YAG 0.25 W with smear of graphite (×1500)

were near the lowest power.

As shown in Table 1, the diameter and number of open dentinal tubules in the group irradiated with Nd:YAG laser at 0.5 W compared to the control group were significantly reduced. Similar changes in diameter occurred in the group treated with Nd:YAG laser at 0.25 W alone. Since the alterations in both number and diameter of DTs causes diminished permeability, Nd:YAG laser is capable of occluding tubules and has the potential for treatment of DH. In this way, our results are in agreement with most literatures[26-29] in which the effectiveness of this laser in occlusion of DTs was established, although the parameters used in this study differ from the others.

In G3 comparison to G1, occluding of DTs did not differ meaningfully, so it is concluded that laser irradiation less than 0.5 W to treat DH without using graphite is not effective. In laser-alone treated groups (G2 and G3), with two by two comparison, significant differences were noted in number and diameter of DTs. Therefore, increase in laser power is associated with decrease in mean of these two factors. Another aspect that must be considered during increasing the power is the risk of pulp damage and its side effects on surface dentin.

In comparing the laser-treated groups with the output powers, significant differences in morphology of DTs were observed. This part of our finding is consistent with other investigations reporting enhanced surface absorption of Nd:YAG laser by using dark smears[30,31]. In the endodontic field, in another study Arisu et al. [30] indicated that the usage of India ink with Nd:YAG laser enhanced the amount of melting and recrystalization of dentin which, in turn, caused the dentine to be less permeable. Goya et al.[31] demonstrated by SEM that Nd:YAG laser irradiation combined with black ink decreased apical microleakage significantly (20% without using black ink versus 0% in association with it).

An interesting point in the assessment of number and diameter of tubules is the absence of significant difference when comparing G2 with G5. Based on obtained results, the efficacy of Nd:YAG laser at 0.25 W on DH is just through the reduction of DT diameter, and dentinal melting is not enough to occlude DTs completely. But at output power 0.5 W, laser operates more effectively and therefore melting of dentin is followed by considerable occlusion and reduction of DTs diameter.

Side effects of Nd:YAG laser irradiation was also evaluated. Craters or valley-like depressions due to laser destruction did not exist in any of the 5 groups, which is inconsistent with the Birang et al. study[32]. A few number of studies pointed out crater formation following the use of Nd:YAG laser. Absence of a crater in this study can be a result of appropriate parameters of laser or continuous motion of handpiece during irradiation on surface of dentin.

Another side effect of laser irradiation is microcrack[33]. In micrographs captured under 100× magnification in non-smeared samples, there were no microcracks, but in graphite used ones there were globules and cracks. SEM micrographs obtained by 1500× magnification demonstrated deeper and more cracks in both graphite used groups as well as rupture of melted dentin in the group with higher power. Laser energy absorption on dentin surface must be controlled precisely to avoid side effects.

In comparing our study with the previous one done by Birang et al.[32] differences in the results of application of the 0.5 W laser on non-smeared teeth was observed (a significant difference in the present study in contrast to a non-significant one in the mentioned investigation). Since laser dose has direct relationship with frequency, increasing frequency from 10 HZ (in the mentioned study) to 15 HZ (in our work) is associated with enhancing dose. Therefore, in our study, the power of 0.5 W with more density was able to occlude dentinal tubules.

Conclusion

Nd:YAG laser irradiation with the output power of 0.25 cannot seal the dentinal tubules significantly. Nevertheless, the use of Nd:YAG laser following graphite application is a suitable method for sealing tubules and management of dentin hypersensitivity, and is more efficient compared with when not staining. It seems that the higher power of Nd:YAG laser alone has a similar effect compared to the lower one associated with graphite.

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