Effects of Diode Laser Alone and in Combination with Indocyanine Green and Graphite Paste on Dentinal Tubule Occlusion: SEM Analysis

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Abstract: ***Aim**: This study aimed to compare the efficacy of Diode Laser alone and in Combination with Indocyanine green at three different concentrations and graphite paste on Dentinal Tubule Occlusion and treatment of Dentin Hypersensitivity. **Methods**: 35 extracted human molars were divided into 7 groups of negative and positive control, 810 nm diode laser irradiation, 810nm diode laser with Indocyanine green (0.5, 1 and 2mg/ml) and 810nm diode laser with graphite paste. Dentinal tubules were evaluated under a scanning electron microscope at x2000 magnification. Statistical analysis-Two-way ANOVA and Tukey's HSD Post hoc Test were used statistical analysis. **Results**: The highest mean percentage of the obstruction of dentinal tubules was noted in the diode laser/Indocyanine green 2mg/ml (64.786 ± 1.897%) while the lowest value was noted in the diode laser group (46.448 ± 2.316%). This mean difference among the 5 study groups was statistically significant at P<0.001. However, no significant difference was noted between diode laser/ICG 1mg/ml & diode laser/graphite paste [P=0.97] and also between diode laser and diode laser/ICG 0.5mg/ml [P=1.00]. **Conclusion**: Indocyanine green increases the tubular occlusion caused by diode lasers. The effect is concentration-dependent. Graphite paste showed results similar to the 1mg/ml ICG group.*

Keywords: Dentin hypersensitivity, Diode laser, Chromophore, Indocyanine green, Graphite paste

1. Introduction

Dentine sensitivity (DS) or dentinal hypersensitivity (DH) is one of the most commonly encountered clinical problems associated with exposed dentinal surfaces. [1] According to international workshop, this condition is defined as follows: [2] "Dentine hypersensitivity is characterized by short, sharp pain arising from exposed dentine in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other dental defect or pathology".

Lasers can be used in effective management of DH as shown by various studies. [3, 4] Laser is a device which transforms light of various frequencies into a chromatic radiation in the visible, infrared and ultraviolet regions with all the waves in phase capable of mobilizing immense heat and power when focused at close range.5 The word Laser is an acronym derived from Light Amplification Stimulated Emission of Radiation.

The role of Laser in decreasing DH is explained by two mechanisms: (1) by directly affecting the electric conduction of pulpal nerve fibers, (2) melting and obstruction of dentinal tubules. [5, 6]

The diode laser is a low-intensity laser with 3 wavelengths of 780, 810, 900 nm used in the treatment of hypersensitivity. This laser was first used at a 780 wavelength by Matsumoto et al in 1985 to treat hypersensitivity, and its therapeutic effect is estimated to be 85%-100%. [5]

Chromophore is an unsaturated organic molecule containing a group of single or multiple chain bonds.7 It is commonly used in photodynamic therapy. [6] These molecules change the light absorbance to longer wavelengths and lower frequencies. [7] Indocyanine green (ICG) is a cyanine dye used in medical diagnostics. At 800nm, its absorption peak is near the emission maximum of diode lasers. Thus, it can be used for greater penetration into periodontal pockets. The combination of the laser and indocyanine green is effective in the treatment of DH. [7]

The effect of diode lasers can also be increased by application of graphite paste. It reduces or closes dentinal tubules by melting effect by increasing the temperature but it can also provoke cracks and destruction at highest energy densities. [8]

Hence, this study aimed to compare the efficacy of the 810 nm diode laser alone and in combination with ICG at three different concentrations i.e., 0.5, 1 and 2 mg/ml and also compare it with combination of diode laser and graphite paste.

2. Materials and Methodology

Preparation of Samples

Thirty-five freshly extracted human molars were collected from department of oral surgery and were kept in balanced salt solution [9] at 4ºC for 1 week.

After removing any tissue remnants, the external surfaces of teeth were cleaned with an ultrasonic scaler to remove stains and calculus. The teeth were then sectioned transversely at the mid-level of the crowns at a low speed (300 rpm) using a diamond disc and straight micromotor handpiece in order to totally expose the dentin. The anatomical crown and root were separated and 3 mm thick dentin discs were obtained. [10]

Dentin surfaces were ground using coarse to fine grit abrasive papers for 60 seconds. [7] The samples were then polished using Shofu super snap polishing discs.

For the exposure of dentinal tubules, all samples except for the negative control group were immersed in 17% ethylene diamine tetra-acetic acid (NEOEDTA liquid) for 60secs. Teeth were rinsed and stored in distilled water. [7]

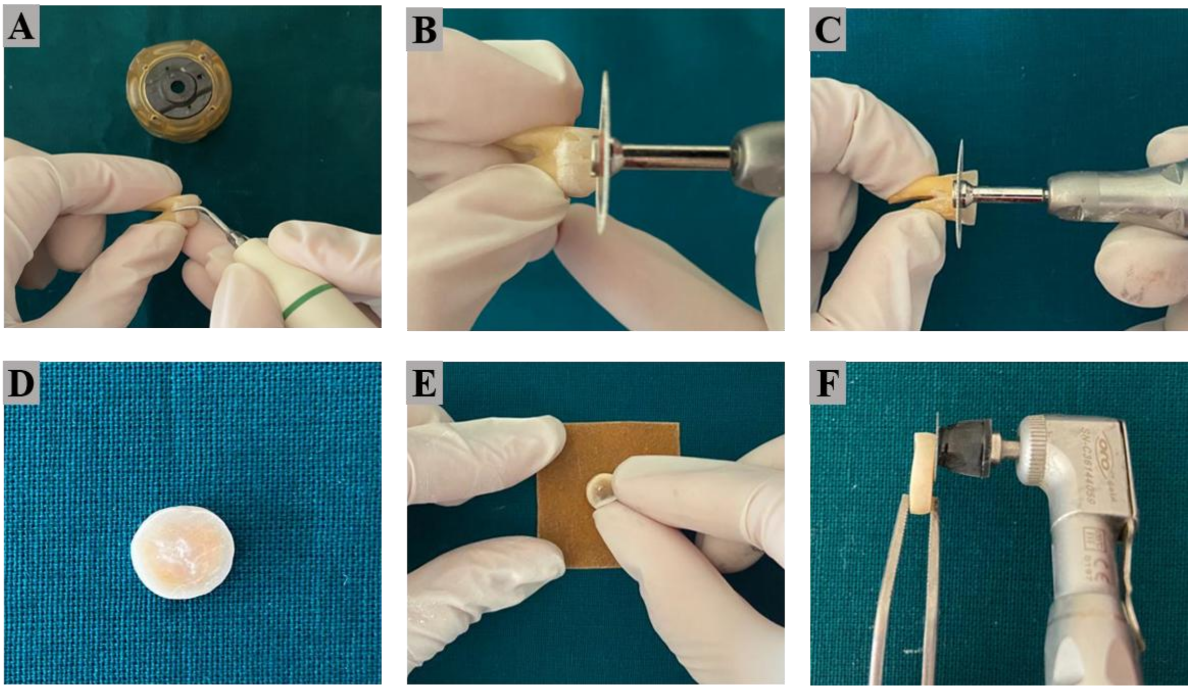


Figure 1: Preparation of dentin discs.
(A) Stains and calculus removed with an ultrasonic scaler, (B) & (C) transverse sectioning at mid-level of crown, (D) 3mm thick dentin discs, (E) & (F) finishing and polishing

3. Therapeutic Intervention

The samples were randomly divided into 7 groups (n=5) as follows [7]:

- Group 1 (negative control): In this group, after using coarse to fine grit abrasive papers, the samples did not undergo any surface treatment.
- Group 2 (positive control): Dentinal tubules in this group were exposed using 17% EDTA. No other intervention was performed.
- Group 3 (diode laser): After surface treatment with 17% EDTA, the teeth samples were subjected to 810 nm diode laser irradiation in sweeping motion with 1 mm distance from the surface. Laser was used with 1W power in a continuous mode for 20 seconds. The fiber diameter was 300 µm.
- Group 4 (diode laser/ ICG 0.5mg/ml): After surface treatment with 17% EDTA, the samples were coated with one layer of chromophore with approximately 20 µm thickness (ICG) and 0.5 mg/mL concentration and were then subjected to diode laser irradiation as explained for group 3.
- Group 5 (diode laser/ICG 1mg/ml): After surface treatment with 17% EDTA, the samples were coated with one layer of chromophore with approximately 20 µm thickness (ICG) and 1 mg/mL concentration and were then subjected to diode laser irradiation as explained for group 3.
- Group 6 (diode laser/ ICG 2mg/ml): After surface treatment with 17% EDTA, the samples were coated with one layer of chromophore with approximately 20 µm thickness (ICG) and 2 mg/mL concentration and were then subjected to diode laser irradiation as explained for group 3.
- Group 7 (diode laser/ Graphite paste): After surface treatment with 17% EDTA, the samples were coated with graphite paste and were then subjected to diode laser irradiation as explained for group 3.

Table 1: Materials and Devices used in this study

Diode Laser	**1 W, 810nm**	**20 seconds, continuous mode, sweeping motion**	**FOX Diode Laser, A. R. C. Laser GmbH Bessemerstraße 14 D-90411 Nürnberg Germany**
Indocyanine Green (ICG)	0.5, 1 and 2mg/ml	Applied to the surface with an approximate thickness of 20 µm using a microbrush	Aurogreen by Aurolabs
Scanning Electron Microscope	sputter-coating with one layer of gold with 10nm thickness	X2000 magnification	Hitachi VP-SEM SU1510 by Hitachi High Technologies America, Inc.

SEM Analysis

The samples were than sputter-coated with one layer of gold with 10nm thickness and inspected under SEM (Hitachi VP-SEM SU1510) at x2000 magnification. [7]

SEM micrographs were analysed in Adobe Photoshop 2021 software to assess the mean percentage of the obstruction of dentinal tubules. The obstructed area of tubule was marked using magic wand tool and object selection tool. The 20um scale given on each micrograph was taken as reference and the cross-sectional area of obstructed and partially obstructed dentinal tubules was calculated in um^2.

Statistical Analysis

The mean percentage of the obstruction of dentinal tubules was reported as mean and standard deviation. Comparison of mean percentage of Dentinal Tubule Occlusion between 5 study groups was carried out using One-way ANOVA Test. Multiple comparison of mean difference in percentage of Dentinal Tubule Occlusion b/w groups was done using Tukey's HSD Post hoc Test.

4. Results

Table no.2 illustrates the comparison of mean Percentage of Dentinal Tubule Occlusion among 5 study groups. The test results demonstrate that the mean Percentage of Dentinal Tubule Occlusion for Group 3 was 46.448 ± 2.316, for Group 4 was 46.696 ± 2.687, for Group 5 was 55.984 ± 2.319, for Group 6 was 64.786 ± 1.897 and for Group 7 was 55.032 ± 2.642. This mean difference in the Percentage of Dentinal Tubule Occlusion among 5 study groups was statistically significant at P<0.001 [***Refer Graph no.1***]

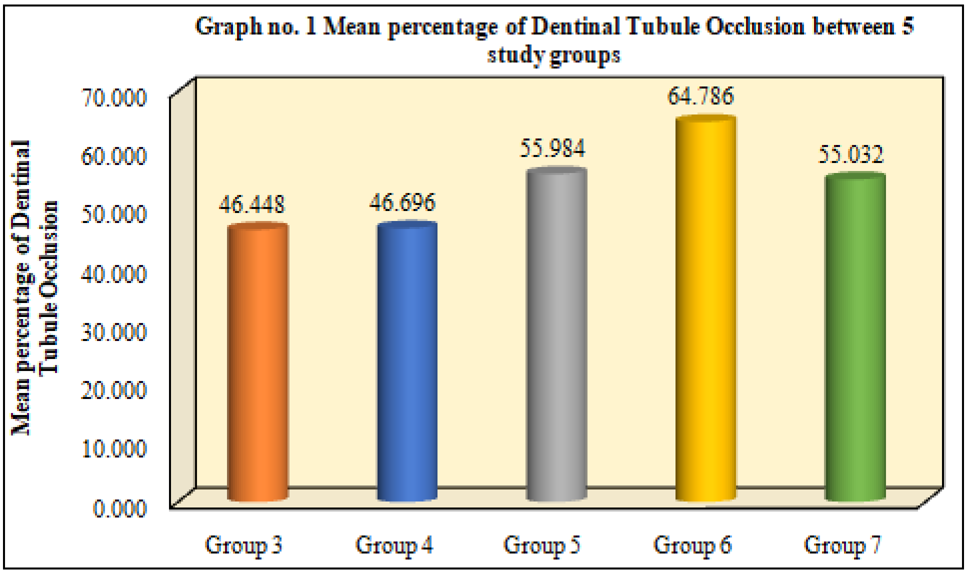
Table no.3 illustrates the multiple comparison of mean differences in Percentage of Dentinal Tubule Occlusion bamong 5 study groups. The test results showed that group 6 exhibited significantly highest Percentage of Dentinal Tubule Occlusion as compared to other study groups at P<0.001. This was next followed by group 5 showing a significantly higher mean percentage of Dentinal Tubule Occlusion as compared to Group 3, & 4 at P<0.001. Later, Group 7 showed a significantly higher mean percentage of dentinal tubule occlusion as compared to Group 3 & 4 at P<0.001. However, no significant difference was noted between Group 5 & 7 [P=0.97] and also between Group 3 & 4 [P=1.00]. This infers that Group 6 demonstrated significantly highest mean percentage of Dentinal Tubule Occlusion, followed Group 5, Group 7, Group 4 and least with Group 3. [***Refer Graphs no.2]***.

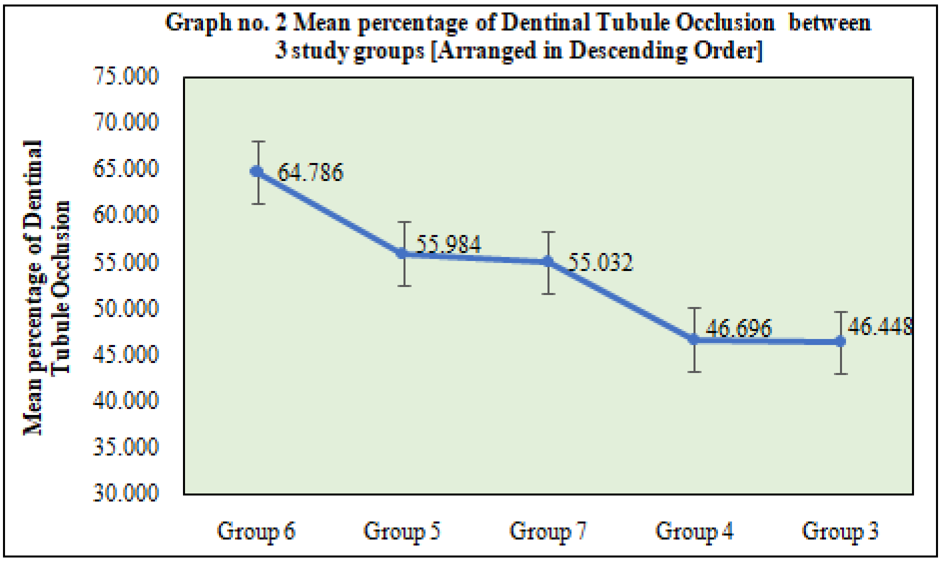
Table 2: Comparison of mean percentage of Dentinal Tubule Occlusion between 5 study groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Group 3	5	46.448	2.316	43.93	49.21	<0.001*
Group 4	5	46.696	2.687	43.37	49.26	
Group 5	5	55.984	2.319	52.16	58.12	
Group 6	5	64.786	1.897	62.18	66.76	
Group 7	5	55.032	2.642	51.51	58.48	

Table 3: Multiple comparison of mean difference in percentage of Dentinal Tubule Occlusion b/w groups using Tukey's HSD Post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Group 3	Group 4	-0.248	-4.769	4.273	1.00
	Group 5	-9.536	-14.057	-5.015	<0.001*
	Group 6	-18.338	-22.859	-13.817	<0.001*
	Group 7	-8.584	-13.105	-4.063	<0.001*
Group 4	Group 5	-9.288	-13.809	-4.767	<0.001*
	Group 6	-18.090	-22.611	-13.569	<0.001*
	Group 7	-8.336	-12.857	-3.815	<0.001*
Group 5	Group 6	-8.802	-13.323	-4.281	<0.001*
	Group 7	0.952	-3.569	5.473	0.97
Group 6	Group 7	9.754	5.233	14.275	<0.001*





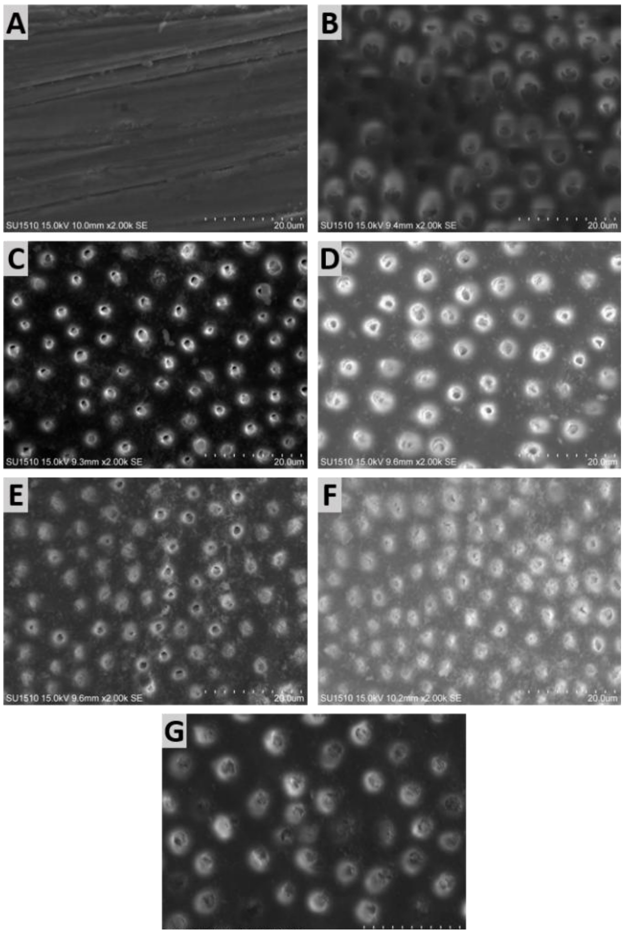


Figure 2: SEM images at x2000 magnification.
(A) Negative control, (B) Positive control, (C) Diode laser alone, (D) Diode laser with 0.5mg/ml ICG, (E) Diode laser with 1mg/ml ICG, (F) Diode laser with 2mg/ml ICG and (G) Diode laser with graphite paste

5. Discussion

Tooth sensitivity is one of the most commonly faced problems causing discomfort and considerable concern to the patient. [11-17] Many studies have investigated its prevalence, with reported frequencies as high as one for every seven individuals. [18] It has been shown that almost all the dentinal tubules are occluded in intact and non-sensitive dentin [15, 19-24] while sensitive radicular dentin has a significant number of open tubules on its surface. [15, 19, 20, 22-24]

In most cases of severe hypersensitivity, active and comprehensive treatment is required. [12] In general, most treatments can exert their desensitization effects by following mechanisms: (a) decreasing tubular fluid flow, and/or (b) ion interactions that affect electrical activity thus decreasing dentinal nerve function. [11, 12, 25-28] Some perfect examples of first type of treatment are various tubule sealants (for example, Arginine 8% with calcium phosphate, [29-32] and Ammonium hexafluorosilicate, [33] etc.), cements, 32 resin bonding agents, and cavity varnishes. [34-36] Examples of the second mechanism include fluoride varnishes, strontium chloride, potassium nitrate in desensitizing toothpastes. [37-40] Unfortunately, these methods can provide only temporary and unpredictable desensitization [12, 41] and they can have delayed effects for as long as several weeks.

Therefore, new treatment methods that do not have the aforementioned complications and are effective for longer time periods are needed. Some studies have reported that laser irradiation alone or in combination with other chemical agents such as sodium fluoride, potassium nitrate and Gluma© yield stable results comparable or superior to different chemical methods used for the treatment of DH. [42, 43]

The advantages in using laser desensitizing treatments include ease and time efficiency of application, higher rates of patient response, early and greater durability and predictable treatment outcome. [4, 5]

In this study, 810nm Diode laser was used alone and in combination with indocyanine green at a concentration of 0.5mg/ml, 1mg/ml and 2mg/ml. Graphite paste was also used to increase the coagulative effect of lasers. Indocyanine green is a chromophore which is believed to play an efficient role in the absorption of laser energy, particularly the diode laser. It is used in different fields such as retinal disease, ocular tumour, systemic disease, infection, and inflammatory disease, owing to its outstanding features and clinically excellent properties that are sufficiently appropriate for treatment. [44] This combination has been successfully used in photodynamic therapy for endodontic and periodontal purposes considering the fact that this photosensitizer is activated with the 810 nm diode laser. [45, 46]

In group I, after using coarse to fine grit abrasive papers, the samples did not undergo any surface treatment. A smear layer was seen on SEM image. This group served as negative control. In group II, the dentin specimens were treated with 17% EDTA for 1 minute to remove the smear layer. SEM image showed completely open dentinal tubules. This was considered as a positive control group for comparison.

In the third group, diode laser was used with 1W power in a continuous mode for 20 seconds.7 The mean percentage of obstruction of tubules was 46.448 ± 2.316. Diode laser acts on DH by two mechanisms. First, it melts and crystallizes dentin's inorganic component and coagulates fluids contained in the dentinal tubules. Second, by reducing the pulpal nerve's pain threshold. [5] Among lasers, diodes are the most studied and gave the best results in several clinical protocols even in severe hypersensitivity cases. [47] In our study, based on the results, this group showed significant occlusion of tubules, which can reduce DH drastically.

In fourth, fifth and sixth group, diode laser was used in combination with Indocyanine green at a concentration of 0.5, 1 and 2mg/ml respectively. Till date, only one study [7] has compared the effect of indocyanine green on tubule occlusion by diode lasers. The effect of different concentrations of ICG on tubular occlusion was not studies in the previous study. In this study, 1mg/ml indocyanine green showed 55.984 ± 2.319% obstruction of dentinal tubules. The results are in agreement with the study conducted by Khoubrouypak Z et. al, [7] and indicated the optimal efficacy of chromophore when used in combination with the diode laser for increasing the absorption of laser energy and the obstruction of dentinal tubules.0.5mg/ml ICG did not show any improvement in tubular occlusion as compared to diode laser group.2mg/ml ICG showed increase in mean tubular obstruction i. e., 64.786 ± 1.897%. These results show that tubular occlusion is directly proportion to the concentration when indocyanine green is used as a chromophore along with Diode laser at 810nm.

Similar results were obtained by Pourhajibagher M et. al. [48] on evaluation of antimicrobial photodynamic therapy with indocyanine green and curcumin on human gingival fibroblast cells. They reported that increasing doses of ICG and decreasing exposure time of light source may be an effective strategy for aPDT as an alternative treatment for periodontal disease.

In this study, graphite paste was also used to evaluate its effect on melting property of diode laser and compare it with ICG. Umana et al [9] used graphite paste as chromophore. In their study, half of the samples were coated with graphite paste and subjected to 810 and 980 nm diode laser irradiation with different powers. The results showed graphite paste increases heat generation and consequently, decreased the diameter of open tubules. Similar results were obtained in this study. Graphite paste group also showed comparable tubular occlusion to 1mg/ml ICG group.

Thus, addition of a chromophore along with lasers can be valuable in management of dentin hypersensitivity. Further studies need to be conducted to evaluate the effect of different chromophores on different lasers and its effect in in-vivo conditions.

6. Conclusion

Considering the limitations of this study, it may be concluded that:

1) Indocyanine green increase the tubular occlusion caused by diode lasers. The effect is concentration dependent.
2) Graphite paste showed results similar to the 1mg/ml ICG group.

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