**Journal of Cardiovascular Disease Research**
**ISSN: 0975-3583, 0976-2833 VOL11, ISSUE 10, 2020**

# EFFECTIVENESS OF Er:YAG LASER AS A DENTINAL TUBULE OCCLUDING AGENT: AN IN-VITRO SEM STUDY

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**Abstract**
*Background:* Dentinal tubules play an important role in transferring stimuli and local irritants to the pulp. *Aim:* This invitro SEM study aims to analyse the occluding effect of Er:YAG Laser on exposed dentinal tubules.
*Materials and Methods:* 60 tooth samples prepared from healthy maxillary first pre-molars extracted due to orthodontic reasons were grouped randomly into two groups of 30 samples each: Group I: control group (C) & Group II: Er:YAG laser group (LG). The group-I samples acted as control to record the diameter of patent dentinal tubules and group-II samples were treated with Er:YAG laser of setting 1.3W, 100mJ, 3Hz, 60s twice to study its occluding effect on the tubules. The samples were then subjected under SEM, to record the data and microphotographs and were then statistically analysed using the unpaired t-test.
*Results:* the results showed that Er:YAG laser (1.3W, 100mJ, 3Hz, 60s twice) can significantly reduce the number and diameter of the open dentinal tubules thus proving to be a promising agent to reduce the patency of open dentinal tubules.
*Conclusion:* The present in-vitro SEM study has demonstrated that Er:YAG laser seems to be an appropriate tool for partially or completely blocking the patent dentinal tubules.

**Keywords:** Dentinal hypersensitivity, laser, dentinal tubule occlusion, scanning electron microscope

## Introduction:

Dentin hypersensitivity (DH) is a specific pain condition originating from exposed dentin with opened patent dentinal tubules (DTs)[1]. It is now one of the most common non-carious diseases as a result of population lifestyle changes. Despite being a common complaint in clinical dentistry practice, it is also one of the least successfully addressed dental issues[2,3]. The major contributing factors for dentin exposure and sensitivity are: loss of enamel and/or denudation of cervical root surface by loss of overlying cementum, resulting due to various reasons such as abrasion, erosion, attrition, abfraction, gingival recession, dental bleaching, periodontal treatment, root exposure with aging and improper brushing habits.[4,5,6]

The hydrodynamic theory presented by Brännström[7] is the most widely accepted explanation for tooth sensitivity. This theory proposes that when a stimulus is applied to opened tubules of dentin (due to non-carious lesions), it causes a rapid shift of fluids within the DTs, resulting in mechanical deformation of sensory nerves, which are located at the inner/pulpal end of the tubules and are responsible for pain production. Based on this theory's notion, DT narrowing or

occlusion to reduce dentin permeability and lower the pulp sensitivity threshold is a viable pain treatment method.[8]

Absi, Addy and Adams[9] (1987) discovered that teeth with DH had eight times more open DTs per surface area than teeth without DH, and sensitive teeth had twice the tubular diameter as insensitive teeth. West10 reported that the diameters of the DTs were substantially greater in a hypersensitive area than in a non-sensitive one. The usual technique for managing DH was to seal DTs exposed to the oral environment with desensitising drugs that prevented contact between tubules and external stimuli.[11-13] For a DH treatment to be effective, desensitizing agents must resist acid challenges and mechanical impediments encountered in the oral cavity. However, many of these agents were not found to have a long-term effect and the use of lasers for DH treatment became currently an efficient alternative, since proved to have an interesting long-term effect. Er:YAG laser gained popularity in the treatment of dental hard tissues after approved by Food and Drug Administration (FDA) in 1997[14]. Er:YAG laser was used for the first time for DH therapy by Schwarz et al.[15], where an immediate laser desensitization from a single session was reported to be effective and maintained a more prolonged positive result compared with conventional desensitizing agents. Therefore, the objective of this in vitro study was to explore the effects of Er:YAG laser parameters suggested for tooth desensitization, on exposed DTs., to provide guidance for the clinical treatment of DH.[16]

## Materials and Methods

***Sample Collection:*** 60 samples of 2x2x1mm section was obtained from just below the cemento-enamel junction of the facial surface of the cervical portion of intact maxillary pre-molars extracted due to orthodontic reasons. Before sectioning, root planing was done to remove the cemental layer, thus exposing the root dentine. The samples were put in 17% aqueous EDTA (Dent Wash, Prime Dental Products PVT. LTD. India) to remove the smear layer and then in 5% sodium hypochlorite solution (Hyposol, Prevest Denpro Ltd. India) for 5 minutes each and finally washed with distilled water and stored in it till further use.

***Group Distribution:*** Finally, 60 samples were divided randomly into two groups of 30 samples each as follows:
Group I : control group (C)
Group II : laser group (LG)

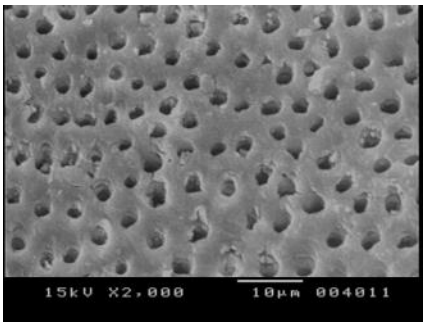
### Group I - Control Group (C)

30 samples of this group served as the control. These were subjected under SEM to study the total number of open dentinal tubule and the tubule diameter which acted as reference to the test group (LG).

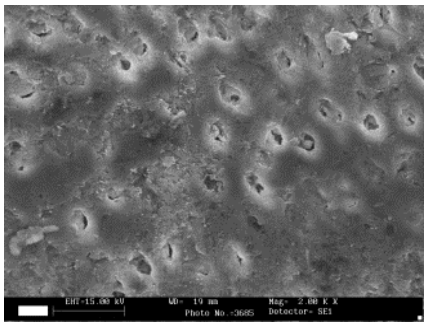
### Group II - Laser Group (LG)

For this evaluation, 30 samples were subjected to a hard-tissue Er:YAG laser (Litetouch, Syneron; version 1.26) with a power setting of 1.30w, 100mJ, 3Hz for 60seconds twice with a water coolant in continuous mode. The Er:YAG laser was used in scanning movements, defocused, perpendicularly at 6 mm from the surface. The Er:YAG laser part of this study was done at Confident Dental Care, Bengaluru; Karnataka.

***SEM preparation of the samples:*** After this, all the samples were first immersed in 2.5% gluteraldehyde (Cidex, Johnson & Johnson, India) for 12 hours at 4$^{o}$C to preserve the sample then chemically dehydrated in ascending grades of ethanol ( 25%, 50%, 70%, 90% and 100% for 5 min each) followed by 24 hours of air-drying at room temperature. The samples were dehydrated, sputter coated with gold and finally subjected under SEM [Leica S440i, Leica Cambridge, Ltd. at CPRI, Bangaluru] at 15kv. Black and white microphotographs were obtained for each sample at 500x, 1000x and 2000x magnification. The number of tubules that were left partially or completely unblocked and the tubule diameter after treatment of the two groups were also obtained using Leica Image Analysis Software.




(a)



(b)

Figure 1: showing (a) control group: open dentinal tubules, (b) Er:YAG laser treated group showing partially/completely occluded dentinal tubules

**Sample Analysis**

**Analysis of reduction in tubule diameter:** Each sample of the two groups was calculated for the mean tubule diameter. In the control group each electron microphotograph taken at 2000x magnification was divided into 5 equal parts and randomly a tubule was chosen from each part. The mean tubule diameter (in microns) for each microphotograph was obtained and thus for the entire control group; and the data noted on a Microsoft excel data sheet (Table:1). This mean tubule diameter of the control group represented the opened tubule diameter at 2000x magnification which acted as the reference value for the test groups. Next, the test group was studied for reduction in the mean tubule diameter following the same criterion as above and tabulated on the data sheet. The mean reduction in the tubule diameter in the test group was calculated by subtracting the mean tubule diameter from the reference value (obtained from the control group). Thus, the mean tubule diameter reduction in the test group was obtained (Table:1). This was followed by calculation and tabulation of the mean percentage reduction of the tubule diameter and statistical analysis using the Unpaired t-test was applied for pair-wise comparison of the groups.

**Analysis of reduction in number of dentinal tubules:** Each dentinal tubule of every microphotograph of the control group was counted using the Leica Image Analysis Software and the values were obtained and tabulated (Table:2). Thus, the mean number of tubules per sample at 2000x magnification was taken as the reference value. Similarly, each microphotograph of the

test group was counted for the left-over partially and completely unblocked dentinal tubules. Thus, to obtain the number of tubules reduced in test group, the mean number of tubules of each test group was subtracted from the mean number of control tubules. The values were tabulated (Table:2) and the mean number of tubule reduction in each group was calculated.The mean percentage reduction in the number of dentinal tubule was then calculated and tabulated by the same method as mentioned above (Table:2) and statistical analysis using the Unpaired t-test was applied for pair-wise comparison of the groups.

**Observation and Results**

- *Statistical analysis of tubule diameter reduction of the test groups with reference to the control group:* The mean tubule diameter for the control group was 1.97 ± 0.49 µm (i.e. 0% reduction) and mean tubule diameter reduction achieved in Er:YAG laser treated group was 0.95 ± 0.33µm (46.71%). The unpaired t-test revealed extreme statistical difference in mean between the test and the control group. Thus, test samples showed significant reduction in the mean tubule diameter as compared to the control group (Table 1&2).

| | **Control Group (C)** | **Laser Group (LG)** | |
|---|---|---|---|
| | Diameter Of Open Dentinal Tubule | Reduction Obtained (In Microns) | Reduction Obtained (In %) |
| **Mean** | 1.97 | 0.95 | 48.2 |
| **Standard Deviation (SD)** | 0.49 | 0.33 | 17.3 |

**Table1: showing dentinal tubule diameter reduction (in microns and in percentage) of the test group with reference to the control group**

| | **Control** | **Control vs Laser** |
|---|---|---|
| **Mean** | 0 | 0.95 |
| **SD** | 0 | 0.33 |
| **P-value** | | less than 0.0001 |

**Table2: showing the unpaired t-test for pair-wise comparison of the test group for tubule diameter reduction with reference to the control group.**

- *Statistical analysis of reduction in number of open dentinal tubules:* The mean number of dentinal tubule observed in the control group was 47.07 ± 15.23 (i.e 0% bocked) and the number of tubules blocked in the laser group was 28.6 ± 10.58 (60.96%). There was an extreme statistical difference in the mean number of tubules blocked in the test group with reference to the control group ($p<0.0001$) (Table 3&4).

Thus, it was observed that Er:YAG laser was effective in reducing the number and diameter of the open dentinal tubules.

| | **Control Group (C)** | **Laser Group (LG)** |
|---|---|---|

| | Total Number Of Open Tubules | Number Of Tubules Blocked | Percentage Of Tubules Blocked |
|---|---|---|---|
| **Mean** | 47 | 28.6 | 60.9 |
| **Standard Deviation (SD)** | 15.2 | 10.6 | 22.5 |

**Table 3: showing number and percentage of dentinal tubules blocked in the test group with reference to the control group**

| | **Control** | **Control vs Laser** |
|---|---|---|
| **Mean** | 47.07 | 28.6 |
| **SD** | 15.23 | 10.58 |
| **P-value** | | 0.0001 |

**Table 4: Unpaired t- test for pair-wise comparison of the groups to statistically evaluate the number of dentinal tubules blocked in the test group with reference to the control group.**

## Discussion

The advent of laser treatment has provided an alternative modality for DH management.[17] The effectiveness of lasers for treating DH varies from 5 to 100%, depending upon the type of laser and the parameters.[18] In this study, we assumed the Er:YAG laser with optimal parameters can effectively treat DH by occluding DT. The parameters of Er:YAG laser considered in this study was kept the same as by Birang et at (2007) in his study. He compared the impact of Er:YAG laser (100mJ, 3Hz, 60s, twice) and Nd:YAG (1W, 15Hz, 60s, twice) laser in dentinal hypersensitivity treatment in 63 patients and stated that both the lasers have an acceptable therapeutic effect.[19] Schwartz et al (2002), first one to try Er:YAG laser for dentin hypersensitivity found that high absorption of the Er:YAG laser emission wavelength in water resulted in evaporation of dentinal fluid from tubules and smear layer especially within first 6 months. Additionally, the probable anti-bacterial feature of the laser may be also bestowed to the desensitising effects.[15]

Our findings were also consistent with the findings of previous other studies exploring the DT occluding effects of the Er:YAG laser, although the parameters used in the present study were different from those used in previous studies. Belal and Yassin[20] (2014) in their SEM study evaluated the effects of an Er:YAG laser on DT occlusion to observe melted areas around exposed DTs. The percentage of occluded tubules was found to be significantly greater in the Er:YAG laser group than in the other groups. Badran et al[21] (2011) also reported complete DT occlusion by 120s of Er:YAG laser irradiation, showing a wrinkled, melted dentin surface with no visible signs of DTs. Overall, the thermo-mechanical ablation of Er:YAG laser may be a major influencing factor for controlling application parameters of the laser. Temperature increase on the irradiated surface can induce melt and recrystallization of the dentin tissue, resulting in obliteration of the tubule orifices.[22]

In summary, we conducted a preliminary *in vitro* study investigating suitable parameters for the successful treatment of DH using the Er:YAG laser. Our findings can, to some extent, serve as a

reference for further clinical trials. Taking the high water absorption of Er:YAG laser energy into account, the fluid in teeth and the blood circulation in the pulp may reduce the increase in temperature, consequently increasing the safety of parameters in actual clinical trials. However, this study has several limitations: first, the sample size was relatively small. Second, it was very difficult to standardize the variations of the DT numbers of dentin even at same depth bellow the dentin due to individual variations. Third, it is an *in vitro* study, and hence clinical trials with long-term follow-up examinations under intraoral conditions like brushing and acidic challenges are required**.**

## Conclusion

Hence, under the present given parameters, Er:YAG laser, was effective in reducing the diameter and number of dentinal tubules. Further in-vitro, experimental and clinical studies including larger samples/subjects can be done to evaluate the long-term stability of the obtained positive results with this test agent.

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