Comparative evaluation of dentinal tubule occlusion ability of commercially available dentifrice and diode laser- A Scanning Electron Microscopic study

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ABSTRACT

The aim of the study was to evaluate and compare the tubule occluding ability of calcium sodium phosphosilicate (Novamine) containing dentifrice, 980nm GaAlAs Diode laser, and their combination by Scanning Electron Microscopy. Total number of 180 Dentine disc specimens were prepared and divided into 4 groups of 45 specimens each. Each group was divided into subgroups as once a week, once a week for two weeks and once a week for three weeks respectively. G1a,1b,1c,2a,2b,2c,3a,3b,3c,4a,4b and 4c The group G1 was brushed without dentifrices. The treatment groups G2, G3 and G4 were subjected to Novamin, 980 nm GaAlAs Diode laser, novamin and laser combination respectively. All the specimens were stored in artificial saliva medium and then observed under SEM to evaluate the tubule occlusion. Data was statistically analyzed using ANOVA test. All the treated groups exhibited higher percentage of tubule occlusion compared to control group. Among the treated groups the specimens brushed with combination group (Laser and Novamin) showed the highest percentage of tubule occlusion followed by GaAlAs laser, and Novamin (P< 0.05). With regard to correlation between frequency of application and tubule occlusion, there was a gradual increase in tubule occlusion with increase in frequency of application. However, the difference in mean occlusion among the different applications was not statistically significant (P>0.05). It was found from the results that these agents can be considered as effective treatment modalities for dentine hypersensitivity therapy.

Key words: Calcium sodium phosphosilicate; Dentine hypersensitivity; Galium Aluminium Arsenide Diode Laser, Dentinal tubules.

INTRODUCTION

Dentinal hypersensitivity (DH), or cervical dentinal sensitivity, is a global oral health problem in the adult population. It is defined as “pain arising from exposed dentine in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical, which cannot be ascribed to any other form of dental defect or pathology” and satisfies all the criteria to be classified as a true pain syndrome. [1] It is clinically described as a brief, sharp, “bright” type of pain with a rapid onset, although it may also be followed by a dull, aching pain. [2] The pain may be localized or generalized, affecting one or multiple tooth surfaces simultaneously. The definition of DH therefore has two aspects: one is describing the clinical presentation and the second identifying the condition by exclusion of other pathologies, highlighting the need for correct differential diagnosis. [3]
Essentially exposure of the dentin may result from one of the processes: [4] either removal of the enamel covering the crown such as tooth brush abrasion, dietary erosion, habits or denudation of the root surface by loss of cementum and overlying periodontal disease. This could be due to gingival recession, chronic periodontal disease, following non-surgical periodontal therapy, incorrect tooth brushing technique, chronic trauma from habits.

Transmission of pain from the dentin to the pulp is not fully understood. The most accepted explanation is the hydrodynamic theory which states that external stimuli causes a rapid flow of fluid in the dentinal tubules, activating mechanoreceptors at the pulp-dentin interface, leading to pain. The flow of dentinal fluid is influenced by configuration of tubules, the tubular diameter, and the number of open tubules.[5]

Various modalities in the treatment of dentinal hypersensitivity include 1. Nerve desensitization: Potassium nitrate. 2. Anti-inflammatory agents: Corticosteroids. 3. Cover or plugging dentinal tubules (a) Plugging (sclerosing) dentinal tubules: Ions/salts, Calcium hydroxide, Potassium oxalate, Sodium Monofluorophosphate, Sodium fluoride, Stannous fluoride, Fluoride iontophoresis (b) Den tine Sealers: Glass ionomer cements, Composites Resins, Varnishes, Sealants, Methyl methacrylate (c) Periodontal soft tissue grafting (d) Crown placement /restorative material (e) Lasers: carbon dioxide laser, diode laser, Nd YAG laser etc. [6]

It has been extensively demonstrated that some agents are able to lower intradental nerve activity in-vivo and reduce dentin permeability in-vitro; however there is little information about their effects under simulated oral environment subsequent to artificial saliva immersion.

The present in-vitro study aimed to evaluate and compare the effects of 980nm GaAlAs (Galium Aluminium Arsinide, SIRO Laser Xtend, Sirona Dental Systems GmbH, Germany) diode laser and bioactive glass containing dentifrice (Sensodyne repair®) alone and in combination in occluding dentinal tubules under the circumstances similar to the oral environment using artificial saliva under scanning electron microscope.

**EXPERIMENTAL SECTION**

The study was conducted in Department of Periodontology D. A. P. M. R.V. Dental College, Bangalore and the Ethical clearance for the study was obtained from the ethical committee and review board of the institution.

**Data collection**

180 premolar teeth extracted due to orthodontic reasons were collected from Department of oral and Maxillofacial Surgery, D. A. P. M. R.V. Dental College, Bangalore. Teeth with fluorosis or hypo calcification, carious tooth, teeth with defective restoration and facets, teeth with cracked structure, teeth with presence of any wasting diseases and teeth extracted from patients who had taken treatment for dentinal sensitivity, were excluded from the study.

**Study design**

Extracted human premolar teeth were cleaned thoroughly with plain water and stored in 10% formalin (pH 7) at room temperature. Materials used in the study are as shown in figure- 1. One hundred and eighty dentin discs each with a thickness of 1mm were carefully cut perpendicular to the long axis of the tooth above the cement enamel junction by means of a diamond disc mounted on hand trimmer. After obtaining teeth sections the occlusal surface of each dentin disc was sanded with 600 grit silicon carbide paper for 30 seconds to create a smear layer. The smear layer was subsequently removed by dipping the dentin discs into 0.5M EDTA solution (pH 7.4) for 2 minutes. The etched dentin discs were rinsed with distilled water and specimens were equally distributed into 4 groups each containing 45 specimens. Each test groups were further subdivided into three subgroups as a, b and c with 15 specimens in each.

**GROUP 1 (n-45):** Control Group (Plain Water)

**GROUP 2 (n-45):** Sensodyne repair® Group (novamin containing dentifrice).

Group 2a (n-15): Sensodyne repair® application once in first week.

Group 2b (n-15): Sensodyne repair® application once a week for two weeks.

Group 2c (n-15): Sensodyne repair® application once a week for three weeks.

**GROUP 3 (n-45):** GaAlAs Diode Laser Group.

Group 3a (n-15): laser application once in first week.
Group 3b (n-15): laser application once a week for two weeks.
Group 3c (n-15): laser application once a week for three weeks.

**GROUP 4(n-45): Sensodyne repair® and Laser Combination Group**

Group 4a (n-15): Sensodyne repair® followed by Laser application once in first week
Group 4b (n-15): Sensodyne repair® followed by Laser application once a week for two weeks.
Group 4c (n-15): Sensodyne repair® followed by Laser application once a week for three weeks.

**Dentifrice application**
Each specimen from Group 2 and Group 4 was brushed with undiluted tooth paste (approximately 1 gram) with a custom made tooth brushing machine and powered tooth brush. Tooth brush with bristles of medium hardness was applied to the dentin surface at an angulation of 90° for 2 minutes. [7] For the control group, specimens were brushed using plain water (Figure-2).

**Diode Laser application**
Each specimen was lased with 980 nm Galium: Aluminum: arsenide (GaAlAs) diode laser for 60 seconds. (Parameters: 0.5 W, non-contact mode with a distance of 2-4 mm and using a fiber of 320-micron diameter) (Figure-3).[8]

Specimens were rinsed with distilled water after each application of respective agents and stored in artificial saliva (pH 7.2) till they were subjected for scanning electron microscopic analysis. Artificial saliva was prepared manually with the following composition. Distilled water (700 ml), Calcium Hydroxide (1.56 mM/L), Potassium Chloride (150 mM/L), Hydrochloric Acid (36 mM/L), Phosphoric acid (0.88 mM/L) and buffer (99.7 mM/L).[9]

**SEM analysis**
SEM analysis of the each specimen was done and percentage of occluded tubules was obtained by dividing the total number of occluded tubules to the total number of tubules in each photomicrography which was then multiplied by 100, to obtain the percentage of occluded tubules for each photograph. Each SEM photograph was assessed as:
- Percentage of Completely occluded tubules,
- Percentage of Partially occluded tubules and
- Percentage of un-occluded tubules. (Figure-4, 5, 6 & 7)

Statistical test: Analysis of Variance (ANOVA).

**RESULTS AND DISCUSSION**

The groups subjected to novamin containing dentifrice (G2), diode laser (G3) and their combination (G4) showed a significantly higher percentage of occluded tubules than control group (G1).

**Completely occluded tubules**
At one week, highest mean completely occluded tubules was observed in Group 4 (73.81%) followed by Group 3 (66.94%), Group 2 (39.88%) and Group 1 (2.07%). At two weeks, highest mean completely occluded tubules was observed in Group 4 (85.63%) followed by Group 3 (76.91%), Group 2 (64.38%) and Group 1 (2.60%). Similarly at three weeks, highest mean completely occluded tubules was observed in Group 4 (96.30%) followed by Group 3 (91.30%), Group 2 (67.19%) and Group 1 (2.93%).

Hence, with the above mentioned data obtained from our results we report that combination treatment group 4 (Novamin and GaAlAs Diode Laser) showed highest mean value of complete tubule occlusion at all intervals followed by group 3 (GaAlAs Diode Laser), group 2 (Novamin) and group 1 (Control).

**Partially occluded tubules**
At one week, highest mean partially occluded tubules was observed in Group 2 (35.27%) followed by Group 3 (22.35%), Group 4 (20.22%) and Group 1 (2.39%). At two weeks, highest mean partially occluded tubules was observed in Group 2 (25.61%) followed by Group 3 (12.48%), Group 4 (12.36%) and Group 1 (3.83%). Similarly at three weeks, highest mean partially occluded tubules was observed in Group 2 (24.74%) followed by Group 3 (2.93%), Group 4 (2.60%) and Group 1 (5.13%).
Hence with the above mentioned data obtained from our results we report that group 2 (Novamin) showed higher percentage of partial occlusion compared to other groups at different applications and difference in mean partial tubule occlusion was statistically significant.

The difference in mean occlusion from pretreatment to post treatment among the groups was found to be statistically significant (P<0.001).

**Unoccluded tubules**

At one week, highest mean unoccluded tubules was observed in Group 1 (96.30%) followed by Group 2 (25.74%), Group 3 (9.78%) and Group 4 (5.13%). At two weeks, highest mean unoccluded tubules was observed in Group 1 (85.86%) followed by Group 2 (10.46%), group 3 (4.21%) and Group 4 (3.83%). Similarly at three weeks, highest mean unoccluded tubules was observed in Group 1 (85.63%) followed by Group 2 (7.66%), Group 4 (2.07%) and Group 3 (1.54%).

Hence with the above mentioned data obtained from our results we report that group 1 (Control) showed higher percentage of unoccluded tubules compared to other groups at different applications and difference in mean partial tubule occlusion was statistically significant.

When mean complete occlusion/ partial occlusion /non occlusion was compared between groups, all the results showed statistically significant difference (P<0.001).

**Comparison of percentage in mean tubule occlusion at different application within each group**

**Comparison of occlusion within Group 1 between different applications:**
Higher mean occlusion was recorded when the application was once in first week followed by once for three weeks and once for two weeks (Graph 1). However, the difference in mean occlusion among the different applications was not statistically significant (P>0.05).

**Comparison of occlusion within Group 2 between different applications:**
Higher mean occlusion was recorded when the application was once in first week followed by once a week for two weeks and once a week for three weeks (Graph 2). However, the difference in mean occlusion among the different applications was not statistically significant (P>0.05).

**Comparison of occlusion within Group 3 between different applications:**
Higher mean occlusion was recorded when the application was once in first week followed by once a week for three weeks and once a week for two weeks (Graph 3). However, the difference in mean occlusion among the different applications was not statistically significant (P>0.05).

**Comparison of occlusion within Group 4 between different applications:**
Higher mean occlusion was recorded when the application was once a week for two weeks followed by once a week for three weeks and once in first week (Graph 4). However, the difference in mean occlusion among the different applications was not statistically significant (P>0.05)

The hydrodynamic theory is widely accepted as a principal mechanism of action for manifestation of dentinal hypersensitivity. There have been 2 basic approaches to the treatment and prevention of DH. 1) An agent that penetrates into the dentinal tubules depolarizes the nerve synapse and, thereby prevents the conduction of pain impulses (e.g., potassium nitrate, Laser). 2) Chemical or physical agent that creates a deposition layer and mechanically occludes dentinal tubules, which prevents pulpal fluid flow (e.g., novamin, lasers, ferric oxalate, strontium chloride). [10 - 12]

Although both approaches are effective at reducing the duration of relief is highly variable. Hypersensitivity usually reappears due to toothbrush abrasion, the presence of acid challenges in the mouth, and/or degradation of the coating material. [13 - 14]

Exposure to saliva or acid may reverse the decrease in permeability caused by the desensitizing agents. Saliva can dissolve the desensitizing material adherent to tooth surface thus reducing their long term effects. Hence for an
agent to be considered as ideal in the treatment of dentinal hypersensitivity, it should not only reduce dentin permeability but should also sustain its occlusion effects in the presence of saliva. [15]

Treatment for dentinal hypersensitivity can be broadly classified as home and office methods. Novamin containing dentifrice comes under the category of home remedies and laser under office methods.

This study aimed at comparative evaluation of potential effects of novamin containing dentifrice, 980nm GaAlAS Diode laser and their combination in dentinal tubule occlusion.

The groups subjected to novamin containing dentifrice, diode laser and their combination showed a significantly higher percentage of occluded tubules than control group (G1). This is in accordance to study by Wang et al. [7] and Alfredo et al [16]

Alfredo et al in their in vitro study showed that following diode laser application, dentin structure was changed because of the thermal effects caused by laser energy. A crystalline arrangement and melting was observed which could be attributed to the fact that some of the energy being absorbed by the dentin’s mineral content, which includes carbonate and phosphate resulting in melting and tubule occlusion.[16]

Difference in sensitivity reduction between novamin containing dentifrice and GaAlAs diode laser can be explained by their mechanism of action. The Calcium sodium phosphosilicate (NovaMin®) bioactive glasses are known to induce the osteogenesis in physiological system and would appear to offer suitable materials for surface reactivity. Such reactive bioglass when exposed to body fluids such as saliva, tended to deposit hydroxycarbonateapatite, a mineral that is chemically similar to the mineral in enamel and dentin, which is supersaturated with respect to artificial saliva. [7]

The SEM photomicrographs revealed that all tested treatments produced morphological modifications to the dentine surfaces. In control group we found that most of the dentinal tubules were open, with neither deposits nor smear layers on the peritubular and intratubular dentin. Small irregular deposits and debris were left in some of the tubules. Brushing dentine with Novamin containing dentifrices and diode laser created a homogeneous layer that completely covered the dentine surface. Only a few open dentinal tubules were visible. This is in accordance with studies by Wang et al [7], Curtis AR et al. [17] Romeo Umberto et al. [8]

Results from the present study showed that combination treatment group 4 (Novamin and GaAlAsDiode Laser) showed highest mean value of complete tubule occlusion at all intervals followed by group 3 (GaAlAsDiode Laser), group 2(Novamin) and group 1(Control). This is in accordance with study by Wang Z. Et al [6],Sauro S. et al [18], Curtis AR et al [17], Romeo Umberto et al. [8]

<table>
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<th>Group</th>
<th>Mean</th>
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<th>95% CI for Mean</th>
<th>P-Value</th>
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*Denotes significant difference

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*Denotes significant difference

Table 3: Comparison of mean completely occluded tubules at 3 weeks

Table 1: Comparison of mean completely occluded tubules and application at first week

Table 2: Comparison of mean completely occluded tubules and application once at 2 weeks

Table 3: Comparison of mean completely occluded tubules at 3 weeks

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### Table 4: Comparison of mean partially occluded tubules at first week

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*Denotes significant difference

### Table 5: Comparison of mean partially occluded tubules and application at 2 weeks

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<td>3 vs 4 (P&lt;0.001)</td>
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<td>12.01</td>
<td>12.71</td>
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*Denotes significant difference

### Table 6: Comparison of mean partially occluded tubules at 3 weeks

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<td>Group 3</td>
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*Denotes significant difference

### Table 7: Comparison of mean unoccluded tubules at first week

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### Table 8: Comparison of mean unoccluded tubules at 2 weeks

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### Table 9: Comparison of mean unoccluded tubules at 3 weeks

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*Denotes significant difference
Table 10: Comparison of occlusion within Group 1 between different applications

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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td></td>
</tr>
<tr>
<td>Once in first week</td>
<td>33.59</td>
<td>44.85</td>
<td>6.69</td>
<td>20.11</td>
<td>47.06</td>
</tr>
<tr>
<td>Once for two weeks</td>
<td>30.76</td>
<td>39.41</td>
<td>5.88</td>
<td>18.92</td>
<td>42.60</td>
</tr>
</tbody>
</table>

Figure :1 Armamentarium for the study
Once for three weeks | 31.23 | 38.93 | 5.80 | 19.53 | 42.92 |  

Table 11: Comparison of occlusion within Group 2 between different applications

<table>
<thead>
<tr>
<th>No of applications</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SE of Mean</th>
<th>95% CI for Mean</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Once in first week</td>
<td>33.63</td>
<td>6.33</td>
<td>0.94</td>
<td>31.73</td>
<td>35.53</td>
</tr>
<tr>
<td>Once for two weeks</td>
<td>33.48</td>
<td>23.05</td>
<td>3.44</td>
<td>26.56</td>
<td>40.41</td>
</tr>
<tr>
<td>Once for three weeks</td>
<td>33.20</td>
<td>25.34</td>
<td>3.78</td>
<td>25.58</td>
<td>40.81</td>
</tr>
</tbody>
</table>

Table 12: Comparison of occlusion within Group 3 between different applications

<table>
<thead>
<tr>
<th>No of applications</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SE of Mean</th>
<th>95% CI for Mean</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Once in first week</td>
<td>33.02</td>
<td>24.83</td>
<td>3.70</td>
<td>25.56</td>
<td>40.48</td>
</tr>
<tr>
<td>Once for two weeks</td>
<td>31.20</td>
<td>32.87</td>
<td>4.90</td>
<td>21.32</td>
<td>41.07</td>
</tr>
<tr>
<td>Once for three weeks</td>
<td>31.92</td>
<td>42.48</td>
<td>6.33</td>
<td>19.16</td>
<td>44.68</td>
</tr>
</tbody>
</table>

Table 13: Comparison of occlusion within Group 4 between different applications

<table>
<thead>
<tr>
<th>No of applications</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SE of</th>
<th>95% CI for Mean</th>
<th>P-Value</th>
</tr>
</thead>
</table>
### Table 1: Summary of Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once in first week</td>
<td>33.05</td>
<td>29.82</td>
<td>4.44</td>
<td>24.09</td>
</tr>
<tr>
<td>Once for two weeks</td>
<td>33.94</td>
<td>37.14</td>
<td>5.54</td>
<td>22.78</td>
</tr>
<tr>
<td>Once for three weeks</td>
<td>33.66</td>
<td>44.90</td>
<td>6.68</td>
<td>20.20</td>
</tr>
</tbody>
</table>

**Figure 4: GROUP 1**
CONTROL GROUP

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 1.

**Figure 5a: GROUP 2**
2A ONE WEEK NOVAMIN®

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 2a.
Figure 5b: GROUP 2
2B: TWO WEEK NOVAMIN®

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 2b.

Figure 5c: GROUP 2C
THREE WEEK NOVAMIN®

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 2c.
Figure 6a: GROUP 3A
ONE WEEK GaAlAs LASER

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 3a.

Fig 4b GROUP
TWO WEEK LASER

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 3b.
Figure 6c: GROUP 3C
THREE WEEK LASER

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 3c.

Figure 7a: GROUP 4A
ONE WEEK LASER AND NOVAMIN

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 4A
Figure 7b: GROUP 4B
TWO WEEK LASER AND NOVAMIN

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 4B

Figure 7c: GROUP 4C
THREE WEEK LASER AND NOVAMIN

SEM Micrographs of the dentine surface morphology group at 1500X (left) and 3000X (right) magnifications in Group 4C
Graph 1: Mean Occlusion in completely occluded tubules with application once in 1st week

Graph 2: Mean Occlusion in completely occluded tubules with application once for 2 weeks

Graph 3: Mean Occlusion in completely occluded tubules with application once for 3 weeks
Graph 4: Mean Occlusion in partially occluded tubules with application once in 1st week

Graph 5: Mean Occlusion in partially occluded tubules with application once for 2 weeks

Graph 6: Mean Occlusion in partially occluded tubules with application once for 3 weeks
Graph 7: Mean Unoccluded tubules with application once in 1st week

Graph 8: Mean Unoccluded tubules with application once for 2 weeks

Graph 9: Mean Unoccluded tubules with application once for 3 weeks
Graph 10: Mean Occlusion recorded in Group 1 with different applications

Graph 11: Mean Occlusion recorded in Group 2 with different applications

Graph 12: Mean Occlusion recorded in Group 3 with different applications
CONCLUSION

The results from our study indicate that all the treatment groups exhibited significantly higher percentage of tubule occlusion compared to control group. Among the treated groups the specimens brushed with combination group (Novamin and Laser) showed the highest percentage of tubule occlusion followed by GaAlAs laser and then Novamin. With regard to correlation between frequency of application and tubule occlusion, there was a gradual increase in tubule occlusion with increase in frequency of application. However differences were not statistically significant.

Hence from the results from our study it can be stated that novamin and GaAlAs laser can be considered as effective treatment modality in dentinal hypersensitivity and combination therapy is superior to treatment with individual agents.

REFERENCES