A Comparative Invitro Study Investigating the Dentinal Changes Caused by Commercially Available Desensitizing Toothpastes and Laser - Original Research Article

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Abstract: *Aim*: The aim of this study was to compare the level of dentinal tubule blocking and mineralization achieved by the use of two commercially available dentifrices (Vantej® and Colgate® sensitive Pro-relief) with or without the use of laser for the management of dentinal hypersensitivity.

Method: Sixty extracted human molar teeth were used for this study. The teeth were sectioned into dentin discs of 2-4mm thickness. These specimens were then randomly divided into 6 groups and were treated with Vantej® tooth paste, Colgate Sensitive Pro-relief® tooth paste, Helium- Neon (He-Ne) laser, combination of He-Ne laser and Vantej® tooth paste and a combination of He-Ne laser and Colgate Sensitive Prorelief® tooth paste. The specimens were then analyzed under scanning electron microscope (SEM) for the evaluation of dentinal tubular blocking. The elemental analysis of the tubular content was evaluated using Energy Dispersive Xray Spectroscopy (EDX).

Results: The SEM analysis showed significant difference in the mean percentage scores within the individual groups. The Laser with Colgate Sensitive Prorelief® tooth paste group showed complete blocking of the dentinal tubules which was statistically significant ($P<0.001$), similar results were also observed with the Pro-relief® alone toothpaste group. The Vantej® tooth paste group also exhibited significant tubular blocking. The Laser alone group and combination of laser and Vantej® tooth paste group showed only partial tubular blocking. EDX analysis of the experimental groups revealed peaks of the elemental contents found in the experimental specimens.

Conclusion: The data obtained from this study demonstrated that the Colgate sensitive Pro-relief® toothpaste alone and /or when used in combination with laser therapy was found to most effective in the blocking of dentinal tubules.

Keywords: Dentinal hypersensitivity, EDX, Helium-neon laser, SEM, Tubular blocking.

INTRODUCTION

Effective management of dentinal hypersensitivity pertaining to the crown or root of a tooth has always been one of the most ceaseless efforts of clinicians. This incensing symptom is often described as a sharp shooting response in the tooth when it is subjected to hot, cold, sweet/ sour items or when air is blown over the surface of the tooth which cannot be recognized as arising from any other form of dental defect or pathology [1]. This condition could arise as a result of various reasons pertaining to faulty tooth brushing, gingival recession, rotated teeth etc. [1-3].

The physiological mechanism of pain sensation, proposed by Brannstrom as the hydrodynamic theory suggests that, the fluid movement within the dentinal tubules, as the basis for the transmission of sensations [4]. Further, Kim modified this theory stating that when potassium nitrate solution was used, it increased the inter-tubular potassium content which resulted in reducing the excitability of the nerve endings by depolarizing the nerve fiber membrane [5]. Based on these proposed mechanisms, the treatment of dentinal hypersensitivity has focused on preventing the triggering stimulus to cause distress either through use of products containing constituents that can block the dentinal tubules or through impediments with the transmission of nerve impulses.

Evidence has shown that lasers are also helpful for the management of dentinal hypersensitivity, with its effect on nerve analgesia and blocking of dentinal tubules [6]. Its application in dentistry has opened a new realm for treatment and research. There are basically two categories of lasers which have been sought for the treatment of dentinal hypersensitivity, this include: low output power (i.e. helium-neon and gallium/aluminium/arsenide lasers) and middle output power lasers, which include the neodymium yttrium-aluminium-garnet (Nd:YAG) and CO_2 lasers [7].

The probable mechanisms being suggested as either by the direct effect of laser irradiation on electric activity of nerve fiber or melting of dentinal surface resulting in blocking of patent dentinal tubules [8].

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Inspite the variant treatment options available for treatment of dentinal hypersensitivity [9] a consistently effective treatment has not been yet achieved. An attempt was made to combine two treatment modalities one, using commercially available desensitizing dentrifices and other using a low output laser along with combination of dentifrice. Hence, prior to performing a clinical trial, an initial work was carried out to evaluate the morphological alterations formed on the dentinal surface after the use of two commercially available dentifrices (Vantej®, Dr. Reddy's Laboratories Limited, Hyderabad, Andhra Pradesh, India and Colgate® sensitive Pro-relief, Colgate-Palmolive, Guildford, GU2 8JZ.) with or without the use of laser for the management of dentinal hypersensitivity. The surface topography of dentine was studied using the scanning electron microscopy [SEM], [LEO 440i, Carl Zeiss, Tokyo, Japan which provides insights on the effectiveness of the blocking action of the desensitizing agents. The chemical characteristics of the blocking agents were analyzed using the energy dispersive x-ray (EDX).

MATERIALS AND METHODS

Sixty extracted human molar teeth were selected for this study. Ethical clearance was obtained from the IRB, Manipal University, Manipal. The teeth were washed with sterile saline to remove loosely adherent soft tissues and the remaining soft tissue was removed using hand instruments. All the teeth were stored in distilled water containing 0.2% thymol at 4^{o}C to prevent bacterial contamination until use.

PREPARATION OF DENTIN DISCS

The crown portion of each tooth was subjected to high speed tungsten carbide fissure bur (Densply; Maillefer Instruments Holding Co., Ballaigues, Switzerland) so as to remove enamel from the surface. The crown were then sectioned into dentin discs of 2-4 mm thickness using a double sided diamond disc. All the discs of the experimental group was etched with 1% citric acid for one minute and washed with distilled water. The specimens were then randomly divided into 6 groups [Group A, B, C, D, E and F] of 10 samples each. In each group, the specimens were treated with the following therapeutic agents, Vantej® tooth paste, Colgate Sensitive Pro-relief® tooth paste, He-Ne Laser, He-Ne Laser and then painted with Vantej® tooth paste, He-Ne Laser and then painted with Colgate Sensitive Prorelief® tooth paste. Control group [F] had specimens washed with distilled water only.

All discs in Group [A, B, D and E] were applied with desensitizing toothpastes using a separate paint brush [Cosmodent, Chicago, IL, USA] for 3 minutes, then washed with distilled water. In the Group [C, D and E], the discs were lased with He-Ne (Melles Griot, Carlsbad, California, US.) laser with a wavelength of 632.80nm, emitting a continuous wave beam at 6 mW. The laser tip was placed as close to the dentinal surface in non-contact mode with the beam parallel to the dentinal tubules and perpendicular to the dentinal surface. The dentin specimens were lased for a time period of 6 minutes.

Following this, all the tooth specimens were prepared to be analyzed using scanning electron microscope (LEO 440i, Carl Zeiss, Tokyo, Japan) for evaluation of dentinal tubular blocking and to determine the presence of chemical contents next to the dentinal tubules of each specimen, an elemental analysis was done using Energy Dispersive X-ray analysis (EDX) (LEO 440i, Carl Zeiss, Tokyo, Japan).

PREPARATION OF TOOTH SPECIMENS FOR SEM

Each specimen was gently washed in 0.2 M potassium phosphate buffer (PBS), pH 7.2. The specimen were then fixed in 2% glutaraldehyde at 4°C to 6°C for 24 h, and then post fixed for12hr at 4°C to 6°C in 1% (wt/vol) osmium tetroxide. Dehydration of the dentin discs were performed with an ascending acetone series (30%, 60%, 100%) for 10 min each. The dentin discs were dried by using a SAMDRI PVT-3 critical point dryer apparatus (Tousimis Research Corp., Rockville, MD). The samples were then mounted on metallic stubs, gold sputtered using an ion sputter, and examined under scanning electron microscope for the surface characteristics and the patency of dentinal tubular blocking which was evaluated at 10 KV and ×1,500 magnification.

The blocked dentinal tubule were evaluated quantitively by the criteria proposed by Rome *et al.* [10] which is as follows: 0- all tubules open, 1- > 50% tubules open, 2- <50% tubules open, 3- all tubules closed.

The results of the present study was analyzed statistically by using one way ANOVA test for intragroup comparison. Bonferroni's post hoc test was

used for intergroup comparison. The level of significance were set as ($p \leq 0.05$).

RESULTS

A total of 10 specimens were divided into 6 groups and each group was treated individually and in combination with desensitizing dentifrices and laser. The SEM analysis showed significant difference in the mean percentage scores within the individual groups when compared to control [Graph **1**]. Among the individual experimental groups, Group B (Pro-relief® toothpaste alone) showed maximum tubular blocking which was statistically significant (mean % = 2.8) compared to Groups A, C, D and F. [Graph **1**, Figure **1B**]. Group A (Vantej® tooth paste alone) exhibited significant tubular blocking (mean % =1.80) compared to Group C, D and F [Graph **1**, Figure **1A**]. Group C (Laser alone) group showed only partial tubular blocking (mean % =1.30) [Graph **1**, Figure **1C**]. Dentin disc specimens in which combination therapy was performed, i.e., Group E (Laser with Colgate Sensitive Prorelief® tooth paste) showed complete blocking of the dentinal tubules (mean % = 2.8) which was statistically significant ($P < 0.001$), (Figure **1E**, Graph **1**) similar to Group B (Pro-relief® alone). Group D (Laser with Vantej® tooth paste) exhibited partial tubular blocking (mean % = 1.30) (Graph **1**, Figure **1D**), similar effects were seen when Laser alone was used. Group F served as control group, in which no treatment was performed, the SEM photomicrograph shows presence of smear layer (Figure **1F**). Intergroup analysis indicated that, Group B (Pro-relief® toothpaste alone) and Group E (Laser with Colgate Sensitive Prorelief® tooth paste) showed significant higher score ($P<0.001$) of tubular blocking (Graph **1**, Figure **1B**, **1E**) compared to Groups A, C and D.

EDX analysis of the experimental groups revealed peaks of the elemental contents found in the experimental specimens. All the groups exhibited peaks of calcium and phosphorous, silicon along with traces of oxygen and carbon except the Laser alone group, which exhibited a raise in level of carbon

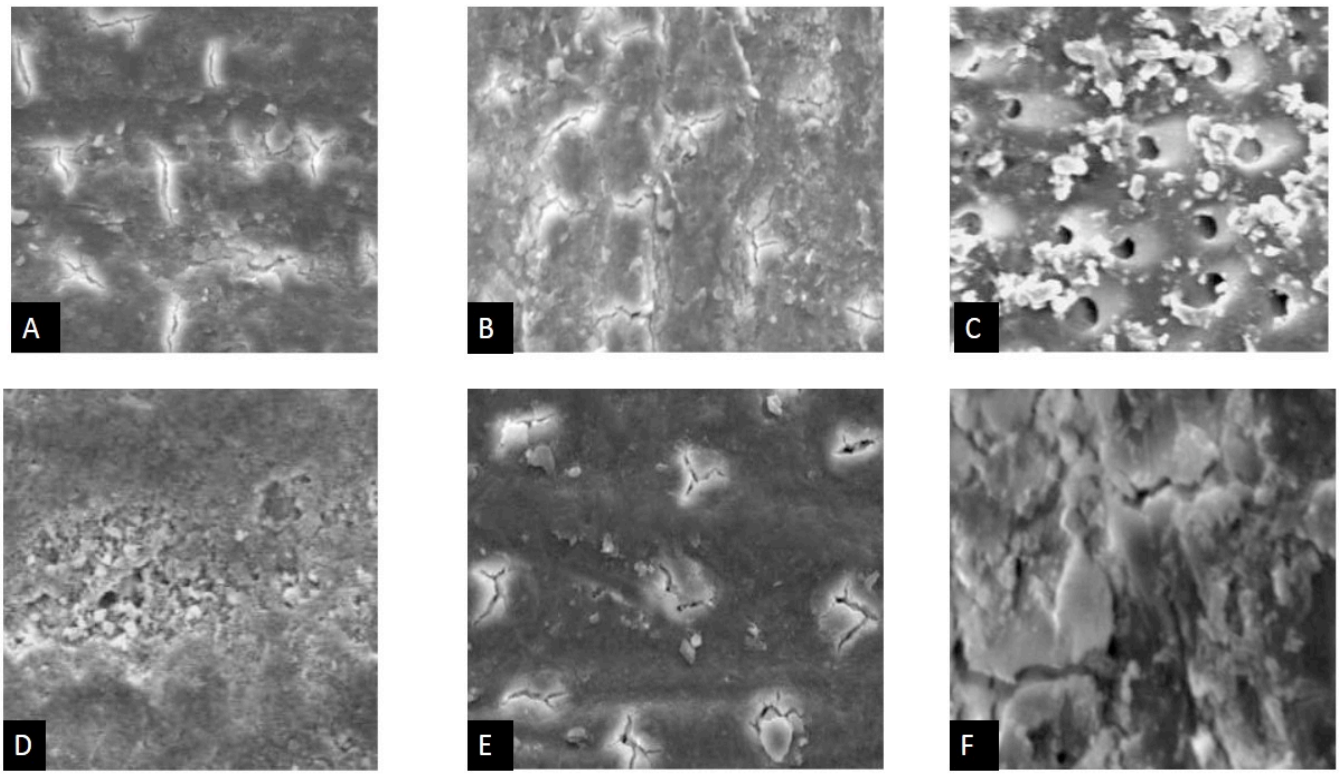


Figure 1: SEM photomicrographs of dentin discs treated with test agents. **A:** Specimens treated with Vantej® tooth paste shows significant tubular blocking. **B**: Specimens treated with Colgate Sensitive Pro-relief® tooth paste complete seal of dentinal tubules. **C:** Specimens irradiated with He-Ne Laser exhibiting partial tubular blocking. **D:** Specimens irradiated with He-Ne Laser and treated with Vantej® tooth paste demonstrate partial blocking of dentinal tubules. **E:** Specimens irradiated with He-Ne Laser and treated with Colgate Sensitive Prorelief® tooth paste exhibits complete blocking of dentinal tubules. **F:** Specimens served as control which shows dentin surface covered with smear layer.

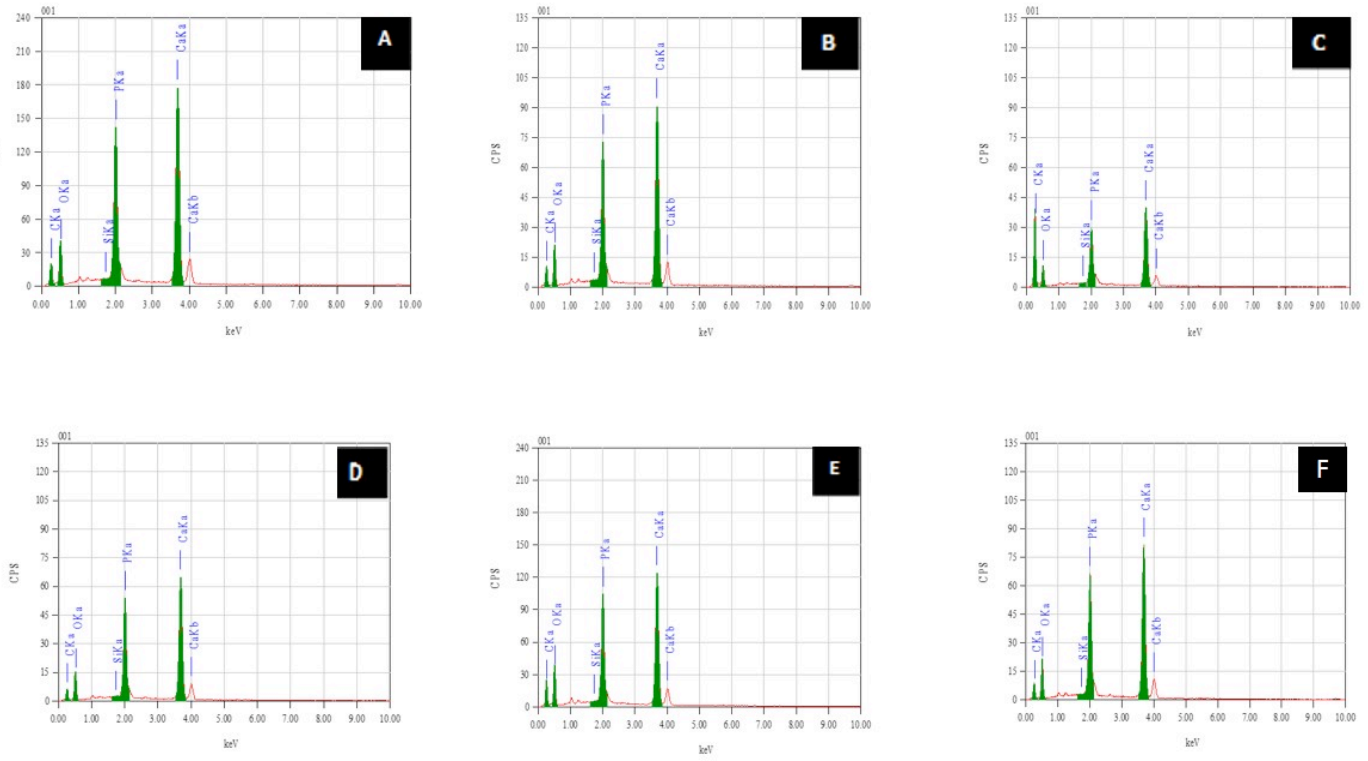
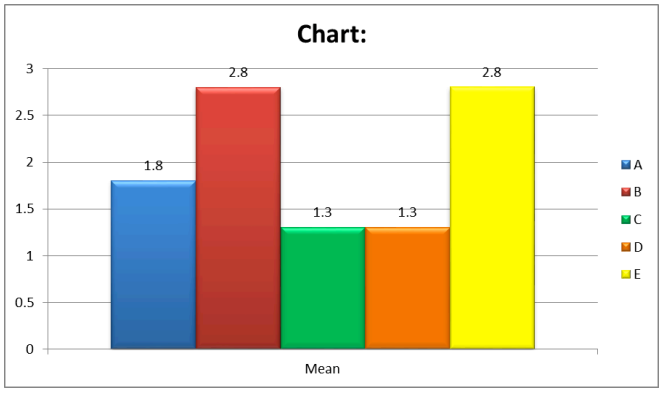


Figure 2: Energy dispersive x-ray (EDX) spectrum of experimental groups.

element along with the presence of other elemental ions (Figure **2**).



Graph 1: Mean percentage score of dentinal tubular blocking in various experimental groups.

DISCUSSION

Dentinal hypersensitivity has always posed the clinician in a perplexing situation, mainly because of the inability in achieving complete resolution of the symptoms. This study was carried out in an attempt to learn the morphological characteristics of the dentinal surface when two different treatment modalities [laser and desensitizing pastes] were tested individually or in combination. Two commercially available desensitizing toothpastes (Vantej® and Pro-relief® toothpaste) have been used to facilitate the blocking of dentinal tubules by the process of surface re-mineralization. A review of clinical evidence on tubule occluding desensitizing dentrifices like strontium-based and stannous fluoride toothpaste have not achieved promising results with the conclusion that these do not induce deposition of “natural” calcium and phosphate [11,12]. The 1% citric acid applied on the dentin discs in all experimental specimens for a time interval of 1 minute, removes the smear layer and exposing the dentinal tubules to approximately 85%, thus clinically simulating the surface as hypersensitive dentin as per the findings of McAndrew and Kourkouta [13]. There were significant difference in the mean scores among all the experimental groups when compared to the control group. In this study, Vantej® toothpaste group [Group A] exhibited significant dentinal tubule blocking formed by the NovaMin compounds (Calcium sodium phosphosilicate, calcium, phosphate and silica). The mechanism of occlusion could be explained by the interaction of the chemical contents of the dentifrice with distilled water, thus releasing Ca and PO_4 ions which brings about natural remineralization, hence resulting in formation of hydroxycarbonate apatite layer which is comparable to the hydroxyapatite layer. This process results in physical blocking of dentinal tubules [14]. The Colgate Pro-relief® toothpaste [Group B]

specimens showed statistically significant tubular blocking compared to other test groups. This could be attributed to the Colgate Pro-relief® toothpaste based on Pro-Argin technology containing arginine (aminoacid), calcium compounds and calcium carbonate. Various elucidative imaging techniques have been conducted on this desensitizing toothpaste, confirming that the technology plugs and blocks dentin tubules effectually, making it acid resistant [15]. In this study, a low output Helium-neon laser (6 mW), was used. Literature review emphasizes the use of low level laser in dentistry for wound healing, analgesic and anti-inflammatory effect [16]. There have been several studies of the effect of LLLT on DH using GaAlAs laser treatment, which have resulted in effective desensitization of hypersensitive cervical dentine [17-19].

There is also supportive evidence that LLLT delivered using He-Ne lasers can reduce DH to air and mechanical stimulation for over three months [20]. The mechanisms of the desensitizing effect have been related to the inhibition of nociceptive signals arising from peripheral nerves [21] which is responsible for it's therapeutic effect. Studies conducted by Featherstone and Nelson [22] have suggested that dentine and enamel are partly transparent to near infrared laser wavelengths and major part of laser energy is transmitted through to the pulp, rather than affecting the tooth surface. Hence, in this present study, there was only partial blocking of dentinal tubules, which was observed in Laser alone group (Figure **1C**). This could also be due to the lasing time period of six minutes, contrary to the irradiation parameter of He-Ne laser stated by Kimura *et al*., stating a irradiation time of 2-5minutes in continuous wave mode for its varying level of effectiveness [7]. This lasing time interval perhaps caused a thermal alteration leading to melting of hydroxyapatite thus resulting in partial blocking of the dentinal tubules. In the He-Ne Laser with Vantej® tooth paste group (Graph I, Figure **1D**), wherein the dentin discs were pre-treated with laser, the thermal effect would have led to water absorption from the dentine thus preventing its interaction with the novamin compounds for remineralization process. Dentin discs belonging to Laser with Colgate Sensitive Prorelief® tooth paste group showed highly significant tubular blocking (Figure **1E**, Graph **1**), which could be attributed to the synergistic outcome of the photothermal effect of laser and the essential components (Arginine-calcium carbonate) present in Prorelief® toothpaste.

Quantitative analysis using EDX (Figure **2**) revealed similar mass percentage of the elements present within the dentin discs. All the specimens showed peak of calcium and phosphorous along with traces of silicon, oxygen and carbon. Only the Laser alone group [Group C] exhibited a raise in level of carbon element, with calcium and phosphorous peaks comparatively lowered. The rise in carbon level could be attributed to the thermal effects of laser which occurred during the time interval.

CONCLUSION

The clinical results of this study demonstrated the use of Colgate sensitive Prorelief® to be effective in blocking the dentinal tubules thus resulting in morphological changes on the dentine surface. The use of lasers prior to the use of desensitizing toothpaste could also provide similar benefits, hence can be used as an effective therapeutic option for management of dentinal hypersensitivity prior to prescribing a desensitizing toothpaste. However, further long term clinical based controlled trials are required to support these results prior to extrapolating into clinical practice.

CLINICAL SIGNIFICANCE

The therapeutic options for dentinal hypersensitivity are wide ranging but a complete resolution of the symptom remains a daunting task. In order to achieve successful and effective results, long term monotherapy either with use of desensitizing dentrifrice, mouthrinse, gels etc and/or a combination therapy (restorations, lasers along with home care methods) may be considered a requisites for management of dentinal hypersensitivity.

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