# Effect of 1440-Nanometer Neodymium:Yttrium-Aluminum-Garnet Laser Irradiation on Pain and Neuropeptide Reduction: A Randomized Prospective Clinical Trial

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## Abstract

**Introduction:** The purpose of this study was to investigate the efficacy of a 1440-nm neodymium:yttrium-aluminum-garnet (Nd:YAG) laser on relieving pain in relation to the levels of inflammatory cytokine and neuropeptides in the root canal exudates of teeth with persistent symptomatic apical periodontitis. **Methods:** Forty teeth with persistent symptomatic apical periodontitis were randomly assigned to treatment groups: group L, intracanal irradiation of 1440-nm Nd:YAG laser with a 300-$\mu$m-diameter fiberoptic tip in addition to conventional root canal retreatment, and group C, conventional root canal re-treatment. The degrees of both spontaneous pain and the pain on percussion before and after treatment were recorded, and root canal exudate samples were collected to quantify the associated levels of substance P, calcitonin gene-related peptide (CGRP), and matrix metalloproteinase (MMP)-8 by immunoassay. **Results:** All of the measured parameters were significantly reduced in group L ($P < .05$), whereas the level of pain on percussion, CGRP, and MMP-8 were significantly reduced in group C ($P < .05$). The 1440-nm Nd:YAG laser had significantly better effect on the relief of pain on percussion and the reduction of substance P, CGRP, and MMP-8 levels. The visual analog scale scores of perceived pain correlated with pain-related neuropeptides and inflammatory cytokine levels in root canal exudates. **Conclusions:** The 1440-nm Nd:YAG laser irradiation via fiberoptic tip to the teeth with persistent apical periodontitis provided promising consequences of pain and inflammation modulation. *(J Endod 2014;40:28–32)*

## Key Words

Fiber-optic tip, Nd:YAG laser, neuropeptides, pain, VAS

A patient undergoing endodontic treatment suffers from varying levels of pain typically expressed as spontaneous pain or pain on percussion at unpredictable point of treatment period. Once the source of inflammation is removed from the root canal system under the proper treatment modality, the associated pain may decrease in intensity or disappear. Thus, the degree of pain expressed by the patient provides clinical confirmation to estimate whether a certain treatment procedure reduced the severity of periapical inflammation. However, the level of pain per se cannot be used as a quantitative diagnostic criterion of the degree of inflammation. In this regard, there have been attempts to visualize the clinical effect of endodontic treatment procedures on pain relief in relation to levels of proinflammatory cytokines and bacterial endotoxins (1–3).

Various techniques have been used for complete debridement and decontamination of the root canal system in attempts to reduce the pain and inflammation associated with endodontic treatment procedures (4–6). Lasers were introduced in endodontics to overcome the limitations of those currently used techniques caused by the inherent anatomic complexity of the root canal system. Most studies (7, 8) used near-infrared lasers such as diode and 1064-nm neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers, medium-infrared erbium-family lasers, or far-infrared $CO_2$ lasers (10,600 nm), and they have confirmed the effectiveness of these lasers in root canal disinfection when used in conjunction with root canal irrigants such as sodium hypochlorite (NaOCl) or EDTA. Because near-infrared lasers have no ablative effect on hard tissue and median-infrared lasers have undesirable ablative and thermal effects on dentinal walls, current efforts to maximize the use of lasers in endodontics are focused on finding the optimal wavelength and appropriate lasing method.

Recently, a new device that delivers high-wavelength near-infrared Nd:YAG laser using flexible fiberoptic tip has been introduced, and Moon et al (9) reported the effectiveness of this 1320-nm Nd:YAG laser when used with a fiberoptic tip for smear layer removal. Consequently, it could be assumed that high-wavelength Nd:YAG laser (1440-nm) may have more diverse effects in relieving pain and alleviating inflammation if irradiated directly to the apical region of the root canal with a flexible fiberoptic tip. However, little is known about the effect of 1440-nm Nd:YAG laser in endodontics in regard to the constituents of root canal exudates and the level of pain experienced by the patient, in particular.

Therefore, the purpose of this randomized prospective clinical trial was to investigate the effect of 1440-nm Nd:YAG laser on reducing pain of teeth with persistent symp-

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tomatic apical periodontitis focused on the pain-related neuropeptides and inflammatory cytokine levels in root canal exudates.

## Materials and Methods

### Patient Selection

The study protocol was approved by the Institutional Review Board (IRB no. CDE12002) of Seoul National University Dental Hospital. Each patient provided written and verbal informed consent to participate in the study. Patients referred to the Department of Conservative Dentistry of Seoul National University Dental Hospital for further management and treatment of teeth with persistent symptomatic apical periodontitis were evaluated as possible candidates for this study. Patients who had teeth with symptomatic apical periodontitis that had previously been initiated primary root canal treatment, with no sinus tract or swelling, no contributory medical history, and no previous systemic administration of antibiotics or analgesics within 2 weeks of enrollment were accepted for the present clinical trial. The patient was excluded if he/she required antibiotic prophylaxis or had uncontrolled diabetes, generalized periodontitis, or if the tooth had periodontal probing depth of more than 3 mm. The teeth with periapical radiolucencies that had typical radiographic characteristics of periapical cyst (well-demarcated corticated margins with more than 10 mm in diameter, evidently showing displacement of adjacent structures or expansion of the outer cortical boundaries of the jaw) were also excluded.

The power analysis was conducted on the basis of the minimum clinically significant difference in the visual analog scale (VAS) score. A sample size of 16 patients in each group was calculated to be sufficient to detect clinically important differences of VAS score (alpha at level 0.05, 90% power, and effect size of 1.2). Ultimately, a sample size of 20 patients in each group was determined, considering the dropout rate such as loss to follow-up. All accepted patients were randomly assigned to treatment groups as follows: group L, laser application in addition to conventional root canal re-treatment, and group C, conventional root canal re-treatment.

### Experimental Procedures and Sample Collection

Each patient's pain level (spontaneous pain and pain on percussion test) was recorded before the initiation of each treatment procedure using the VAS score (0–10 scale: 0, no pain; 10, extremely severe pain). For percussion test, the cusps of each tooth were percussed 3 times using the shaft of periodontal probe. First, the normal asymptomatic tooth was tested to make the patient experience the normal tapped feeling of the percussion test and to rule out the possible false-positive response. Then, the subjected tooth and its adjacent teeth were tested in a randomized order, and the pain of the subjected tooth that the patient perceived was recorded using VAS scores.

For the routine root canal re-treatment procedure, the tooth was isolated with a rubber dam, and the access cavity was refined. If necessary, topical anesthetic agent was applied to prevent immediate increase of substance P (SP) release as a result of needle injection. If the symptom was too severe to place the clamp even with the aid of topical anesthesia, the whole quadrant was isolated, and the clamp was placed on another tooth (usually the most distal tooth of the isolated quadrant).

Upon access, teeth with confirmed crack line under microscopic evaluation were also excluded. The operator carefully removed previous root canal filling material if present, confirmed patency of the root canals with ISO #10 K-files, and determined the working length using an electronic apex locator (Dentaport ZX II; Morita Co, Kyoto, Japan) and radiographs.

The root canals were gently irrigated with sterile saline using a 25-gauge side-vent needle. After the gross irrigant was removed by aspiration, sterile paper points were inserted into the root canals and left in place for 2 minutes to collect the root canal exudate. The paper points soaked with root canal exudate were placed into a 1.5-mL Eppendorf tube containing 100 $\mu$L Tris-HCl buffer, pH 7.5 with 0.15 mol/L NaCl and 1 mmol/L $CaCl_2$. The tubes were placed on a shaker at room temperature for 3 hours and then stored at −70°C for further analysis. The root canals were then enlarged to 3 sizes larger than the initial apical file, at least up to size ISO #35, using nickel-titanium rotary instruments (ProFile; Dentsply Maillefer, Bellaigues, Switzerland), Gates Glidden burs, stainless steel K-files, and RC Prep (Premier Dental Products, Norristown, PA). All instrumentations were accompanied by careful and copious irrigation with a sufficient volume of 3.5% NaOCl. Then, the root canals were dried with sterile paper points.

For the experimental treatment procedure in group L, the Nd-YAG laser (Slimlift MPX; B&B Systems, Seoul, Korea) was applied for 10 seconds to the apical 3-mm level of the root canals with 300-$\mu$m-diameter fiberoptic tip. The laser was set to 1440-nm wavelength, 200 mJ/$cm^2$ energy, and 1 Hz frequency. If the patient was allocated to group C, the fiberoptic tip of the laser was focused on the other side of the rubber dam sheet, mimicking laser irradiation sound to prevent placebo effect. The root canals were filled with calcium hydroxide (Metapaste; Meta Biomed, Osong, Korea), and the access cavity was sealed with temporary filling material (Caviton; GC, Tokyo, Japan). The participants were provided escape medication (ibuprofen/arginine) in case of flare-up with severe pain and instructed for emergency visit.

The patient was recalled 3 days after the experimental treatment procedure for the post-treatment evaluation. Post-treatment pain levels were recorded (VAS), and root canal exudates were collected again as described above. After root canal exudate sample collection, patients received routine root canal treatment procedure. Subsequently, all canals were filled with gutta-percha and root canal sealers.

### Substance P and Calcitonin Gene-related Peptide Analyses

Quantification of neuropeptides was completed with immunoassay kits according to the manufacturers' instructions: SP, enzyme-linked immunosorbent assay kit (Parameter, Human SP assay; R&D Systems Inc, Minneapolis, MN) and calcitonin gene-related peptide (CGRP), EIA kit (Human CGRP enzyme immunoassay kit; SPUbio, Massy, France). Fifty microliters of each sample, standards, and controls were pipetted into 96-well plates coated with specific antibodies. All samples were analyzed in duplicate. The absorbency of each sample at 450 nm ($OD_{450}$) was read using a microplate reader (Model 680; Bio-Rad, Hercules, CA) and analyzed with Microplate Manager (version 5.2.1; Bio-Rad). The data were expressed as the total amount (ng) per sample.

### Matrix Metalloproteinase-8 Analysis

Matrix metalloproteinase (MMP)-8 was assayed with an assay kit from Millipore (Human sepsis magnetic bead panel 2; Billerica, MA). All specimens were assayed in duplicate according to the manufacturer's protocols. Multiplex immunoassays were performed using the Luminex 100 IS System (Luminex Corp, Austin, TX). The standard curve for sample cytokine concentration determination was used. MMP-8 concentrations were calculated on the basis of the standard curves using Bio-Plex Manager 6.1 (Bio-Rad Laboratories). The data were expressed as the total amount (ng) per sample.

### Statistical Analysis

The differences among experimental groups were analyzed using a nonparametric Kruskal-Wallis analysis. A series of Mann-Whitney tests were used for multiple comparisons. The effect of experimental