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ORIGINAL RESEARCH

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Effects of an Nd:YAP laser used for root canal disinfection in pulp regenerative therapy: a pilot study

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Abstract

This study aimed to compare the disinfection effects between a triple antibiotic paste and neodymium-doped yttrium aluminum perovskite (Nd:YAP) laser in pulp regenerative therapy and evaluate corresponding therapeutic effects based on apical radiographs and cone-beam computed tomography (CBCT). Sixty-six immature permanent teeth in 66 patients diagnosed with acute or chronic apical periodontitis were analyzed. All teeth were given pulp regenerative therapy. The patients were categorized into a control (triple antibiotic paste) and an experimental (Nd:YAP laser) group. Teeth in the experimental group were disinfected using an Nd:YAP laser, while those in the control group were disinfected using a triple antibiotic paste. Clinical and radiological examinations were performed every 3–6 months after treatment and followed up for 24 months. Statistical analysis was performed after clinical examination and showed that after one week of treatment, symptoms persisted in two teeth in the control group and two teeth in the experimental group. Two weeks later, the clinical symptoms disappeared in all teeth ($p > 0.05$). After 24 months of follow-up, the clinical symptoms recurred in two teeth in the control group and one tooth in the experimental group. On radiographic examination, 31 and 27 teeth showed continued root development, and three and two teeth showed no obvious root development in the control and experimental groups, respectively. The pulp sensibility test was positive in four teeth in both groups, with no significant difference between the two groups ($p > 0.05$). The results of this study suggest that endodontic irradiation with an Nd:YAP laser could be an effective alternative to triple antibiotic paste during disinfection of pulp regenerative therapy. Treatment outcomes were assessed using apical radiographs and CBCT, and no negative prognostic effects of the Nd:YAP laser on pulp regenerative therapy were found.

Keywords

Immature permanent tooth; Nd:YAP laser; Pulp regenerative therapy; Triple antibiotic paste; Root canal disinfection

1. Introduction

In clinical pediatric dentistry, pulp regenerative therapy is considered a valuable treatment option for immature teeth with root development arrest, not only to eliminate clinical symptoms of the affected tooth but also to promote further root development and achieve apical closure [1, 2]. Both the American Association of Endodontists and the European Society of Endodontology have published guidelines for pulp regenerative therapy. According to these guidelines [3, 4], an important step in pulp regenerative therapy is the complete disinfection of the root canal while providing a good environment for subsequent pulp regenerative therapy. During this procedure, the root canal should be physically prepared without applying a root canal file or minimally prepared and is required to be disinfected with a triple antibiotic paste or a calcium hydroxide ($Ca(OH)_2$) paste. Unfortunately, the absence of physical preparation leaves a bacterial biofilm on the root canal wall. The use of antibiotics and $Ca(OH)_2$ can be associated with adverse effects such as bacterial resistance, antibiotic allergy, dental staining and reduced resistance of the root canal wall [5]. Considering the potential health risks of these adverse effects, researchers are continuously researching new disinfection methods, and the current focus is on laser disinfection. Commonly used lasers include neodymium-doped yttrium aluminum garnet (Nd:YAG) and neodymium-doped yttrium aluminum perovskite (Nd:YAP) [6–10], among which, the Nd:YAP wavelength is better absorbed in water, and has shown better disinfection effects in *in vitro* experiments [11]. However, few studies have compared the disinfection efficacies of Nd:YAP laser with triple antibiotic paste in pulp regenerative therapy. Additionally, literature on its impact on the prognosis of pulp regenerative therapy remains limited [10, 12]. Here, we hypothesized that Nd:YAP laser could provide

complete disinfection and replace triple antibiotic paste in pulp regenerative therapy. Thus, in this study, we used apical radiographs and cone-beam computed tomography (CBCT) to evaluate the effects of Nd:YAP laser on the prognosis of pulp regenerative therapy.

2. Materials and methods

The data of 66 patients (totaling 66 affected teeth) who met the inclusion criteria and were treated at the Department of Pediatric Dentistry of Changzhou Hospital of Stomatology (Changzhou, Jiangsu Province, China) from January 2018 to June 2021 were retrieved and assessed. The inclusion criteria were as follows: (1) Anterior or premolar teeth with acute or chronic periapical periodontitis and a history of spontaneous chewing discomfort, which revealed pain on percussion or abnormal mobility on clinical examination and showed widening and blurring of the periapical membrane or periapical hypodense areas on radiographs. (2) Immature teeth in Nolla's developmental stages 7, 8, or 9. Stage 7 indicated one-third root formation, stage 8 indicated two-thirds root formation, stage 9 indicated root near formation without closure of the apical foramen, and stage 10 indicated complete root formation with a closed apical foramen. (3) Patients with no history of antibiotic allergy, in good health and were satisfactorily compliant.

In this pilot study, we numbered patients according to the time of their visit and assigned odd-numbered patients to the control group and even-numbered patients to the experimental group as randomization. We expected that from 2018 to 2021, the study would include approximately 70–80 teeth. Ultimately, a total of 66 patients underwent pulp regenerative therapy. The guardians of three patients in the experimental group did not agree to be included in the experimental group and were transferred to the control group for treatment. Therefore, the control group comprised 36 teeth, while the experimental group comprised 30 teeth.

Preoperative intraoral apical radiographs of all teeth were obtained to assess the health of periapical tissues and root development. The working length was determined using apical slice image processing software. The pulp chamber was opened after adequate isolation using a rubber dam (Dahui Medical Instruments, Changzhou, China) for all teeth, and linear access to the root canal was established. The pulp status was evaluated, and the presence of viable pulpal and periapical tissues was investigated with a root canal file. Local infiltration anesthesia with lidocaine (Lidocaine Hydrochloride Injection, H13022313 Hebei Tiancheng Pharmaceutical Co., Ltd, Cangzhou, Hebei, China) was performed if viable pulp tissue was present. Briefly, the root canal was gently irrigated with 20 mL of 2.5% sodium hypochlorite (NaOCl) (Longly Biotechnology, Wuhan, Hubei, China) for 5 min, followed by 20 mL of 17% ethylenediaminetetraacetic acid (EDTA, Longly Biotechnology) for 5 min and 50 mL of 0.9% sodium chloride injection (Kelun Pharmaceutical, Chengdu, Sichuan, China), without root canal preparation.

For teeth in the control group, an antibiotic paste was prepared using the method proposed by Hoshino *et al*. [13]. Briefly, ciprofloxacin, metronidazole and minotetracycline were removed from their sugar coating or capsule, ground into powder, mixed in a 1:1:1 ratio, and mixed with distilled water to make a freshly mixed paste (triple antibiotic paste), with a final concentration of 1 mg/mL. The triple antibiotic paste was introduced into the root canal with a syringe, and the pulp cavity was sealed with a small, sterile cotton ball (Dahui Medical Instruments) and a temporary sealing material in the form of glass ionomer cement (Fuji IX, GC Corporation, Tokyo, Japan).

For teeth in the experimental group, the root canal was also gently irrigated with 20 mL of 2.5% NaOCl for 5 min, followed by 20 mL of 17% EDTA for 5 min and 50 mL of 0.9% sodium chloride injection, without root canal preparation. An Nd:YAP laser (Lokki dt, Vienne, France; wavelength: 1.34 μm, power: 3 W, and optical fiber diameter: 0.32 mm) was used and tuned to the root canal disinfection submenu (frequency: 30 Hz, power: 100 mJ). The open root canal was filled with a 0.9% sodium chloride injection, and a light-guiding fiber was inserted into the root canal approximately 1 mm from the root tip and lifted up and down along the root canal wall for 30 s. After laser irradiation, the pulp cavity was blotted dry with sterile paper tips and sealed with a small sterile cotton ball and glass ionomer cement.

Teeth in both the experimental and control groups were reviewed 1 week after the procedure. In addition, routine clinical examination was performed, and the results were recorded. The procedure was repeated 1 week later, and the results were recorded. All examinations were performed by the same pediatric dentist. If the clinical symptoms persisted, the root canal disinfection was judged to have failed; if no symptoms persisted, the pulp cavities of the teeth in the experimental and control groups were re-irrigated with 2.5% NaOCl, dried with sterile paper tips, and the pulp or periapical tissue was gently pierced with a sterile root canal file to cause bleeding approximately 2–3 mm from the root canal orifice. Then, we waited 15 minutes for a blood clot to form in the root canal. Thereafter, the root canal orifice was sealed with mineral trioxide aggregate (MTA; ProRoot MTA, Dentsply, York, PA, USA), and the coronal portion was filled with a light-curing composite resin (3M ESPE Filtek Z250, 3M, St. Paul, MN, USA).

The patients were reviewed every 3–6 months and followed up for 24 months. Postoperative results were evaluated via clinical and radiographic examinations.

Clinical examination included the evaluation of patients' complaints of pain, pain on percussion, abnormal mobility, and gingival condition. Pulp temperature and sensibility tests were performed on the affected teeth and compared with the contralateral teeth. The test procedure was as follows. (1) temperature test: the affected and control teeth were isolated with a cotton ball, the tooth surfaces were dried, and a popsicle and heat-softened gutta-percha was placed on one-third of the necks of the teeth, on the buccal surfaces. The control tooth was assessed first, followed by the affected tooth, and the patient's response was recorded, and (2) pulp sensibility test: the affected and control teeth were isolated with a cotton ball, the tooth surfaces were dried, and a layer of toothpaste was applied to one-third of the necks of the teeth, on the buccal surfaces. A pulp sensibility tester (Zhijia Pulp Tester,

Denjoy Dental, Changsha, Hunan, China) was used to assess the control tooth, followed by the affected tooth, and the results were recorded.

Apical radiographs (Planmeca INTRA, Helsinki, Finland) and CBCT images (NewTom, VG, Genova, Italy) were used to evaluate postoperative healing of the periapical tissue, including the reduction or disappearance of periapical lesions and root development, including root lengthening, root canal cavity narrowing, and apical foramen closure.

Clinical evaluation criteria were as follows: (1) primary goal: the disappearance of clinical symptoms, with radiographic evidence of a reduction in the size or disappearance of the periapical lesion; (2) secondary goal: continued development and increase in length of the root, thickening of the root canal wall, and formation of satisfactory apical closure; and (3) tertiary goal: positive results on the pulp sensibility test. Achievement of all these goals was considered clinical treatment success, while the recurrence of clinical symptoms or enlargement of lesions in the apical area on radiological images was considered as treatment failure.

The Statistical Product Service Solutions (SPSS) 22.0 software package (IBM Corp., Armonk, NY, USA) was employed for statistical analyses. The normality of the distribution for continuous numeric variables was assessed using the Kolmogorov-Smirnov test. Normally distributed variables are presented as means with standard deviations, and non-normally distributed variables as medians with interquartile ranges (95% Confidence intervals (CIs)). The baseline values and results of the two groups were compared using the Student *t*-test or the chi-square test. Statistical significance was set at $p < 0.05$.

3. Results

For this study, we attempted to include the maximum number of teeth possible. Consequently, we included 66 teeth from 66 patients. All patients were randomly assigned to one of the two groups, considering the guardian's wishes. Statistical evaluation was performed for patients with positive consent. The study cohort comprised 35 boys and 31 girls aged between 8–14 (mean: 12.1 $\pm$ 1.6) years. No differences in baseline values were found between the two groups ($p < 0.05$).

The study results showed that clinical symptoms disappeared after one week of treatment with triple antibiotic paste in 34 of 36 teeth in the control group. Two teeth exhibited pain on percussion and discomfort on palpation, although these clinical symptoms disappeared after one week of further observation.

For the experimental group, we observed that the clinical symptoms disappeared after one week of Nd:YAP laser treatment in 28 of 30 teeth. Two teeth exhibited discomfort on percussion and palpation but disappeared after one week of further observation (Table 1).

The results of the 24-month follow-up after pulp regenerative therapy showed that 34 teeth in the control group and 29 teeth in the experimental group achieved the primary goal, with no statistically significant differences on clinical examination. The clinical symptoms recurred in two teeth in the control group and one tooth in the experimental group, and the difference was not statistically different. Secondary goals were achieved in 31 and 27 teeth in the control and experimental groups, respectively, with the disappearance of periapical lesions, root lengthening, root canal wall thickening and apical foramen closure on radiographs, and the disappearance of clinical symptoms in three and two teeth, respectively. No obvious root development was observed on radiographs. Four teeth in total achieved the tertiary goal, and no statistically significant difference was observed between the two groups (Table 2). Different degrees of calcification in the root canal were evident on radiological examination (Figs. 1,2).

According to the clinical efficacy criteria, the pulp tissues of 66 teeth were regenerated, 63 teeth achieved the primary goal, 58 teeth achieved the secondary goal, and the treatment failed in three teeth.

4. Discussion

Lesions in immature permanent teeth are common and frequently encountered in clinical pediatric dentistry and may jeopardize the physical and mental health of adolescents. Compared with mature permanent teeth, the roots of immature permanent teeth are not fully developed; hence, their lesions are difficult to treat clinically. Currently, the main guiding principles of clinical treatment for immature permanent tooth lesions are to eliminate the inflammation, promote the root's continued development, and ensure long-term normal tooth function [14, 15]. The apical induction shaping procedure employed in the middle of the last century and MTA apical closure proposed in recent years are two classical treatment methods for these lesions. The main disadvantage of these treatment methods is that, although they control inflammation in the affected tooth and induce apical closure, the root formation does not occur in three dimensions, and the canal wall is thin and prone to break. The first case of persistent root development was reported by Iwaya *et al*. [1] and is called pulp regenerative therapy [16–18].

At present, NaOCl root canal irrigation + triple antibiotic paste sealer + root canal coagulation is the standard procedure for pulp regenerative therapy. The combination of three antibiotics, ciprofloxacin, metronidazole and minocycline, is reportedly effective in controlling infections [19–21]. However, various reports have revealed the limitations of triple antibiotic pastes, which include the need for a longer sealed time, possible allergies in some patients, antibiotic resistance that can lead to less effective outcomes, and teeth staining. The specific wavelength of the Nd:YAP laser is 1.34 μm, and it has specific water and hemoglobin absorption rates, which can provide adequate sterilization [11]. The principle of action is that the laser's high energy instantly vaporizes water in the root canal to form a small explosion, which is also effective against bacteria in the dentinal tubules or smear layer, making it suitable for disinfection of root canals [22, 23]. It is equally effective and well-suited for endodontic applications with additional analgesia compared to other high-power laser instruments [24]. In this study, for clinical pediatric dentistry, the use of the Nd:YAP laser for pulp irradiation achieved root canal disinfection equivalent to that of triple antibiotic paste in the control group, effectively avoiding the negative effects of

TABLE 1. Clinical evaluation of 66 teeth with periapical periodontitis after disinfection.

Group	n	One week after disinfection		Two weeks after disinfection	
		Elimination of symptoms	Presence of symptoms	Elimination of symptoms	Presence of symptoms
Control	36	34	2	36	0
Experimental	30	28	2	30	0

**There was no statistical difference between the control and experimental groups ($p = 0.368$).*

TABLE 2. Clinical evaluation of 66 teeth with periapical periodontitis after pulp regenerative therapy of 24 months.

Group	n	Twenty-four months of follow-up				
		Primary goal	Recurrence of symptoms	Secondary goal	Undeveloped tooth roots	Tertiary goal
Control	36	34	2	31	3	4
Experimental	30	29	1	27	2	4

**There was no statistical difference between the control and experimental groups ($p = 0.406$).*

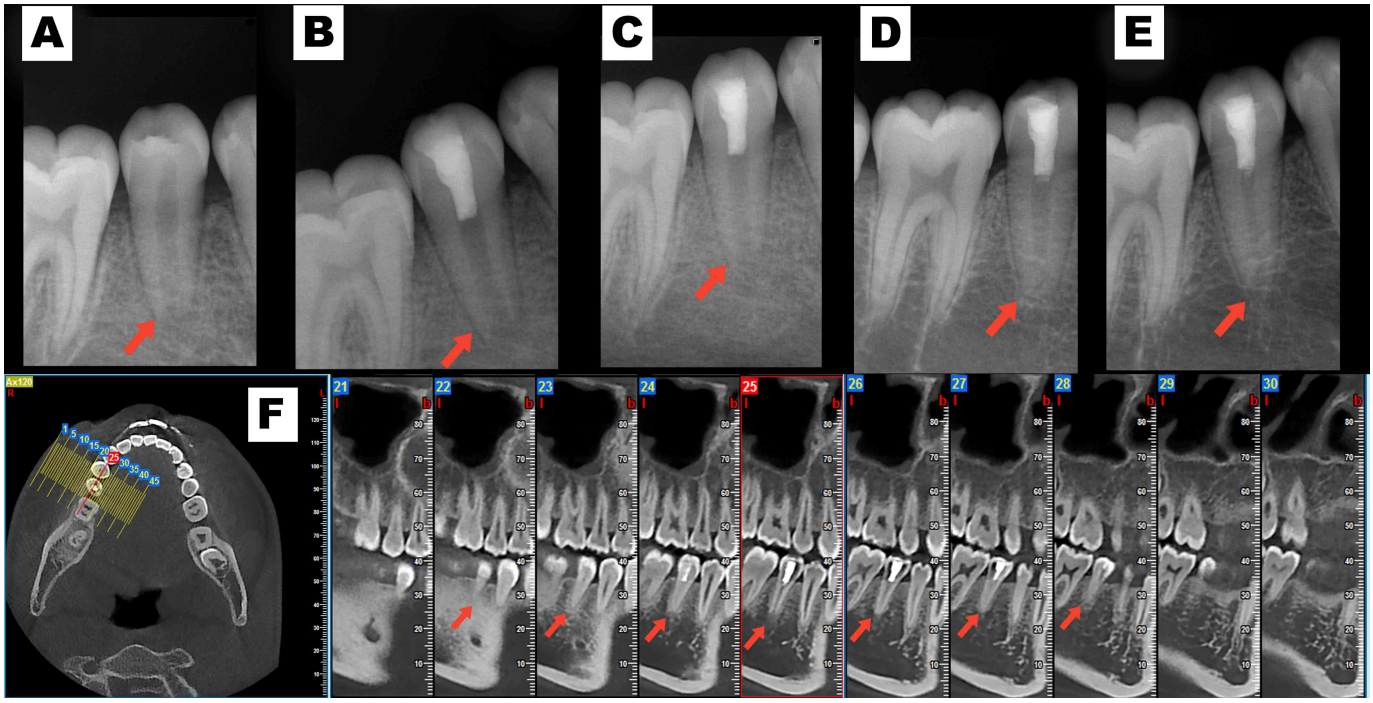

FIGURE 1. Radiographs of teeth in the control group obtained. (A) preoperatively. (B) 6 months post-therapy. (C) 12 months post-therapy. (D) 18 months post-therapy, and (E) 24 months post-therapy after 24 months of pulp regenerative therapy. (F) Cone-beam computed tomography images 24 months after therapy.

antibiotic application.

Triple-antibiotic-paste sealing is one of the basic steps of pulp regenerative therapy. The application of Nd:YAP lasers remains in the exploratory stage, and its principle of disinfection is based on the high-energy characteristics of the laser, which act not only on microorganisms but also on the pulp and periapical tissues. Different light-emitting characteristics, irradiation power, frequency, time, and operation methods have different effects on the pulp and periapical tissues [12]. In this study, relevant parameters were selected based on the results of previous research and the characteristics of Nd:YAP lasers [25–27]. However, there is currently no unified standard operating procedure for the application of lasers in root canal disinfection. Such standardization is required for reproducibility.

In this study, two teeth in the control group and one tooth in the experimental group showed clinical symptoms during the 24-month observation period. The result was not statistically significant, indicating that the long-term disinfection effect of the Nd:YAP laser was equal to that of the triple antibiotic paste. Further, the roots of 58 affected teeth continued to develop with satisfactory apical closure and thickening of the root canal walls, indicating that the pulp regenerative therapy was successful [28]. In five cases, although the clinical symptoms disappeared, the roots did not continue to develop, and no signs of apical closure were evident. The thickness of the root canal cavity did not change significantly. According to statistics from previous reports, most treated teeth (91%–94%) achieved the primary goal after pulp regenerative therapy and showed satisfactory curative effects, according to doctors

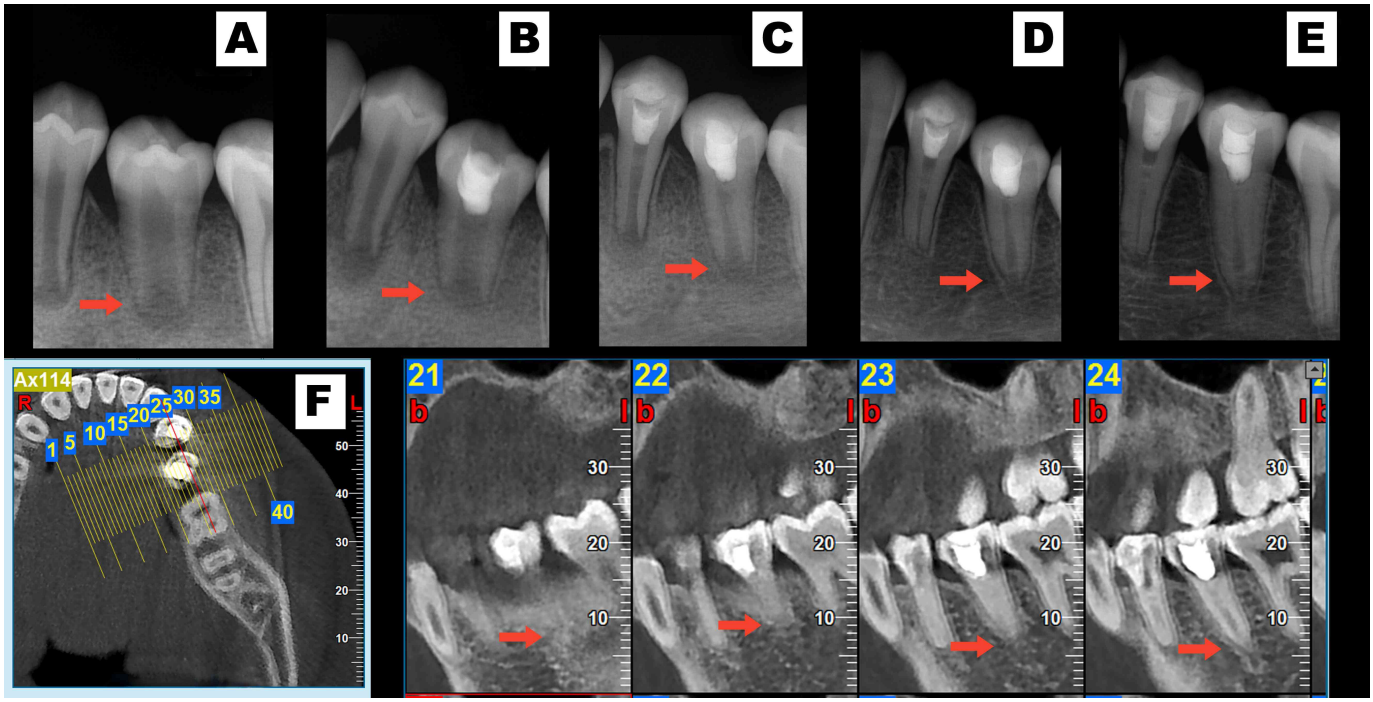

FIGURE 2. Radiographs of teeth in the experimental group. (A) preoperatively. (B) 6 months post-therapy. (C) 12 months post-therapy. (D) 18 months post-therapy, and (E) 24 months post-therapy after 24 months of pulp regenerative therapy. (F) Cone-beam computed tomography images 24 months after therapy.

and patients [29, 30]. Most teeth achieved root canal wall thickening (79.2%) and continued root development (76.2%) after treatment [31], demonstrating clinical success rates of 71.4% and 97.9% [32], respectively. However, an analysis of these reports showed that the achievement of secondary goals was uncertain and difficult to predict compared with primary goals [29]. Nearly 60% of young permanent teeth had positive pulp sensibility after pulp regenerative therapy [33]. The treatment success rates in this study were consistent with the results of previous reports, except for the success rate of the tertiary goal. In this present study, the success rates of the tertiary goal in the experimental and control groups were 11.1% and 13.3%, respectively, which might be because all cases in this study were diagnosed with apical periodontitis, and the probability of a viable pulp was low [32, 34, 35].

Both apical radiographs and CBCT images showed heterogeneous high-density shadows in the pulp cavity, indicating pulp calcification, consistent with previously reported results of pulp regenerative therapy and the presence of bone-like tissue on histological examination [36].

This study was limited by the small number of cases with anterior or premolar teeth. Therefore, the definitive role of lasers in the pulp disinfection of pulp regenerative therapy could not be confirmed. The results could also be the effect of a large extent of NaOCl irrigation, which should be further confirmed in *in vivo* experiments. In addition, the biological effects of lasers on the pulp-dentin complex and periapical tissue have not been confirmed, and further studies are needed to clarify the effects of lasers on pulp regenerative therapy or bone-like tissue growth.

Apical radiographs and CBCT images can reflect the clinical effects of pulp regenerative therapy and effectively show the length of the roots and the thickening of the root canal wall. The advantage of CBCT is that it can display the actual condition of the roots in three dimensions and more effectively show the closure of the apical foramen [37]. The evaluation of treatment outcomes using apical radiographs and CBCT images did not show any negative effect of the Nd:YAP laser on the prognosis of pulp regenerative therapy. However, it should also be noted that CBCT is relatively time-consuming and expensive, and patients' guardians are often concerned about radiation exposure. Further, satisfactory CBCT images were sometimes difficult to achieve because the patients were poorly cooperative minors and due to swallowing movements, which often resulted in blurred images.

5. Conclusions

This study showed that endodontic irradiation with Nd:YAP laser could be an effective alternative to triple antibiotic paste during disinfection of pulp regenerative therapy, although further studies are needed to confirm these findings. In addition, both apical radiographs and CBCT images were clinically useful in evaluating the outcomes of pulp regenerative therapy. No obvious negative effects of Nd:YAP laser on the prognosis of pulp regenerative therapy were observed. CBCT was better than apical radiographs for detailed analyses, though some difficulties in obtaining CBCT images in pediatric patients were unavoidable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

YY and XL—designed the research study. YY, XL and QL—performed the research. XL and QS—analyzed the data. XL, QS and YY—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Changzhou Hospital of Traditional Chinese Medicine (committee reference number: 2020-LL-04(L)). All patients and their guardians provided written informed consent to participate.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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