INTRODUCTION

Endodontic is a branch of dentistry that focuses on the prevention, diagnosis, and treatment of dental pulp pathosis [1]. Pulp and periapical tissue infections occur when microorganisms invade the hard tissues in teeth and progress into the root canal [2]. The main objective of a root canal treatment is to eliminate microorganisms, toxins, and pathological debris from the root canal, preventing further infection to the surrounding bone [3,4]. At least 300 different microorganisms species found in root canal infections, including fungal organisms, are found in pure culture or with bacteria in primary, secondary, and persistent root canal infections. Candida albicans is the most common fungi in the oral cavity and the root canal [5]. The incidence of C. albicans in a healthy oral cavity is approximately 30–45%, but its incidence can be as high as 95% in immune compromised individuals [6]. The prevalence of C. albicans in root canal infections is approximately 1–17% [7]. C. albicans is more frequently found in teeth with failed root canal treatments [8].

C. albicans dentin colonization plays an important role in persistent root canal infection, and the invasion of the C. albicans into the dentinal tubules can protect it from intracanal procedures. C. albicans is a dimorphic fungus that can alter its morphology in response to a host’s attempts to fend off infection. C. albicans has hyphae morphology with a diameter around 1.9–2.6 μm, which suggests that it has the ability to penetrate dentinal tubules [8].

Endodontic treatment procedures rely on mechanical instrumentation, irrigants, and medicaments to disinfect the root canal [5]. Irrigants help remove microorganisms that are untouched by mechanical instrumentation [9]. The most commonly used endodontic irrigant is sodium hypochlorite (NaOCl), which works by turning the fatty acid in microbes’ cell membranes into fatty salt and glycerol, damaging the cell membranes [10]. Another endodontic irrigant is CHX. Cationic molecules from CHX bind to microbes’ cell membranes, which are negatively charged, causing cell lysis. These irrigants must be in direct contact with the microorganisms to be effective, but they have limited penetration into dentinal tubules [11].

Root canal disinfection is one of the basic principles to retain tooth treated root canals in the long term. Root canal disinfection is the primary challenge in endodontic procedures, and although the use of irrigants may decrease the number of microbes in infected root canals, they do not achieve the total disinfection of entire root canal systems. Lasers are one method used to overcome this challenge because lasers can access tubular systems that irrigants cannot [5].

The antimicrobial effect of lasers depends on the dosage of the heat delivered to the target [12]. Various types of lasers, such as diode lasers, are used in dentistry. The use of diode lasers in endodontics is an innovative approach to root canal disinfection because these lasers have the ability to penetrate deeper into dentinal tubules [3]. Diode lasers have become the method of choice due to their advantages including their ease of use and relatively small size compared to other types of lasers [13].

The antimicrobial effect of lasers has been shown in previous studies. According to some studies, laser alone is not more effective than irrigants [12]. Baz et al. conducted an in vitro study on the disinfection of 60 single root canals and found that disinfection by NaOCl irrigants is significantly better than disinfection using diode lasers alone. However, disinfection with a combination of irrigants and diode lasers resulted in the greatest bactericidal effect. Therefore, the diode laser is considered as an adjunct to enhance the bactericidal effect of endodontic irrigants [10].

Kaiwar et al. found similar results when they studied the use of diode lasers combined with irrigants. This combination resulted in the highest root canal disinfection rate when compared with disinfection...
by irrigants or lasers alone. Differences in the disinfection rates were statistically significant [13].

Gerek et al. conducted an ex vivo study on 176 single-rooted teeth contaminated by bacteria and C. albicans. These teeth were treated using endodontic irrigants and an 810-nm diode laser. The method did not achieve complete sterilization of the root canal system, but it achieved a statistically significant decrease in the bacterial and C. albicans counts [14].

Many studies on the effect of diode lasers in root canal bacterial disinfection treatments have been conducted, but studies on the effect of 980-nm diode lasers on fungal organisms are rare. The objective of this study is to evaluate a 980-nm diode laser’s enhancement of the antifungal properties of 2.5% NaOCl and 2% CHX against a C. albicans biofilm.

METHODS

This is a laboratory experimental study. C. albicans strain ATCC 10231 — provided by the Oral Biology Laboratory in the Faculty of Dentistry, University of Indonesia — was the sample used in this study. The C. albicans biofilms were created on a well plate. After the biofilms were created, they were subjected to 2.5% NaOCl or 2% CHX solutions followed by 980-nm diode laser treatments. A swab of biofilm that has been treated was taken and put inside an Eppendorf tube filled with phosphate-buffered saline (PBS) solution and diluted to –6. Each sample was cultured in a Sabouraud dextrose agar medium (SDA). The culture was incubated for 24 h at 37°C.

An assessment of the diode lasers’ ability to enhance the antifungal properties of 2.5% NaOCl and 2% CHX against the C. albicans biofilms was completed by visually counting the colony-forming units (CFU) of C. albicans. The living and colonized C. albicans on the agar were manually counter after it was exposed to the irrigants and the 980-nm diode laser. If more colonies were formed, the CFU/ml score increased, and the antifungal effect of the irrigant and diode laser was lower on the testing materials.

Statistical analysis was conducted using SPSS 22.0 (IBM, United States). Normality tests were conducted using the Sapiro-Wilk test, which found that the data had a normal distribution (p>0.05). Homogeneity test discovered that the data were homogeneous (p<0.05). Therefore, data qualify for the parametric test (one-way ANOVA), which was calculated at p<0.05 (p=0.000). Finally, a post hoc Bonferroni test was conducted.

RESULTS

The mean values of the C. albicans count from the five experimental groups can be seen in Table 1. The 2.5% NaOCl only and 2% CHX only groups showed lower C. albicans counts than the biofilm C. albicans group. Therefore, both 2.5% NaOCl and the 2% CHX have antifungal properties. The 2.5% NaOCl plus diode laser group had the lowest C. albicans count (5.67 CFU/mL), which suggests that the use of 2.5% NaOCl with subsequent diode laser radiation has stronger antifungal effects than the use of 2.5% NaOCl alone (154.00 CFU/mL).

The post hoc Bonferroni test results (Table 2) found a statistically significant difference in C. albicans count between the biofilm C. albicans group, 2.5% NaOCl group, and the 2% CHX group (p<0.001). No significant difference between the 2.5% NaOCl group and the 2% CHX group was found (p=1.000), which suggests that both the 2.5% NaOCl and 2% CHX have similar antifungal effects on the C. albicans biofilm.

A statistically significant difference in C. albicans count was found between the 2.5% NaOCl only group and 2.5% NaOCl plus diode laser group (p=0.001). Similar results were found between the 2% CHX only group and 2% CHX plus diode laser group (p=0.001). This suggests that the diode laser enhances the antifungal effects of both the 2.5% NaOCl and the 2% CHX.

DISCUSSION

The CFU counts in the 2.5% NaOCl and 2% CHX groups were lower than the control group, which suggests that both 2.5% NaOCl and 2% CHX have antifungal properties with regard to C. albicans. Siquirra and Sen also found that these irrigants had antifungal effects on C. albicans [8]. The 2.5% NaOCl group and 2% CHX group’s CFU counts had no statistically significant difference, which suggests that 2% CHX antifungal properties not comparable to 2.5% NaOCl’s antifungal properties against the C. albicans biofilm. Sena et al. found similar result that both NaOCl and CHX have antifungal properties but do not differ significantly, supporting the findings of this study [15].

Gopikrishna et al. found that a 60°C increase in the 1.25% NaOCl temperature can significantly reduce the viscosity of NaOCl and may influence the movement of NaOCl solution on root canal in clinical applications [16]. Gukabi also found that a 37°C increase in the 2.5% NaOCl temperature can increase the effectiveness of NaOCl against C. albicans [17].

The use of a diode laser is an innovative approach to root canal disinfection. Diode lasers are able to create heat increasing the temperature of the irrigants [18]. An increase in the temperature of low concentrations of NaOCl solutions can enhance the solution’s antimicrobial properties [16,17,19]. Table 1 summarizes a reduction in the CFU in the 2.5% NaOCl plus diode laser radiation group when compared to the 2.5% NaOCl, only group. Table 2 summarizes the statistically significant difference between the two groups.

Table 1: The mean values of the C. albicans count before and after the application of the 2.5% NaOCl and 2% CHX with and without diode laser radiation (CFU/ml)

| Experimental group                  | n   | Mean±SD | 95% CI Lower value | 95% CI Highest value |
|-------------------------------------|-----|---------|--------------------|----------------------|
| C. albicans biofilm                 | 3   | 228.00±9.00 | 219               | 237                  |
| NaOCl 2.5%                          | 3   | 154.00±4.00 | 150               | 158                  |
| CHX 2%                              | 3   | 151.67±22.50 | 129               | 174                  |
| NaOCl 2.5%+diode laser              | 3   | 5.67±3.22   | 2                   | 8                    |
| CHX 2%+diode laser                  | 3   | 6.00±1.00   | 5                   | 7                    |

| C. albicans: Candida albicans, CHX, Chlorhexidine, CFU: Colony-forming units, SD: Standard deviation, CI: Confidence interval |

Table 2: The p value of the C. albicans count before and after the application of the 2.5% NaOCl and 2% CHX with and without the application of the diode laser at 980 nm of radiation

| Experimental group                  | p    |
|-------------------------------------|------|
| C. albicans biofilm versus NaOCl 2.5% | 0.001 |
| C. albicans biofilm versus CHX 2%   | 0.001 |
| C. albicans biofilm versus CHX 2%+diode laser | 0.001 |
| C. albicans biofilm versus CHX 2%+diode laser | 0.001 |
| NaOCl 2.5% versus CHX 2%            | 1.000 |
| NaOCl 2.5% versus NaOCl 2.5%+diode laser | 0.001 |
| NaOCl 2.5% versus CHX 2%+diode laser | 0.001 |
| CHX 2% versus NaOCl 2.5%+diode laser | 0.001 |
| CHX 2% versus CHX 2%+diode laser    | 0.001 |
| CHX 2%+diode laser versus CHX 2%+diode laser | 1.000 |

Post hoc Bonferroni statistic test with p<0.05. C. albicans: Candida albicans, CHX: Chlorhexidine
No studies or literature have been found regarding the use of a 980-nm diode laser combined with 2% CHX against C. albicans the effect of changing the 2% CHX temperature in root canal disinfection. Hamud et al. found that a 980-nm diode laser can induce cavitation on a water-based medium by the formation and implosion of water vapor. Bubble pressure from cavitation can increase the breakdown of the irrigation solution, which enhances disinfection. In the root canal, the bubble pressure can potentially destroy microorganisms’ biofilms, rupture cell membranes, and to clear the smear layer and debris [20]. The 2% CHX solution is thought to create cavitation after subsequent diode laser radiation, supporting the findings of this study, which showed an increase in the antifungal properties of 2% CHX after diode laser radiation.

An explanation of the effect of a 980-nm diode laser on the C. albicans biofilm has not been discussed in previous literature. However, many studies researched the effect of diode lasers on bacteria are assumed to be applicable to C. albicans. Bago et al. and Baz et al. studied E. faecalis and concluded that diode lasers have a better effect if they are used in conjunction with endodontic irrigants [10,12]. Similar results were found in this study. The CFU of C. albicans was significantly lower in the irrigants plus diode laser groups than the irrigants only groups. Therefore, we concluded that the diode laser activates the antifungal properties of the 2.5% NaOCl and the 2% CHX against C. albicans.

CONCLUSION
This study found that the 2.5% NaOCl and 2% CHX endodontic irrigants have antifungal properties. The use of a diode laser in addition to the irrigants can activate the antifungal properties of the 2.5% NaOCl and 2% CHX.

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