ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Current Research in Dental Sciences

Research Article *Araştırma Makalesi* DOI: 10.5152/CRDS.2023.22094

Disinfection Effect of Gaseous Ozone on *Candida albicans* and *Enterococcus faecalis*: A Randomized Clinical Trial in Infected Primary Molars

Gaz Halindeki Ozonun *Candida albicans* ve *Enterococcus faecalis* Üzerindeki Dezenfekte Edici Etkisi: Enfekte Süt Azı Dişlerinde Randomize Bir Klinik Çalışma

Efsun SONGÜR[1]
Merve AKSOY[2]
Berrin ÖZÇELİK[3]
Firdevs TULGA ÖZ[4]

[1]Pediatric Dentistry, Dentarya Oral Health Center, Ankara, Turkey
[2]Department of Pediatric Dentistry, University of Health Sciences, Gülhane Faculty of Dental Medicine, Ankara, Turkey
[3]Faculty of Pharmacy, Gazi University, Ankara, Turkey
[4]Department of Pediatric Dentistry, Ankara University, Faculty of Dentistry, Ankara, Turkey

ABSTRACT

Objective: The current clinical randomized study's primary purpose was to investigate the antimicrobial efficiency of gaseous ozone on primary teeth root canals.

Methods: The included teeth were randomly divided into three groups, and in each group, the root canal therapies of 12 primary teeth were done. In group 1, 2.5% sodium hypochlorite was applied as an irrigation agent. In group 2, 0.9% sterile saline solution was combined with 80 seconds OzonyTronX® application. In group 3, 2.5% sodium hypochlorite was applied as an intracanal medicament in combination with 80 seconds OzonyTronX®. Microbial analyses assessed the colonization of *Candida albicans* and *Enterococcus faecalis* before and after the procedure.

Results: Thirty-six primary molars of 36 completely healthy patients (aged 5-10) were included in the study. The results revealed that the percentage decrease in the number of *C. albicans* was higher in group 3 (99.3%). This decrease was statistically higher than group 1 (96.1%) and group 2 (94.2%). Group 1 showed better results in eliminating the viable *C. albicans* versus group 2 ($P = .033$). The decrease in the number of *E. faecalis* was higher in group 3 (99.4%) compared to group 1 (99.1%) and group 2 (98.7%) ($P = .006$). Although the percentage of the unviable microorganisms was higher in group 1, there was no statistical difference versus group 2 ($P > .05$).

Conclusion: Using gaseous ozone alone or in combination with sodium hypochlorite may enhance the success of the antimicrobial stage of primary root canal therapies. It could be an alternative to toxic chemical agents such as highly concentrated sodium hypochlorite.

Keywords: Dentistry, disinfection agents, ozone, pediatric dentistry, primary teeth, root canal treatment

ÖZ

Amaç: Bu çalışmada ozon gazının süt dişi kök kanalları üzerindeki antimikrobiyal etkinliğinin incelenmesi amaçlanmıştır.

Yöntemler: Dahil edilen dişler rastgele three gruba ayrıldı ve her grupta 12 süt dişinin kanal tedavisi yapıldı. Grup 1'de irrigasyon ajanı olarak %2,5 NaOCl uygulandı. Grup 2'de %0,9 steril salin solüsyonu 80 saniye OzonyTronX® uygulaması ile kombine edildi. Grup 3'te %2,5 NaOCl kanal içi ilaç olarak 80 saniye OzonyTronX® ile kombinasyon halinde uygulandı. Mikrobiyal analizler ile işlemden önce ve sonra *C. albicans* ve *E. faecalis*'in kolonizasyonu değerlendirdi.

Bulgular: Tamamen sağlıklı 36 hastanın (5-10 yaş arası) 36 süt azı dişi çalışmaya dahil edildi. Sonuçlar, *Candida albicans* sayısındaki azalmanın Grup 3'te (%99,3) daha yüksek olduğunu ortaya koydu. Bu azalma Grup 1 (%96,1) ve Grup 2'ye (%94,2) göre istatistiksel olarak daha yüksekti. Grup 1'in Candida Albicans üzerindeki antimikrobiyal etkisi Grup 2'ye kıyasla daha yüksek bulundu ($P = ,033$). *Enterococcus faecalis* sayısındaki azalma Grup 3'te (%99,4) Grup 1 (%99,1) ve Grup 2'ye

Received/Geliş Tarihi: 28.06.2022
Accepted/Kabul Tarihi: 20.03.2023
Publication Date/Yayın Tarihi: 10.10.2023

Corresponding Author/Sorumlu Yazar: Merve AKSOY
E-mail: merve.aksoy@sbu.edu.tr

Cite this article as: Songür E, Aksoy M, Özçelik B, Tulga Öz F. Disinfection effect of gaseous ozone on candida albicans and enterococcus faecalis: A randomised clinical trial in infected primary molars. *Curr Res Dent Sci.* 2023;33(4):219-225.

(%98,7) göre daha fazlaydı (P = ,006). Canlı olmayan mikroorganizmaların yüzdesi Grup 1'de daha yüksek olmasına rağmen, Grup 2'ye göre istatistiksel olarak anlamlı bir fark tespit edilemedi (P > ,05).

Sonuç: Ozone gazının yalnız ya da sodyum hipoklorit ile kombine kullanımının süt dişi kanal tedavilerinin antimikrobiyal etkinliğini arttırabileceği ve bu uygulamanın toksik kimyasal kanal içi dezenfektanlara alternatif olabileceği düşünülmektedir.

Anahtar Kelimeler: Diş hekimliği, dezenfekte edici ajanlar, ozon, çocuk diş hekimliği, süt dişleri, kök kanal tedavisi

INTRODUCTION

Tooth caries in primary teeth may occur rapidly, and the progressive character of the lesions reveals pulpal damage in a shorter time compared to permanent teeth. Once the pulp is infected, removing the pulp tissue to avoid disseminating the infection through the surrounding tissue and mainly to the germ of the permanent tooth in the neighborhood is the preferable choice in appropriate cases.[1,2] This treatment, named root canal therapies in primary teeth, reveals differences compared to permanent dentition due to the root canal anatomies, larger and enhanced dentinal tubules, accessory canals, and root tip bifurcation. Furthermore, these differences make it impossible to disinfect the root canal of a primary tooth through instrumentation.[3] The root resorption degrees and the germ in the neighborhood of the periapical area are also challenging parts of primary root canal treatments.[4]

Numerous types of infectious agents can colonize in root canals, and the studies assessing primary root canal microbiota revealed that anaerobic strains form the main microorganism population of primary teeth with necrotic pulp.[3,5] Accordingly, *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. albicans*) are the main strains isolated from the infected root canals.[6,7] An appropriate disinfection procedure should be applied to achieve a successful root canal treatment, and this protocol consists of 2 main components: mechanical preparation and chemical irrigation.[8,9] In primary teeth, the presence of the lateral and accessory canals and the complexity of root canal anatomy make it impossible to reach all the microbial residuals and remove them by mechanical approaches.[3] By this means, chemical irrigants take a significant part of the root canal disinfection procedure in the primary dentition.[4,7,10,11] Various disinfection and irrigation agents have been used, and sodium hypochlorite (NaOCl) has been preferred as a gold standard in this area.[12-15] Though this agent shows an effective antimicrobial activity, it still has some disadvantages that restrict its use in pediatric patients. The excessive use of NaOCl causes cytotoxic effects and inflammatory responses such as edema, severe pain, ecchymosis, and paresthesia.[3,16] Hence, the search for ideal root canal disinfection agents remains to be a necessity in root canal treatment of primary teeth.[3,6,17]

Ozone is an alternative non-invasive antibacterial, antiviral, and antifungal agent preferred in reducing the number of caries-causing bacteria. Ozone reveals the antimicrobial effect by effectively oxidizing the bacterial cell wall and cytoplasmic membrane.[18-22] Accordingly, it has been used in medicine and dentistry in various areas such as wound healing, caries management, cavity disinfection, root canal therapies, tooth hypersensitivity therapies, temporomandibular joint disorders, and oral diseases.[23]

In literature searches, studies investigating the antimicrobial effect of gaseous, aqueous, and oiled forms of ozone versus NaOCl, and various disinfection agents have been detected.[2-4,24] However, these studies were mainly held on ex vivo conditions, and the application was held on permanent teeth.[17,25-29] In the literature review, no study was detected to assess the antimicrobial effect of gaseous ozone and combined use of it with NaOCl versus the use of NaOCl alone in primary teeth, in vivo. The main purpose of the current study was to investigate the antimicrobial effect of gaseous ozone and the combined use of ozone gas with NaOCl in the disinfection of primary root canals, in vivo, and to find a safe and effective alternative to NaOCl disinfection agents used in deciduous root canal therapies. The study hypothesized that the antimicrobial efficiency of gaseous ozone would be higher than the other groups compared.

MATERIAL AND METHODS

Ethical Approval

The study protocol was approved by Ethical Committee of Ankara University Faculty of Dentistry (Date: 26.10.2010, Number: 3/6). The research was conducted in full accordance with the World Medical Association Declaration of Helsinki, with all amendments. The study was designed in accordance with STROBE guidelines. The legal representatives of the participants and the patients were informed of the study design, and informed consent was obtained from them. All treatment procedures were completed for each patient admitted to our clinics, whether they were a part of the study or not. All the participants were informed that they were free to leave the study if they did not want to attempt the treatment sessions at any time interval.

Case Selection

The study population was detected by G*Power Software based on an effect size of 0.5, an alpha significance level of 5% (0.05), and a beta of 20% (0.20) to achieve an 80%.[30] Accordingly, a total of 36 mandibular second primary molars of 36 children aged between 5 and 10, with no systemic illnesses [American Society of Anesthesiologists -I] were included in the study. Including criteria were as follows: pediatric patients with no use of an antimicrobial agent in the last 3 months, teeth with positive responses to percussion and palpation, no sinus tract and intra-oral, extra-oral swelling, teeth with no pathological and physiological mobility, and restorable with stainless steel crown. Additionally, in radiographical examinations, the selected teeth needed to show the Res(i) and Res (1/4) scores according to the root resorption scoring system (Figure 1).[31] No radiolucency in the peri-radicular tissue and no internal–external root resorption were also among the including criteria. However, the teeth with lesions only in 1/3 percent of the bifurcation area were also included in the study

The patients who are not compatible with including criteria, who cannot adopt the dental treatments, and with whom rubber-dam isolation was not possible were excluded from the study.

The included teeth were randomly divided into three groups, and in each group, the root canal therapies of 12 primary teeth were done. The flowchart of the study design is described in Figure 2.

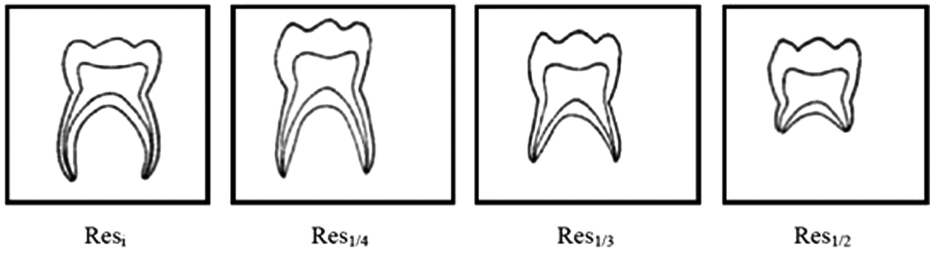

Figure 1. The root resorption degrees for mandibular deciduous molars.

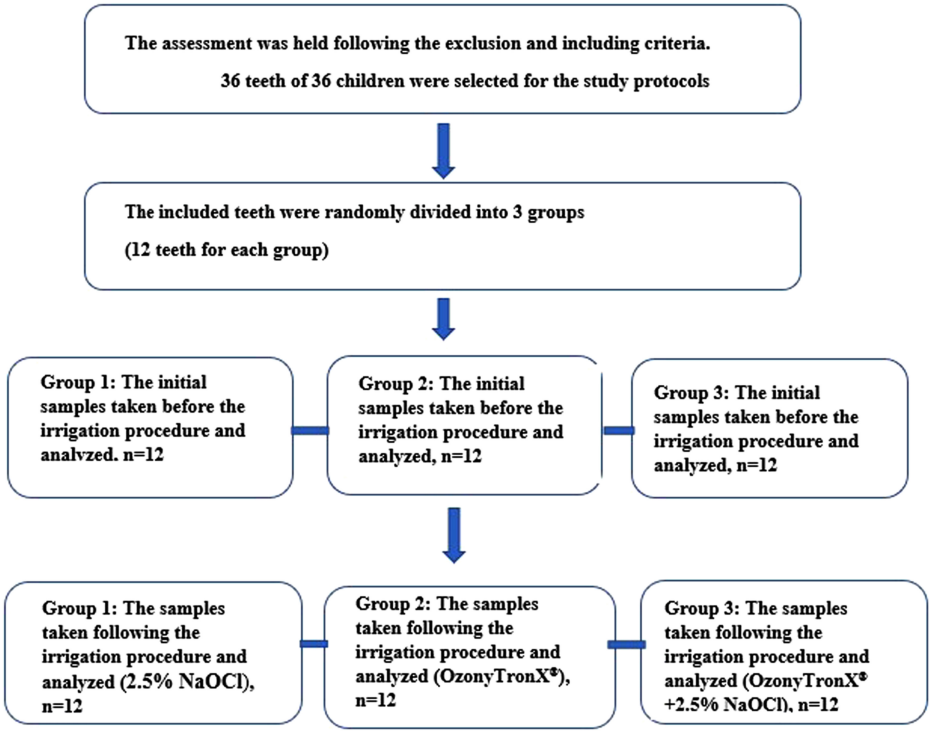

Figure 2. Flowchart of study design.

In group 1 (control group), 2.5% NaOCl was applied as an irrigation agent.

In group 2 (study group), 0.9% sterile saline solution was combined with 80 seconds OzonyTronX® application.

In group 3 (study group), 2.5% NaOCl was applied as an intracanal medicament in combination with 80 seconds OzonyTronX®.

Ozone System

OzonyTronX®, developed in 2005 in Germany, was formed by a central unit, a plasma probe, and an applicator. High-frequency impulses are sent from the central unit to the applicator via a connector. OzonyTronX® is not a generator that produces gaseous ozone. The oxygen molecules (O_2) in the atmosphere are divided into atomic oxygen when the probe contacts the application area. This atomic oxygen combines with O_2 and forms O_3, a highly effective disinfection agent.[32] OzonyTronX® has different probes that differ by application areas. The probe used in narrow (2 cm^3) and curved areas such as root canals and periodontal defects was preferred in the study. According to company recommendations, ozone at a concentration of 100 µg/mL (level 5, 40 seconds) was used with a blue probe for disinfection procedures of root canals. The use of gaseous ozone for 20-120 seconds in dental practices was approved by Medical Device Authority (MDA) and Technical Inspection Association (TUV). Accordingly, in the current study, the application was made twice (80 seconds) to obtain the maximum disinfection effect.

Clinical Procedures

The root canal length was assessed with a phosphor plaque sensory system using a standard parallel technique (Digora® Soredex, Soredex Medical Systems, Helsinki, Finland) (Figure 3). Regional alveolar inferior block anesthesia was applied, and rubber-dam isolation was done. The plaque and other bacterial accumulations were cleaned off with pumice and a micro-brush. The external surfaces of the teeth, clamps, and rubber liner were also wiped using 0.12% chlorhexidine (CHX; Klorhex™, Drogsan, Ankara, Turkey), 3% H_2O_2 (60 seconds) (Carlo Erba, Emmendingen, Germany), 5.25% NaOCl (60 seconds) (Wizard™, İstanbul, Turkey). The remedies of NaOCl were removed using 5% sodium thiosulfate (HT1005, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), and sterile saline solution was used to wash the operation place. The coronal cavity access was performed using a sterile diamond bur (Meisinger 801-16, Germany). The first samples of microorganisms were taken by sterile paper points placed 2 mm shorter than the root canal length for 60 seconds. The samples

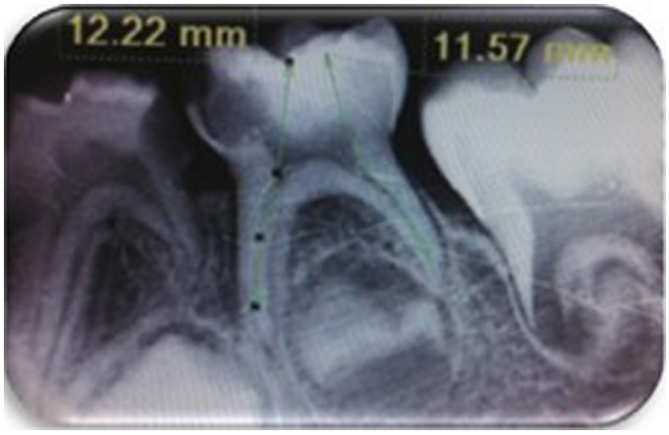

Figure 3. Determining the root length using standard parallel technique (Digora® Soredex, Soredex Medical Systems, Helsinki, Finland).

were taken from the largest (distal) canal of the tooth, and meanwhile, the other canal orifices were closed by sterile cotton pellets. The paper points were placed in sterile tubes containing 2 mL reduced transport fluid and transported to Gazi University Faculty of Pharmacy Department of Pharmaceutical Microbiology laboratories. After the first samples were obtained, the mechanical preparation was done with ISO 30 K files (Golden Star Medical, Guangdong). The chemical applications were performed after the mechanical applications were made in both study and control groups.

In Group 1, 10 mL 2.5% NaOCl application followed the mechanical preparation. After 2 mL 0.9% saline application, the second samples were taken by placing a sterile cone in the largest canal of the tooth (distal canal for 60 seconds). The mesiobuccal and distobuccal canals were closed with a sterile cotton pellet while the samples were taken. After the application, the root canal filling was completed with a calcium hydroxide-containing paste (Tg-Pex™, London, England), glass ionomer cement (Ionofil U, Voco, Cuxhaven, Germany) was placed, and the teeth were restored with a Stainless-Steel Crown (3M ESPE, Seefeld, Germany).

In Group 2, the canals were irrigated with 10 mL 0.9% sterile saline. Ozone treatment (OzonyTronX with CA probe) was applied for 80 seconds, and 2 mL 0.9% saline application was performed. Finally, the second samples were taken by the method described in group 1, and the canal treatment was completed the same as mentioned in group 1.

In Group 3, the disinfection of root canals was done using 10 mL, 2.5% NaOCl, and ozone treatment for 80 seconds, and 2 mL 0.9% saline application was performed. Finally, the second samples were taken by the method described in group 1, and the canal treatment was completed in the same manner as mentioned in group 1. The materials used in the study are listed in Table 1. The clinical procedures are shown in Figure 4**.**

The Microbial Analyses

The microbial samples were obtained by using a sterile paper point. After the coronal cavity was prepared and the disinfection agents were applied, the initial and the last root canal microbiota was obtained by placing a paper point in the root canals for 60 seconds. These paper points were immediately placed in sterile tubes containing RTF and transported to the pharmaceutical microbiology laboratories.

The colonization of *C. albicans* and *E. faecalis* was assessed by 10 times diluting the samples in PBS (Phosphate buffered saline) and inoculating the samples in MacConkey agar in 3 parallel lines. The identification of microorganisms was made by API (microorganism identification) kits (Biomerieux, Marcy-l'Étoile, France), and the values of alive microorganisms were assessed macroscopically in colony-forming unit (CFU)/mL, in a microaerophilic atmosphere (Anaerocult C), 37°C, following the incubation of 48 hours (Arendorf & Walker 1979).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM Corp.; Armonk, NY, USA) was used to analyze the study results. Whether the distribution of continuous-measure variables was close to normal was investigated using the Shapiro–Wilk test, and the homogeneity of variances was investigated with the Levene test. Descriptive statistics are shown as mean (standard deviation). The effectiveness of the irrigation agent was evaluated with the "Wilcoxon Sequential Sign Test" using pre-post-irrigation data for in-group comparison. The difference between the groups was also evaluated by performing the "Kruskal–Wallis test" separately on the microbiological data obtained before and after the application. If there was a significant difference due to the Kruskal–Wallis test, the groups causing the difference were determined by Conover's non-parametric multiple comparison test.

RESULTS

In Table 2, the results of the descriptive statistical analyses of the values of *C. albicans* and *E. faecalis* prior to the application and following the applications were shown in Log CFU. According to the study results, the number of viable microorganisms was significantly reduced compared to the initial samples in all groups ($P < .05$).

However, according to the Kruskal–Wallis analyses in which the initial microorganism values versus the values obtained following

Table 1. The Materials and Chemical Agents Used in the Study

Material	Ingredients	Producer Firm
Xylocaine 10% spray	Lidocain 10%	AstraZeneca, Switzerland
Ultracain D-S ampul	40 mg articain hydrochloride, 0.006 mg epinephrine hydrochloride, 0. 5 mg sodium metabisulfite, 1 mg sodium chloride, 0.3162 mg 0.1 N hydrochloric acid, and 1 mL water for injection	Aventis Farma, Turkey
Saline solution	0.9% isotonic sodium chloride, 0.9 g sodium chloride (154 mEq/L sodium and 154 mEq/L chloride), 100 mL water for injection	İ.E Ulagay, Istanbul, Turkey
Sodium hypochlorite (NaOCl)	5% NaOCl and sterile saline	Wizard, Rehber Kimya San. ve Tic, Istanbul, Turkey
$Ca(OH)_2$ paste	30% $Ca(OH)_2$, 40.4% iodoform, 22.4% silicon oil	Tg-Pex™, London, England
Glass ionomer cement	Florosilicate glass, polyacrylic acid, PHB esters	Ionofil U, Voco GmbH, Cuxhaven, Germany
Gaseous ozone		OzonyTronX®, Mymed, Germany
Stainless steel crown	72% iron, 18% crom, 10 % nickel manganese, silicon, and carbon	3M ESPE, Seefeld, Germany
Reduce transport fluid	NaCl, $(NH_4)_2SO_4$, KH_2PO_4, $KHPO_4$, Mg_2SO_4, NaEDTA, L-cysteine	Merck, Turkey

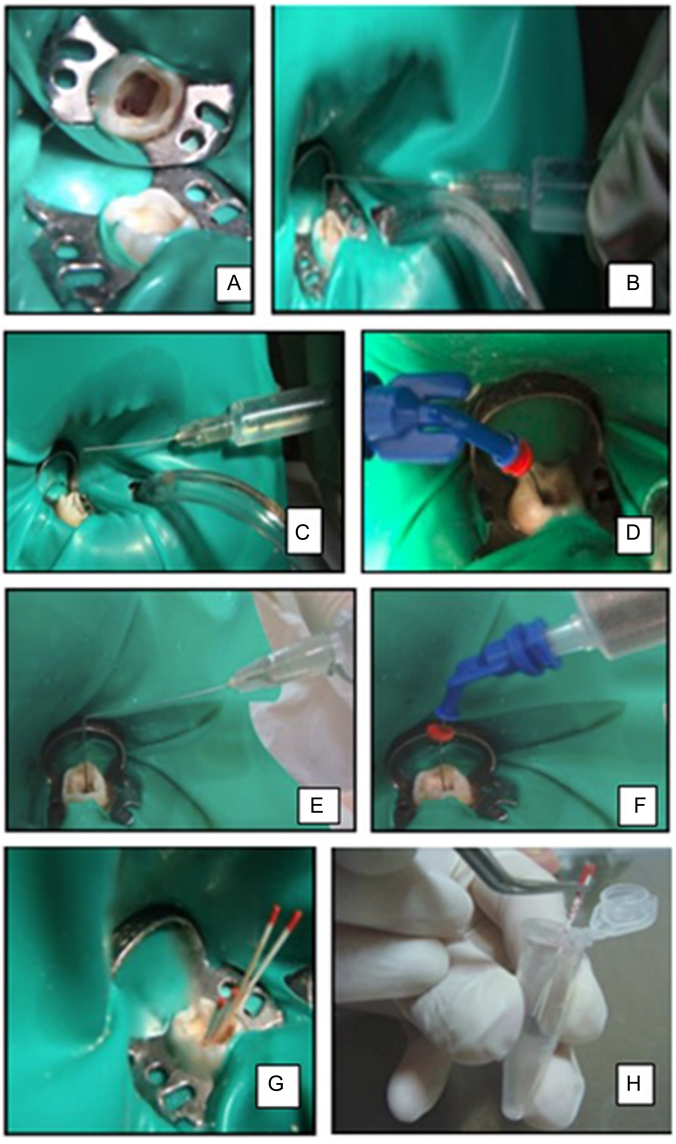

Figure 4. The clinical procedures of different disinfection agents' applications. (A) The coronal access cavity was performed under rubber dam isolation. (B) A patient from Group 1—NaOCl irrigation was performed. (C) A sample from Group 2—saline application. (D). Group 2—ozone application. (E) Group 3—NaOCl application. (F) Group 3—ozone application. (G) The microbial samples were taken by sterile cotton pellets. (H) The microbial samples were placed into RTF-containing tubes and transferred to laboratories.

the antimicrobial treatment applications were compared, the number of viable microorganisms revealed higher values in the ozone group (Table 3, Figures 5 and 6).

The Wilcoxon signed-rank test determined the decrease of the microorganisms in root canals in percentage to compare the effect of irrigation procedures (*E. faecalis* and *C. albicans)* (inter-group comparisons-between the groups).

Group 1 showed better results in eliminating the viable *C. albicans* versus group 2 ($P = .033$). The percentage decrease in the number of *C. albicans* was higher in group 3 (99.3%). This decrease was statistically higher than group 1 (96.1%) and group 2 (94.2%).

The decrease in the number of *E. faecalis* was higher in group 3 (99.4%) compared to group 1 (99.1%) and group 2 (98.7%) ($P = .006$). Although the percent of the unviable microorganisms was higher in group 1, there was no statistical difference versus group 2 ($P > .05$).

Groups 1, 2, and 3 revealed better results in eliminating the viable *E. faecalis* versus eliminating the viable *C. albicans* ($P = .008$, $P = .015$, $P = .002$).

Table 2. The Mean Values of the Microorganisms Obtained Before/After the Irrigation/Disinfection Procedures (Log CFU)

Before Irrigation	n	(Log CFU) Mean (Standard Deviation)	After Irrigation	n	(Log CFU) Mean (Standard Deviation)
C. albicans			*C. albicans*		
Group 1	12	3.6675 (0.41784)	Group 1	12	.014417 (0.0036546)
Group 2	12	3.4892 (0.54773)	Group 2	12	.022167 (.0156253)
Group 3	12	4.4514 (0.08507)	Group 3	12	.014917 (.014917)
E. faecalis			*E. faecalis*		
Group 1	12	4.1350 (0.63110)	Group 1	12	.015333 (.0025346)
Group 2	12	4.3233 (0.89024)	Group 2	12	.028667 (.0338213)
Group 3	12	4.5417 (0.02488)	Group 3	12	.013500 (.0018340)

Table 3. Decrease in the Amount of *C. albicans* and *E. faecalis* in Percentage Following Irrigation/Disinfection Procedures

Groups	*C. albicans*	*E. faecalis*	*P*
Group 1	96.1 (5.15)^{A}	99.1 (1.06)^{D}	.008*
Group 2	94.2 (7.40)^{B}	98.7 (1.21)^{D}	.015*
Group 3	99.3 (0.82)^{C}	99.4 (0.63)^{E}	.002*
P	.033*	.006*	

*$P < .05$.**Same upperletters means no statistical difference

DISCUSSION

In the current study, in which the antibacterial activity of ozone application on *E. faecalis* and *C. albicans* in infected primary tooth's root canals was compared with NaOCl, all agents revealed an antimicrobial activity on both *E. faecalis* and *C. albicans* colonization and the number of the viable microorganism decreased in all groups compared after the applications of irrigation agents. Ozone's combined use with NaOCl was found to be superior to NaOCl alone and the hypothesis of the study was rejected.

For *E. faecalis*, ozone gas application (98.7%) provides a numerically lower effect than the application of NaOCl alone (99.1%); the difference is not statistically significant. However, the effect in the group (99.4%) in which NaOCl and ozone were applied together was significantly higher than the other groups. For *C. albicans*, although the application of ozone gas (94.2%) provides a numerically lower effect than the application of NaOCl alone (96.1%), the difference is not statistically significant. However, the effect seen in the group (99.3%) in which NaOCl and ozone were applied together was significantly higher than the other groups.

In the literature review, according to previous in vitro and in vivo studies, the antimicrobial effect of NaOCl showed better results versus the various antimicrobial agents used (e.g., ozone, saline, laser applications, green tea).[2,3,5] Kapdan et al[2] held a similar study, aimed to assess the effect of different irrigation procedures on the viability of *E. faecalis* colonized in primary incisors' roots (n = 60), in vitro. Gaseous ozone (150 seconds), NaOCl, KTP (potassium titanyl phosphate) laser, and saline solution were used as disinfection agents and techniques. Results of the study showed that NaOCl was superior to all groups compared. The results of the study were similar to the current study we conducted. Öter et al[4] studied the antimicrobial effects of Endosafe, PAD (photo-activated disinfection), diode laser, ozone, and NaOCl on microbial colonization on 100 extracted human primary molars. According

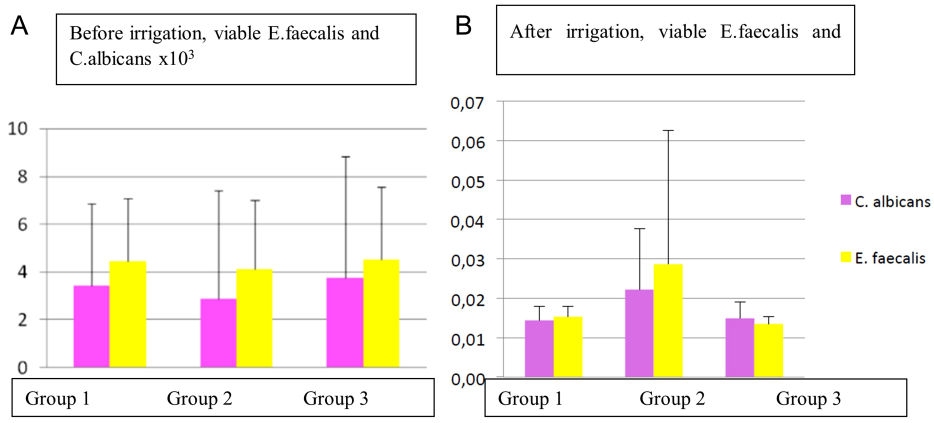

Figure 5. (A) *C. albicans* and *E. faecalis* amounts isolated from root canals before irrigation. (B) *C. albicans* and *E. faecalis* amounts isolated from root canals after irrigation.

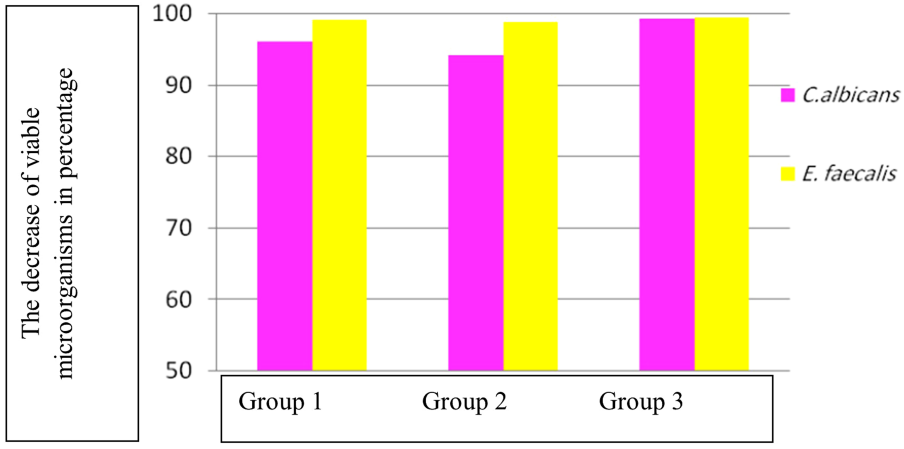

Figure 6. The decrease of viable microorganisms in percentage after the final irrigation compared to before irrigation.

to the results, NaOCl revealed better results than diode laser application. Diode laser was superior to ozone, Endosafe, and PAD groups. The efficiency of ozone was similar to the Endosafe and PAD applications. This study has also revealed that NaOCl application was superior to ozone application by means of root canal disinfection efficiencies.

However, in an in vivo study, Ajeti et al[24] (2018) assessed the disinfection efficiencies of 0.9% NaCl, 2.5% NaOCl, and 2% CHX combined with gaseous ozone. The last group (2% CHX combined with gaseous ozone) revealed superior results compared to the groups studied. The results of this study were different from our findings. However, Ajeti et al[24] studied the effect of the combined use of CHX and ozone versus NaOCl. The additional antimicrobial effect of CHX may enhance the efficiency of ozone gas, and the success of ozone-CHX versus NaOCl could be attributed to the effect of CHX use.

In another previous study, Agarwal et al[3] (2020) compared the antimicrobial efficiency of ozone, green tea, and saline in 60 primary teeth with a single root, in vivo. The microbial samples were taken before treatment, after the irrigation, and on the third day after the treatment. The results showed that the antimicrobial effect of ozone application was superior to green tea and saline treatments. The result of this study was different from the findings of the current study we held. This difference might be attributed to the fact that, in the study by Agarwal et al,[3] the antimicrobial effect of ozone was compared to saline and green tea. The comparison between ozone and NaOCl was not made in this study, and the results might be changed if the study groups were enlarged, including the application of NaOCl.

The study was held on clinical conditions; although clinical studies are more respectable than in vitro studies, environmental and biological factors may affect the study results. The findings might differ if the study was supported with an in vitro trial stage. Repeating the treatment in a larger patient population may enhance the reliability of the study. Furthermore, only the gaseous form of ozone was obtained and used in the current study. However, different ozone forms, such as oiled ozone and ozonated water, could affect the study results. These could be mentioned as the limitations of the current study

Based on our findings, ozone gas could be an alternative agent in pediatric patients with fear and anxiety, thanks to the disinfection effect with short application time in infected root canals. Furthermore, ozone gas could be an aid to NaOCl in endodontic treatment, thanks to its enhanced antibacterial efficiency when used following mechanical instrumentation. However, long-term follow-up clinical studies are needed to assess the effect of ozone gas application on the success rate of endodontic treatment of primary teeth with periapical/furcation lesions

Ethics Committee Approval: The study protocol was approved by Ankara University Faculty of Dentistry Ethical Committee (Date: 26.10.2010, Number: 3/6).

Informed Consent: The legal representatives of the participants and the patients were informed of the study design, and written informed consent was obtained from them.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – F.T.O.; Design – F.T.O, E.S.; Supervision – F.T.O.; Resources – M.A., E.S.; Materials – E.S.; Data Collection and/or Processing – E.S.; Analysis and/or Interpretation – B.O.; Literature Search – M.A., E.S.; Writing Manuscript – M.A., E.S.; Critical Review – F.T.O.

Declaration of Interests: The authors declare that they have no competing interest

Funding: This study was funded by Ankara University Scientific Research Committee, with project number 12B3334001.

Etik Komite Onayı: Bu çalışma için etik komite onayı Ankara Üniversitesi'nden (Tarih: 26.10.2010, Sayı: 3/6) alınmıştır.

Hasta Onamı: Katılımcıların ve hastaların yasal temsilcileri çalışma tasarımı hakkında bilgilendirilmiş ve kendilerinden yazılı bilgilendirilmiş onam alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız

Yazar Katkıları: Fikir – F.T.O.; Tasarım – F.T.O, E.S.; Denetleme – F.T.O.; Kaynaklar – M.A., E.S.; Malzemeler – E.S.; Veri Toplanması ve/veya İşlemesi – E.S.; Analiz ve/veya Yorum – B.O.; Literatür Taraması – M.A., E.S.; Yazıyı Yazan – M.A., E.S.; Eleştirel İnceleme – F.T.O.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir

Finansal Destek: Bu çalışma Ankara Üniversitesi Bilimsel Araştırma Kurulu tarafından 12B3334001 proje numarası ile desteklenmiştir.

REFERENCES

1. American Academy of Pediatric Dentistry. *Pulp Therapy for Primary and Immature Permanent Teeth. The Reference Manual of Pediatric Dentistry*. American Academy of Pediatric Dentistry; 2022:415-423
2. Kapdan A, Kustarci A, Tunç T, Sumer Z, Arslan S. Which is the most effective disinfection method in primary root canals: conventional or newly developed ones? *Niger J Clin Pract*. 2015;18(4):538-543. [CrossRef]
3. Agarwal S, Tyagi P, Deshpande A, Yadav S, Jain V, Rana KS. Comparison of antimicrobial efficacy of aqueous ozone, green tea, and normal saline as irrigants in pulpectomy procedures of primary teeth. *J Indian Soc Pedod Prev Dent*. 2020;38(2):164-170. [CrossRef]
4. Öter B, Topçuog Lu N, Tank MK, Çehreli SB. Evaluation of antibacterial efficiency of different root canal disinfection techniques in primary teeth. *Photomed Laser Surg*. 2018;36(4):179-184. [CrossRef]
5. da Silva LA, Nelson-Filho P, Faria G, de Souza-Gugelmin MC, Ito IY. Bacterial profile in primary teeth with necrotic pulp and periapical lesions. *Braz Dent J*. 2006;17(2):144-148. [CrossRef]
6. Silva EJNL, Prado MC, Soares DN, Hecksher F, Martins JNR, Fidalgo TKS. The effect of ozone therapy in root canal disinfection: a systematic review. *Int Endod J*. 2020;53(3):317-332. [CrossRef]
7. Gomes BPFA, Herrera DR. Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology. *Braz Oral Res*. 2018;32(suppl 1):e69. [CrossRef]
8. Vasavada K, Kapoor S. Evaluation of ozonized calcium hydroxide as an effective intracanal medicament during root canal procedures: an in vitro observational study. *Med Gas Res*. 2020;10(3):122-124. [CrossRef]
9. Zan R, Alacam T, Hubbezoglu I, et al. Antibacterial efficacy of superoxidized water on Enterococcus faecalis biofilms in root canal. *Jundishapur J Microbiol*. 2016;9(9):e3000
10. Kapdan A, Öztaş N, Sümer Z. Comparing the antibacterial activity of gaseous ozone and chlorhexidine solution on a tooth cavity model. *J Clin Exp Dent*. 2013;5(3):e133-e137. [CrossRef]
11. Estrela C, Holland R, Estrela CRDA, Alencar AH, Sousa-Neto MD, Pécora JD. Characterization of successful root canal treatment. *Braz Dent J*. 2014;25(1):3-11. [CrossRef]
12. Trope M, Debelian G. Microbial control: the first stage of root canal treatment. *Gen Dent*. 2009;57(6):580-588
13. Zehnder M. Root canal irrigants. *J Endod*. 2006;32(5):389-398. [CrossRef]
14. Fidalgo TKDS, Barcelos R, Portela MB, Soares RM, Gleiser R, Silva-Filho FC. Inhibitory activity of root canal irrigants against Candida albicans, Enterococcus faecalis and Staphylococcus aureus. *Braz Oral Res*. 2010;24(4):406-412. [CrossRef]
15. Neelakantan P, Herrera DR, Pecorari VGA, Gomes BPFA. Endotoxin levels after chemomechanical preparation of root canal s with sodium hypochlorite or chlorhexidine: a systematic review of clinical trials and meta-analysis. *Int Endod J*. 2019;52(1):19-27. [CrossRef]
16. Goswami M, Chhabra N, Kumar G, Verma M, Chhabra A. Sodium hypochlorite dental accidents. *Paediatr Int Child Health*. 2014;34(1):66-69. [CrossRef]
17. Kist S, Kollmuss M, Jung J, Schubert S, Hickel R, Huth KC. Comparison of ozone gas and sodium hypochlorite/chlorhexidine two-visit disinfection protocols in treating apical periodontitis: a randomized controlled clinical trial. *Clin Oral Investig*. 2017;21(4):995-1005. [CrossRef]
18. Çolak H, Tokay U, Uzgur R. What role does ozone play in preventing dental caries? An evidence-based review. *Ozone Sci Eng*. 2015;37(6):563-567. [CrossRef]
19. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent*. 2008;36(2):104-116. [CrossRef]
20. Baysan A, Lynch E. The use of ozone in dentistry and medicine. *Prim Dent Care*. 2005;12(2):47-52. [CrossRef]
21. Çetİnkaya M, Aksoy M, Tulga Öz F. F. Better with ozone, or not? An in-vivo study of ozone therapy as A pre-treatment before fissure sealant application. *Cumhuriyet Dent J*. 2020;23(4):262-272. [CrossRef]
22. Polydorou O, Halili A, Wittmer A, Pelz K, Hahn P. The antibacterial effect of gas ozone after 2 months of in vitro evaluation. *Clin Oral Investig*. 2012;16(2):545-550. [CrossRef]
23. Srinivasan SR, Amaechi BT. Ozone: A paradigm shift in dental therapy. *J Glob Oral Health*. 2019;2(1):68-77. [CrossRef]
24. Ajeti NN, Pustina-Krasniqi T, Apostolska S. The effect of gaseous ozone in infected root canal. *Open Access Maced J Med Sci*. 2018;6(2):389-396. [CrossRef]
25. Noites R, Pina-Vaz C, Rocha R, Carvalho MF, Gonçalves A, Pina-Vaz I. Synergistic antimicrobial action of chlorhexidine and ozone in endodontic treatment. *BioMed Res Int*. 2014;2014:592423. [CrossRef]
26. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. *J Endod*. 2004;30(11):778-781. [CrossRef]
27. Üreyen Kaya B, Kececi AD, Güldaş HE, et al. Efficacy of endodontic applications of ozone and low-temperature atmospheric pressure plasma on root canals infected with Enterococcus faecalis. *Lett Appl Microbiol*. 2014;58(1):8-15. [CrossRef]
28. Hubbezoglu I, Zan R, Tunc T, Sumer Z. Antibacterial efficacy of aqueous ozone in root canals infected by Enterococcus faecalis. *Jundishapur J Microbiol*. 2014;7(7):e11411. [CrossRef]
29. Boch T, Tennert C, Vach K, Al-Ahmad A, Hellwig E, Polydorou O. Effect of gaseous Ozone on Enterococcus faecalis biofilm–an in vitro study. *Clin Oral Investig*. 2016;20(7):1733-1739. [CrossRef]
30. Faul F, Erdfelder E, Lang AG, Buchner AG. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175-191. [CrossRef]
31. Fanning EA. The relationship of dental caries and root resorption of deciduous molars. *Arch Oral Biol*. 1962;7(5):595-601. [CrossRef]
32. Bezgin T, Oz FT, Ozalp N. Efficacy of Ozone Pretreatment on Fissure Sealant Adaptation and Retention. *Ozone Sci Eng*. 2022;44(3):317-323. [CrossRef]