

# Evaluation of the antimicrobial effects of ozonated water on the sanitization of endodontic files contaminated with *C. Albicans*



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## Abstract

**Objectives:** The aim of this study was to evaluate the effectiveness of 0.5 ppm ozonated water in the elimination of *Candida albicans*, from endodontic files.

**Materials and Methods:** The study was performed on 20 K-files, 21 mm long and of size 25. Of these, five files were taken as a control group, and the remaining 15 files were divided into three groups of 5 files each and they were tested for the efficacy of sanitization with different disinfecting material: sodium hypochlorite alone, sodium hypochlorite followed by ozonated water and ozonated water alone. From the prepared sample of *C. albicans*, the 20 K-files were contaminated.

**Results:** Antimicrobial effectiveness was evaluated by the reduction of microbial counts. Incomplete disinfection of ozonated water up to 20% was observed (%80 sterilization).

**Conclusions:** The results showed that disinfecting and exposing to ozonated water alone cannot give complete sterilization. Sodium hypochlorite followed by ozonated water can be used as an alternative method for using ozonated water alone.

**Keywords:** Ozonated water, endodontic files, *Candida Albicans*.

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## Introduction:

Most endodontic infections have polymicrobial etiology; *Enterococcus faecalis* and *C. Albicans* are considered the most resistant species and possible causes of root canal treatment failures<sup>(1)</sup>. Microorganisms induce a variety of infections and diseases in the human body and are largely ubiquitous in nature. Contamination directly or indirectly leads to transmission of infectious agents<sup>(2)</sup>. In endodontics, various instruments like files, reamers, gates glidden drill and peeso reamers are used for cleaning and shaping the root canal system and to eliminate the bacterial population in pulp canal space. Various methods are followed to sterilize and disinfect these instruments, such as dry heat sterilizer, autoclave, sodium hypochlorite, ethylene oxide gas, glass bead sterilizer or hot-salt sterilizer, etc.<sup>(3)</sup>. Ozone is a trivalent form of oxygen found in nature and can be produced by generators. It presents antimicrobial properties and low level of cytotoxicity<sup>(4)</sup>. Ozonated water is a new option that is being studied. Recently, ozone has become one of the most important disinfecting agents used in dentistry. It can be administered in either gaseous or aqueous form. Both of them may act like powerful antimicrobial agents that are strong and fast oxidizers of cell walls and cytoplasmic membranes of microorganisms. For these reasons, ozone is considered as one of the best bactericidal, antiviral, and antifungal

agents<sup>(5,6)</sup>. Ozone is highly indicated in root canal therapy as an irrigant due to its strong disinfection property and absence of cytotoxicity. Other interesting biological characteristics include: bactericidal action, debriding effect, angiogenesis stimulation capacity and high oxidizing power<sup>(7,8)</sup>.

The use of ozonated water showed that following ozone therapy there was the high metabolic activity of the associated fibroblasts indicating an increase in the healing process<sup>(9)</sup>. Ozonated water has some potential in removing the smear layer, and it has certain ability for the smear layer removal in combination with sodium hypochlorite<sup>(10)</sup>.

Recent investigations of aqueous ozone have indicated that it is a powerful antimicrobial agent against oral pathogens. This suggests that aqueous ozone at different doses might eliminate the oral resistant microorganisms too<sup>(11,12)</sup>. One of the crucial properties of aqueous ozone is its nontoxicity to oral cells in vitro. On the other hand, it is less toxic than all other known antiseptics<sup>(13)</sup>. However, the most important disadvantage of aqueous ozone is its unstable concentration in a long time. Consequently, aqueous ozone should be used as soon as possible after obtaining the ozone generator, these properties indicated that aqueous ozone could be beneficial in

many branches of dentistry, and its use has been recommended by some researchers for the treatment of endodontic infections<sup>(14)</sup>.

### Materials and Methods:

The study was performed on 20 K-files, 21 mm long and of size 25. Of these, five files were taken as a control group, and the remaining 15 files were divided into three groups of 5 files each and they were tested for the efficacy of disinfection with a different disinfecting agent: sodium hypochlorite alone, sodium hypochlorite followed by ozonated water and ozonated water alone.

All the 20 files included in the study were pre-sterilized in an endodontic instrument box by autoclaving for 30 minutes at 121°C at a pressure of 15 pounds.

The test files were divided into four groups of 5 files each and labeled as:

1. Group A: normal saline (control group).
2. Group B: ozonated water alone.
3. Group C: sodium hypochlorite followed by ozonated water.
4. Group D: sodium hypochlorite alone.

### Isolation of sample:

A swab of a lesional site from the oral cavity of leukemic patients (symptomatic patients) is a relatively simple method of detecting growth of *Candida* can be obtained. The sampling approach involves gently rubbing a sterile cotton swab over the lesional tissue of oral patient, and then subsequently inoculating a primary isolation and differential culture medium such as Sabouraud Dextrose Agar (SDA) were white to cream, round, curved, soft and smooth to wrinkled (figure 1), with a characteristic yeast odor, it was growing rapidly and incubated at 37°C for 24-48 hrs.<sup>(15)</sup>



Figure1: growth of *Candida* spp. On SDA.

The isolates corresponding to yeasts morphology were inoculated in CHROMagar *Candida* and incubated in the oven at 37°C for 48-72 hrs.<sup>(16)</sup>

CHROMagar *Candida* medium (CHROMagar) has been reported to achieve the goal of rapid and reliable direct isolation and in some cases identification of *Candida* species<sup>(17)</sup>. Typically this media incubated aerobically at 37°C for 48–72 hrs. The colony morphology (color, size, and texture) assessed to interpret the identification of species. The interpretation was based on published appearance of various species on chromogenic agar. *C. albicans* produce Parrot green colonies (figure2)<sup>(18,19)</sup>.

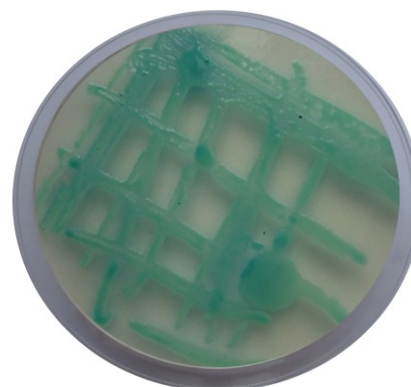


Figure 2: Growth of *Candida albicans* on CHROMagar *Candida* medium.

### Procedure:

All the pre-sterilized files were contaminated with 10ml (150 x10<sup>6</sup>CFU/ml) *C. Albicans* in a sterile test tube for 5 minutes. Then the files were transferred to another sterile test tube with the help of a sterile tweezer.

Each one of the 5 contaminated files in group A placed in a sterile test tube and disinfected with normal saline for 5 minutes (control group) and after completion of disinfection of the files each file placed in separate tubes with the help of a sterile tweezer.

Each one of the 5 contaminated files in Group B placed in a sterile test tube and were disinfected with 0.5ppm ozonated water for 5 minutes (Aqueous ozone was obtained from the custom-made ozone generator (OPURA/CANADA) (figure 3), After completion of disinfection of the files, each file placed in separate tubes with the help of a sterile tweezer.

Each one of The 5 contaminated files in Group C placed in a sterile test tube and were disinfected with sodium hypochlorite 5% followed by ozonated water for 10 minutes. After completion of disinfection of the files, each file placed in separate tubes with the help of a sterile tweezer.

Each one of The 5 contaminated files in Group D placed in a sterile test tube and disinfected with 5% sodium hypochlorite for 5 minutes. After completion of



**Figure 3: OPURA - Ozone generator.**

disinfection of the files, each file placed in separate tubes with the help of a sterile tweezer.

The test tubes containing files were labeled with the date and were kept for incubation at 37°C for two days. After two days, the test tubes were removed from the incubator, and each test tube was inoculated, a test tube which contains Sabouraud Dextrose Broth (Sabouraud Liquid Medium) were kept for incubation at 37°C for 24-48 hrs. After 48 hrs. The test tubes were subcultured on Chrome agar media for about two days at 37°C. The presence of the green color colony on Chrome agar indicated the presence of *C. Albicans* and that the particular file was not sterilized completely.

### Results:

Antimicrobial effectiveness was evaluated by the reduction of microbial counts. Table 1 showed that the endodontic files disinfected with sodium hypochlorite followed by ozonated water (group C) and by sodium hypochlorite alone (group D) total sterility %100.

**Table 1: The Data Values of Log CFU(colony forming unit) Enumeration after the Application to *C. Albicans*.**

Samples	Group A(control)	Group B (Ozonated water)	Group C sodium hypochlorite followed by ozonated water)	Group D Sodium hypochlorite
1	130 x10 <sup>6</sup> CFU/ml	No growth	No growth	No growth
2	98x10 <sup>6</sup> CFU/ml	1x10 <sup>6</sup> CFU/ml	No growth	No growth
3	100x10 <sup>6</sup> CFU/ml	No growth	No growth	No growth
4	140 x10 <sup>6</sup> CFU/ml	No growth	No growth	No growth
5	100 x10 <sup>6</sup> CFU/ml	No growth	No growth	No growth

The files subjected to disinfection by ozonated water alone showed the presence of turbidity in one test tube. Incomplete disinfection up to 20% was observed (%80 sterilization).

The endodontic files sterilized with distilled water (control group) showed growth in all the test tubes %100 not sterilized.

### Discussion:

Ozone has many uses in medicine and dentistry particularly as irrigant in endodontic in the form of Ozone gas but ozonated water can also be used in endodontic as intracanal irrigant because it has strong antibacterial antifungal and antiviral activities [20,21], with the highest level of biocompatibility and hemostatic effect without affecting micromechanical properties of dentin<sup>(22,23)</sup>. Because it's liquid, it acts as a Lubricant for canal walls and instruments<sup>(24)</sup>.

However, aqueous ozone cannot achieve the same antibacterial effect as NaOCl, which is commonly preferred to use in root canal disinfection. Many researchers have already investigated the antimicrobial efficacy of various concentrations of NaOCl against resistant microorganisms<sup>(25,26)</sup>. In particular; 5.25% NaOCl solution has shown the strongest bactericidal efficacy in eliminating all microorganisms in root canals and deeper dentinal tubules. Therefore, 5.25% NaOCl is recommended as an effective solution in the treatment of infected root canals due to its well-known antimicrobial effects<sup>(27)</sup>.

Many methods have been advocated for disinfecting of endodontic instruments. Ozonated water and sodium hypochlorite are among the commonly recommended methods of disinfection. The present study indicated that complete sterilization was possible by disinfecting the instruments with sodium hypochlorite and sodium hypochlorite followed by ozonated water.

For evaluating the antimicrobial effect of aqueous ozone (3.3 mg/L, 300 s) against *C. Albicans* and *Enterococcus faecalis*. A significant reduction was observed in the number of *C. Albicans* and *E. faecalis* when samples were immediately taken from the root canals. Furthermore, aqueous ozone as an irrigant agent was effective against *C. Albicans* and *E. faecalis*, but it was not able to neutralize all the microorganisms<sup>[28]</sup>.

The antimicrobial effect of aqueous ozone (4 mg/L) applied with ultrasonic techniques at different times against *C. albicans* on acrylic resin plates showed there was a slight reduction in the number of fungi after 60 s, it took more than 30 min to achieve complete microbial elimination<sup>(29)</sup>.

This study showed that disinfection by ozonated water was 80% by immersing the files in ozonated water solution for 5 minutes and cannot be relied upon completely to sterilize endodontic instruments.

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## Conclusions:

1. The present study indicates that disinfecting and exposing to ozonated water alone cannot give complete sterilization. Sodium hypochlorite followed by ozonated water can be used as an alternative method rather than using ozonated water alone.
2. As an option, endodontic instruments should be considered as single-use as this would reduce the risk of transmission of infectious agents.
3. The ozonated water machine used in this study can be used for all units in the dental clinic not only as an endodontic instruments sanitizer but also as intracanal irrigant, for cavity cleaning, mouth irrigation because it considers cheaper than gaseous ozone generator and can be used easily with much less toxic effect.

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