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RESEARCH ARTICLE

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF CHLORHEXIDINE AND OZONE GAS AGAINST *C. ALBICANS*

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ABSTRACT

Background: Success of endodontic treatment depends on complete debridement and disinfection of root canal space. ***Objective:*** The evaluation of the efficiency of the Chlorhexidine and ozone gas in the elimination of *Candida Albicans* in the root canals. ***Hypothesis****:* Is Chlorexidine treatment more effective than ozone gas treatment? ***Materials and methods:*** In this study we inoculated 20 teeth with *C. albicans*. All teeth were washed with 10 cc of sterile saline solution. The root canals were incubated with Brain-Heart infusion broth at 37°C for 24 hour. 20 single-rooted human teeth were prepared using Mtwo instrument. In the first group (n=10) as an irrigating agent we used 10 cc of 2% CHX solution and in the second group (n=10) as an irrigating agent we used ozone gas. Statistical analysis was performed using IBM SPSS Statistics 23.0. Data were analyzed by one-sample t-tests. The significance level (α) was set at 0.05, with a confidence interval (CI) of 95%, whereas P-value and analysis of variance (ANOVA) are used to compare independent samples. ***Results:*** Chlorhexidine treatment reduced significantly the amount of *C.albicans* (P=0.002) compared with the group treated with ozone gas (P=0.005). By reducing the number of treatments in time period, the difference between the effects of two irrigants is increased, dominating the chlorhexidine treatment. ***Conclusion:*** The efficiency of the treatment with chlorhexidine is maximal and the colony forming units falls significantly and we recommend using this treatment in comparison to the ozone gas treatment.

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INTRODUCTION

Microorganisms play an important role in the etiology of the pulpar and periapical diseases (Sakamoto *et al*., 2008). Post-treatment apical periodontitis is typically caused by diverse pathogens, including Gram-positive, Gram-negative and C. albicans (Siqueira *et al.,* 2009). Elimination of microorganisms and their by-products from the root canal system is a main goal of endodontic treatment (He *et al*., 2009, Nair 2006). Success of endodontic treatment depends on complete debridement and disinfection of root canal space. This is not always achieved completely because microorganisms may be found in root canals, dentinal tubules, apical ramifications, cementum or areas of root resorption, thereby limiting the access of root canal systems by instruments and irrigants (Nair 2006). The irrigants have mechanical and biological action on the root canal (Sen *et al*., 1999). The mechanical action includes the detrite removing, canal lubrification, removing the organic and inorganic components and tooth whitening. The biological action is strongly related to its antimicrobial effect (Siquiera *et al.,* 2002).

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During the endodontic treatment of root canal are used many different types of irrigants such as Chlorhexidine and Ozone gas. It is suggested that CHX should be used for the irrigation of the root canal and intracanalicular medication during the time treatment periods of the root canal (Mohammadi *et al*., 2008).This study aims at providing data about the effectiveness of Chlorhexidine treatment compared with that of ozone gas. The hypothesis raised is that Chlorhexidine treatment is more effective that the treatment with ozone gas. In order to prove our hypothesis we did an experiment and the data collected proved our hypothesis. Different researchers recommend chlorhexidine at a 2% concentration to be used for the removal of organic detritions (Noites *et al*., 2014). Chlorhexidine is mainly used as a preventive measure and in the treatment of periodontal diseases and dental caries (Mahendra *et al*., 2014). Chlorhexidine is a broad spectrum antimicrobial agent that has substantive antimicrobial activity and relatively low toxic effects (Estrela *et al.,* 2007). CHX has been suggested as a root canal irrigant owing to its unique ability to bind to dentin, its effectiveness as an antimicrobial agent and its substantivity in the root canal system (Sena *et al.,* 2006). It is an effective antifungal agent, especially against C. albicans and has the unique property of antimicrobial substantivity owing to its

cationic structure (Delany *et al.,* 1982). Ozone is a triatomic molecule and its application in medicine and dentistry has been indicated for the treatment of 260 different pathologies and is the most powerful oxidant (Azarpazhooh *et al*., 2008, Bocci 2006, Nogales *et al*., 2008). It is an allotropic form of oxygen that possesses unique properties, which are being defined and applied to biological systems as well as to clinical practice (Cardoso *et al*., 2008, Nagayoshi *et al.,* 2004). Ozone is being presented as a possible alternative antiseptic agent due to its antimicrobial power and low cytotoxicity (Stoll *et al.,* 2008). However, there is little information regarding the time and concentration that it should be used (Saini 2011, Thanomsub *et al.,* 2002).

MATERIALS AND METHODS

20 teeth were inoculated with C. albicans ATCC90028, which were cultured for 24 hours at 37°C in Brain-Heart agar (Liofilchem, Italy). We used Chlorhexidine and ozone as irrigating agents. In the first group (n=10) as an irrigating agent we used 10 cc of 2% CHX solution (Evonik Technochemie GMBH, Germany) for 1, 3, 4 and 6 minutes and in the second group (n=10) as an irrigating agent we used ozone. Gaseous ozone (2100 ppm/ min) was applied with an ozone generator (Prozone, W &H) for 6, 12, 24, 30, 60, 120 and 150 seconds. The antimicrobial efficiency was evaluated based on the reduction of the bacterial flora. After exploring the canal to the apex with a manual file Nr.10 in stainless we continued with the following sequence of Mtwo instrument (*Sweden* & Martina, Due Carrare (PD), Italia): 10/.04, 15/.05,20/.06,25/.06. Mtwo endodontic instruments are a new generation of NiTi rotary instruments (Malagnino *et al.,* 2008). A consistent rotation speed of 300 rpm was used in a crown-down manner involving a gentle in-and-out motion according to manufacturer's instructions. Teeth were irrigated with 2 mL of saline solution at each change of file. Smear layer was removed with 10% acid citric solution (10 mL) and a final irrigation of saline solution is done. Statistical analysis was performed using IBM SPSS Statistics 23.0. Data were analyzed by one-sample t-tests. The significance level (α) was set at 0.05, with a confidence interval (CI) of 95%, whereas P-value and analysis of variance (ANOVA) are used to compare independent samples.

RESULTS

Chlorhexidine treatment reduced significantly the amount of *C.albicans* (P=0.002) compared to the group treated with ozone gas (P=0.005). Referring the values of $P_{(CHX)}$ and $P(o_3)$ for both groups we found out that the number of microbe is reduced more after the treatment with Chlorhexidine rather than with the treatment with ozone gas (Table 1). The antimicrobial-time variability in the ozone gas treated group gives a strong negative correlation (R = -0.965). The colony forming units in chlorhexidine treatment falls much faster up to an insignificant value (R = -0.985). There was a statistically significant decrease in total cell counts after an exposure to ozone gas and the confidence interval was CI(%):-.994 (-.812), while cells treatment with chlorhexidine was more significant based on this confidence interval (%):-.997-(-.915) (Table 2). Based on our statistical results we state that ozone gas treatment reduces *C. albicans* in short time intervals, but the number of *C. albicans* reduced was not at the same level compared to chlorhexidine treatment. By reducing the number of treatments in time period, the difference between the effects of two irrigants is increased dominating the chlorhexidine treatment (Graphic 1, 2, 3)

DISCUSSION

Fungus constitute a small part of the oral microbiota. Candida albicans is the fungal species most commonly detected in the oral cavity (Kaur *et al.,* 2014). According to Kaur *et al.,* who studied the incidence of *C.albicans* in the oral cavity, reported that this fungus was present from 30% to 45% in healthy adults and 95% in patients infected with Human immunodeficiency virus. Their findings claimed that *C.albicans* is commonly found in the root canals of obturated teeth in which treatment has failed. *C. albicans* has been associated with cases of persistent root canal infections, because this yeast can be resistant to some intracanal medications (Kaur *et al.,* 2014). There is a direct relation between disinfection of root canal system and success of endodontic treatments. The purpose of endodontic therapy is disinfection of the root canal space. Ability of *C. albicans* to invade into dentinal tubules, limits penetration of medicaments into dentin complicating the cleaning of the root canal system. An ideal irrigating solution should have maximal antimicrobial toxic effect (Tronstad *et al.,* 2003).

Candida Albicans seem to be more common in the root canals of obturated teeth in which the treatment has failed (Foulkes *et al.,* 1973). The present study has shown that canals that received a final rinse with a 2% Chlorhexidine solution were significantly more often free of cultivable microorganisms. Barbosa, Lenet and Vianna clearly stated that Chlorhexidine gluconate at concentrations 2%, presented tissue biocompatibility and an effective antifungal action (Barbosa *et al.,* 1997; Lenet *et al.,* 2000; Vianna *et al*., 2004). According to them this feature makes it an agent indicated worldwide for use in endodontic therapy. The present study relates to previous studies conducted by Sena, Ferraz and Ferguson, who stated that Chlorhexidine is effective against fungus particularly Candida albicans (Sena *et al.,* 2006; Ferraz *et al*., 2007; Ferguson *et al.,* 2002). Based on our findings ozone gas would take a longer time period to eliminate Candida albicans, but we are not presenting further evidence for the ozone treated group. Similar results to ours came from Huth who evaluated the effect of the ozone gas against *C. albicans,* where most of them were eliminated after the application of gaseous ozone for 60 (Huth *et al.,* 2006). Polydorou found that ozone gas applications were not effective in short time periods in eliminating *C.albicans* (Polydorou *et al.,* 2012).

Kustarci *et al*. evaluated the antimicrobial activity of gaseous ozone in root canals for 120 s(Kustarci *et al.,*2009). Whereas Muller explored the resistance of ozone against microorganisms. They applied gaseous ozone for 60 s. However, complete elimination of *C. albicans* could not be achieved in both these studies (Muller *et al.,* 2007). After reviewing the results of the application time, Hubbezoglu[35] investigated the antifungal effect of gaseous ozone obtained from Heal Ozone on root canals infected with *C. albicans* for 300 s. According to the results of the his study, gaseous ozone eliminated most of the *C. albicans* (Hubbezoglu *et al.,* 2013). Arita examinated the antimicrobial effect of aqueous ozone (4 mg/L) applied with ultrasonic techniques at different times against *C. albicans.*

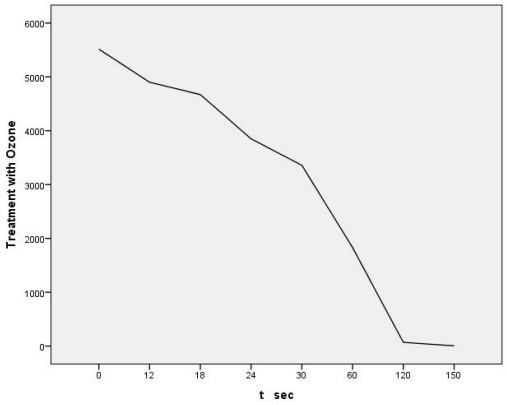
Table 1. Treatment with two irrigants is statistically significant because of the value P <0.05. [1]

Irrigants	t	SD	Mean	P
CHX	4.930	2068.885	3605.750	0.002
O3	3.970	2155.194	3024.875	0.005

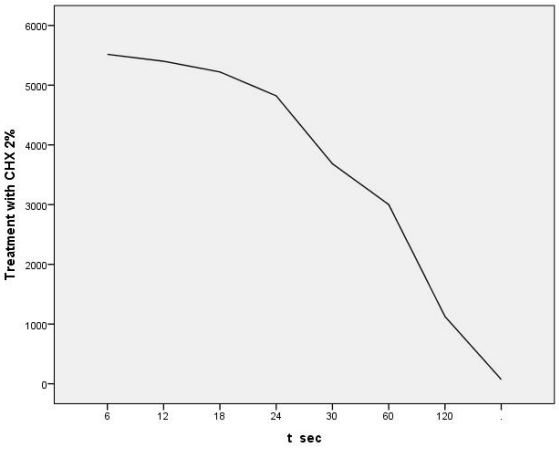
[1]*t= t-test
P= P-value
SD=Standard Deviation

Table 2. It shows a high degree of correlation between two irrigants CHX and O3

	Pearson	Count	Lower C.I.	Upper C.I.
CHX	-.985	8	-.997	-.915
O3	-.965	8	-.994	-.812

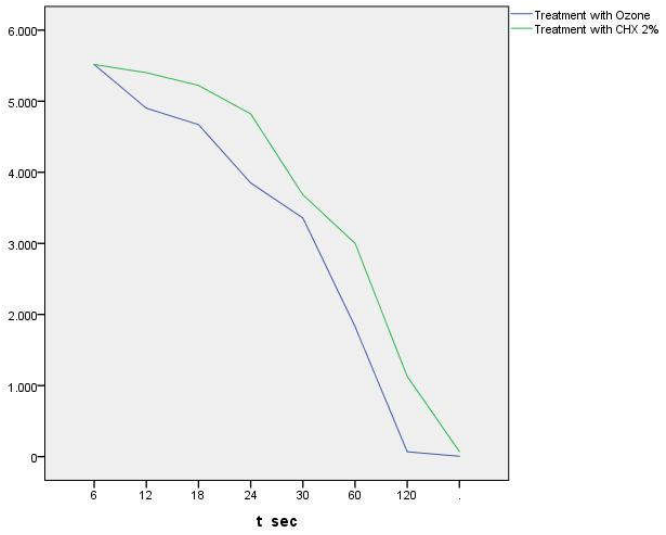


Graphic 1. The dependence of *C. albicans* and time in the treatment with Ozone gas



Graphic 2. The dependence of *C. albicans* and time in the treatment with Chlorhexidine

Although there was a slight reduction in the number of fungi after 60 s, it took more than 30 min to achieve complete microbial elimination (Arita *et al.,* 2005). In the present study the verification intervals have been from 6 sec to 150 sec. Based on our results we can say that the application of gaseous ozone during short periods (6, 12, 18,24,30 and 60 seconds) was not sufficient to eliminate *C. albicans*. On the other hand higher periods (120 s and 150 s) demonstrated a significant decline of *C. albicans* (p=0.005). Chlorhexidine treatment showed to have statistically superior antimicrobial efficacy against *C. albicans* (p=0.002). The colony forming units in chlorhexidine treatment falls much faster up to an insignificant value. This clearly states the most important finding of the present study that Chlorhexidine has a broad spectrum of substantivity properties as an antimicrobial agent and it also showed an excellent correlation **(**R = -0.985). The duration of action can be an important consideration in ozone gas antifungal effect.



Graphic 3. The difference of fungal load in both treatments

There have been no protocol reasons to respect these control intervals, but we are inclined to provide possible statistical data for the study. So the time intervals have been our choice of selection. Graphs show an increasing rate of *C. albicans* destruction over time. As is it often the case in epidemiology, the effect of the drug action is felt over time and in our study we found similar data. The effect shown in both graphs is higher in relation to time. According to the findings of the current study, gaseous ozone can be used for disinfection of infected root canals, but it is not adequate when used alone for root canal sterilization. Our study has less deviations and the statistical errors were smaller in Chlorhexidine treatment compared with ozone gas treatment, where deviations were major and the errors, as well. In conclusion the findings of this study were that the efficiency of the treatment with Chlorhexidine is maximal and the colony forming units falls significantly. Based on this we claim that using Chlorhexidine treatment is more effective in comparison to the ozone gas treatment.

Conclusion

In conclusion, we claim that although the treatment with ozone gas was effective, the number of *C. albicans* reduced were not at the same level with the number of *C. albicans* reduced with chlorhexidine treatment. So, chlorhexidine treatment was more effective than ozone gas treatment, but none of these irrigants was able to completely remove *Candida albicans.*

Clinical significance: This study proves that Chlorhexidine treatment is more effective than Ozone gas treatment, in reducing significantly the amount of C.albicans in the root canals.

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