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Antibacterial Effect of Gaseous Ozone In Infected Root Canal. *In-Vivo* Study.

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ABSTRACT

The irrigation is a central role in endodontic treatment. The aim of this clinical study was: to determine the anti-bacterial effect of gaseous ozone, combined with 0.9% NaCl, 2.5% NaOCl and 2% CHX, in an infected root canal. The research was performed in the University Dentistry Clinical Centre of Kosovo, respectively in the Department of Endodontic and Dental Pathology, Prishtina, Kosovo. In this research were included 40 patients of both genders, in age between 15-65 years. The sample selection was random. Upon taking theanamnesis and diagnosing for each patient, radiography was taken of the suspected retroalveolar tooth. To disinfect the root canal, the following irrigants were used: 2.5% NaOCl (Sodium Hypochlorite Solution, Sigma Aldrich-Germany), 2% CHX (Chlorhexidine Digluconate Solution, Sigma Aldrich-Spain) and gaseous ozone (Prozone WH, Austria). The study only included patients diagnosed with Parodontitis apicalischronica and Necrosis pulpae. The Kruskall-Vallis test, X-test, DF=3, $r<0.01$ showed that exists the significance difference in the isolated average number of the aerobe and anaerobe bacteria colonies, when gaseous ozone was used. In treating the infected root canal with gaseous ozone, combined with irrigants 0.9%, 2.5% NaOCl and 2% CHX, reduce the number of colonies of aerobic and anaerobic bacteria.

Keywords: Gaseous Ozone, NaCl, NaOCl, CHX.

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INTRODUCTION

The successes of endodontic treatments are influenced by elimination of microorganisms from root canals (1).Residual pulp tissue, bacteria, and dentine debris may persist in the irregularities of root canal system after meticulous mechanical preparation (2).Also after, mechanical instrumentation, ex vivo in vivo evidence has revealed significant portions of the root canal walls untouched (3).During and after instrumentation, the irrigants facilitate the removal of micro-organisms, residual tissue and dentine debris from the root canal, using a driving mechanism (4).

Several irrigants solutions have antimicrobial activity, and actively kill the bacteria and smear layer when in direct contact with micro-organisms. There are also other irrigating solutions with a cytotoxic potential, when meeting periapical tissue, thereby causing severe pain (5).

Sodium hypochlorite is the most commonly used irrigation solution. It is an excellent antibacterial agent able to dissolve necrotic and vital pulp tissue the organic components of dentin as well as biofilm. The flaws of NaOCl may be unpleasant flabour,cyto-toxicity (6), a potential of corrosion (7), but also possible allergic effects (8).

Chlorhexidinedy-glyconate is also widely used in dentistry, for its anti-microbial effect. One of the reasons for the CHX is the uniqueness of its use, namely the sustained anti-bacterial effect. (9). Nevertheless, similar to other agents, the impact of CHX is depend on the pH, and largely reduces in the presence of organic matter (10).

CHX 2% may cause desquamation of the oral cavity mycosis, discolouration of teeth, and it may have a toxic effect on epithelial cells (11, 12, 13).

For such reason, in endodontic treatment, one must use anti-septic means with anti-bacterial properties, but with the least side effects possible (12).

Irrigants may be used also in combination with other means of disinfecting the infected canal of the tooth. Ozone has brought about a revolution in endodontic practice, in terms of disinfecting the root canal infected. The anti-bacterial effect of the ozone is a result of its action on cells, thereby damaging the cytoplasm membrane, as a consequence of an osmolysis of a dual bond, and the ozone effect on intracellular content, as a result of oxidisation.

Ozone is very efficient in anti-biotic resistant strains, and its effect increases in acidic pH. Ozone influences the cell immunity and humeral systems of the human organism. Ozone stimulates the proliferation of immune-competent cells and the immunoglobulin synthesis. It also activates the microphage function against phagocytosis (13).

A higher concentration of ozone kills bacteria much faster, and it is 1000 times more powerful than any other agents against bacteria. One ozone molecule is equal to 3000-10000 chlorine molecules, thereby acting against micro-organisms around 3500 times faster (14).The aim of this clinical study was: to determine the anti-bacterial effect of gaseous ozone, combined with 0.9% NaCl, 2.5% NaOCl and 2% CHX, in an infected root canal.

METHODS

The research was performed in the University Dentistry Clinical Centre of Kosovo, respectively in the Department of Endodontic and Dental Pathology, Prishtina, Kosovo.

In this research were included 40 patients of both genders, in an age between 15-65 years. The sample selection was random. Upon taking theanamnesis and diagnosing for each patient, radiography was taken of the suspected retroalveolar tooth. To disinfect the root canal, the following irrigants were used: 2.5% NaOCl (Sodium Hypochlorite Solution, Sigma Aldrich-Germany), 2%CHX (ChlorhexidineDigluconate Solution, Sigma Aldrich-Spain) and gaseous ozone (Prozone WH, Austria).

Criteria for including patients in the study:

The study only included patients diagnosed with Parodontitisapicalis chronica and Necrosis pulpae. Patients included within the study must not be suffering from any other diseases such as: allergic diseases, systemic diseases, respiratory systems, cardiovascular system, endocrines disorders of the thyroid gland. Further, the patients must not be under the effect of any other therapy, including antibiotics in the last 6 months, or be under treatment of chemotherapy.

The clinical study included patients in which the root canal was disinfected by applying gaseous ozone, combined with the following irrigants: 0.9% NaCl, 2.5% NaOCl and 2% CHX.

The group was divided into 3 experimental groups and one control group.

Experimental group:

Gr.1 (n=10) - disinfecting the root canal with gaseous ozone, combined with 0.9% NaCl.
Gr.2 (n=10) - disinfecting the root canal with gaseous ozone, combined with 2.5% NaOCl.
Gr.3 (n=10) - disinfecting the root canal with gaseous ozone, combined with 2% CHX.

Control group

Gr.1 (n=10) the root canal was irrigated only with 0.9% NaCl.

Experimental group

Since in the experimental group, three types of irrigants were used (0.9% NaCl, 2.5% NaOCl and 2% CHX). The technique was the same for all three groups, only the irrigation protocol for the three groups was changed.
For this reason, this protocol of root canal irrigation shall be described specifically for each experimental group.

Gaseous ozone working technique and irrigation protocol using 0.9% NaCl

This research group included 10 patients. Upon diagnosing the sterile cofferdam was placed. After the trepanning of the pulpar cavity and before the instrumentation of the root canal, a primary sample of aerobic and anaerobic bacteria on the root canal is undertaken, with the aid of a sterile paper point. (First measurement).

With the aim of cultivating the aerobic bacteria the sample was taken from the root canal by sterile paper point. The sterile paper point placed in the root canal in at duration of 1 minute. This sample was rooted in agar and further dipped in a tube containing 9 ml Thioglycolate *(Thioglycolatemedium, Liofilchem Italy)*. For cultivating anaerobic bacteria, another sample was taken of the root canal, using a sterile paper point, placed in the root canal at a duration time of 1 min, further planted in Schadler agar (*bioMerieux Sa, France*) and after dipped in a BHI-containing tube 9ml (*Brain Heart Infusion Broth Biolife, Italy)*.

Upon the first sample, length of the root canal was determined to be 1mm shorter than the real length of canal.

Further, instrumentation of the root canal was made by a conventional technique, with instruments K-files ≠15-60, depending on the volume of the root canal. After each instrumentation, the canal was irrigated with 5 ml of 0.9% NaCl, and the final irrigation again with 5ml of 0.9% NaCl was made. After irrigating, the canal was drained with a sterile paper point, while the desinfectation of the root canal used gaseous ozone (Prozone, WH Austria), at a duration time of 6", 12", 18" and 24". (second, third, fourth and fifth measurement). Upon every gaseous ozone exposure, two samples from the canal are taken, one for the aerobic bacteria and the other for anaerobic bacteria, in the same conditions as in making the first sample of the root canal. (Fifth measurement)

After instrumentation root canal, the 0.9%, NaCl solution, is placed and the canal temporarily filled with phosphate cement.

Schaedler plates, together with BHI-containing tubes, are placed in plastic bags, together with an indicator for anaerobic bacteria identification *(Anaerob Indicator, bio-Mérieux SA, France)* and a Gen Bag generator. *(Gen Bag bio-MérieuxSa, France).*

This bag was hermetically closed with clips, and immediately sent to the microbiological laboratory of the National Public Health Institute of Kosovo, for culturing of bacteria colonies. Further, samples were placed in a thermo state at a temperature of 37°, thereby incubating for 24-48 hours. The gram-positive and gram-negative anaerobic bacteria were determined by special cards *(Bio-MérieuxSa, France)*, while their reading was made possible by the digital device *Vitek2 (Bio-Mérieux Sa, France)*.

3 days later, the patient was called for an examination, thereby removing the temporary backfill, removing also the 0.9% NaCl dipped fuse. Further, the root canal was drained with the sterile paper point, thereby taking the third sample from the root canal, under the same conditions of other samples. (Sixth measurement)

Gaseous ozone working technique and the irrigation protocol using 2.5% NaOCl

The group of this study involved 10 patients. The same technique and procedure for instrumentation of the root canal and the sampling and submission to the micro-biological laboratory, were used. The differed of this group was the protocol of irrigating the root canal. Upon diagnosing the sterile cofferdam was placed. After the trepanning of the pulpar cavity and before the instrumentation of the root canal, a primary sample of aerobic and anaerobic bacteria on the root canal is undertaken, with the aid of a sterile paper point. (First measurement).

In this group, the root canal was irrigated with 5ml of 2.5% NaOCl. Inorganic tissue was removed using 5ml of 17% EDTA *(Ethyilendiamine tetra acetic, acid disodium salt dehydrate, Czech Republic*), and duration of time was 1min. The final irrigation again used 5 ml of 2.5% NaOCl. To neutralize the root canal against the NaOClsolution, was used irrigant 5ml of 0.9% NaCl. Upon irrigating the root canal, the canal was drained with the use of a sterile paper point, while the canal was disinfected with gaseous ozone, at periods of 6", 12", 18" and 24". (second, third, fourth and fifth measurement). Upon every gaseous ozone exposure, two samples from the canal were taken, one for the aerobic bacteria and the other for anaerobic bacteria, in the same conditions as in making the first sample of the root canal. And 2 similar samples were taken 3 days after the instrumentation of the root canal. (Sixth measurement)

Gaseous ozone working technique and the irrigation protocol using 2% CHX

The group of this study involved 10 patients. The same technique and procedure for instrumentation of the root canal and the sampling and submission to the micro-biological laboratory were used. This group only differed with the difference in the protocol of irrigating the root canal. Upon diagnosing the sterile cofferdam was placed. After the trepanning of the pulpar cavity and before the instrumentation of the root canal, a primary sample of aerobic and anaerobic bacteria on the root canal is undertaken, with the aid of a sterile paper point. (First measurement).

In this group, the root canal irrigator 5 ml of 2% CHX was used. In this group as well, the inorganic component was removed by using 5 ml of EDTA 17%.

Upon irrigating the root canal, the canal was drained with the use of a sterile paper point, while the canal was disinfected with gaseous ozone, at periods of 6", 12", 18" and 24". (second, third, fourth and fifth measurement). Upon every gaseous ozone exposure, two samples from the canal were taken, one for the aerobic bacteria, and the other for anaerobic bacteria, in the same conditions as in making the first sample of the root canal, and 2 similar samples were taken 3 days after the instrumentation of the root canal. (Sixth measurement)

Control group

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The group of this study involved 10 patients. The same technique and procedure for instrumentation of the root canal and the sampling and submission to the micro-biological laboratory were used. This group only differed with the difference in the protocol of irrigating the root canal.

In this group, only the root canal irrigator of 10ml of 0.9% NaCl was used. Two samples were taken prior to instrumentation: one for aerobic and one for anaerobic bacteria. (First measurement) Immediately after the instrumentation were taken other two samples for aerobic and anaerobic bacteria (Second measurement), and three days after the instrumentation the similar two samples, as previous. (Third measurement)

RESULTS

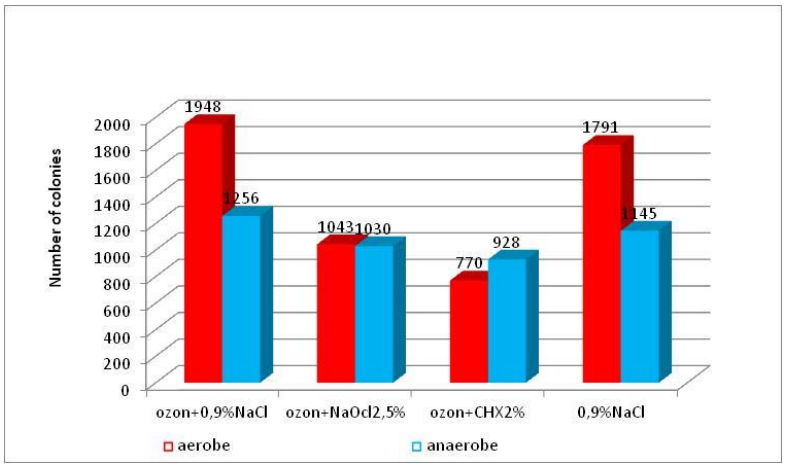
In this study were included 40 patients of both genders and different ages from 15-65 years.

The root canal was disinfected by applying gaseous ozone combined with the following irrigants: 0.9% NaCl, 2.5% NaOCl and 2% CHX. For every patient were taken before the instrumentation of the root canal (for aerobe and anaerobe bacteria). This was the first sampling.

Eight samples were taken after the instrumentation of the root canal. Two of the eight samples were taken after the application of the gaseous ozone (for aerobe and anaerobe bacteria), with the duration time of the 6". This was the second sampling. The third sampling was 12", the fourth sampling was 18" and the fifth sampling was 24". The last two samples (for aerobe and anaerobe bacteria), were taken only after instrumentation of the root canal. This was the sixth sample.

In total for 40 patients were taken 480 samples from the infected root canal.

Based on Kruskall-Vallis test, r>0.05, X-test=7.748. DF=3, showed that there was not any statistical significance in the average number of the isolated colonies of the aerobe bacteria between the clinical tested groups. Also, Kruskall-Vallis test, r>0.05, X-test=0.426, DF=3, showed that there was not a statistical difference in the average number of isolated colonies in the anaerobe bacteria. (First measurement, Graph 1.)



Graph 1: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at first measurement.

After the application of the gaseous ozone (for 6 sec) mixed with the other different irrigants, the statistical results as follows were founded:Kruskall-Vallis test, p>0.05, X-test=19.304, DF=3, p<0.01 showed that there was a high statistical significance for the number of bacteria, isolated in the root canal. Whereas, the detailed analyze of Mann-Whitney test with inversion showed that there was not a statistical significance in between the group 1-2 and group 3-4 compared with the group 2-3 and 2-4 where the statistical significance were found.

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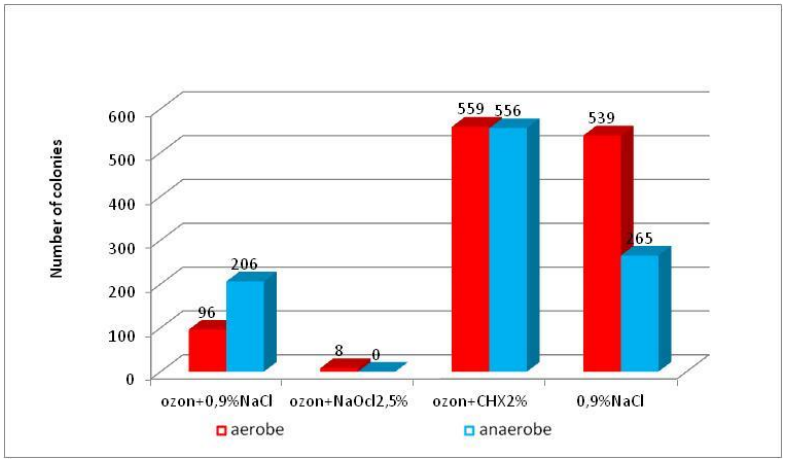
Gaseous ozone combined with 2.5% NaOCl was mostly efficient in the reduction of aerobe bacteria compared with other groups.

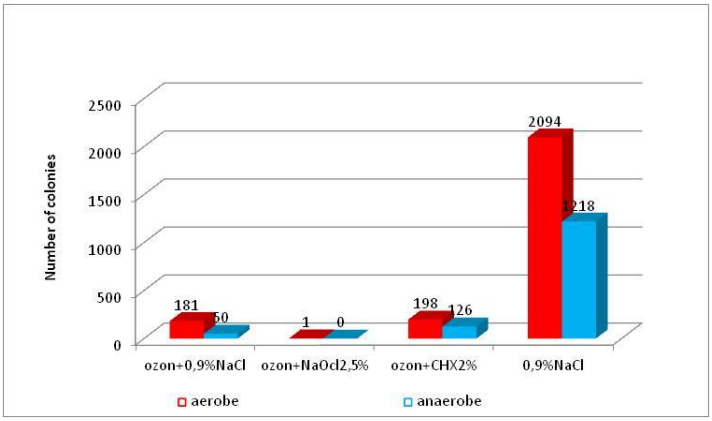
As concerned the anaerobe bacteria the Kruskall-Vallis test, X-test=10.495, DF=3, p<0.01 showed that exists the significance difference in the isolated average number of the anaerobe bacteria colonies. Whereas the detailed analysis with the Mann-Whitney test showed that does not exists any significant difference in between the groups 1-2, 1-3, 1-4, 2-3 and 3-4, compared with the group 2-4 where the significant statistical difference were found. The second measurement showed that when is gaseous ozone is combined with 2.5% NaOCl, affect in decreasing the number of isolated colonies of the anaerobe bacteria, compared with other testing groups. (Second measurement, Graph 2.)



Graph 2: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at second measurement.

Kruskall-Vallis test, X-test=17.29, DF=3, p<0.01 showed also that in the third measurement (gaseous ozone application at duration time 12"), exists the high statistical difference in the average number of aerobe bacteria, especially in the group 2. The statistical significance between the group 1-4, 2-3, 2-4 and 3-4 was shown and during the detailed analysis of Mann-Whitney test, compared with the group 1-2 and 1-3, which did not have any statistical significance in between.

As concern, the colonies of anaerobe bactrias, the statistical results with the tests: Kruskall-Vallis test, X-test=110.724, DF=3, p<0.01 showed that exists significant difference between the test group especially the group 2. The Mann-Whitney test showed that exists signicant difference only between the group 2-4, compared with the groups 1-2, 1-3, 1-4, 2-3 and 3-4, where the statistical significance was not found. This measurement, also showed that the application of the gaseous ozone combined with 2.5% NaOCl was more efficient in the reduction of the number of the colonies of anaerobe bacteria. (Third measurement, Graph 3.)



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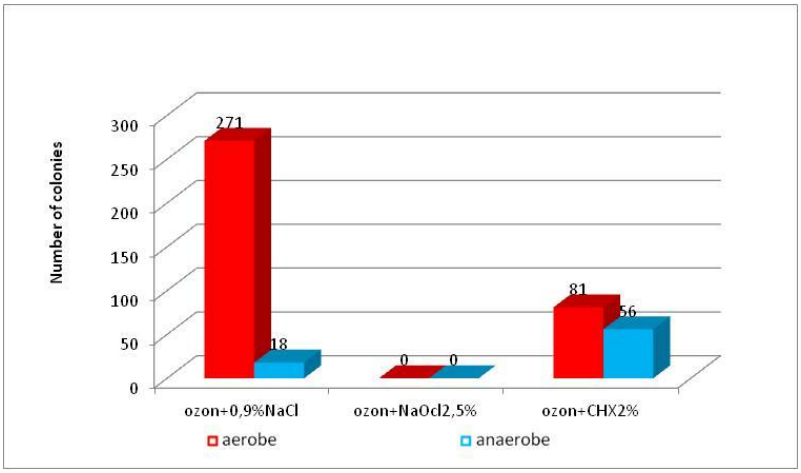
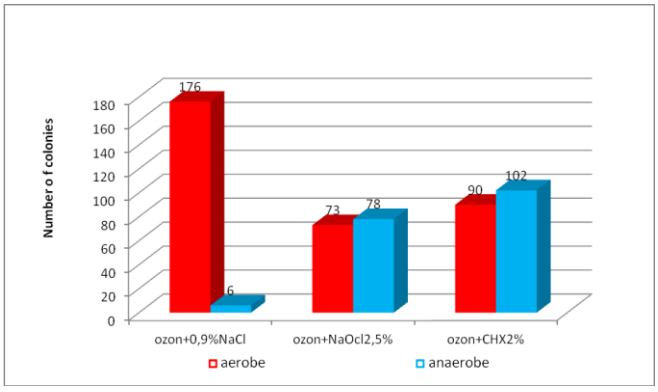
Graph 3: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at three measurement.

After the application of the gaseous ozone combined with the different irrigants, the statistical results were found:Kruskall-Vallis test, X-test=5.352, DF=2, p>0.05 showed that does not exist any statistical difference in the average number of the colonies of aerobe bacterias. Also Kruskall-Vallis test, X-test=8.116, DF=2, p>0.05 showed that does not exist any statistical difference in the average number of the colonies of anaerobe bacterias between the tested groups. (Fourth measurement, Graph 4.)



Graph 4: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at fourth measurement.

After the application of the gaseous ozone for 24" combined with different irrigants these statistical results were found:Kruskall-Vallis test, X-test=0.886, DF=2, p>0.05 showed that does not exist any statistical difference in the average number of the colonies of aerobe and anaerobe bacterias. (Fifth measurment, Graph 5.)



Graph 5: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at fifth measurement.

After the instrumentation of the root canal the statistical test: Kruskall-Vallis test, X-test=7.23, DF=2, p<0.05 showed that exists the statistical significance, between the tested groups, especially in the second group. Mann-Whitney test showed that exists the statistical significant difference between the group 1-2 compared with the group 2-3 in the average number of the colonies of aerobe bacteria. Also, during this measurement gaseous ozone combined with 2.5% NaOCl, affected in the reduction of the number of aerobe bacterias.

Whereas, as conceren the anaerobe bacterias the Kruskall-Vallis test, X-test=1.496, DF=2, p>0.05 did not give any statistical difference between the tested groups.(Sixth measurement, Graph 6.)

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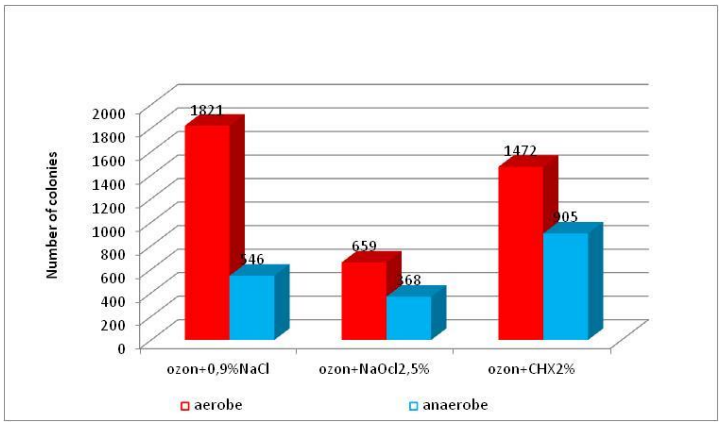

Graph 6: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at sixth measurement.

DISCUSSION

Ozon is currently being discussed as a possible alternative antiseptic agent in dentistry because of its reported high antimicrobial power without the development of drug resistance [15].Gaseous ozone in concentration of ~4gm^{-3}. (Heal Ozone: Kavo, Biberach, Geremany) is already being used clinically for endodontic treatment. However, results of studies into its efficacy against endodontic pathogens has been inconsistent and there is a less literature information regarding the most appropriate application time, concentration[16] and species of the bacteria. In our study, the antibacterial effect of the gaseous ozone was estimated at periods of 6", 12", 18" and 24", combined with 0.9% NaCl, 2.5% NaOCl and 2% CHX. The results of this study showed that the gaseous ozone desinfectation of the root canal, combined with 2.5% NaOCl, demonstrates a significant difference in reducing the aerobic and anaerobic bacteria colonies, compared to the use of gaseous ozone combined with 0.9% NaCl and 2% CHX. In a study of Alwadiet al (17)., in vivo conditions, antibacterial effect is reported in the use of 0.5%, NaOCl, with or without using gaseous ozone in the root canal. In such a study, they included 100 patients and the root canal samples were taken before and after instrumentation of the root canal. According to the scholars, NaOCl and gaseous ozone influence the reduction of the bacteria colonies number in the infected root canal and that the ozone combined with NaOCl marks a significant difference, when compared with the sole use of NaOCl. In this study, the gaseous ozone, at a concentration of 5 gm^{-3} entirely eliminated the number of aerobic and anaerobic bacteria colonies in the infected root canal, which also matches our own study results. On the other hand, according to a study by Müller et al. (18), it was concluded that 5% NaOCl may reduce all bacteria from the infected root canal, for a difference form of the application of gaseous ozone, photo-dynamic therapy and 2% CHX. The anti-bacterial effect of gaseous ozone was further confirmed by Virtej et al. (19).

The solution of 2.5% NaOCl, combined with gaseous ozone, at a duration time of 40", significantly reduced the number of aerobic and anaerobic colonies from the infected root canal, Also an in vivo study of Jankovic et al.(20), which again matches our own study results, are similar.

In terms of duration of gaseous ozone application, our results have shown that the application of gaseous ozone at durations of 6" and 12" marks a significant reduction of aerobic and anaerobic bacteria colonies, when compared with the application of gaseous ozone at durations of 18" and 24" and compared with the number of bacteria colonies sampled before the instrumentation of the root canal.

It is worth mentioning, that with the extension of the application period of gaseous ozone, combined with 2.5% NaOCl, we came to entirely extinct the number of colonies of aerobic and anaerobic bacteria in the infected root canal, when compared with gaseous ozone combined with 0.9% NaCl and 2% CHX.

The number of bacteria colonies increased again after three days of disinfecting the root canal by using gaseous ozone. The increasing of colonie, came as a result of failure to apply solutions for curing the infected root canal, which would help in further canal desinfectation.

CONCLUSION

Based on the results of our research, it may be concluded that:

In treating the infected root canal with gaseous ozone, combined with irrigants 0.9%, 2.5% NaOCl and 2% CHX, reduce the number of colonies of aerobic and anaerobic bacteria.

Statistical data show that the application of gaseous ozone, combined with 2.5%, NaOCl, has a better anti-bacterial effect against the number of aerobe and anaerobe bacteria colonies in the infected root canal, when compared with 0.9% NaCl and 2% CHX.

Abbreviations

NaOCl-Sodium Hypochlorite Solution
CHX -ChlorhexidineDigluconate
NaCl- Sodium chloride
EDTA -Ethyilendiamine tetra acetic-acid disodium salt dehydrate

Competing Interest

The authors declare that they have no competing interests.

Financial interests

Authors declare that they have non-financial competing interests.

Authors contributions

NexhmijeAjeti, as an author have made substantial contributions to the study and have given final approval of the version to be published.
TeutaPustina-Krasniqi, as a co-author have been involved in drafting the manuscript.
Sonja Apostolska, as a co-author made analysis and interpretations of datas.
VioletaVula, as a co-author made collection of the patients and datas.
TringaKelmendi, as a co-author made collection of the patients and datas.
LindihanaEmini, as a co-author made language corrections.

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