Open Access Maced J Med Sci **electronic publication ahead of print,**
published on February 14, 2018 as https://doi.org/10.3889/oamjms.2018.102

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
https://doi.org/10.3889/oamjms.2018.102
eISSN: 1857-9655
Dental Science

The Effect of Gaseous Ozone in Infected Root Canal

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Citation: Ajeti NN, Pustina-Krasniqi T, Apostolska S. The Effect of Gaseous Ozone in Infected Root Canal. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2018.102

Keywords: Antibacterial Effect; CHX; Gaseous Ozone; NaCl; NaOCl; Root Canal

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Received: 09-Oct-2017; **Revised:** 30-Oct-2017; **Accepted:** 31-Jan-2018; **Online first:** 14-Feb-2018

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

OBJECTIVES: During the treatment of chronic apical periodontitis and pulp necrosis the main role is to irrigate the root canal.

AIM: The aim of this in vivo study was to irrigate with 0.9% NaCl (Natrium Chloride), 2.5 % NaOCl (Sodium Hypochlorite Solution, Sigma Aldrich - Germany) and 2% CHX (Chlorhexidine Digluconate Solution, Sigma Aldrich - Spain) combined with Gaseous Ozone (Prozone WH, Austria).

MATERIAL AND METHODS: This study was realised in the University Dentistry Clinical Centre of Kosovo (UDCCK), respectively in the Department of Endodontic and Dental Pathology, Dental Branch, Faculty of Medicine, Prishtina, Kosovo. The 40 subjects involved in this study belonged to both genders, in age between 15 - 65 years. The sample selection was randomised. The retroalveolar radiography for each patient was taken in the suspected tooth. As a therapeutic plan the authors decided to disinfect the root canal with the irrigants, as follows: 2.5 % NaOCl, 2 % CHX and gaseous ozone.

RESULTS: The statistical analyses were based on Kruskal - Vallis test, X - test, DF = 3, $r < 0.01$. In the isolated average number of the aerobe and anaerobe bacteria colonies, when gaseous ozone was used, there was the significant statistical difference.

CONCLUSIONS: When gaseous ozone was combined with irrigants 0.9%, 2.5 % NaOCl and 2% CHX, it was concluded that the number of colonies of aerobic and anaerobic bacteria was reduced.

Introduction

The successes of endodontic treatments are influenced by the 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 microorganisms, 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 microorganisms. 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 a biofilm. The adverse effects of NaOCl may include unpleasant flavour, cytotoxicity [6], a potential of corrosion [7], but also possible allergic effects [8]. CHX by-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 antibacterial effect [9]. Nevertheless, similar to other agents, the impact of CHX is depended on the pH, and largely reduce 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 antiseptic means with antibacterial properties, but with the least side effects possible [12]. Irrigants may also be used in combination with other means of disinfection. Ozone has brought about a revolution in endodontic practice, regarding disinfection. The antibacterial effect of the ozone is a result of its action on cells, thereby damaging the cytoplasm membrane, as a consequence of osmosis of a dual bond, and the ozone effect on intracellular content, as a result of oxidisation [13].

Ozone is very efficient in antibiotic-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 macrophage function against phagocytosis [14]. 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 microorganisms around 3500 times faster [15]. The aim of this clinical research study was: to determine the antibacterial effect of, gaseous ozone combined with 0.9 % NaCl, 2.5 % NaOCl and 2 CHX, in an infected root canal.

The aim of this study was to the determinate antibacterial effect of Gaseous Ozone, combined with 0.9% NaCl, 2 % CHX and 2.5% NaOCl.

Material and 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 40 patients of both genders, in age between 15 - 65 years, were included. The sample selection was random. Upon taking the anamnesis 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).

Criteria for including patients in the study

The study only included patients diagnosed with Parodontitis apicalis chronic and Necrosis pulpae. To put diagnose and to come to the therapeutic plan, the retroalveolar radiography for each patient was taken in the suspected tooth. Patients included in 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 six months, or be under treatment of chemotherapy.

The group was divided into three 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.

In each 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.

Control group

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

Gaseous ozone working technique and irrigation protocol using 0.9% NaCl

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

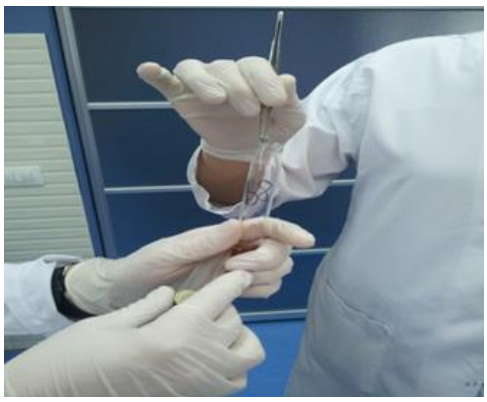

Figure 1: Placement of sterile paper point in tube

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 a duration of 1 minute. This sample was rooted in agar and further dipped in a tube containing 9 ml Thioglycolate *(Thioglycolate медиум, Liofilchем Italy),* (Figure 1).

For cultivating anaerobic bacteria, another sample was taken from 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 9 ml (*Brain Heart Infusion Broth Biolife, Italy)*. Upon the first sample, length of the root canal was determined to be 1 mm shorter than the real length of the 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 disinfection of the root canal used gaseous e ozone (Prozone, WH Austria), at a duration time of 6", 12", 18" and 24" (second, third, fourth and fifth measurement), (Figure 2, Figure 3 and Figure 4).

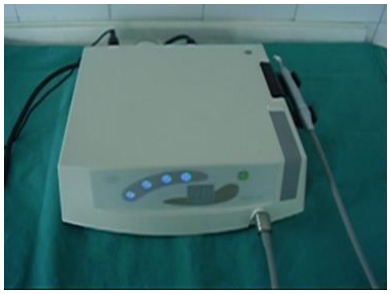

Figure 2: Prozone apart

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)*

Figure 3: Surgical aspirator

After instrumentation root canal, the 0.9 %, NaCl solution, was 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 *(Anaerobic Indicator, bio - Mérieux SA, France)* and a Gen Bag generator. *(Gen Bag bio - Mérieux Sa, France).*

Figure 4: Application of Gaseous Ozone in root canal

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 thermal 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 - Mérieux Sa, France)*, while their reading was made possible by the digital device *Vitek2 (Bio - Mérieux Sa, France)*.

Three days later, the patient was called for an examination, thereby removing the temporary backfill, also removing 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 ten patients. Upon diagnosing the sterile Rubber Dam was placed. After the trepanning of the pulp cavity and before the instrumentation of the root canal, a primary sample of aerobic and anaerobic bacteria on the root canal was 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 a duration of 1 minute. This sample was rooted in agar and further dipped in a tube containing 9 ml Thioglycolate *(Thioglycolate медиум, Liofilchем Italy)*. For cultivating anaerobic bacteria, another sample was taken from 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 BHIcontaining tube 9 ml (*Brain Heart Infusion Broth Biolife, Italy)*.

Upon the first sample, length of the root canal was determined to be 1 mm shorter than the real length of the 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 2.5 % NaOCl. Inorganic tissue was removed using 5ml of 17 % EDTA *(Ethylenediamine tetraacetic, acid disodium salt dehydrate, Czech Republic*), and duration of time was 1 min and the final irrigation again with 5 ml of 0.9 % NaCl was made. After irrigating, the canal was drained with a sterile paper point, while the disinfection of the root canal used gaseous e ozone (Prozone, WH Austria), at a duration time of 6", 12", 18" and 24" *(second, third, fourth and fifth measurement)* (Figure 2, Figure 3 and Figure 4). 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, was 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 *(Anaerobic Indicator, bio - Mérieux SA, France)* and a Gen Bag generator. *(Gen Bag bio - Mérieux Sa, 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 thermal state at a temperature of 37°C, thereby incubating for 24 - 48 hours. The gram-positive and gram-negative anaerobic bacteria were determined by special cards *(Bio - Mérieux Sa, France)*, while their reading was made possible by the digital device *Vitek2 (Bio - Mérieux Sa, France)*.

Three days later, the patient was called for an examination, thereby removing the temporary backfill, also removing 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% CHX

The group of this study involved ten patients. Upon diagnosing the sterile Rubber Dam was placed. After the trepanning of the pulp cavity and before the instrumentation of the root canal, a primary sample of aerobic and anaerobic bacteria on the root canal was 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 a duration of 1 minute. This sample was rooted in agar and further dipped in a tube containing 9 ml Thioglycolate *(Thioglycolate медиум, Liofilchем Italy)*. For cultivating anaerobic bacteria, another sample was taken from 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 BHIcontaining tube 9 ml (*Brain Heart Infusion Broth Biolife, Italy)*. Upon the first sample, length of the root canal was determined to be 1 mm shorter than the real length of the 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 2% CHX. Inorganic tissue was removed using 5ml of 17% EDTA *(Ethylenediamine tetraacetic, acid disodium salt dehydrate, Czech Republic*), and duration of time was 1 min 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 for the disinfection of the root canal was used gaseous ozone (Prozone, WH Austria), at a duration time of 6", 12", 18" and 24" (second, third, fourth and fifth measurement) (Figure 2, figure 3 and Figure 4). 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).*

Figure 5: Bacterial colonies

After instrumentation root canal, the 0.9%, NaCl solution, was 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 *(Anaerobic Indicator, bio - Mérieux SA, France)* and a Gen Bag generator *(Gen*

Bag bio - Mérieux Sa, 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 thermal state at a temperature of 37°C, thereby incubating for 24 - 48 hours. The gram - positive and gram - negative anaerobic bacteria were determined by special cards *(Bio - Mérieux Sa, France)*, while their reading was made possible by the digital device *Vitek2 (Bio - Mérieux Sa, France)* (Figure 5).

Three days later, the patient was reexamined, thereby removing the temporary backfill, also removing 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)

Control group

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 microbiological 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 10 ml of 0.9% NaCl was used. Two samples were taken before instrumentation: one for aerobic and one for anaerobic bacteria (First measurement). Immediately after the instrumentation was 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 GO combined with the following irritants: 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 GO (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 Kruskal - Vallis test, $r > 0.05$, χ – 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 clinically tested groups. Also, Kruskal - Vallis test, $r > 0.05$, χ - 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, Figure 6).

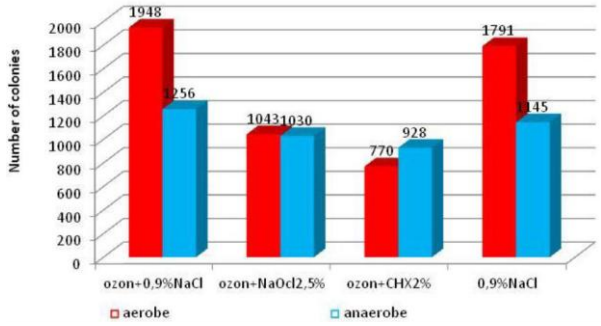

Figure 6: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at first measurement

After the application of the GO (for 6 sec) mixed with the other different irritants, the statistical results as follows were founded: Kruskal - Vallis test, $p > 0.05$, χ – 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 analysis 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 was found. Gaseous Ozone combined with 2.5% NaOCl was most efficient in the reduction of aerobe bacteria compared with other groups.

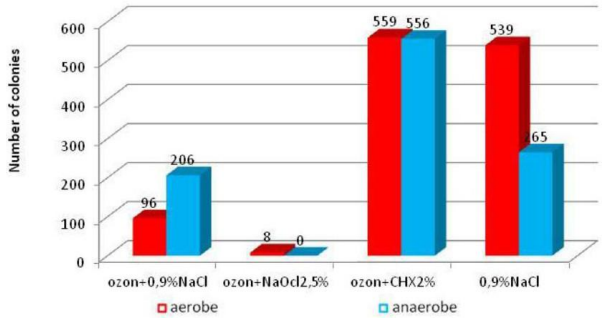

Figure 7: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at second measurement

As concerned the anaerobe bacteria the Kruskal - Vallis test, χ –test = 10.495, DF = 3, $p < 0.01$ showed that exists the significant difference in the isolated average number of the anaerobe bacteria colonies. Whereas the detailed analysis with the Mann - Whitney test showed, that does not exist 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 was found. The second measurement showed that when Gaseous Ozone was combined with 2.5 % NaOCl, decreased the number of isolated colonies of the anaerobe bacteria, compared with other testing groups (Second measurement, Figure 7).

Kruskal - Vallis test, χ – test = 17.29, DF = 3, $p < 0.01$ also showed 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 a concern, the colonies of anaerobe bacteria, the statistical results with the tests: Kruskal - Vallis test, χ – test = 110.724, DF = 3, $p < 0.01$ showed that exists a significant difference between the test group especially the group 2. The Mann - Whitney test showed that exists significant 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, Figure 8).

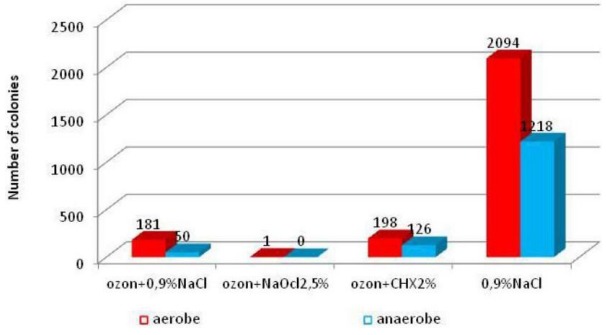

Figure 8: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at third measurement

After the application of the GO combined with the different irritants, the statistical results were found: Kruskal - Vallis test, χ – 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, Kruskal - Vallis test, χ – 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, Figure 9).

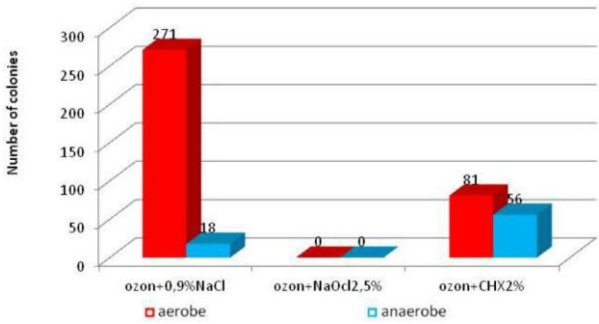

Figure 9: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at fourth measurement

After the application of the Gaseous Ozone for 24" combined with different irrigants these statistical results were found: Kruskal - 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 measurement, Figure 10).

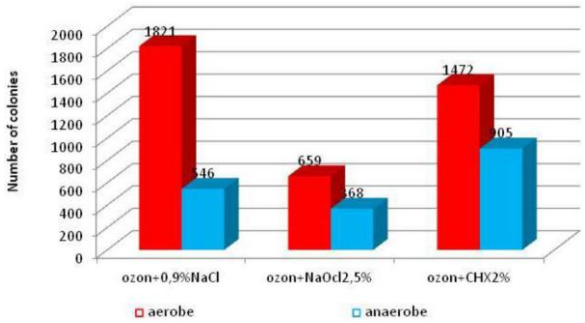

Figure 10: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at fifth measurement

After the instrumentation of the root canal the statistical test: Kruskal - 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 statistically significant difference between the group 1 - 2 compared with the group 2 - 3 in the average number of the colonies of aerobe bacteria.

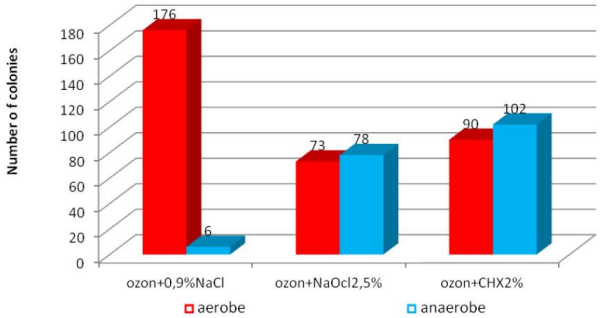

Figure 11: Average number of isolated bacteria colonies, aerobic and anaerobic bacteria, measured in all patients at sixth measurement

Also, during this measurement GO combined with 2.5% NaOCl affected in the reduction

of the number of aerobe bacterias. Whereas, as a concern, the anaerobe bacterias the Kruskal - Vallis test, X –test = 1.496, DF = 2, p > 0.05 did not show any statistical difference between the tested groups (Sixth measurement, Figure 11).

Discussion

In the literature, ozone 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 [16]. Gaseous Ozone in the concentration of ~4 gm¯3. (Heal Ozone: Kavo, Biberach, Germany) 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 [17] and species of the bacteria. In our study, the antibacterial effect of the GO 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 disinfection 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 Alwadi et al., [18], in vivo conditions, the antibacterial effect was reported in the use of 0.5%, NaOCl, with or without using GO 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 eliminated the number of aerobic and anaerobic bacteria colonies in the infected root canal, which also matches our study results. On the other hand, according to a study by Müller et al., [19], it was concluded that 5% NaOCl might reduce all bacteria from the infected root canal, for a different form of the application of Gaseous Ozone, photodynamic therapy and 2% CHX. The antibacterial effect of Gaseous Ozone was further confirmed by Virtej et al. [20]. 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. [21], which again matches our study results, is 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 colonies came as a result of failure to apply solutions for curing the infected root canal, which would help in further canal disinfection. Before the instrumentation, irrigation with NaOCl 2.5% and application of Gaseous Ozone, from the infected root canal was isolated four types of anaerobic bacterias; Clostridium clostridioforme, Clostridium bifermentans, Clostridium baratii and Actinomyces meyeri.

Whereas, after the application of these procedures, it was concluded that Clostridium bifermentans persisted in the root canal, even the application of Gaseous Ozone in a time interval of 6", 12" and 18". The disappearance was noted after application in a time interval of 24", whereas Actinomyces meyeri disappeared after 6" and Clostridium baratii completely disappeared after the application of Gaseous Ozone. Before the instrumentation, irrigation with CHX 2% and application of Gaseous Ozone, from the infected root canal was isolated four types of anaerobic bacterias; Lactobacillus, Actinomyces meyeri, Clostridium subterminale, Clostridium bifermentans and Clostridium butyricum. After the application of Gaseous Ozone in a time interval of 6" persisted only Clostridium butyricum, whereas the other bacterias completely disappeared.

Based on the results of this 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 antibacterial effect against the number of aerobe and anaerobe bacteria colonies in the infected root canal when compared with 0.9% NaCl and 2% CHX.

Acknowledgements

We acknowledge Prof. Vladimir Nikolov, from Faculty of Medicine, University of Skopje, Macedonia for statistical analysis and interpreting the data.

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