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EVALUATION OF ANTI-MICROBIAL EFFICACY OF OZONATED WATER ON MICROORGANISMS PRESENT IN ROOT CANAL SYSTEM-AN IN VIVO STUDY

Dental Science

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ABSTRACT

Background: the present study was undertaken for evaluating Anti-Microbial Efficacy of Ozonated Water on Microorganisms Present in Root Canal System.
Materials & methods: 45 patients with primary endodontic infection were treated with ozonated water and samples were analysed for microorganisms. All the results were recorded in Microsoft excel sheet and were analysed by SPSS software.
Results: patients with microbes after treatment with ozonated water reduced from 97.78% to 62.22%. Gram Negative bacteria reduced from 82.22% and 51.11%. Gram positive bacteria reduced from 15.56% to 11.11%. aerobic bacteria reduced from 66.67% to 42.22%. anaerobic bacteria reduced from 31.11% to 20%.
Conclusion: Ozonated water is an effective but not potent antimicrobial irrigating solution and is more effective against anaerobic than aerobic bacteria.

KEYWORDS

Infected root canal, Ozonated water

INTRODUCTION

The success of endodontic treatment depends on the eradication of these micro-organisms from the root canal system and prevention of reinfection. Mechanical instrumentation alone does not result in micro-organisms –free root canal system, when the complex anatomy is considered. The complexity of the root canal system is because of the presence of numerous dentinal tubules in the roots, more over formation of smear layer during instrumentation and presence of dentin as a tissue are the major obstacles in achieving the primary objectives of complete cleaning and shaping of root canal systems. Microscopic examinations of root canals show that they are irregular and complex systems with many cul-de-sacs, fins and lateral canals.[1-3]

Thus, irrigants are essential to ensure microbial minimization and elimination of organic tissue remnants.1To effectively clean and disinfect the root canal system, an irrigant should be able to disinfect and penetrate dentin and its tubules, offer long-term antibacterial effect (substantivity), remove the smear layer, and are nonantigenic, nontoxic and noncarcinogenic.[4]

The ideal endodontic irrigant should possess the following characteristics: be an effective germicide and fungicide, be non-irritating to the periapical tissues, remain stable in solution, have a prolonged antimicrobial effect, be active in the presence of blood, serum, and protein derivate of tissue, have low surface tension, should not interfere with repair of periapical tissues, not stain tooth structure, be capable of inactivation in a culture medium, should not induce a cell mediated immune response. None of the available irrigating solutions can be regarded as optimal. Using a combination of products in the correct irrigation sequence and technique contributes to a successful treatment outcome.[5]

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. Ozone gas in a concentration of 4 g/m3 (Heal Ozone; KaVo, Biberach, Germany) is already being used clinically for endodontic treatment. Regarding the demand on relative non-toxicity toward periapical and oral mucosal tissue for the endodontic irrigants, the ozone gas concentration currently used in endodontics (4 g/m3) has been shown to be slightly less cytotoxic than NaOCl (2.5%) and aqueous ozone (up to 20 µg/mL) showed essentially no toxicity to oral cells in vitro studies.[6]

Hence; the present study was undertaken for evaluating Anti-Microbial Efficacy of Ozonated Water on Microorganisms Present in Root Canal System.

MATERIALS & METHODS

The present study was conducted in the department of conservative dentistry of the dental institute and it included assessment of Anti-Microbial Efficacy of Ozonated Water on Microorganisms Present in Root Canal System. A total of 45 patients with primary endodontic infection were enrolled in the present study. Complete clinical and demographic details of all the patients were obtained. Access cavity was prepared manual irrigation with sterile saline solution was done. Measurement of the root canal was done using electronic apex locator. Introduction of the sterile saline solution was done in the canal with a sterile syringe. Samples were taken with a sterile paper point followed by their insertion in the aerobic and anaerobic BACTEC 9050 bottles. 500 ml of distilled water is taken in a sterile plastic container then Ozonater device (Kent vegetable purifier) is plugged in and operated for 15 minutes and then the concentration of ozonated water is measured with the help of dissolved ozone concentration meter. When the required concentration of 12 ppm formed then this freshly prepared ozonated water is used as intra canal irrigant.

All the samples were sent to microbiology department. Turbidity of the media was used for assessing the bacterial growth. A sterile loop was used to pick up the colonies of microorganism from the media. This colony of microorganisms was taken and put in a 3ml of 100% PCR grade water [0.45% at PH 5 to 7.2] . The Turbidity of the mixture was checked to ensure correct density of 0.5 to 0.6 m.f.u. This mixture was then inoculated into Vitek 2 Microbial Identification system with respective gram +ve / gram –ve cartridges. The Vitek 2 Microbial Identification system was analysing the solution and identifies the various microorganisms in the solution. Separate cartridges were used for gram +ve or gram –ve microorganisms.

The material and method used in this study is consistent with one of the best technology available today. Infact the system used in this study including the culture and bacterial identification system are used and recommended by National Aeronautics and space Administration (NASA).

The BACTEC 9050 automated culture instrument and the Vitek 2 microbial identification system represent the state of the art technology & are some of the best available today. The BACTEC 9050 automated culture instrument is designed for the rapid detection of the microorganism in clinical specimens. The sample to be tested is inoculated into the vial which is entered into the BACTEC 9050

instrument for incubation and periodic reading. Each vial contains the sensor which responds to the concentration of CO2 produced by the metabolism of the microorganisms or the consumption of O2 needed for the growth of microorganisms. The sensor is monitored by the instrument every 10 minutes for increases in fluorescence, which is proportional to the increasing amount of CO2 or the decreasing amount of O2 present in the vial. A Positive reading indicates the presumptive presence of viable microorganisms in the Vial.

The innovative Vitek 2 microbial identification systems is the next generation platform which provides fast, accurate microbial identification and antibiotics susceptibility testing. Identification of microorganisms is accomplished by biochemical methods. A turbid metrically controlled suspension of pure colonies in saline is inoculated into identification cards. These cards contain 29 different biochemical broths in reaction cells and one negative control cell to assess growth and viability of the suspension. Conventional catalase, coagulase, and oxidase tests (where appropriate) and the results of a Gram stain are required before inoculation of cards. The Vitek programmed computer determines whether each well is positive or negative by measuring light attenuation with an optical scanner. When the incubation period is completed, the reactions are analysed automatically and the identification is printed. Antimicrobial susceptibility tests are run similarly on cards which contain dilutions of antimicrobials to determine the breakpoint minimum inhibitory concentration (MIC) against the organisms.[7] There are separate cards for Gram positive and Gram negative organisms. The MIC cut off values differentiating sensitive, moderate, and resistant status for an organism against appropriate antimicrobials are programmed into the system and are based on the National Aeronautics and space Administration (NCCLS), now this changed into Clinical and laboratory Standards Institute (CLSI), United States of America (USA) guidelines.[8] All the results were recorded in Microsoft excel sheet and were analysed by SPSS software.

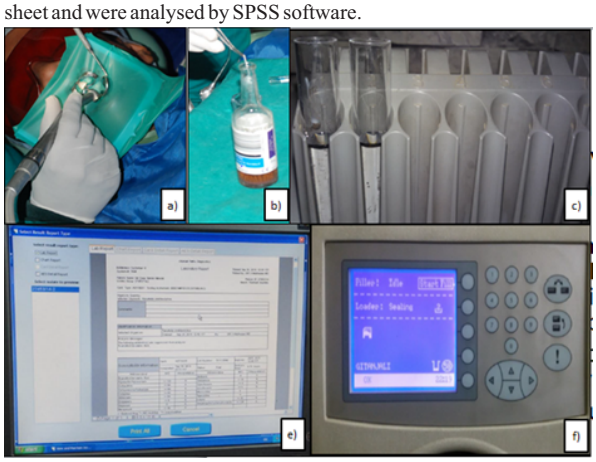

Figure 1(a): Access cavity preparation, Figure 1(b): Taking sample with paper point, Figure 1(c): Inoculating the sample in sterile PCR grade water with gram positive and gram negative pipette, Figure 1(d): Image showing sealing in Vitek 2 system, Figure 1(e): Result shown in Vitek 2 system

RESULTS

In the present study, the overall growth in root canal system before and after treatment with ozonated water was found to be present in 97.78% and 62.22% of the patients respectively. Sphingomonas paucimobilis and Entrobacter cloacae were the mostly commonly found micro-organisms found in the infected root canal before treatment with ozonated water. However; Sphingomonas Paucimobilis and Staphylococcus hominis were the mostly commonly found micro-organisms found in the infected root canal after treatment with ozonated water.

In the present study, the prevalence of Gram Negative bacteria in root canal system before and after treatment with ozonated water was found to be 82.22% and 51.11% respectively. The prevalence of Gram positive bacteria in root canal system before and after treatment with ozonated water was found to be 15.56% and 11.11% respectively. Significant results were obtained while comparing the overall prevalence of Gram Positive and Gram Negative bacteria in root canal system before and after treatment with ozonated water.

In the present study, the prevalence of aerobic bacteria in root canal system before and after treatment with ozonated water was found to be 66.67% and 42.22% respectively. The prevalence of anaerobic bacteria in root canal system before and after treatment with ozonated water was found to be 31.11% and 20% respectively. Significant results were obtained while comparing the overall prevalence of aerobic and anaerobic bacteria in root canal system before and after treatment with ozonated water.

Table 1: Comparison of microbial profile found in root canal system of patients, before and after treatment with ozonated water

Microbial organism	Before treatment with ozonated water		After treatment with ozonated water	
	N	%	N	%
Aeromonas sobria	1	2.22%	1	2.22%
Enterobacter aerogenes	5	11.11%	2	4.44%
Enterococcus faecalis	2	4.44%	2	4.44%
Entrobacter cloacae	6	13.33%	3	6.67%
Pseudomonas aeruginosa	4	8.89%	3	6.67%
Pseudomonas luteola	2	4.44%	1	2.22%
Raoutella planticola	1	2.22%	1	2.22%
Serratia fonticola	1	2.22%	0	0.00%
Sphingomonas Paucimobilis	10	22.22%	6	13.33%
Staphylococcus aureus	4	8.89%	2	4.44%
Staphylococcus hominis	5	11.11%	4	8.89%
Staphylococcus lentus	3	6.67%	3	6.67%
No Growth	1	2.22%	17	37.78%
Total	45	100%	45	100%

Table 2: Comparison of prevalence of Gram Positive and Gram Negative bacteria in root canal system before and after treatment with ozonated water

Bacteria	Before ozonated water		After ozonated water	
	n	%	n	%
Gram +ve	7	15.56%	5	11.11%
Gram -ve	37	82.22%	23	51.11%
No Growth	1	2.22%	17	37.78%
Total	45	100.00%	45	100.00%
Chi- square value	17.82			
p- value	0.0001 (Significant)			

Table 3: Comparison of prevalence of aerobic and anaerobic bacteria in root canal system before and after treatment with ozonated water

Bacteria	Before ozonated water		After ozonated water	
	n	%	n	%
Aerobic	30	66.67%	19	42.22%
Anaerobic	14	31.11%	9	20.00%
No Growth	1	2.22%	17	37.78%
Chi- square value	17.78			
p- value	0.0001 (Significant)			

DISCUSSION

In endodontic treatment, major goal is to disinfect the root canal system before canal obturation.[9] The root canal system is complex anatomy as it consists of accessory features such as fins, cul de sacs, and intercanal communications which are colonized by microorganisms once the tooth becomes infected.[10, 11] The quest to get an ideal root canal irrigant is a continuous evolving process.

Smear layer is one of the important factors for disinfection in root canal. A smear layer is formed during biomechanical preparation of the root canal. The smear layer consists of both an organic and an inorganic component. No clear scientifically based understanding exists on whether this layer must be removed or can be left. However, a multitude of opinions have been offered on both sides of this question. In addition to weak acids, solutions for the removal of the smear layer include carbamide peroxide, aminoquinaldinium diacetate (i.e., Salvizol), and EDTA.[12] EDTA is normally used in a concentration of 17%. It removes smear layers in less than 1 minute if the fluid is able to reach the surface of the root canal wall. The decalcifying process is self-limiting, because the chelator is used up. Although citric acid appears to be slightly more potent at similar concentration than EDTA,

both agents show high efficiency in removing the smear layer. Due to the drawbacks of existing irrigating solution researchers are continuously looking for better and improved irrigating solutions. One such material is ozone. Ozone is currently being tried as a possible alternative antiseptic agent in dentistry because of its reported high antimicrobial power without the development of drug resistance. Ozone gas in a concentration of 4 g/m^3 is already being used clinically for endodontic treatment. Regarding the demand on relative non-toxicity toward periapical and oral mucosal tissue for the endodontic irrigants, the ozone gas concentration currently used in endodontics (4 g/m^3) has been shown to be slightly less cytotoxic than NaOCl (2.5%). And also in in vitro study , aqueous ozone (up to 20 µg/mL) showed essentially no toxicity to oral cells.[13] Cardoso et al. investigated the effectiveness of aqueous ozone to eradicate E. faecalis and Candida albicans from root canals. They demonstrated that aqueous ozone can eliminate bacteria.[14]

Thus in this study we have evaluated the irrigating and anti-microbial efficacy of 12 ppm of freshly prepared ozonated water for 4 min on microorganisms present in root canal systems of patients reporting to the department of Conservative Dentistry And Endodontics in Buddha Institute of Dental Sciences And Hospital, Kankarbagh, Patna 800020.

In the present study we evaluated the effect of freshly prepared ozonated water on microorganisms present in root canal systems on patients reporting to our department by using vitek 2 system for identification of microorganism. Vitek 2 system used in this study includes the culture and bacterial identification system used and recommended by National Aeronautics and space Administration (NASA).

Analysing the results in our study it was clear that ozonated water has antimicrobial property as there was reduction in the count of bacteria after the use of ozonated water but this reduction was not uniform, as our study was done in a in- vivo environment. As the in vivo environment may significantly have altered the efficacy of the solution which is quite effective in vitro. In earlier studies the efficacy of ozonated water was only tested in –vitro environment like by Nagayoshi et al (2004)[13], Hems et al (2005)[15], Polydoru et al (2006)[16], Huth et al (2006)[17], Estrela et al (2007)[18], Stoll R et al (2008)[19], Cardoso M.G et al (2008)[14], Bialoszewski D et al (2011)[20], HubbezogluI et al (2014)[21], Noites R et al (2014)[22], Tuncay O et al (2015)[23] etc. which showed it as good antimicrobial agent in experimentally controlled condition and environment.

With our result we found out that found that ozonated water was more effective on anaerobic and gram negative organism than aerobic and gram positive organism, which was similar as other studies conducted by Nagayoshi et al(2004)[13] which showed that (0.5–4 mg/L) was highly effective in killing both gram positive and negative micro-organisms. Gram negative bacteria, such as Porphyromonas (P.)endodontalis and P. gingivalis were substantially more sensitive to ozonated water than gram positive oral Streptococci and C. albicans in pure culture.[13]

This was because the antimicrobial effect of ozone results from oxidation of microbial cellular components. Ozone is a highly reactive form of oxygen that is generated by passing oxygen through high-voltage. Several biological oxidations involve the loss of hydrogen atoms (dehydrogenation reactions). Oxygen is essential for the survival of cells that follow aerobic metabolism, although it has a dramatically toxic effect on microaerophiles and anaerobic bacteria. Aerobic respiration involves ATP generation at specific sites in the electron transport chain via oxidative phosphorylation (the final electron acceptors include oxygen).

Although aerobic bacteria contain a variety of enzymes that protect them from oxygen toxicity, microaerophilic and anaerobic bacteria are devoid of these protective mechanisms. The final electron acceptors in anaerobic respiration include inorganic substrate – sulphate ions, nitrate ions and carbonate ions. Oxygen is not used in fermentation process; anaerobic bacteria use organic compounds as final oxygen acceptor during its energetic metabolism.

Our result also showed that there was no reduction in E. faecalis count after the use of ozonated water which was similar as Estrela et al who evaluated the antimicrobial efficacy of aqueous ozone and NaOCl in root canals inoculated with E. faecalis which showed that aqueous ozone was unable to achieve the complete elimination of E. faecalis after 20 minutes use of aqueous ozone.[18]

CONCLUSION

Under the light of above obtained results, the authors conclude that ozonated water is an effective but not potent antimicrobial irrigating solution and is more effective against anaerobic than aerobic bacteria as an irrigating solution. Therefore; it can be used as an adjunct to other available irrigating solution solutions.

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