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Microbial Study of Antimicrobial Effect of Ozone on Carious Dentine of First Permanent Molars in Children

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Abstract

Introduction: *Ozone may provide a new alternative in arresting caries lesions and to promote their remineralization. In cavitated lesions,the use of ozone to eradicate the microbial acidic niche and its by-products and toxins due to its oxiding power, thus promoting remineralization of the leathery affected dentin, would to contribute to keep the structure integrity and strength of the tooth as high as possible. the objective of this study was to evaluate the antimicrobial effects of ozone on bacteria invading dentin of first permanent molars in children.*

Materials and Methods: *In vivo study based on 30 patient* ***(****age 6-9 years****)****, the patient were from Damascus dental schools, each has one first permanent molars with deep enamel-dentin occlusal caries. Each tooth was dried using sterile cotton wool rolls and air syring .Half of each lesions was removed using a sterile excavator. The excavator blade was used to traverse the lesion in line with the long axis of tooth across the maximum gingival/occlusal dimension. the remaining lesion were treated with Ozone* ***(****HealOzone, concentration 2100 ±5% PPM and flow rate 615ml/min for40 seconds****)*** *and further sampl was taken .each sample was immediately put into sterile vacuum tube filled with 1 ml of fastidious anaerob broth* ***(****Bactec plus Anaerobic / F,Ireland****)*** *and vortxed for 30 seconds to facilitate the extraction of any micro-organism from carious dentin and disperse any aggregates .Dilution were performed and 100µl aliquots* ***(*** *for both test and control groups****)*** *were spread on blood agar at 37C for 48 hours .the total number of colony-forming units cfu was calculated. The test of T -test was used to compare the test and control groups.*

Results: *The total cultivable microflora was assessed by counts of the colony-forming units for all samples before and after ozone treatment. there was a significant* ***(****$p<0.005$****)*** *difference between the control and test samples for 40 seconds of Ozone application.*

Conclusion: *Ozone exposure for 40 seconds reduced micro-organism in the enamel dentin occlusal carious lesions.*

Key Words: *Ozone.*

Introduction

The most common bacterial diseases in the mouth are dental caries and gum disease as well.

For the consequences resulting from it, and the caries of the first permanent molars is a large part of the caries **(**Sunnia that we encounter frequently2,1**)**. Many treatment modalities are currently available **(**2**)** To deal with caries, starting with preventive measures and ending with preparing the dental holes, has tomorrow Early diagnosis and treatment based on identifying the patient's risk factors and causes **(**3**)** The modern method adopted as an alternative treatment system to the traditional system. This system is known What's new is Dentistry Invasive Minimally, as it requires all the traditional "aggressive" methods. Removing even a small amount of dental tissue, while less destructive methods are available to structures Teeth such as the Abrasio Air, hard laser, and tooth-based dredging **(**4,3**)**

Transforming the process of degradation of mineral salts that occurred as a result of an acidic environment into a process Remineralization requires the elimination of all caries-causing germs responsible for This acidic environment, with the aim of establishing a healthy and sound environment.

And with the strong entry of ozone into the medical fields, dentistry had a large share of it Its uses, the most important of which was its preventive effectiveness and its ability to stop the progression of dental caries Especially in children, as ozone treatment is considered a new method that works to destroy Germs that cause dental caries and affect its organic structure.

Ozone provides a new alternative for controlling necrotic lesions and inducing remineralization When using ozone in the treatment of necrotic pits, it works to cancel the environment bacterial acidosis and the elimination of its products and toxins through their oxidation, which incites To remineralize the injured dentin, which maintains the integrity and hardness of the dental material if possible **(**8**)**.

The aim of the research:

A laboratory bacteriological study aimed at studying the effect of ozone on the general bacterial population Bacteria present in the dentin cause necrosis in the permanent first molars in children Using the HealOzone device with a concentration of 2100 ± 5% ppm and a flow rate 15 milliliters per minute for 40 seconds.

Materials and Methods

Study Sample:

The laboratory study sample included 30 children attending the Pediatric Dentistry Department

Damascus University, their ages ranged between 6-9 years, and they were chosen so that each of them had One of them is a permanent first molar, upper or lower, right or left, with port necrosis

Deep, impermeable, milled ivory **(**first class**)**, and the degree of dental caries is determined depending. On the DIAGNOdent laser fluorescence device due to the ability of this device to determine The severity of dental caries, so that the readable values of the intensity of necrosis on the screen of the device are at Grade D4 **(**between 30-40**)** indicates the presence of deep, impenetrable dentin necrosis What is needed in this study, as molars with penetrating dentinal caries were excluded. And then we take a sample of dentine from the molar affected by dentin necrosis before applying it Ozone and another dentin sample of the same molar after applying ozone directly to the tooth without any time interval; So that the total ivory samples are 60 samples, and so on To study the effect of ozone on bacteria present in the necrotic dentin of the first molars permanent in children.

Study Methods

Sampling method:

The selected molars were clinically examined before ozone was applied to the dentist's chair In the supine position, the light of the dental apparatus was shone into the mouth using examination instruments A single-use mirror, probe and forceps were selected Permanent first molars with deep, impenetrable occlusal enamel-dentinal necrosis **(**class First**)** based on the DIAGNOdent laser fluorescence device produced by K German**)**, and the degree of dental caries was determined by recording the degree shown on the screen of the device, It is the highest value read from multiple sites on the molar surface The values read for the intensity of necrosis on the screen of the device are between **(**30-40**)**, which indicates the presence of Deep, impermeable dentin enamel necrosis. Salivary isolation was done using sterile cotton rolls and sucking saliva without preparing the molar-crushing weapon with spikes, then the teeth were dried with a roller sterile cotton swabs, and the carious lesion was swept away with a sterile double-headed curette, where the scoop with a line parallel to the longitudinal axis of the tooth across the maxillary occlusal gingival dimension, When the entire cubic millimeter head of the shovel was covered with necrotic dentin, it was removed directly to a sterile, airtight, airtight 5 mL tube containing broth medium Nutrient for growing germs **(**Bottles Nutrient

Broth / Anaerobic + bactec plus Ireland,F BD 25 mL**)** so that the proportion of expansion of the sample to the nutrient broth is 1/1000, and anaerobic incubation was done to study selective aerobic and anaerobic bacteria, and continued Incubation for 48 hours. The method for taking the dentine sample and transferring it to the sterile tube is similar **(**For the method used by Munson and colleagues in 2004**(**5**)** and Draghincescu and colleagues in 2004 **(**10**)**.

Before taking the second dentin sample of the same molar, ozone was applied using the ozone device. HealOzone **(**produced by the German company Kavo located in the Department of Oral Medicine at the Faculty of Medicine**)**.

5 ± % part in 2100 This device generates ozone in concentration Damascus University. teeth, million, at a flow rate of 615 milliliters **(**40 seconds**)**, and only once directly on the tooth.

The same sample from which the first dentin necrotic sample was taken, then a second dentine necrotic sample was taken after Ozone is applied in the same way as before, to directly investigate the presence of germs in it.

In the same way; Then the comparison between the two results of the bacterial examination before the application of ozone year and after.

Method of dealing with samples:

Samples were dealt with in three stages: 1- Expansion of samples: dilation aims to reduce the density of bacterial cells in the sample.

Then separate countable colonies were cultured. The samples were diluted to a ratio of 1/ 1,000,000 milliliters, in two stages:

The first stage: dilution at a ratio of 1/1000, by adding a volume of the sample to 1000 volumes of nutritious broth, and shaking it well and sufficiently to ensure its homogeneity, and vibrator was used for 30 minutes. again, to give homogeneity to the new solution.

The second stage: spread 1/1000 ml, i.e. one microliter, on the blood agar tray. **(**Petri boxes with a diameter of 10 cm in which the bactericidal medium, Agar Blood, is poured**)** And make an enumeration of Colony Forming Units CFUs After incubation for 48 hours, then The number of bacteria per milliliter is the number of growing colonies 610×2. Bacterial culture: One microliter was taken from

the diluted solution 1/1000 using Ghana. The planting was carried out by moving the agar on the surface of the bloody agar in the form of horizontal lines. on the entire surface with the aim of distributing the sample over the entire surface and distributing the colonies as much as possible.

As possible, the petri dishes were covered and placed inside the incubator at a temperature of 35-37 degrees. degree and a period of 48 hours. After this period, the bacterial enumeration process was carried out. The users have been counted.

Colony Forming Units: CFUs 3 The number of colonies or growing on the surface of the agar **(**all colonies without exception**)** and called the general census Using a light microscope, a 100× submersible lens is used.

Results

The statistical tests used:

Student's t-test was used for correlated samples to study the significance of the binomial differences in the mean of The general number of bacterial colonies **(**in thousands**)** between the two stages **(**before the application of ozone, after application of ozone**)** in the study sample.

Results of the statistical study:

Standard deviation	Arithmetic mean	Number of dentinal necrotic specimens	Millstone site	The studied stage	Studied variable
162948.5	157013.1	13	Upper millstone	Before ozone application	General population of bacterial colonies (in thousands)
66274.1	82007.9	13		After ozone application	
108627.6	82619.3	17	Lower millstone	Before ozone application	
106623.5	62241.8	17		After ozone application	
137489.3	114856.6	30	Complete laboratory study sample	Before ozone application	
90493.4	70807.1	30		After ozone application	

Table No. (1) The arithmetic mean of the general population of bacterial colonies **(**in thousands**)** in a sample Laboratory study according to the stage studied and the location of the molar

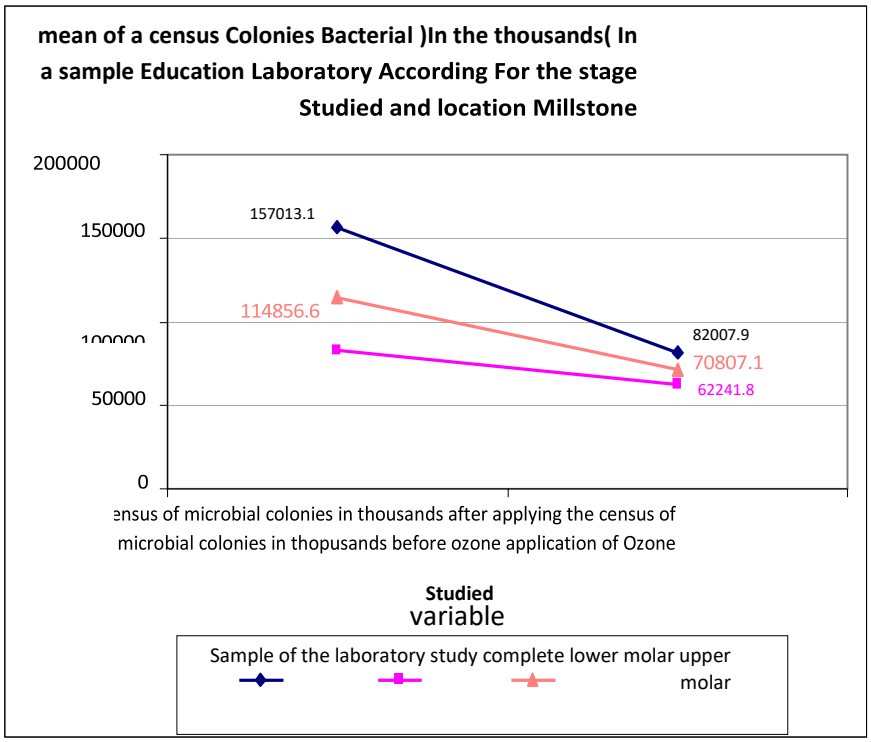

Chart No. (1) represents the arithmetic average of the general population of bacterial colonies **(**in thousands**)** In the laboratory study sample according to the stage studied and the location of the molar.

Student T test results for correlated samples:

Table No. (2) It shows the results of the student's T test for correlated samples to study the significance of the binary differences in the average general bacterial colony count **(**in thousands**)** between the two stages **(**before the application of ozone, and after applying ozone **(**according to the location of the millstone in the laboratory study sample.

Significance of differences	gnificance level value	Degrees of freedom	Calculated T value	Differences between the two averages	Millstone site	Comparison in the severity of necrosis between periods
There are no significant differences	0.066	12	-2.024	-75005.2	Upper millstone	After ozone application – Before Ozone application
There are significant differences	0.021	16	-2.559	-20377.5	Lower millstone	
There are significant differences	0.015	29	-2.580	-44049.5	Study Sample Complete laboratory	

Table (2) shows that the value of the estimated significance level is greater than the value 0.05 in the upper molars group, meaning that at the 95% confidence level there are no statistically significant differences.

In the average of the general bacterial colony count **(**in thousands**)** between the two studied stages **(**before applying ozone, and after applying ozone**)** in the laboratory study sample.

In the group of mandibular molars and in the entire laboratory study sample, it is noted that the value of the estimated significance level is smaller than the value 0.05, that is, at the level of confidence of 95%, there is Statistically significant differences in the average general bacterial colony count **(**in thousands**)** between the two stages studied **(**before ozone application, and after ozone application**)**.

The algebraic sign of the differences between the means is negative; We conclude that the values of the general bacterial colony count **(**in thousands**)** after ozone application are smaller than they were before it was applied, meaning that ozone led to a decrease in the general bacterial colony count in the study sample Laboratory.

Discussion

The values of the general bacterial colony count **(**in thousands**)** after the application of ozone are smaller than those before its application, and then we conclude that ozone led to a decrease in the number of bacterial colonies year in the laboratory study sample. This effect is due to the fact that ozone is a strong oxidant capable of rapidly stopping the activity of germs responsible for causing dental caries, as it dissolves directly with water and penetrates the cell wall of germs, killing them directly **(**7**)**

It was difficult to compare the results of the current study with other studies. comparable to them, the results were compared with studies that conducted bacterial tests on palm trees PRCLs Lesions Caries Root Primary, through a population study

General bacterial colonies of dentin samples taken before ozone application and after Apply it. Baysan and her colleagues found in 2003 in a study of the antibacterial effect of ozone on primitive root rot PRCLS that ozone is able to penetrate such pests and kill most of the micro-organisms responsible for them **(**11**)**, taken with caution, as samples taken from these areas of the tooth surface are not representative of samples taken from inside the mouth, as well as the accumulation of plaque.The germ in such areas differs from the grinding surface of the tooth. **(**1**)**

Baysan also mentioned in another study of the effect of ozone on the bacteria present in the primitive root rot PRCLS that the decrease in the general population of bacterial colonies.

After application of ozone for 10 seconds in small, non-cavitated non-cavitated lesions, It was greater than the decrease in the larger lesions **(**6**)**. Small lesions had fewer cavitated lesions compared to larger lesions. The limited response is due to the application of ozone in the lesions. the widest Cavitated Lesions to an increase in the amount of demineralized dentin causing an increase in The amount of organic content needed to feed these microbes, which leads to an increase in their number **(**11, 8**)**

The selection of dentine samples in this study was for the excavated grinding necrotic lesions, and the readings of the laser fluorescence device were adopted to determine the degree of necrotic lesions and to standardize The criterion for judging the ability of ozone to penetrate dentinal necrotic damage, where the values of the readings of the laser fluorescence device ranged between **(**30-40**)** Lussi stated that due to the ability of the device The laser fluorescence test for the severity of dental caries is a suitable tool for a longitudinal study to monitor the progression of dental caries, and to re-evaluate the caries during the follow-up periods. **(**9**)** The readings were the upper molars selected in this study are all over 35, and it is possible to explain the limited response to ozone application in the upper molars to our selection of samples from Extensive cavitated necrotic lesions in which the deep bacterial groups are diverse and different, As the value of the estimated significance level is greater than the value of 0.05 in the upper molar group, that is, at the 95% confidence level, there are no statistically significant differences in the average of the General bacterial colonies **(**in thousands**)** between the two stages studied **(**before the application of ozone, and after the application of ozone **(**in the group of upper molars.

Also, Baysan and Lynch studied the effect of ozone on the bacteria present in the primordial root rots, by studying the general bacterial population and studying the population of Streptococcus aureus, Sobrinus and Mutans, and there was a difference in the two rates. The control and the tested subjects before applying ozone and after applying ozone for 10 or 20 seconds, and there was a significant difference in the general bacterial count between the two times of application.

Ozone 10 or 20 seconds, and logarithmic counting was used in calculating the results, A clear decrease was observed in the number of Streptococcus Sobrinus and Mutans, which is of significant significance. % after 10 seconds with a percentage before and after applying ozone, and the decrease was 99%, 99.9% after 20 seconds of applying ozone. **(**12**)**

Bocci's study in 1999 showed that treating carious dentin with ozone for 10-20 seconds causes a decrease in the number of bacteria within the primary carious lesions up to limits.

99%, especially the number of Streptococcus mutans, as the use of ozone in a dose correct makes it considered as a therapeutic agent and a strong antibacterial. **(**13**)**

The difference in the results of the previous studies and ours was attributed to the difference in the method and place of taking dentin samples **(**in the previous studies, the place of sampling was from small and large necrotic radical injuries in either Cavitation-North Cavitation In our study, the samples were taken from the grinding surfaces **(**wide cavitated Lesions**)**, as well as the difference in the method of calculating the number of bacterial colonies.

The previous calculation method was based on the decimal logarithm, while in our study it was use thousands.

In the comprehensive review conducted by Library Cochrance to evaluate the effectiveness of ozone In the treatment of dental caries, the available studies published in the medical literature and in all university doctoral theses, and many criticisms were made of the approach to conducting these

The number of pests that were required to studies and data that have been presented, especially for Application of other traditional or dental treatments and approved statistical methods. Whereas Rickard and colleagues stated that there is no reliable logical evidence that the application of ozone on the surfaces of decayed teeth can stop or reverse the role of dental caries, and it is necessary to More evidence is needed before the use of ozone becomes accepted as an alternative method of smoke management.

The philosophy of applying ozone to dental palms is highly controversial. Therefore, amend Sunni. **(**14**)** Many factors contribute to the success of this technology

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