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Original article

PREVENTION OF DENTAL CARIES IN CARIES-ACTIVE INDIVIDUALS BY GASEOUS OZONE

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ABSTRACT

Introduction: The action of ozone is associated with the prevention of dental plaque formation, and the reduction of bacterial development supports the prevention of dental caries. This paper aims to establish the effect of gaseous ozone on developing new caries lesions two years after its application for caries-active patients.

Material and Methods: Patients aged 30 to 50 were selected with no common diseases. They all have five or more caries on the teeth, without missing teeth. Patients were divided into two groups of 10 participants. One group had ozonation of all teeth, and another is a control group without ozonation.

Results: After two years, the number of caries lesions in the group without prophylactic ozonation is more significant. The results showed that the patients in the ozonation group had significantly fewer primary caries than those in the control group($p \leq 0.0001$).

Conclusions: Repeated ozonation of all teeth according to the applied methodology (10 times, distributed twice a week by 24 seconds exposure to ozone in a gaseous state) has a caries-protective effect against new caries lesions.

Keywords: prevention of dental caries, ozone therapy, Streptococcus mutans, Candida albicans, gaseous ozone.

INTRODUCTION

The prevention of dental caries is a task of paramount importance[1, 2]. Restoration of teeth with caries, improvement of oral-hygienic activities, and level of oral-hygienic knowledge is essential [3]. Nevertheless, the development of new caries is not always prevented in caries-active patients. To improve the condition and prevent the development of new cavities, it is recommended to use remineralizing solutions, fluoride preparations, hydroxylapatite solutions, and others [4, 5].

Another means of influencing cariogenic microorganisms in the development of caries is the application of ozone [5, 6, 7]. The action of ozone to treat dental caries is used in the UK in half a million patients to destroy caries-causing bacteria and stage its development [8]. Ozone destroys microorganisms by rupturing their cell membrane in just two seconds [9]. Studies by Kirilova and co-authors have shown that administering gaseous ozone for 24 seconds before restoration in caries-active patients (acute proximal deep caries) statistically significantly reduces cariogenic microorganisms [10]. In addition, various studies have shown the beneficial effects of ozone, such as its influence on the cellular and humoral immune system, stimulation of collagen production, hyaluronic acid, and chondroitin sulfate synthesis [11]. After ozonation on dentin, exposed to bacterial attacks, no dense biofilm is formed [12]. Ozone interferes the cellular polysaccharide synthesis by Streptococcus mutans by inactivating the enzyme glycosyltransferase [13]. According to Knight, the action of ozone associated with the prevention of dental plaque formation and the reduction of bacterial development supports the prevention of dental caries [13]. Grocholewicz et al. studies proved that gaseous' ozone application influences remineralization processes in superficial carious lesions [5]. It also improves the hardness of enamel and dentin [5, 8].

The accumulated evidences for the action of gaseous ozone raises the question: in addition to the effects of gaseous ozone for treating carious lesions, whether ozone can be applied to prevent the development of caries.

This paper aims to establish the effect of gaseous ozone on developing new caries lesions two years after its application for caries-active patients.

MATERIAL AND METHODS

Patients aged 30 to 50 years were selected, with no common diseases. They all have five or more cavities on the teeth, without missing teeth. The wisdom teeth are not included in this study. After the initial examination, professional oral hygiene was carried out. The investigation was made with DIAGNOcam (KAVO, Germany) for accurate diagnostics of the existing carious lesions.

Ethics statement. The Scientific Ethical Committee approved the study protocol at the Medical University of Sofia, Bulgaria, before the initiation of the study (KENIMUS –No. BK-297/14.04.2015 and No. 13/23.4.2015). According to the Helsinki Declaration II, all subjects gave written informed consent for the study.

Patients were divided into two groups of 10 participants. All carious teeth were treated in both groups by restorations. The treatment was completed with glass-ionomer cement (Fuji LC II, GC Int. Corp, Japan) as a liner and composite material (Diamond, Kulzer, Germany) as a restorative material.

The difference between the groups is in the medication of the cavity before restoration.

Group 1. For the medication of the dentin, wound was used 3% oxygen peroxide.

Group 2. For the medication of the dentin, wound were used 3% oxygen peroxide and gaseous ozone for 24 seconds.

Device for gaseous ozone procedures. Ozone Generator Prozone/ OZOTOP (TIP TOP TIPS Sarl, Switzerland) produces gaseous ozone. The unit processes 2 liters of air per minute. The use of ozone gas for therapeutic purposes has virtually no contraindication. However, the manufacturer still offers a maximum permissible dose: 140 ppm/ 45 = 1.5 ppm or 280 mg/ m^3 x 0.002 m^3/ min = 0.56 mg/min = 35 mg/hour. The maximum ozone production for the whole day for ten patients is: each patient is treated five times with a 24-second application. The total ozone dose is 10 x 24 x 5 x 0.56 mg/min/60=11 mg ozone for the dental office. In addition, the manufacturer offers a device for securing and removing the ozone gas during operation [14].

After treatment of the existing carious lesions, the examined patients were checked again with DIAGNOcam for missed caries lesions. If such were diagnosed, they were obturated.

All patients underwent oral hygiene training with two control sessions. Instructions were given for proper maintenance of personal oral hygiene.

Participants in the second group underwent prophylactic application of gaseous ozone for six weeks. The procedures were carried out two times a week, according to the patient's availability.

Gaseous Ozone procedure. Before starting the ozonation procedure, patients rinse their mouths with saline. At each visit, patients are ozonated five times for 24 seconds each, the maximum permissible dose for one visit when applying this device. All proximal, cervical surfaces of the teeth (buccal and lingual) are sequentially ozonated by slowly sliding the coro-tip of the ozone generator (figure 1).

Fig. 1. Gaseous Ozone procedure and generator with coro-tips.

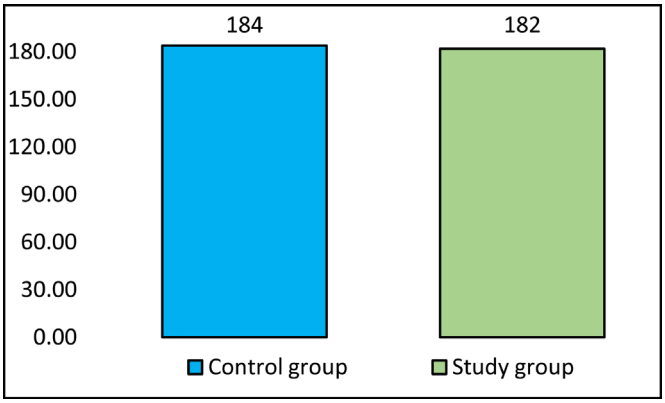
After two years, the patients of the two groups were examined with a DIAGNOcam to identify newly occurring caries. The caries cases were divided into primary and secondary. Fractured teeth, fractured or missing restorations without carious destruction, and endodontic retreatments were excluded from the examination.

The results obtained were processed statistically by the Mann-Whitney test method.

RESULTS

The total number of caries is shown in the groups - Figure 2.

Fig. 2. The total number of caries affected in group 1(control) and group 2(study) at the beginning of the study.



The Mann-Whitney U test was applied to test the hypothesis that the degree of caries activity in the two groups of the second year do not differ statistically significantly with the effects of the gaseous ozone and without it. The results obtained are presented in Table 1. There is a statistically significant difference in the number of reported caries in the two study groups. Their number in the group without prophylactic ozonation is more significant. The results showed that the patients in the ozonation group were statistically significantly less than those in the control group. No statistically significant difference exists in the number of reported secondary caries (figure 3, table 1).

Patients in group 2 with ozonation were noted to have visible teeth whitening.

Fig. 3. The average number of caries checked in both groups after the second year.

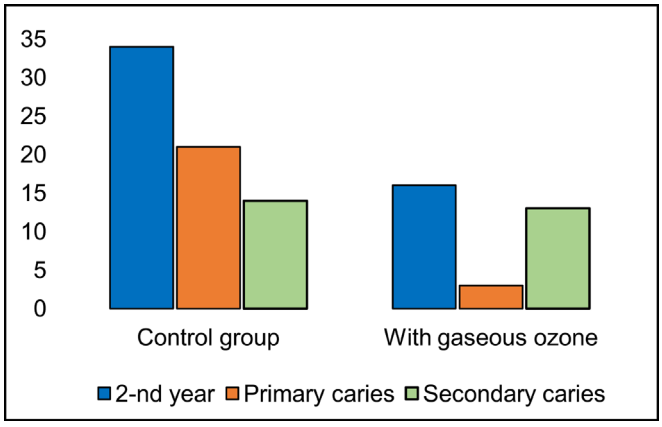


Table 1. Comparison between tested groups using Mann–Whitney test.

	2 year	Primary caries	Secondary caries
Control group	34	21	14
With gaseous ozone	16	3	13
Mann-Whitney U Test	*Z=3.34069*	*Z=4.6255*	*Z=0.24345*
	*P=0.00084**	*P≤0.0001**	*P=0.81034*

**The mean difference is significant at the 0.05 level.*

The results of the Mann-Whitney U test showed a statistically significant difference in caries activity in the two study groups in the second year p = 0.00084, z = 3.34069. The differences between the two groups are mainly associated with primary caries. Statistically significant, the number of primary caries is less in the group with prophylactic gaseous ozone treatment. There are no statistically significant differences between the number of secondary caries in the groups.

DISCUSSION

The proximal surfaces of the teeth are the most preferred place for caries development in adult patients. Dental cervixes are also among the places where dental caries develop more frequently. For this reason, we have chosen to conduct the ozonation of these surfaces.

Lynch et al. apply the gaseous ozone through an apparatus with special tips so that its pressure application is directed precisely on the selected surface [8]. The apparatus applied by us does not concentrate the application of ozone only on the treated surface. But since these areas of the dental crown are located near the gingival tissues, ozonation also affects these tissues. Ozone brings oxygen into tissues, enhances oxidation processes such as glycolysis and the Krebs cycle at the cellular level, changes the electrical charge of cell membranes and cellular energy, and activates cellular metabolism [8]. It acts on blood elements (erythrocytes, leukocytes, platelets, endothelial cells, blood vessels) and affects oxygen metabolism, immunomoduleted processes, the protective antioxidant system in cells, and activates blood circulation [14]. Ozone affects the cellular and humoral immune system. Ozone-induced blood oxidation activates blood cells (leukocytes, lymphocytes, monocytes), enhances hemopoiesis, and forms cytokines, interleukins, interferon, tumor necrosis factor TNF-α, and the transforming growth factor TGF-β. Thus, the immunostimulating effect of ozone is manifested [15]. It stimulates the proliferation of immunocompetent cells and the synthesis of

immunoglobulins. It activates macrophages and makes microorganisms susceptible to phagocytosis. Stimulating immune processes in gingival tissues is an advantage in this type of ozonation.

Procedures for treating caries do not always guarantee a successful removal of all microorganisms that cause the disease.

The development of dental caries is associated with a predominance of streptococcal microorganisms [11]. The antimicrobial action of ozone has been demonstrated against staphylococcus aureus, streptococcus mutans, streptococcus sobrinos, mycobacterium, escherichia coli, pseudomonas spp., enterococcus faecalis, candida albicans. The process of ozonating attacks glycoproteins, glycolipids, lipids, lipoproteins, phospholipids, and amino acids and blocks enzyme systems. This leads to an increase in the membrane permeability of cells, an essential element for their viability opens the door for the ozone to the bacterial cells, and their function immediately stops. Entering bacterial cells, ozone causes their death [16]. Ozone can attack many biomolecules, such as cysteine, methionine, histidine, and other amino acids. In fact, by oxidizing biomolecules, it damages various microorganisms, including cariogenic bacteria [8].

Studies by Kirilova and co-authors found in addition to streptococci in caries-active individuals (with good oral hygiene), candida albicans in 33% of cases [11]. Azmanova, through clinical, laboratory, and experimental studies found a destructive effect of candida albicans on the enamel surface in the zone of the tooth cervical line [11]. Other authors proved that candida albicans have a supporting role, especially in the early phases of caries lesion formation, as it is involved in bacterial biofilm formation and acid formation [17].

It has been shown that ozone destroys candida albicans. The application of gaseous ozone destroys this microorganism [11, 17], and also ozonated water shows excellent results against candida albicans, reducing colony formation more effectively than a local antifungal agent [17]. In this sense, the application of ozone gas in caries-active individuals is justified, and the results of this study confirm its preventive effect against the formation of new caries.

Besides reducing or eliminating microbial flora, ozonating tooth surfaces is essential for healing enamel surfaces and remineralizing them. Enamel is a mineralized tooth structure that can be damaged throughout the life of individuals. Therefore, to protect against caries, it is necessary to control the formation of dental plaque and remineralize the enamel. Such are the possibilities provided by ozone gas. Floare and co-authors proved that after treatment of the enamel surface with ozone gas (40-50 seconds laboratory study), the microhardness of the enamel increased, and a remineralization rate between 96.82–97.38% was achieved. The authors conclude that gaseous ozone is an alternative to preventing dental caries [18]. The authors apply another type of ozonation apparatus. With the results of this clinical study, we confirm a decrease in the formation of primary cavities in patients with prophylactic ozonation of tooth surfaces compared to a control group.

A study by Nardi and co-authors proved that ozonized olive oil has antibacterial activity against str. mutans and reduces the formation of bacterial dental plaque. With the results of this clinical study, we confirmed the long-term prophylactic action of ozone gas to reduce new cases of dental caries in the second year after their conduction [19].

So far, there are no proven indications of the use of ozone gas in treating and preventing dental caries. But a careful review of the literature found that to achieve an optimal effect in practical terms, it is essential to comply with the methodical requirements for work regarding duration, concentration, pressure, and targeting of ozone on the treated area [8]. The borderline between therapeutic and toxic doses is narrow [11]. Therefore it is necessary to control the ozonation process precisely. Although ozone is more stable in an aqueous solution, its gaseous use in dentistry ensures safety and control [19]. However, the duration of the application and the preliminary bacterial contamination affect the antimicrobial effect of gaseous ozone. Therefore, gaseous ozone therapy must be carried out according to the manufacturer's recommendations for each device.

The application of gaseous ozone to all teeth of caries-active individuals is an approach that can be successfully used in preventing dental caries.

CONCLUSIONS

Repeated ozonation of all teeth according to the applied methodology (10 times, distributed twice a week by 24 seconds exposure to ozone in a gaseous state) has a caries-protective effect.

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