Clinical Evaluation of Ozone on Dentinal Lesions in Young Permanent Molars using the Stepwise Excavation

Osama Safwat*/ Mona Elkateb **/ Karin Dowidar ***/Omar El Meligy ****

Aim: *To evaluate the clinical changes in dentin of deep carious lesions in young permanent molars, following ozone application with and without the use of a remineralizing solution, using the stepwise excavation.* ***Study design:*** *The sample included 162 first permanent immature molars, showing deep occlusal carious cavities that were indicated for indirect pulp capping. Teeth were divided into 2 main groups according to the method of ozone treatment. Each group was further subdivided equally into test and control subgroups. Following caries excavation, color, consistency and DIAGNOdent assessments of dentin were evaluated after 6 and 12 months.* ***Results:*** *Regarding dentin color and consistency, no significant differences were observed following ozone application, with and without a remineralizing solution. There were no significant differences between ozone treatment, and calcium hydroxide during the different evaluation periods, except in group I cases after 6 months, concerning the dentin color. The DIAGNOdent values were significantly reduced following ozone application, with or without a remineralizing solution, as well as between test and control cases in group I after 6 months.* ***Conclusions:*** *Ozone application through the stepwise excavation had no significant effect on dentin color and consistency in young permanent molars. DIAGNOdent was unreliable in monitoring caries activity.*

Key words: *Ozone, Stepwise excavation, Young permanent molars.*

INTRODUCTION

The maintenance of integrity and health of the young permanent tooth and its supporting tissue in cases with deep carious lesions is based on careful diagnosis and the appropriate treatment procedure which varies from indirect pulp treatment, direct pulp capping, and pulpotomy for both carious and traumatic exposures, to allow the continued physiologic development and formation of the root apex [1].

Different methods were used to diagnose carious lesions such as visual, tactile and radiographic examinations. The development of new technology, has characterized these methods over the years and resulted in various non-invasive devices [2,3]. Among them, a laser or light-induced fluorescence device; the DIAGNOdent was developed [4].

The traditional management of carious lesions dictates the removal of all infected and affected dentin to prevent further cariogenic activity and provide a well mineralized base of dentin for restoration [5].

A less invasive caries excavation approach was proposed for management of deep dentinal lesions, focusing on changing an active lesion into an arrested one, even without performing an excavation close to the pulp. It involves a two stage excavation procedure "stepwise excavation" aiming to reduce the risk of pulpal exposure during the first excavation and to promote dentin-pulp complex reaction [6].

Ozone has been introduced to medicine since 1885 [7]. As regard the biological effect of ozone, this gas is considered a very effective disinfecting agent [8]. Ozone has the unique feature of decomposing to

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a harmless, non-toxic and environmentally safe material (oxygen O_3). In view of its powerful oxidizing properties, ozone disrupts the cell walls of microorganisms within seconds, leading to immediate functional cessation. This effect within a very short time is of great clinical significance, as the potential for microbial resistance to this treatment modality is insignificant [9]. Technical advances have made it possible for ozone to be applied to small areas of dental hard tissues using a device known as the HealOzone and considered as a pharmaceutical approach for treatment of caries [10], and tooth sensitivity [11].

The use of remineralizing solution as an adjunct method to the treatment of deep caries has proved to be successful [12-14]. Preliminary studies have indicated that remineralizing solutions can effectively aid in the remineralization process of deep carious dentin due to its different ingredients as Xylitol which decreases acidity associated with bacteria. Also fluoride, calcium and phosphate ions aid in remineralization of tooth structure [15].

Based on the reported antibacterial effect and dentinal sealing ability of ozone, the use of Healozone in combination with a remineralizing solution in treatment of deep carious lesions seems to be promising.

The aim of this study was to evaluate the clinical changes in dentin of deep carious lesions in young permanent molars following ozone application with and without the use of a remineralizing solution using the stepwise excavation.

MATERIALS AND METHOD

The Ethical Committee at the Faculty of Dentistry, Alexandria University approved the research protocol.

Study Design

A randomized, split-mouth, controlled clinical trial was performed.

Sample Size Calculation

Sample size was estimated using the following assumptions: alpha error= 5%, beta error= 20%, DIAGNOdent reading in control group= 30, DIAGNOdent reading in test group= 20, common standard deviation= 10 [16]. The minimum required sample size was calculated (http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-Equality) to be 16 which was increased to 20 to make up for cases lost to follow up. The minimum required sample size per subgroup was thus set at 20.

Study Sample

A sample of 81 healthy children (46 males and 35 females) aged 7–9 years with a mean age of 8.25±0.69 years were selected for the present study. Each child had contralateral immature first permanent molars, with deep class I carious lesions. A total of 162 teeth (109 mandibular first permanent molars and 53 maxillary first permanent molars) were included in the study. Children were recruited from either those attending the clinic of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University, or children from different junior schools in Alexandria.

Inclusion Criteria

1. Bilateral vital asymptomatic carious first permanent molars with no pulp involvement, as evident by clinical examination (no spontaneous throbbing pain, no pain on percussion, no gingival redness or swelling, and no tooth mobility).
2. Cavitated class I carious lesions with opaque or discolored enamel exposing the dentin beneath according to the clinical severity index (CSI) score 4 of Ekstrand et al [17].
3. DIAGNOdent reading equal or more than 31(score 4) indicating deep dentinal caries [18].
4. Immature teeth with open apex, having no periapical pathosis as evident by periapical radiographs.
5. No proximal caries as evident by bitewing radiographs.
6. Patients are free from any systemic diseases that may affect the treatment prognosis.

Table 1 shows the used equipments, materials and their manufacturers.

Table 1. Equipments, materials and their manufacturers.

Equipments and materials	Manufacturers
HealOzone device	HealOzone KaVo Co., GmbH, D-88400 biberach/Riss-Germany
DIAGNOdent	DIAGNOdent KaVo Co, Biberach/ Riss, GmbH, D-88400 Germany
Remineralizing solution-pH balancer	PH Balancer:CurazoneInc USA, Inc.
Calcium Hydroxide paste -Dycal	Dycal: Dentsply Co. Rua Alice Herve, 86-25665-010-Petropolis-RJ.Brasile
OpalDam	Opaldam:Ultradent products USA.505 West 10200 South South Jordan, UT 84095 1.888.230.1420
One-step self-etching adhesive	Xeno III:Dentsply Co. De-Trey-Str.1 78467 Konstanz, Germany
Light activated composite	TPH Spectrum: Dentsply Co. Rua Alice Herve,86-25665-010-Petropolis-RJ.Brasile
Light activated glass ionomer cement lining	Vivaglass Liner. Ivoclar Vivadent AG FL-9494 Schaan/Liechtenstein. Germany

For each patient one tooth was treated with ozone gas only or the ozone gas followed by a remineralizing solution and the contralateral tooth was treated using calcium hydroxide ($Ca(OH)_2$) base material and served as control.

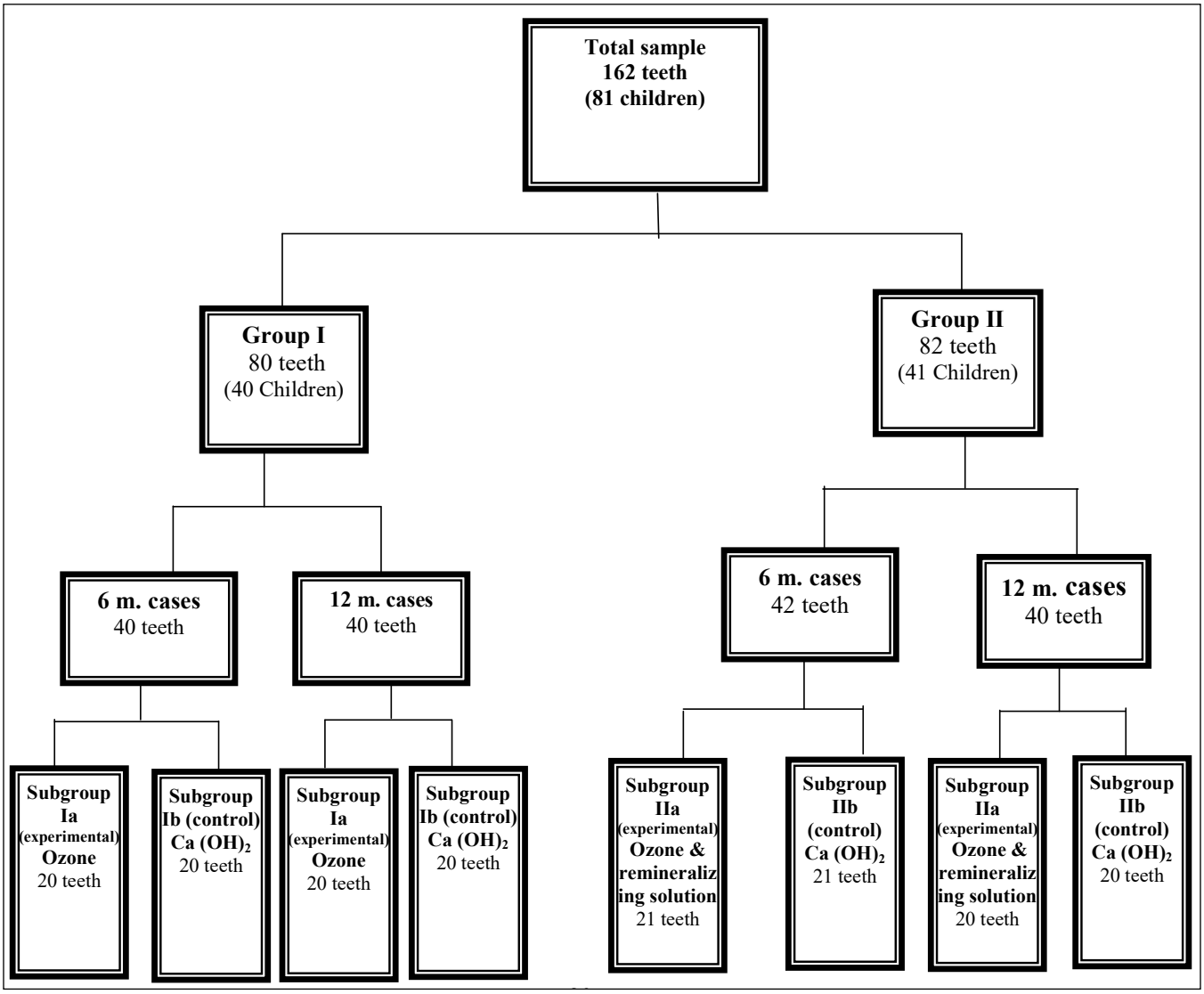
The selected teeth were randomly divided into 2 main groups according to the method of ozone treatment (Figure 1).

Group I: Consisted of 80 teeth, it was subdivided into 2 equal subgroups of 40 teeth each.

Subgroup Ia: Carious lesions were exposed to ozone gas (experimental group). Subgroup Ib: Carious lesions on the contralateral side were treated using $Ca(OH)_2$ base material (control group).

Group II: Consisted of 82 teeth, it was subdivided into 2 equal subgroups of 41 teeth each. Subgroup IIa: Carious lesions were exposed to ozone gas followed by the application of a

Figure 1. Description of the sample according to method of treatment and follow-up periods.



remineralizing solution (experimental group). Subgroup IIb: Carious lesions on the contralateral side were treated using $Ca(OH)_2$ base material (control group).

For each group, half of the cases were monitored for the clinical changes after 6 months and the other half after 12 months.

Prior to treatment, a written informed consent was obtained from the parents.

An operator performed the clinical procedures and an examiner who was blinded to the method of treatment performed the assessment of dentin color and consistency. The examiner was a faculty staff member from the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University. Intra-examiner agreement was determined using the Kappa statistic and was considered excellent (K=0.92).

Clinical Procedure

After application of topical anesthesia, local anesthesia was administrated; infiltration for the upper molars and nerve block for the lower molars. Complete isolation was performed using rubber dam and saliva ejector. All selected teeth were cleaned using a rotating bristle brush with copious of water. Stepwise excavation according to Bjorndal et al [6]: Caries from the enamel walls and dentino-enamel junction (DEJ) was removed using a sterile diamond fissure bur No.3 in a high speed handpiece. The central cariogenic biomass and only the superficial layer of the necrotic and infected dentin were removed, using a sharp excavator.

Baseline Assessment of Dentin Color and Consistency

After carious excavation, the color and consistency of the dentin were assessed for both experimental and control groups according to Bjorndal et al criteria [6].

The color of the demineralized central dentin was classified as either: (a) Light yellow, (b) Yellow, (c) Light brown, (d) Dark brown, (e) Black. The color classification was made by comparing the visual observation with photographs illustrating the five typical dentin color classes.

The consistency of the dentin was assessed using a sterile straight probe, according to the following criteria: (a) Very soft: probe penetrates dentin with easy fragment loss of demineralized tissue, (b) Soft: probe penetrates tissue with no resistance when removing probe, (c) Medium hard: slight resistance when removing probe, (d) Hard: comparable to unaffected dentin.

The DIAGNOdent device was used to assess the residual carious status before ozone treatment (baseline) according to the manufacturer's instructions [16]. A laser probe was used for scanning the dentin surface with slight pendulum movements in a buccolingual and mesiodistal direction. The probe was placed without pressure against the dentin surface. The value of the current measurement and the maximum value were displayed. The peak value was only recorded in the patient examination chart.

For subgroups Ia and IIa, ozone gas was applied for 40 seconds according to the manufacturer's instructions [19]. The unit was turned on using the power switch located on the left side of the rear panel. The appropriate delivery cup was selected according to the size of molar. The selected delivery cup was fixed to hand-piece head. The reset button was pressed to bring up cycle time screen. A 40 second dosage time was selected by pressing the TIME/PRIME button. A seal with the cup was created and maintained over the area of caries to be treated. The START button or foot control was pressed to start the vacuum pump inside the unit. If the integrity of the seal around the lesion was intact, then air was drawing through the hose, which in turn switched on ozone production. If the seal broke down for any reason, production of ozone ceased. The cup's position could be slightly repositioned, and once the seal had been re-established, the delivery of ozone would recommence. The count down on the display started. The unit bleeped; emitting brief, constant beeping sound every second. At the end of the timed ozone delivery, the unit continued to suck for 10 seconds before the complete sequence ends. A longer beep sounded when the ozone dosage time and the evacuation cycle was completed.

The DIAGNOdent device was used to evaluate the direct effect of ozone on the residual carious dentin (second reading).

In subgroup IIa, a dose of 2 ml HealOzone remineralizing solution was applied in the cavity for 5 seconds, and then dried briefly by air from a syringe [20].

In both subgroups Ia and IIa a temporary seal of a thin layer of OpalDam, light activated resin material, was applied to isolate the dentin surface for further examinations. It was cured for 20 seconds using a high intensity visible light source device. In both subgroups Ib and IIb, cavities were lined with $Ca(OH)_2$ base materials. The teeth were sealed temporarily using one-step self-etching adhesive and light activated composite in all subgroups according to the manufacturer's instructions.

Assessment of Dentin Color and Consistency After 6 and 12 Months

For both experimental and control subgroups, following local anesthesia administration, complete isolation and tooth polishing were performed. Composite restoration was completely removed and the cavity was re-opened using No. 3 sterile diamond fissure bur and sharp excavators. In the experimental subgroups, the OpalDam was removed to expose the carious dentin, while in the control subgroups, the $Ca(OH)_2$ was removed. The color and consistency of the residual carious dentin were reassessed.

The DIAGNOdent was used to evaluate the residual caries status at the end of the follow-up period of the 6th and 12th month cases (third reading in the experimental subgroups and second reading in the control subgroups).

If there was any soft dentin, final excavation was performed using sharp excavators or low speed rotating round carbide burs. The ozone gas was reapplied for 40 seconds and the cavity was sealed with light activated glass ionomer lining cement. Then, the cavity was permanently restored with one-step self-etching adhesive and the light activated composite.

Statistical Analysis

Descriptive statistics were displayed as frequencies and percents for qualitative variables (color and consistency), and means and standard deviations for quantitative variables (DIAGNOdent readings). Comparison of nominal and ordinal variables between the two groups was done using chi square, and Mann Whitney U test respectively, while comparison of the quantitative variables was done using t test, or Mann Whitney (after Kolmogrov Smirnov test proved they were not normally distributed). Comparisons of subgroups and changes across time in the same subgroup was done using Wilcoxon signed ranks test (for ordinal variables or non-normally distributed quantitative variables), or paired t test (for normally distributed quantitative variables) [21].

RESULTS

Sample Distribution

Table 2 shows sample distribution according to method of treatment and time of evaluation. Group I consisted of 80 teeth. It was subdivided into 2 equal subgroups of 40 teeth each, in which 40 carious teeth were treated using ozone (test subgroup Ia) and 40 teeth (control subgroup Ib) were treated using $Ca(OH)_2$. For each subgroup, 20 cases were monitored for the clinical after 6 months and the other 20 cases for 12 months. Group II consisted of 82 teeth. It was subdivided into 2 equal subgroups of 41 teeth each, in which 41 carious teeth were treated using ozone followed by remineralizing solution (test subgroup IIa) and the other 41 teeth (control subgroup IIb) were treated using $Ca(OH)_2$. For each subgroup, 21 cases were monitored after 6 months and the other 20 cases after 12 months.

Table 2. Sample distribution according to method of treatment and time of evaluation.

Description			Group I 40 cases (80 teeth)				Group II 41 cases (82 teeth)	
Subgroups	Time of evaluation	Tooth No.	n (%)				n (%)	
Test subgroup a	6 months	16	Ozone Cases	20 teeth	2 (10)	Ozone / remineralizing Cases	21 teeth	6 (28.6)
		26			1 (5)			2 (9.5)
		36			10 (50)			3 (14.3)
		46			7 (35)			10 (47.6)
	12 months	16		20 teeth	1 (5)		20 teeth	6 (30)
		26			4 (20)			5 (25)
		36			6 (30)			6 (30)
		46			9 (45)			3 (15)
Control subgroup b	6 months	16	$Ca(OH)_2$ Cases	20 teeth	1 (5)	$Ca(OH)_2$ Cases	21 teeth	2 (9.5)
		26			2 (10)			6 (28.6)
		36			7 (35)			10 (47.6)
		46			10 (50)			3 (14.3)
	12 months	16		20 teeth	4 (20)		20 teeth	4 (20)
		26			1 (5)			6 (30)
		36			9 (45)			4 (20)
		46			6 (30)			6 (30)

Table 3 shows sample distribution according to DIAGNOdent readings. There were no significant differences between group I and II among test and control 6 and 12 month-subgroups in the initial DIAGNOdent readings recorded during selection of the cases ($p > 0.05$). This insignificant difference between the subgroups in group I and II indicated that the selected teeth were comparable concerning caries extension.

Table 3. Sample distribution according to DIAGNOdent readings.

Subgroups	Time of evaluation	Group I (Mean ± SD)	Group II (Mean ± SD)	T test P value
Test subgroup a DIAGNOdent readings	6 months	73.65 ± 24.44	74.19 ± 22.11	0.69 0.50 NS
	12months	77.55 ± 25.42	82.70 ± 21.67	0.07 0.94NS
Control subgroup b	6 months	66.35 ± 23.26	61.48 ± 24.36	0.66 0.52 NS
DIAGNOdent readings	12 months	72.10 ± 26.01	76.00 ± 23.24	0.50 0.62 NS

T test: between group I and group II.

NS: Not statistically significant.

Dentin Color Assessment

Figure 2 shows the distribution of group I cases (test and control) with different dentin color at baseline directly after cavity preparation and first step of caries excavation (First dentin assessment) and at the final assessment of the 6 and 12 month-cases. For 6-month test-cases, the percentage of ozone-treated teeth recorded of yellow color dentin increased from 15% at base line to 20% after 6 months, that of brown color from 60% to 65%. However, teeth having black color decreased from 25% to 15%. The difference in dentin color between baseline and after 6 months was not statistically significant (P=0.13).

As regards the $Ca(OH)_2$ control subgroup Ib, the percentage of cases showing yellow dentin color decreased from 15% at base line to 10% after 6 months, that of the brown from 50% to 45%. Cases with black dentin color increased from 35% to 45%. There was no statistically significant difference between base line and after 6 months regarding different dentin colors (P=0.06). When comparing between experimental and control cases, the difference was not statistically significant at baseline (P= 0.85), whereas it was significant after 6 months (P=0.03).

For 12 month-test subgroup, cases showing yellow dentin color decreased from 30% at base line to 20% after 12 months. Cases with brown dentin color increased from 30% to 45%. Cases with black dentin color decreased from 40% to 35%. There was no statistically significant difference between base line and after 12 months regarding different dentin colors (P=0.34).

Concerning the control subgroup, cases showing yellow dentin color decreased from 35% cases at base line to 30% after 12 months, while the brown color increased from 40% to 50%. Cases recorded with black dentin color decreased from 25% to 20%. There was no statistically significant difference between base line and after 12 months as regards distribution of cases with different dentin colors (P=0.13). The differences between experimental and control for 12 month-cases were not statistically significant at base line (P=0.43) and after 12 months (P=0.27).

Figure 3 shows the distribution of group II cases (test and control) with different dentin color at baseline directly after cavity preparation and first step of caries excavation (First dentin assessment) and at the final assessment of the 6 and 12 month-cases. For 6 month-test cases, the percentage of ozone/remineralizing solution-treated teeth with yellow dentin color increased from 23.81% at base line to 28.6% after 6 months, while that of the brown color decreased from 57.14% to 47.6%. Cases having black color increased from 19.05% at baseline to 23.8% after 6 months. There was no statistically significant difference between base line and after 6 months regarding different dentin colors (P=0.26).

Figure 2. The distribution of group I cases (test and control) regarding the different dentin color at baseline and after 6 and 12 months.

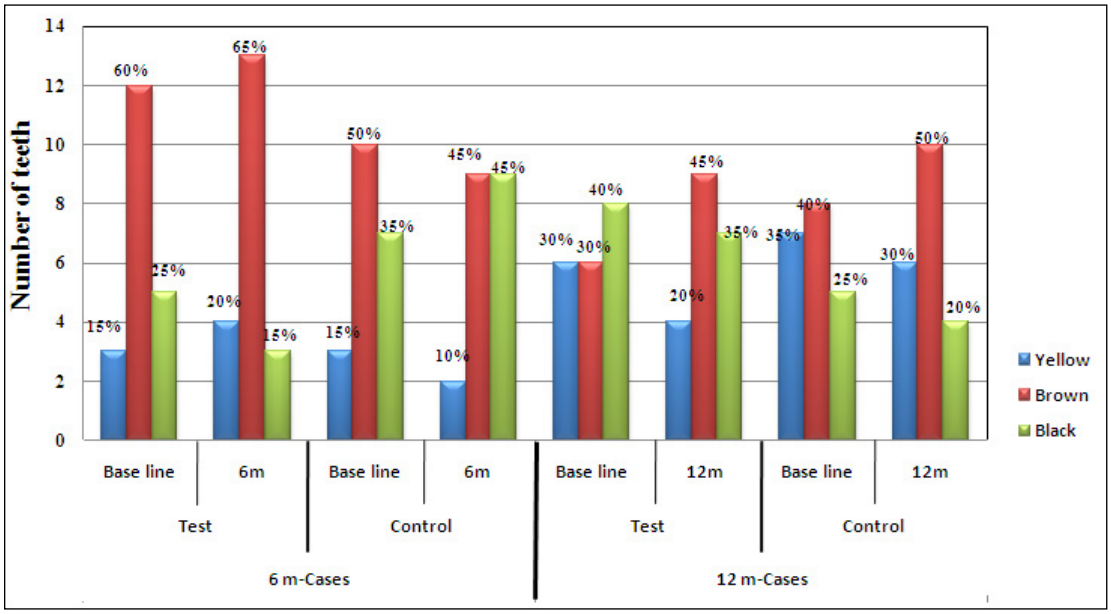
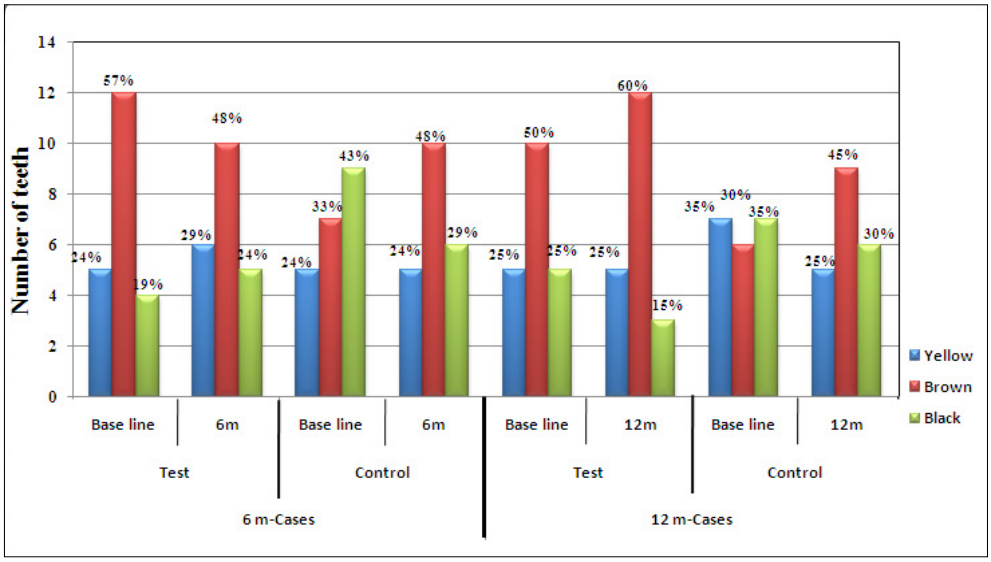


Figure 3. The distribution of group II cases (test and control) regarding the different dentin color at baseline and after 6 and 12 months.



Regarding the $Ca(OH)_2$ control subgroup IIb, the percentage of cases showing yellow dentin color at baseline (23.8%) did not change after 6 months. Cases with brown dentin color increased from 33.3% at base line to 47.6% after 6 months, while they decreased from 42.9 % to 28.6% for the black color. The difference in color distribution between baseline and after 12 months was not statistically significant (P=0.20). When comparing the experimental subgroup with its control in group II, there were no statistically significant differences at baseline (P=0.47) and after 6 months (P=0.84).

For 12 month-test cases, the percentage of teeth (25%) showing yellow dentin color did not change after 12 months. Cases showing brown dentin color increased from 50% at baseline to 60% after 12 months, while the black color ones decreased from 25% to 15%. There was no statistically significant difference between base line and after 12 months regarding different dentin colors (P=0.53).

Concerning the control subgroup IIb, cases showing yellow dentin color decreased from 35% at base line to 25% after 12 months. Cases showing brown dentin color increased from 30% to 45%. Black dentin color decreased from 35 % to 30%. The difference in dentin color distribution between baseline and after 12 months was not statistically significant (P=0.10). When comparing the experimental subgroup with its control in group II, there were no statistically significant differences at baseline (P= 0.82) and after 12 months (P=0.45).

Regarding comparison of dentin color between experimental subgroups Ia and IIa at baseline and at 6 and 12 month-follow up, the percentages of teeth recorded with different dentin color were not significantly different at the first dentin assessment between the 6 month-cases and the 12 month ones in subgroups Ia (P=0.18) and subgroups IIa (P=0.32).

When comparing between the second dentin color assessment, no significant differences were observed between the 6 month and 12 month-cases in test subgroup Ia (P=0.66) and subgroup IIa (P=1.00). In addition, no significant differences were also found between experimental subgroups Ia and IIa at baseline (P=0.12 and 0.14), after 6 months (P=0.37) and after 12 months (P=0.76).

Dentin Consistency Assessment

Figure 4 shows the distribution of group I cases (test and control) according to dentin consistency at base line after first step of caries excavation (First dentin assessment) and after 6 and 12 months. For 6 month-subgroup Ia, the percentages of ozone-treated teeth recorded with medium hard dentin decreased from 80% at the base line to 55% after 6 months. Cases having hard dentin consistency increased from 20% at the base line to 45% after 6 months. No soft dentin cases were reported at base line or after 6 months. There was no statistically significant difference between base line and after 6 months regarding different dentin consistency (P= 0.16).

As regards the control subgroup Ib, soft dentin was not observed 6 months after $Ca(OH_2)$ treatment, although 5% of cases were soft at the base line. For medium hard dentin, no change was noted after 6 months. Cases with hard dentin increased from 40% to 45% after 6 months. There was no statistically significant difference between base line and after 6 months as regards the distribution of cases with different dentin consistency (P=0.41). The differences between the experimental and control for 6 month-cases were not statistically significant at baseline (P= 0.32) and after 6 months (P=0.76).

For 12 month-test subgroup Ia, soft dentin was not recorded at base line or after 12 months. The percentage of medium hard dentin increased from 40% at base line to 55% after 12 months. Cases with hard dentin decreased from 60% to 45% at the end of the follow-up. The difference was not statistically significant between baseline and after 12 months (P=0.08).

Concerning the control subgroup Ib, soft dentin was not reported at base line or after 12 months. The medium hard dentin increased from 45% at base line to 50% after 12 months. Cases with hard dentin decreased from 55% to 50%. There was no statistically significant difference between base line and after 12 months as regards the distribution of cases with different dentin consistency (P=0.56). The differences between experimental and control after 12 months were not statistically significant at baseline (P= 0.56) and after 12 months (P=0.32).

Figure 5 show the distribution of group II cases (test and control) regarding dentin consistency at base line and after 6 and 12 months. For 6-month test subgroup II, soft dentin was not reported at base line and after 6 months. Cases having medium hard dentin decreased from 57.1% at base line to 42.9% after 6 months. Cases with hard dentin increased from 42.9% at baseline to 57.1% after 6 months. There was no statistically significant difference between base line and after 6 months regarding different dentin consistency (P= 0.08).

Concerning the control subgroup II, soft dentin cases remained unchanged till 6 months (4.8%). Cases with medium hard dentin increased from 47.6% at base line to 52.3% after 6 months, while hard dentin cases decreased from 47.6% to 42.9%. There was no statistically significant difference between base line and after 6 months as regards the distribution of cases with different dentin consistency (P=0.74). The differences between experimental and control for 6 month cases were not statistically significant at baseline (P= 1.00) and after 6 months (P=0.66).

For 12 month-test sub group IIa, although 10% of the cases had soft dentin at base line, no soft dentin was reported after 12 months. Medium hard dentin remained unchanged, while hard dentin cases increased from 40% at baseline to 50% after 12 months. There was no statistically significant difference between base line and after 12 months regarding different dentin consistency (P= 0.013).

As regards the control subgroup IIb, although 5% of the cases had soft dentin at base line, it was not detected at the end of the follow-up. Cases with medium hard dentin increased from 45% to 55%, while the hard dentin decreased from 50% to 45%. There was no statistically significant difference between base line and after 12 months as regards distribution of cases with different dentin consistency (P=0.76). The differences between experimental and control for 12 month-cases in subgroups II were no statistically significant at baseline (P= 0.26) and after 12 months (P=0.66).

Regarding comparison of dentin consistency between test subgroups Ia and IIa at baseline and at 6 and 12 month-follow-up,

Figure 4. The distribution of group I cases (test and control) regarding dentin consistency at baseline and after 6 and 12 months.

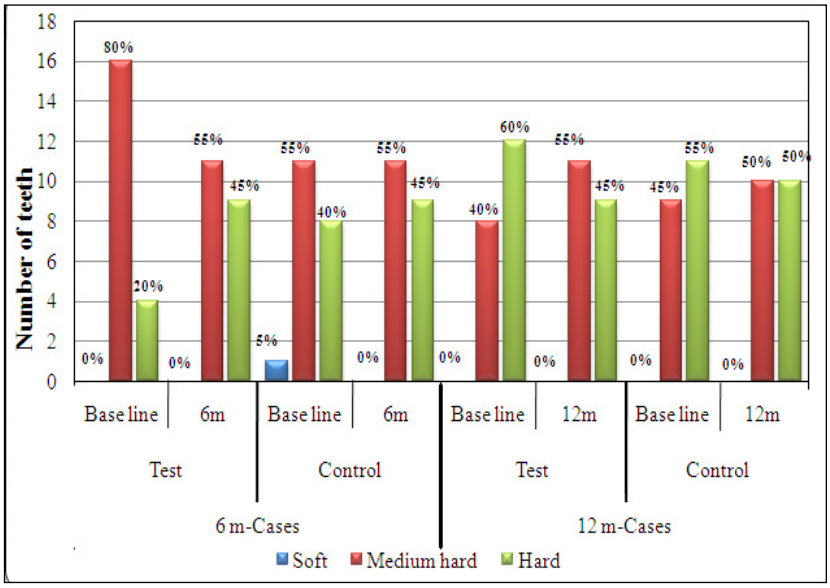
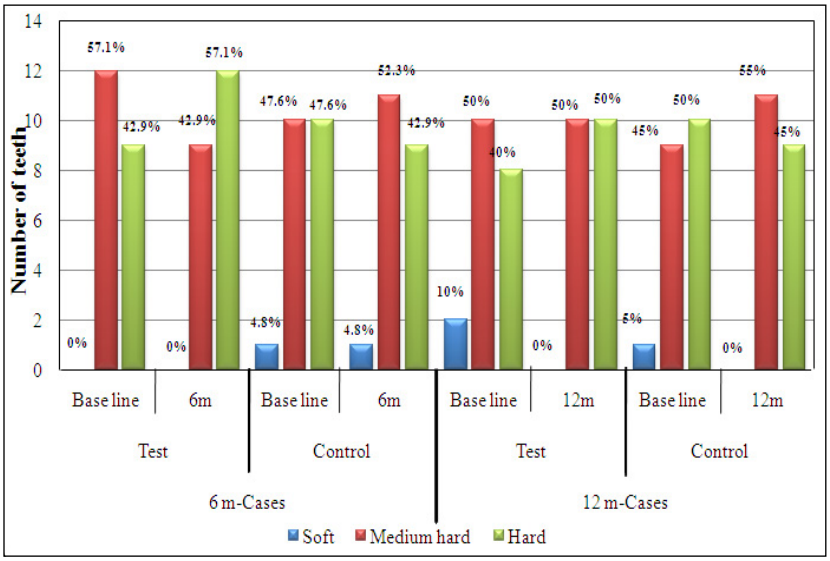


Figure 5. The distribution of group II cases (test and control) regarding dentin consistency at baseline and after 6 and 12 months.



the percentages of teeth recorded with different dentin consistency were not significantly different at the first dentin assessment between the 6 month-cases and the 12 month ones in subgroups Ia (P=0.08) and subgroup IIa (P=0.13).

When comparing between the second dentin consistency assessment, no significant differences were observed between the 6 month and 12 month-cases in test subgroup Ia (P=0.16) and subgroup IIa (P=0.08). In addition, no significant differences were also found between experimental subgroups Ia and IIa at baseline (P=0.12 and 0.14), after 6 months (P=0.37) and after 12 months (P=0.76).

DIAGNOdent Assessment

Table 4 shows comparison of mean DIAGNOdent readings between experimental and control in group I at baseline after the first step of caries excavation and at the final assessment of the 6 and 12 month-cases. For 6 month-test subgroup Ia, the mean DIAGNOdent readings decreased from 81.85±20.43 at the base line before ozone application to 58.35±29.89 after 6 months. There was a statistically significant difference between base line and at the final assessment after 6 months as regards to DIAGNOdent readings (P=0.003). Concerning the control subgroup Ib, the mean of DIAGNOdent readings decreased from 84.65±21.13 at the base line to 72.95±31.18 after 6 months. There was no statistically significant difference between base line and after 6 months (P= 0.16). When comparing the mean DIAGNOdent values between experimental and control cases, no significant difference was observed at base line (P=0.68), while the difference was statistically significant at the final assessment after 6 months (P=0.04).

In experimental subgroup Ia, the mean DIAGNOdent values of the 12 month-cases decreased from 75.70±25.22 at the base line to 67.10±26.13 after 12 months. There was no statistically significant difference between the mean DIAGNOdent readings at base line and after 12 months (P=0.19). Concerning the control subgroup Ib, the mean DIAGNOdent values decreased from 75.50±23.67 at the base line to 68.10±28.34 after 12 months. There was no statistically significant difference between base line and after 12 months (P= 0.28). The differences between experimental and control cases were not statistically significant at baseline (P= 0.98) and after 12 months (P= 0.91).

Table 5 shows comparison of mean DIAGNOdent readings between experimental and control in group II at baseline and after 6 and 12 months. For 6 month-test subgroup IIa, the mean DIAGNOdent readings decreased from 80.29±25.34 at the base line to 63.00±28.61 after 6 months. There was a statistically significant difference between the mean DIAGNOdent readings at base line and after 6 months (P=0.042). Regarding the control subgroup IIa, the mean DIAGNOdent values decreased from 77.38±24.63 at the base line to 73.48±35.35 after 6 months. There was no statistically significant difference between base line and after 6 months (P= 0.39). The differences between test and control cases were not statistically significant at baseline (P= 0.69) and after 6 months (P= 0.14).

In experimental subgroup IIa, the mean DIAGNOdent readings decreased from 76.40±24.21 at the base line to 67.70±32.20 after 12 months. No significant difference was observed between the mean DIAGNOdent readings at base line and at the final assessment after 12 months (P=0.13). As for the control subgroup IIb, the mean DIAGNOdent readings increased from 70.15±27.27 at the base line to 74.85±30.09 after 12 months. There was no statistically significant difference between base line and after 12 months (P= 0.41). The differences between experimental and control cases was no statistically significant at baseline (P= 0.20) and after 12 months (P= 0.34).

Table 4. Comparison of mean DIAGNOdent readings between experimental and control in group I at different evaluation periods.

Group I	Baseline DIAGNOdent readings Mean ± SD	Final DIAGNOdent readings Mean ± SD	Paired t test$_1$ P value
6m-cases			
Experimental subgroup Ia (ozone –treatment)	81.85 ± 20.43	58.35 ± 29.89	3.35 0.003*
Control subgroup Ib ($Ca(OH)_2$)	84.65 ± 21.13	72.95 ± 31.18	1.45 0.16NS
Paired t test$_2$ P value	0.42 0.68NS	2.17 0.04*	
12m-cases			
Experimental subgroup Ia (ozone –treatment)	75.70 ± 25.22	67.10 ± 26.13	1.38 0.19 NS
Control subgroup Ib ($Ca(OH)_2$)	75.50 ± 23.67	68.10 ± 28.34	1.10 0.28 NS
Paired t test$_3$ P value	0.03 0.98 NS	0.12 0.91 NS	

Paired t test$_1$: between the baseline and final DIAGNOdent values.

Paired t test$_2$: between subgroup Ia and subgroup Ib 6m-cases.

Paired t test$_3$: between subgroup Ia and subgroup Ib 12 m-cases.

NS: Not statistically significant.

* Statistically significant at P≤0.05.

Regarding comparison between mean DIAGNOdent readings before, directly after ozone application, and at the final assessment in ozone and ozone/remineralizing solution-treated teeth; in subgroup Ia 6 month-cases, the mean DIAGNOdent reading decreased from 81.85±20.43 at the base line to 74.55±26.06 directly after ozone application and 58.35±29.89 after 6 months. There was no significant difference between base line DIAGNOdent reading and directly after application of ozone (P=0.15), whereas the difference was statistically significant between the reading directly following ozone treatment and after 6 months (P= 0.03). As for subgroup Ia 12 month-cases, the mean DIAGNOdent reading decreased from 75.70±25.22 at the base line to 71.50±25.22 directly after ozone application and 67.10±26.13 after 12 months. No significant differences were found between DIAGNOdent readings at different evaluation time, where P=0.32 and 0.51. When comparing the DIAGNOdent values between the 6 month and 12 month-cases, no significant differences were observed at baseline (P= 0.40), directly after ozone application (P= 0.71) and at the final assessment (P= 0.33).

For subgroup IIa 6 month-cases, the mean DIAGNOdent values increased from 80.29±25.34 at the base line to 83.33±25.65 directly after ozone application, while it decreased to 63.00±28.61 after 6 months. There was no significant difference between base line DIAGNOdent reading and directly after application of ozone (P=0.63), whereas the difference was statistically significant between the reading directly following ozone treatment and after 6 months (P= 0.03). For subgroup IIa 12 month-cases, the mean DIAGNOdent readings increased from 76.40±24.21 at base line to 80.55±25.58 directly after ozone application, while it decreased to 67.70±32.20 after 12 months with no significant differences between the readings (P=0.17 and 0.08). The differences between 6 and 12 month-cases in experimental subgroups IIa were not statistically significant difference at baseline (P= 0.62), directly after ozone application (P= 0.73) and after 12 months (P= 0.62).

Table 6 shows comparison of percent change between mean DIAGNOdent values among test subgroups of the 6 and 12 month-cases. For 6 month-cases, the percent changes between mean DIAGNOdent values before and directly after ozone application were -7.84±26.24% for test subgroup Ia, and -1.11 ± 28.27% for test subgroup IIa, with no significant difference between the subgroups (P=0.45). When comparing between DIAGNOdent values directly following ozone application and after 6 months, the percent changes were -26.62 ±37.26% for test subgroup Ia, and -21.14 ± 51.72% for test subgroup IIa with no significant difference between the subgroups (P=0.86). The differences between the percent change 1 and 2 in mean DIAGNOdent values were statistically significant for both test subgroups Ia and IIa (P=0.02 and 0.03 respectively). For 12 month-cases, the percent changes between mean DIAGNOdent values before and directly after ozone application were -2.39 ± 25.20% for test subgroup Ia, and 6.40 ± 21.55% for test subgroup IIa, with no significant difference between the subgroups (P=0.14). When comparing between DIAGNOdent values directly following ozone application and after 12 months, the percent changes were 1.89 ±80.73% for test subgroup Ia, and -10.80±44.78% for test subgroup IIa, with no significant difference between the subgroups (P=0.78). The differences between the percent change 1 and 2 in mean DIAGNOdent values reveals no significant changes for both test subgroups Ia and IIa (P=0.33 and 0.08 respectively).

Table 5. Comparison of mean DIAGNOdent readings between experimental and control in group II at different evaluation periods.

Group II	Baseline DIAGNOdent readings Mean ± SD	Final DIAGNOdent readings Mean ± SD	Paired t test$_1$ P value
6m-cases			
Experimental subgroup IIa (ozone / remineralizing solution)	80.29 ± 25.34	63.00 ± 28.61	2.02 0.042*
Control subgroup IIb ($Ca(OH)_2$)	77.38 ± 24.63	73.48 ± 35.35	0.66 0.39 NS
Paired t test$_2$ P value	0.41 0.69NS	1.56 0.14NS	
12m-cases			
Experimental subgroup IIa (ozone / remineralizing solution)	76.40 ± 24.21	67.70 ± 32.20	1.62 0.13 NS
Control subgroup IIb ($Ca(OH)_2$)	70.15 ± 27.27	74.85 ± 30.09	0.84 0.41NS
Paired t test$_3$ P value	1.32 0.20NS	0.98 0.34NS	

Paired t test$_1$: between the baseline and final DIAGNOdent values.

Paired t test$_2$: between subgroup IIa and subgroup IIb 6m-cases.

Paired t test$_3$: between subgroup IIa and subgroup IIb 12 m-cases.

NS: Not statistically significant.

* Statistically significant at P≤0.05.

Table 6. Comparison of percent change between mean DIAGNOdent values of 6 and 12m-cases among test subgroups.

% change in DIAGNOdent values	Test subgroup Ia Mean ± SD	Test subgroup IIa Mean ± SD	MWU test P value
6m-cases			
% change 1	-7.84 ± 26.24	-1.11 ± 28.27	0.75 0.45NS
% change 2	-26.62 ± 37.26	-21.14 ± 51.72	0.18 0.86NS
WSR Test	2.39	2.21	
P value	0.02*	0.03*	
12m-cases			
% change 1	-2.39 ± 25.20	6.40 ± 21.55	1.47 0.14NS
% change 2	1.89 ± 80.73	-10.80 ± 44.78	0.29 0.78NS
WSR Test	0.97	1.73	
P value	0.33NS	0.08NS	

Percent change$_1$: between values before and directly after ozone application.

Percent change$_2$: between values directly following ozone application and at the final assessment.

NS: Not statistically significant.

* Statistically significant at P≤0.05.

MWU test: Mann Whitney U test.

WSR test: Wilcoxon signed ranks test.

DISCUSSION

The operative tradition or the classical caries excavation is to remove the softened dentin in order to eliminate infected tissue. This approach assumes that both the biofilms and the microorganisms within the carious dentin drive the caries process. In fact, it is not possible to eliminate all the microorganisms because a few will remain, even if all soft dentin is removed [22]. Over the pulpal surface, contemporary trends recommended that carious dentin that is 'firm and leathery' should be left, where its removal might expose the pulp [23]. For this reason, other new techniques such as the use of ozone may help in elimination of infection in this firm dentin layer; prevent pulp exposure and simplify the treatment plan.

For all patients in this study, deep dentinal lesions were diagnosed initially by visual examination using score 4 CSI according to Ekstrand *et al* [16]. They found that, when the carious lesion appeared clinically as cavitations in opaque or discolored enamel with exposing dentin beneath, the histological picture was demineralization and heavily infected dentin, up to its inner third layer. Visual examination was selected as it is non-invasive, clinically acceptable and the least expensive diagnostic method. It also preserves the surface structure of the tooth comparing to tactile examination, which may transfer microorganisms from one site to another, with possibility of further spread of the disease [24].

A visual diagnosis alone has low sensitivity (ability to correctly identify decayed surfaces) for detection of carious lesions, even though the specificity (ability to correctly identify sound surfaces) is high [25]. For this reason, the DIAGNOdent was selected as an adjunctive diagnostic method to the visual examination, to combine the high specificity of visual examination with the high sensitivity of DIAGNOdent [26]. In addition, both visual examination and DIAGNOdent were also used to evaluate the clinical changes in the deep dentinal lesions, following ozone treatment in the different evaluation periods.

In the present study, DIAGNOdent was used to choose cases with reading equal or more than 31 indicating deep dentinal caries [17]. No significant differences were found among the experimental and control subgroups in both groups, in the initial DIAGNOdent values recorded during case selection. These results indicated that cases selected had comparable degree of caries involvement.

The concept of this study in using the stepwise excavation was based on the results of several previous studies [6,27]. They found that, stepwise technique reduces the risk of pulp exposure, controls caries progression and promotes dentin-pulp complex reaction. According to Bjorndal *et al* [6], the first important step in declaration of lesion progression was the initial removal of the cariogenic microbial biomasses in the cavity, together with infected superficial necrotic dentin.

This study was conducted on young first permanent molars, with deep dentinal caries of children, aged from 7-9 year-old. Young permanent teeth in this age are cellular and have a better healing potential than such teeth in adult patients [28]. Accordingly, the pulp-dentin complex would better respond to all procedures performed in the dentin [29].

The ability to treat a carious lesion without the need of amputation of the diseased tissue would be one of the greatest achievements in the history of dentistry. Ozone therapy has the potential to move toward this goal. The ozone's oxidative reaction can destroy bacteria by oxidizing bacterial cell walls and membranes. This has a disruptive effect on the bacterial population in the carious lesion, which may result in swinging the equilibrium in favor of remineralization [30,31].

Ozone was used alone or followed by a remineralizing solution. The rational of its use was to neutralize any possible residual ozone, and to act as initiating agent to facilitate the remineralization process. This might be useful for the conditioning of the tooth substrate, after elimination of the microflora to start the healing process by remineralization [32].

Split-mouth design was used, so that patient served as their own control. In the control subgroups, $Ca(OH)_2$ was used as a gold standard capping material of indirect pulp treatment [33]. Stepwise excavation technique using calcium hydroxide has proved to be a suitable and safe method in management of deep dentinal lesions without pulpal symptoms [6].

Change in Dentin Color and Consistency

While analyzing the results of dentin color and consistency, the light yellow and yellow dentin cases were combined together, as well as the light brown and brown cases to render the results of the statistical analysis more relevant. For the same reason, the very soft and soft dentin consistencies were combined together.

After ozone application with or without using a remineralizing solution in subgroups Ia and IIa, there were no significant differences between the baseline and after 6 or 12 month periods, regarding dentin color and consistency. This might be due to the different remineralized and demineralized reactions of the remaining carious

dentin. The changes in the dentin color and consistency might be contributed to the oxidative effect of ozone on the protein biomolecules in the carious dentin [34], amongst these cariogenic molecules are formic and pyruvic acids. These acids contribute to the decreased pH values associated by inhibiting the precipitation of the minerals [30]. Pyruvic acid, that is oxidized by ozone forms acetate and carbon dioxide compounds that cause rise in pH values of the lesion [31] thus enhance the remineralization of dentin and caries reversal.

The improvement in dentin consistency might also be related to the stepwise or biologically based excavation and sealing of the cavity. Control of the local environment has been found to reduce the substrate for bacteria, decrease lesion progression, thus promoting dentin sclerosis and a reactionary response with tertiary dentin formation [35]. The deposition of tertiary dentin increases the distance between the affected dentin and the pulp, while the deposition of peritubular (sclerotic) dentin, decreases its permeability [36]. Moreover, Wambier *et al* [37], suggested that sealing the cavity contributed to the remineralization process. He added that remineralization occurred in the inner carious dentin, where the living odontoblasts provided calcium phosphate to the vital pulp tissue.

Regarding the $Ca(OH)_2$ control group, no significant differences were also reported between base line and after 6 or 12 month-follow-up periods, concerning dentin color and consistency. Even though, there was no significant change in the dentin, the remaining carious dentin improved and hardened. Possible explanations for this observation are the increase in the mineral content of the underlying dentin, as well as the chemical effect of $Ca(OH)_2$ on the soft demineralized dentin [38]. The therapeutic effect of $Ca(OH)_2$ liner is dependent on its dissociation into calcium and hydroxide ions. This causes high alkalinity, which would activate adenosine triphosphate (ATP) activity [39] which could be responsible for subsequent remineralization and healing of the remaining affected dentin [40]. A study by Graham et al [41], found that $Ca(OH)_2$ liners are able to release the transforming growth factors (i.e.TGF-β) superfamily, trapped within the dentin matrix. These can then diffuse down the dentinal tubules, promoting a reactionary response in the underlying odontoblasts.

When comparing the effect of ozone application with $Ca(OH)_2$ treatment, there was no significant difference after 12 months. The reported changes in dentin color and consistency throughout the study might be due to the combination of the control of the local environment, as well as the remineralization of dentin. These changes in clinical dentin criteria appeared to be effective in improving the remaining remineralizable dentin, whether treated with ozone with or without a remineralizing agent, as well as the use of $Ca(OH)_2$ liners.

However, a significant difference was observed only in dentin color between the experimental subgroup Ia (ozone per se) and its control (subgroup Ib) after 6 months. This may indicate that $Ca(OH)_2$ had an effect on arresting the caries process, and enhancing dentin remineralization in contrast to the ozone. According to De Munck *et al* [42], ozone has a dehydration effect on dentin that may result in decreased dentin wettability, thus altering its substrates. Other explanation could be related to the inhibitory effect of ozone gas on metabolic activity of the cells [43], thus affecting the reactionary response of the pulp-dentin complex.

In contrast to our results, Dahnhardt *et al* [44], found significant improvement in the hardness of open single-surface lesion in primary teeth after 2, 4, 6 and 8 months in the ozone treated lesions compared with the base line and control lesions. This may be related to differences in the type of dentitions, depth and size of the cavity, and repeated application of the ozone five times during the procedure.

In the experimental subgroup II, when a remineralizing solution was used with the ozone gas, dentin still did not show significant changes. This might be due to insufficient time of ozone and remineralizing solution application on the dentin surface, in relation to the size of the carious lesion. Polydorous *et al* [45], found that 80 seconds ozone application was more effective on the carious dentin than 40 seconds. According to AbuNaba'a *et al* [11], ozone has the ability to remove organic materials such as proteins from demineralizing dentin, thus improving the permeability of the lesion to the remineralizing solution, and allowing caries reversal.

Changes in DIAGNOdent Value Following Ozone Application

When analyzing the results of DIAGNOdent readings directly after ozone application, as well as after 6 and 12 months in comparison to the base line, no significant differences were observed. However, the DIAGNOdent readings significantly decreased 6 months after ozone application in subgroups Ia and IIa in comparison to baseline values. Similar findings were also reported when comparing the values between readings directly after ozone application, and those after 6 months in subgroups Ia and IIa with -26.62±37.26 and -21.14±51.72 percent reduction respectively. This might be the result of increase dentin hardness. This finding was also observed clinically, where cases having hard dentin increased from 20% at base line to 45%, 6 months after ozone treatment. For cases treated with ozone and remineralizing solution, they increased from 42.9% at base line to 57.1%, after 6 months.

In the present study, the insignificant difference in the DIAGNOdent values in most subgroups might be attributed to proximity of the residual dentin to the pulp of the young permanent teeth. Iwami *et al* [46], found that DIAGNOdent values were influenced by the internal structure of dentin, when the thickness of sound dentin was less than 0.2-0.3 mm. Iwami *et al* [47] and Krause *et al* [48] suggested that laser fluorescence reading increases as the resistance of the residual dentin decreases through tooth demineralization leading to increase porosity, in addition to the influence of pulp tissue itself in deep cavities. The laser fluorescence values could also be affected by the transmission and scattering properties of the deep dentin, which differ according to the direction of the tubules [46], the diameter of apatite crystals [49], and the higher organic contents of dentin near the pulp [48]. The denaturing organic materials in the carious dentin, in addition to that emitted from bacterial metabolites [47] would therefore reflect the increased fluorescence reported in deep lesions.

Iwami *et al* [47] also reported that the DIAGNOdent values may not always correspond to the presence of bacteria in the dentinal tissues. The results of their study showed that, when the DIAGNOdent values were between 10 and 50, the rates of bacterial detection increased, as the DIAGNOdent values increased. When the DIAGNOdent values are less than 10 and more than 50, the rates of bacterial detection are 0% and 100% respectively. In the present study the mean DIAGNOdent values were higher than 50 in the four subgroups, indicated that the correlation between the DIAGNOdent values and the amount of bacteria would be unreliable.

The current study showed a decrease in DIAGNOdent readings in the final assessment, which might be related to the increase in dentin hardness. Other possibility is that tertiary dentin formation increases the distance between the surface of the lesion and the pulp [35]. Also, the deposition of sclerotic dentin decreases the permeability [50] and lead to decrease in DIAGNOdent readings.

The results of the present study are in line with Dahnhardt *et al* , [44] who found decreased DIAGNOdent values over different periods of time in open single surface lesions following ozone application. This decrease was not statistically significant. Furthermore, laser fluorescence measurements demonstrate low specificity, meaning that sound sites may be incorrectly diagnosed as carious [51].

In the present study, there was a significant difference in DIAGNOdent readings between the ozone treated teeth and their $Ca(OH)_2$ controls after 6 months. This difference might be related to the presence of more black dentin cases in control subgroups (9 out of 20) than in experimental ones (3 out of 20) after 6 months. A possible explanation for the increase discoloration observed with the $Ca(OH)_2$ subgroups could be related to its effect on dentin remineralization. According to clinical observations by Bjorndal *et al* [6], the arrested lesions are characterized by deep pigmentation and a hard surface. The correlation between the increase in DIAGNOdent reading with increase in darkness of the dentin color is in agreement with Bamzahim *et al* [52] and Souza-Zaroni *et al* [53], who reported that discoloration or stains could be the source of high rate of false positives obtained with laser fluorescence and the consequent reduction in its specificity.

CONCLUSIONS

Ozone application with or without a remineralizing solution through the stepwise excavation had no significant effect on dentin color, and consistency in young permanent molars. DIAGNOdent was unreliable as a diagnostic tool in monitoring caries activity, following treatment of deep dentinal lesions.

RECOMMENDATIONS

A long-term evaluation of cultivable microflora following ozone application in dentinal lesions needs to be done.

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