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ORIGINAL ARTICLE

# A clinical and microbiological comparative study of deep carious lesion treatment in deciduous and young permanent molars

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**Abstract** The aim of this study was to compare one-visit indirect pulp treatment (IPT), two-visit IPT, and direct complete excavation (DCE) of deciduous and young permanent molars with deep carious lesions from clinical and microbiological points of view. One hundred thirty-five teeth (83 deciduous molars and 52 young permanent molars) were included in the study. The teeth were randomly selected and treated either with one-visit IPT, two-visit IPT, or DCE. For two-visit IPT, the final excavation was performed after a period of 3 months. The color, consistency, and humidity of the dentin at the cavity floor were recorded for clinical assessment, and dentin samples were obtained from all teeth. Dentin samples were microbiologically investigated for the total number of colony forming units, mutans streptococci, and lactobacilli. The results showed bacterial growth in 63.8% of the dentin samples in one-visit IPT, while in two-visit IPT, bacterial growth was observed in all of the samples (100%) after the first excavation. When the cavities were reopened before the final excavation, the number of samples with positive growth had decreased significantly (44.4%), and after the final excavation, the number of the samples with positive growth had decreased to 2.2%. In the DCE group, only 25.6% of the samples revealed bacterial growth. No statistical difference was found between deciduous and permanent molars in any of the treatment groups in terms of microbiologic results ($p>0.05$). In conclusion, although none of the treatment methods completely eliminated the viable microorganisms during the initial excavations, a dramatic reduction in bacterial growth was detected during the treatment stages of two-visit IPT.

**Keywords** Indirect pulp treatment · Deep carious lesions · Microbiology · Stepwise excavation · Clinical study

## Introduction

Children and young adults who have not received early and adequate dental care and optimal systemic fluoride and lack adequate oral hygiene often develop deep carious lesions in the primary and permanent teeth [28]. A major area of concern regarding deep caries lesions is the effective treatment. Frequently, complete caries removal of these lesions causes a pulp exposure that endangers the vitality of the pulp. The principal objective of the management of primary and young permanent dentition is to preserve the vitality of the teeth while causing as little trauma as possible to the pulp [30]. Therefore, a technique referred to as indirect pulp capping has been advocated for more than 200 years as a conservative pulp therapy [1]. The term indirect pulp capping was recently replaced with the term indirect pulp treatment (IPT) [2].

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IPT is described as a procedure which includes the incomplete removal of carious dentin to avoid pulp exposure and the treatment of decay using biocompatible material [2, 12, 28]. Some describe IPT as a procedure which can be performed as a single- or a double-visit treatment [12, 17], while others refer to the single-visit procedure as IPT and the double-visit procedure as stepwise excavation [10, 18]. In a one-visit approach, a permanent restoration is carried out. In the two-visit approach, an intermediate restoration is done before reentry. The cavity is then reopened after a period of time and the final excavation performed [9, 17].

Besides the IPT technique in which the caries is intentionally left in the cavity to prevent pulp exposure, a recently published study by Innes et al. [16] defined a novel technique named Hall's technique in which carious primary molars were treated using preformed metal crowns without any caries removal, cavity preparation, or any local anesthesia. In addition, tooth-saving preparation and restoration techniques such as tunnel, box only, preventive resin restorations, and the atraumatic restorative treatment approach could result in remaining caries in the cavity [40]. The fate of the remaining caries is thought to be suspicious and has directed researchers to speculate on the question “how clean must a cavity be before restoration [5, 18, 40]?”

Several bacteriological studies were conducted to find out “whether the caries progresses or not when the infected and demineralized dentin is sealed with restorative material?” In these studies, bacteriological samples were taken from incompletely excavated cavities before the application of a restorative material. After a period of time, the restorative materials were removed and the bacteriological examination repeated [8, 9, 21, 25, 30, 32, 36]. Among these studies, differences in the depth of the lesions, the amount of carious dentin removed at the initial excavation stage, the restorative materials utilized, and the reentry time lead to different results. However, in general, a decrease both in the numbers and types of bacteria in the cultivable flora was detected. Moreover, the dentin became a darker, drier, and harder tissue. Briefly, these studies revealed evidence pointing to the inactivity of the remaining caries in the cavity [18]. The inactivation of caries with the initial excavation procedure brought up the question of the necessity of the second stage for removing the remaining caries. In other words, whether to perform the IPT as a one-visit or a two-visit procedure led to a controversy [5, 7, 18].

Hence, it was considered worthwhile to assess and compare the clinical and microbiological characteristics of the dentin at the cavity base during the procedures of one-visit IPT, two-visit IPT, and direct complete excavation (DCE) in both deciduous molars and young permanent molars with deep carious lesions to get insight with regard to the necessity of the second stage.

## Materials and methods

The material consisted of 154 teeth (94 mandibular second deciduous molars and 60 mandibular permanent first molars) with deep carious lesions in 123 patients admitted to the outpatient clinic at the Department of Pediatric Dentistry at Ankara University.

The inclusion criteria for the study were: (1) deep dentin carious lesions according to clinical and radiographic examinations and a risk of pulp exposure if completely excavated in one step (the demineralized dentin penetrated three fourths or more of the entire dentine thickness as determined on a radiograph [7, 10]); (2) the absence of fistula, swelling in periodontal tissues, and abnormal tooth mobility; (3) the absence of clinical symptoms of irreversible pulpitis such as spontaneous pain or pain persisting after the disappearance of the existing stimulus or sensitivity to pressure; (4) the absence of radiolucencies at the interradicular or periapical regions or thickening of the periodontal spaces, which would indicate the presence of irreversible pathologies or necrosis, absence of internal and external root resorption, absence of calcification in pulp tissue as determined by radiographic examinations, and (5) pulp vitality, which was confirmed by a cold stimulation tester (Chloraethyl; Wehr, Baden, Germany) and/or electric pulp tester (Digitest; Parkell, Farmingdale, NY, USA).

This study has been approved by the University Scientific Ethics Committee, and the informed consent was prepared and obtained according to the Helsinki Declaration II. The patients were only included if the parents/legal guardians had read and signed the informed consent form for this study.

Teeth were randomly assigned for the treatment groups as: one-visit IPT, two-visit IPT, and DCE group (Table 1). For the random selection, lots were drawn by the investigator who was blinded to the treatments (FTO). A pediatric dental consultant (AIO) who carried out the treatments performed all the clinical and radiographic examinations and microbiological sampling. Except this

**Table 1** The distribution of the teeth according to the treatment groups

| Treatment groups | Deciduous teeth | Permanent teeth | Total |
|---|---|---|---|
| One visit IPT | 29 | 18 | 47 |
| Two visit IPT | 29 | 16 | 45 |
| DCE | 25 | 18 | 43 |
| Total | 83 | 52 | 135 |

pediatric consultant, the other investigators were blinded to the treatments.

During the treatments, pulp exposure occurred in 19 of the teeth, which were thus excluded from the study group. The final study group comprised 135 teeth (83 mandibular second deciduous molars and 52 mandibular permanent first molars) with deep carious lesions in 111 patients. Of all patients, 51 were women and 60 were men, aged 4–15 years with a mean age of 8.5 years.

## Treatment procedures

After the administration of anesthetics and the rubber dam isolation of the area to be treated, only the surfaces without caries were cleaned with Savlon (5% savlon concentration, 95% ethanol). Then, the tooth was washed with saline and dried using sterile swabs. The first clinical step in all teeth comprised the opening of the cavity and the removal of undermined enamel using high-speed equipment with copious air/water spray and diamond burs (KG Sorensen, Zenith Dental ApS, Denmark). Caries at the lateral walls of the cavity and at the enamel–dentin junction was completely removed with excavators and/or round tungsten carbide burs (Komet Germany No.1.204.018) at low speed. Subsequently, the central cariogenic biomasses and superficial part of the necrotic and demineralized dentin were removed.

One-visit IPT was performed on 47 teeth. The treatment protocol for this group was as follows: After the elimination of the superficial part of the necrotic dentin, excavation continued until the operator thought pulp exposure would occur with further excavation; thus, a layer of soft carious dentin was left on the cavity floor adjacent to the pulp wall. The remaining innermost layer of carious dentin was covered with calcium hydroxide (Dycal, Dentsply/Caulk, Dentsply International Inc., Milford, DE, USA). The cavity was then sealed with restorative material (Fig. 1).

Two-visit IPT was applied on 45 teeth. In this group, the same treatment protocols were carried out as in the previous group. However, after the application of calcium hydroxide, cavities were sealed using reinforced zinc oxide eugenol cement (IRM, the L.D. Caulk Division, Dentsply International Inc.) for a 3-month period. This time was considered adequate for calcium hydroxide to exert its effect and for the pulp to respond with possible formation of tertiary dentin. The teeth were followed up every month and the integrity of the temporary restoration checked. After this interval of 3 months, the clinical and radiographic examinations were repeated. In the clinical examination procedure, history of pain, sensitivity to percussion and palpation, mobility, and absence or existence of fistula or edema were recorded. The pulp sensitivity was evaluated again by means of the cold stimulation tester and electric pulp tester. After the rubber dam application, the temporary filling and the remaining carious dentin was removed with excavators and slowly rotating burs. The floor of the cavity was sealed with calcium hydroxide, and the teeth were finally restored (Fig. 2).

The rest of the 43 teeth underwent DCE. All carious dentin was removed using excavators and slowly rotating burs until hard dentin was reached or pulp exposure occurred (Fig. 3). A caries-free cavity was defined as one without softening in the remaining dentin upon the examination of the cavity floor with a blunt probe using moderate pressure.

For the permanent restoration, first, glass ionomer cement (Ionofil V, Voco GmbH, Cuxhaven, Germany) was placed at the floor of the cavity on the calcium hydroxide as a cavity base, after which, the deciduous molars were restored using compomer (Dyract Extra, Dentsply, DeTrey

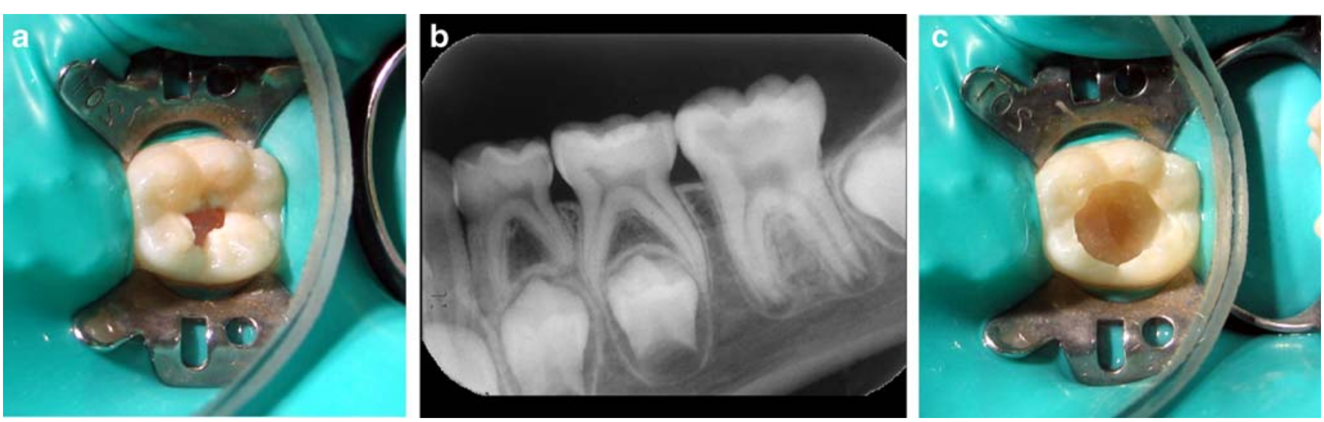


**Fig. 1** Clinical photographs and radiographs depicting one-visit indirect pulp treatment of mandibular left first permanent molar: **a** pretreatment view of the tooth with a deep carious lesion with large accumulations of cariogenic biomasses, **b** the pretreatment radiograph shows no apical pathosis, but a deep carious lesion, **c** clinical photograph showing the cavity preparation (caries was not completely removed at areas close to the pulp) before final restoration

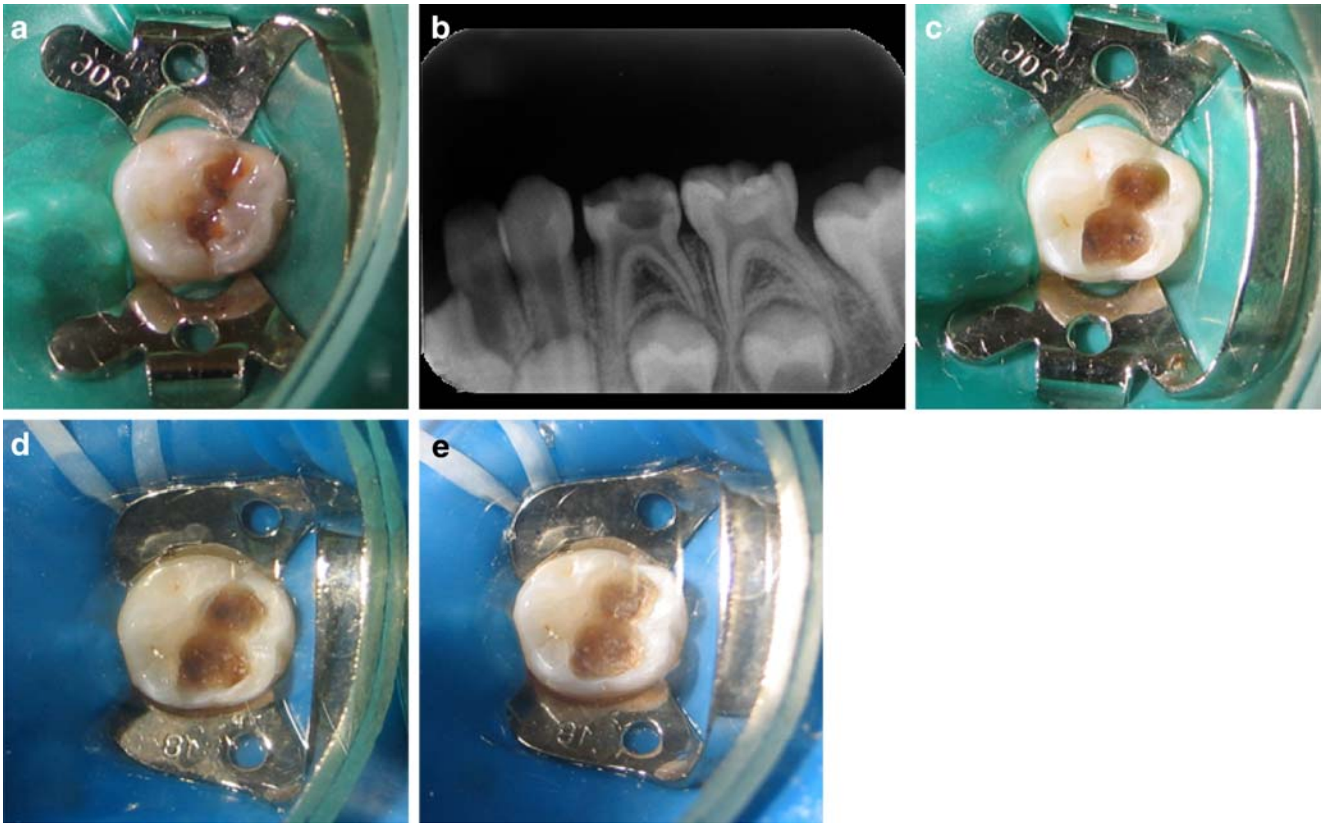


**Fig. 2** Clinical photographs and radiographs depicting two-visit indirect pulp treatment of mandibular left second deciduous molar: **a** close-up view of the tooth with a deep carious lesion, **b** the pretreatment radiograph shows no apical pathosis, but a deep carious lesion, **c** photograph showing the cavity preparation (incomplete caries removal) in the first visit before the placement of calcium hydroxide and temporary filling, **d** the cavity immediately after removal of temporary filling at the end of the 3-month interval period, **e** the cavity after the final excavation

GmbH, Konstanz, Germany) and the permanent molars restored with composite resin (Grandio Voco, 27457, Cuxhaven, Germany).

## Clinical and radiographic examinations

A number of clinical criteria (color, consistency, and humidity of the dentin) were recorded from the sites where the microbiological samples were collected. In the one-visit IPT and DCE groups, these clinical evaluations were done before the permanent restorations were placed, while in the two-visit IPT group, the evaluations were done at stage 1 (after initial excavation), stage 2 (before final excavation), and stage 3 (after final excavation).

The color of the dentin was classified according to the criteria of Bjørndal et al. [9] as either light yellow, yellow,


**Fig. 3** **a** Deep carious lesion in mandibular left first permanent molar, **b** the pretreatment radiograph shows no apical pathosis, but a deep carious lesion, **c** cavity after direct complete excavation

light brown, dark brown, or black. The classification was done by comparing the clinical observation with photographs illustrating the typical five dentin color classes. The consistency of the dentin was classified as either very soft (probe penetrates dentin with easy fragment loss of demineralized tissue), soft (probe penetrates tissue with no resistance when removing probe), medium hard (slight resistance when removing probe), or hard (comparable to unaffected dentin) [9]. In addition, the sample site was entered gently with a probe. If the tissue oozed moisture, it was classified as wet, and if it did not, it was assessed as dry [19].

The radiographs were taken before treatments and after 3 months before the final excavation for the two-visit IPT. The procedure was as follows: Standardized periapical radiographs with parallel technique were used as a diagnostic aid and to exclude teeth with apical pathosis. To obtain standardization in the films, periapical radiographs were obtained with an intra-oral X-ray system operating at 60 kVp, 7 mA by Heliodent DS (Sirona DentalSystem GmbH, Bensheim, Germany), and Ekstaspeed plus no. 2 films (Eastman Kodak, NY, USA). Exposure time was 0.4 s. These were taken using parallel technique with a XCP system (Rinn Co., IL, USA) device with a 12-in. cone attached. Exposed films were processed according to the manufacturer's instructions using an automatic film processor (XR 24, Dürr Dental GmbH& Co. KG, Bietigheim-Bissingen, Germany) with Kodak ReadyMatic chemistry. All radiographs were examined by an oral and maxillofacial radiologist (KO) and a pediatric dental consultant (AIO) using a standard viewing box in a darkened room. The final diagnosis and radiographic condition of each tooth was obtained with consensus between the two professionals.

## Microbiological sample collecting and processing

The samples were taken subsequently to the clinical recordings. In the one-visit IPT and DCE treatment groups, the dentin samples were collected before permanent restorations were placed, while in the two-visit IPT group, samples were recorded during three different stages (stages 1, 2, 3). In this group, clinical photographs of the initial sampling area were taken to identify the same central sampling site during the final excavation.

The cavity was thoroughly rinsed with saline and then dried with sterile swabs. Sampling was done [9] by slowly rotating a sterile tungsten carbide round bur (Komet No.1.204.18, Lemgo, Germany) wetted in reduced transport fluid (RTF) [39]. The bur was then placed into the sterile vial containing 3 ml RTF with glass beads and transported to the microbiological laboratory within a few hours.

The samples were dispersed ultrasonically for 5 s (Ultrasonic, LC30, Germany) and vortexed for 15 s (Firlabo.S.A., 230/50, Lyon, France). The samples were diluted tenfold in the Todd–Hewitt broth; (Oxoid, Basingstoke, UK). Subsequently, 0.1-ml quantities obtained from the dilutions were inoculated onto various media. For the isolation of mutans streptococci (MS), Mitis Salivarius agar (Difco Laboratories, Detroit, MI, USA) supplemented with sucrose and bacitracin was used [13]. Rogosa agar (Merck, Darmstadt, Germany) was used for the isolation of lactobacilli [35]. A total count of colony-forming units (CFU) was obtained through a culture in Brain Heart Infusion agar (Merck) supplemented with 5% blood. These media were incubated in microaerofilic atmosphere (Anaerocult C, Merck) at 37°C for 2 days. Then, the colonies were counted macroscopically based on their colony morphology. Counts below 20 CFU were beyond detectable limits and recorded as 0 (undetectable). All microbiological processes were carried out by a microbiologist experienced in oral microbiology (BO).

## Statistical analysis

The total CFU, MS, and lactobacilli were recorded as CFU/ml of sample. The Mann–Whitney *U* nonparametric test was used to check for statistical significant differences between the bacterial counts of deciduous and permanent teeth, between the bacterial counts of two-visit IPT stage 1 and one-visit IPT (to compare the bacterial counts in the remaining caries), and between the bacterial counts of two-visit IPT stage 3 and DCE (to compare the bacterial counts in cavity floors thought to be caries-free according to hardness criteria). The same statistical test was used to analyze the amount of bacteria in relation to the humidity of dentin.

The chi-square test was used to see if there was any relationship in terms of the color, consistency, and humidity. A $p$ value of less than 0.05 was considered statistically significant. The Kruskal–Wallis test was used to analyze the amount of bacteria in relation to the color of dentin and the amount of bacteria in relation to the consistency of dentin. Friedman's parametric test was used to compare the bacteriological counts of two-visit IPT at three stages. The same test was also used to compare the color and consistency of dentin in these three stages. Moreover, the dentin color and consistency between two-visit IPT stage 1 and one-visit IPT and between two-visit IPT stage 3 and DCE were compared using the same test. The Cochran test was used for comparing the humidity of the dentin among the treatment groups. Statistical analyses were performed using the SPSS 11.0 program (SPSS, Chicago, IL, USA) for Windows.

## Results

### Clinical observations

Of all 47 teeth which were treated with one-visit IPT, the most frequent color was light brown with 46.8% ($n$=22) followed by dark brown with a percentage of 34% ($n$=16). In the 45 teeth which were treated with two-visit IPT, the most frequent color in stage 1 was dark brown with 40% ($n$=18) followed by light brown with 35.6% ($n$=16), whereas in stage 2, the dark brown was observed the most with 62.2% ($n$=28), and the most frequent colors in stage 3 were light brown with 37.7% ($n$=17) followed by light yellow with 28.9% ($n$=13). For the 43 teeth in the DCE group, the most frequent color was light brown with 37.2% ($n$=16) followed by yellow with 30.2% ($n$=13; Table 2).

The consistency and humidity in the one-visit IPT was detected mostly as medium hard and dry in 33 teeth (70.2%). In the 45 teeth which were treated with two-visit IPT, the most frequent consistency in stage 1 was soft in 22 teeth with a ratio of 48.9% followed by medium hard in 14 teeth (31.1%), while medium hard (48.9%; $n$=22) and hard (36.6%; $n$=16) were detected as the most frequent consistency in stage 2, respectively. However, the ratio of teeth with a consistency rating of hard increased in the third stage (after the final excavation) to 93.3% ($n$=42). The humidity for the two-visit IPT group was 66.7% ($n$=30) wet (stage 1), 88.9% ($n$=40) dry (stage 2), 97.8% ($n$=44) dry (stage 3). For the DCE group, due to complete caries removal until the sound dentin was reached, the consistency was hard and humidity was dry in all of the teeth (Tables 3 and 4).

Statistical analysis revealed a significant difference between two-visit IPT stage 1 and one-visit IPT before the permanent restoration in terms of humidity and consistency ($p<0.05$), whereas no statistical difference was found according to color ($p>0.05$). No statistical difference was found between two-visit IPT stage 3 and DCE before the permanent restoration in terms of color, consistency, and humidity ($p>0.05$).

The chi-square test revealed that the relationship between humidity and consistency was statistically significant ($p=0.001$). As consistency changes from soft towards hard, the humidity of the dentin altered from wet to dry. No statistical significance was found between deciduous and permanent molars according to dentin characteristics ($p>0.05$).

### Microbiological results

All of the patients were available for recall in the two-visit IPT group and were included in the microbiological analysis. Table 5 shows the total CFU, MS, and lactobacilli counts for the deciduous and permanent molars in treatment groups, namely, one-visit IPT, two-visit IPT stages 1, 2, 3, and DCE. For the deciduous teeth in the one-visit IPT group, the mean of the total CFU was 8,310.3, while the means of MS and lactobacilli were 3,965.5 and 2,731, respectively. For permanent teeth in the same group, the mean of total CFU was 5,722.2, while the means of MS and lactobacilli were 1,555.6 and 1,905.6, respectively. In this group, the number of samples without bacterial growth for total CFU, MS, and lactobacilli were 17 (36.2%), 22 (46.8%), and 21 (44.7%) teeth, respectively (Table 6).

The results indicated a gradual decrease in the counts of the total CFU, MS, and lactobacilli during the treatment (stage 1, 2, 3) of two-visit IPT. For deciduous teeth, the mean of total CFU in stage 1 was 52,727.6, while the mean of total CFU decreased to 772.4 in stage 2, and no bacterial growth was detected in stage 3. For permanent teeth, the mean of total CFU in stage 1 was 293,419, while the mean of total CFU decreased to 740 in the stage 2 and to 31.3 in stage 3 (Table 5). The decrease was statistically significant between stage 1 and stage 2 and between stage 1 and stage 3 ($p<0.05$), whereas no statistical difference was observed between stage 2 and stage 3 ($p>0.05$) for both deciduous and permanent teeth. In stage 1, bacterial growth was detected in all of the samples (100%), while in stage 2, the number of samples with bacterial growth decreased to 20

**Table 2** Dentin color in the treatment groups

| Treatment groups | Dentin color | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Light yellow | | Yellow | | Light brown | | Dark brown | | Black | |
| | $n$ | Percentage | $n$ | Percentage | $n$ | Percentage | $n$ | Percentage | $n$ | Percentage |
| One-visit IPT | 0 | 0 | 8 | 17 | 22 | 46.8 | 16 | 34 | 1 | 2.2 |
| Two-visit IPT (stage 1) | 2 | 4.4 | 8 | 17.8 | 16 | 35.6 | 18 | 40 | 1 | 2.2 |
| Two-visit IPT (stage 2) | 0 | 0 | 0 | 0 | 13 | 28.9 | 28 | 62.2 | 4 | 8.9 |
| Two-visit IPT (stage 3) | 13 | 28.9 | 7 | 15.6 | 17 | 37.7 | 7 | 15.6 | 1 | 2.2 |
| DCE | 7 | 16.3 | 13 | 30.2 | 16 | 37.2 | 7 | 16.3 | 0 | 0 |

**Table 3** Dentin consistency in the treatment groups

| Treatment groups | Dentin consistency | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Very soft | | Soft | | Medium hard | | Hard | |
| | *n* | Percentage | *n* | Percentage | *n* | Percentage | *n* | Percentage |
| One-visit IPT | 2 | 4.3 | 11 | 23.4 | 33 | 70.2 | 1 | 2.1 |
| Two-visit IPT (stage 1) | 7 | 15.6 | 22 | 48.9 | 14 | 31.1 | 2 | 4.4 |
| Two-visit IPT (stage 2) | 0 | 0 | 7 | 15.6 | 22 | 48.9 | 16 | 35.5 |
| Two-visit IPT (stage 3) | 0 | 0 | 0 | 0 | 3 | 6.7 | 42 | 93.3 |
| DCE | 0 | 0 | 0 | 0 | 0 | 0 | 43 | 100 |

(44.4%), and in stage 3, only one tooth showed bacterial growth (2.2%; Table 6).

For the DCE group, the mean of total CFU was 420.7 and the mean of MS was 177.9, with a mean of 133.5 for the lactobacilli (Table 5). In this group, only 11 of the samples had bacterial growth (25.6%; Table 6).

No statistically significant difference was found between deciduous and permanent teeth in terms of total CFU, MS, and lactobacilli ($p>0.05$). The statistical analysis carried out to compare the microbiological status of the dentin at the cavity bottom between two-visit IPT stage 1 and one-visit IPT revealed a significant difference. The number of all of the bacteria (total CFU, MS, lactobacilli) was higher in the group of two-visit IPT ($p<0.05$). No statistical difference was found between the bacterial counts (total CFU, MS, lactobacilli) of two-visit IPT in stage 3 and DCE ($p>0.05$).

### Association between clinical observations and microbiological results

Overall, lesions classified on the basis of their color did not harbor significantly different levels of bacteria (total CFU, MS, lactobacilli; $p>0.05$). Harder lesions yielded significantly less bacteria (total CFU, MS, lactobacilli) than softer lesions ($p<0.05$). However, the bacterial counts of lesions which were recorded as soft and very soft were not significantly different from each other ($p>0.05$). The mean number of bacteria (total CFU, MS, lactobacilli) recovered from wet lesions were significantly higher than those in dry lesions ($p<0.05$).

## Discussion

The main objective of IPT is to maintain the vitality of teeth with reversible pulp injury [12, 32, 34]. The traditional technique for this treatment utilizes three strategies: the restriction of nutrient supply by way of isolation from the oral cavity, the removal of the infected dentin by operative treatment, and the use of cariostatic filling material [40]. A careful diagnosis of the preoperative pulp status is essential for the success of the treatment. The indication for IPT is limited to teeth that have no signs of irreversible pulp pathology based on a thorough clinical and radiographic examination [2, 12, 34].

Although IPT has been suggested for the treatment of deep dentin carious lesions for many years, there is no precise conclusion as to whether it should be performed in a single visit or two. In other words, the need for reentry to remove residual caries has not been dwelled upon. Advocates of the one-visit approach have drawn attention to the success of this procedure and claimed that there is insufficient evidence to support reentry. Moreover, they told that the second visit may lead to pulp exposure and further damage to the pulp [11, 17]. According to Kidd [18], the

**Table 4** Dentin humidity in the treatment groups

| Treatment groups | Dentin humidity | | | |
|---|---|---|---|---|
| | Wet | | Dry | |
| | *n* | Percentage | *n* | Percentage |
| One-visit IPT | 14 | 29.8 | 33 | 70.2 |
| Two-visit IPT (stage 1) | 30 | 66.7 | 15 | 33.3 |
| Two-visit IPT (stage 2) | 5 | 11.1 | 40 | 88.9 |
| Two-visit IPT (stage 3) | 1 | 2.2 | 44 | 97.8 |
| DCE | 0 | 0 | 43 | 100 |

**Table 5** Bacteriological data (CFU/ml) of microorganisms in the treatment groups

| Treatment groups | Deciduous teeth | | | Permanent teeth | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total CFU (mean) | MS (mean) | Lactobacilli (mean) | Total CFU (mean) | MS (mean) | Lactobacilli (mean) | Total CFU (mean) | MS (mean) | Lactobacilli (mean) |
| One-visit IPT | 8,310.3 | 3,965.5 | 2,731 | 5,722.2 | 1,555.6 | 1,905.6 | 7,319.1 | 3,042.5 | 2,414.9 |
| Two-visit IPT (stage 1) | 52,727.6 | 11,889.7 | 7,300 | 293,419 | 9,187.5 | 20,429.4 | 138,307 | 10,928.9 | 11,968.2 |
| Two-visit IPT (stage 2) | 772.4 | 248.3 | 55.2 | 740 | 241.2 | 73.1 | 760.9 | 245.8 | 61.6 |
| Two-visit IPT (stage 3) | 0 | 0 | 0 | 31.3 | 0 | 0 | 11.1 | 0 | 0 |
| DCE | 523.6 | 226 | 149.6 | 277.8 | 111.1 | 111.1 | 420.7 | 177.9 | 133.5 |

caries process is driven by the activity in the biofilm, so the process should be arrested simply by sealing the cavity. It was stated that the persistence of a few microorganisms might therefore be irrelevant. On the other hand, those who support the two-visit approach emphasize that in the event that the restoration fails and is not detected, the potentially reactivated lesion would already be in an advanced stage [33]. Moreover, there may not be completely sterile conditions, and no long-term data support the avoidance of the final excavation resulting in deep lesions. The final excavation helps in clinically controlling the tooth's reaction and enables the removal of the carious/demineralized dentin before carrying out the permanent restoration [7]. The analysis of clinical studies shows high success rates of both one-visit IPT [1, 15, 26, 38] and two-visit IPT [8–10, 23, 30]. However, there are no randomized clinical studies comparing these two approaches in deep carious lesions. The lack of comparative studies resulting in controversy has led us to carry out this study.

There are some difficulties when conducting studies on deep carious lesions such as precise measurement of lesion depth. The clinical caries lesion might be deeper than the given lesion's radiographic appearance [17]. What often appears to be an intact barrier of secondary dentin protecting the pulp may actually be a perforated mass of irregularly calcified and carious material [28]. As a result, this difficulty in determining the thickness of sound dentin between the caries and the pulp by clinical and radiographic means might have caused different baseline lesion depths in this study. Moreover, in IPT, there are no precise methods to determine how much caries should be removed. According to Massler [27], the infected outer carious dentin must be removed and the affected inner carious dentin beneath this must be protected. However, it is hard to distinguish the infected and affected dentin clinically. The distinction must still be made by the clinician who should be guided by the quality of the dentin, the depth of the lesion, and the clinical symptoms along with the radiographic appearance [6, 34, 37]. The differentiation of the infected and effected dentin in this study was carried out according to this description.

In our study, clinical alterations of the remaining carious dentin during two-visit IPT were characterized with darker, harder, and drier tissue after the treatment interval. This finding is indicative of arrested lesion, also defined by Miller and Massler as darkly pigmented and having hard or leathery consistency [29]. After the final excavation, the hardness of the dentin increased and the color got lighter.

**Table 6** Number of samples with and without bacterial growth in the treatment groups

| Treatment groups | Total CFU | | | | MS | | | | Lactobacilli | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | | − | | + | | − | | + | | − | |
| | *n* | Percentage | *n* | Percentage | *n* | Percentage | *n* | Percentage | *n* | Percentage | *n* | Percentage |
| One-visit IPT | 30 | 63.8 | 17 | 36.2 | 25 | 53.2 | 22 | 46.8 | 26 | 55.3 | 21 | 44.7 |
| Two-visit IPT (stage 1) | 45 | 100 | 0 | 0 | 44 | 97.8 | 1 | 2.2 | 37 | 82.2 | 8 | 17.8 |
| Two-visit IPT (stage 2) | 20 | 44.4 | 25 | 55.6 | 17 | 37.8 | 28 | 62.2 | 7 | 15.6 | 38 | 84.4 |
| Two-visit IPT (stage 3) | 1 | 2.2 | 44 | 97.8 | 0 | 0 | 45 | 100 | 0 | 0 | 45 | 100 |
| DCE | 11 | 25.6 | 32 | 74.4 | 10 | 23.3 | 33 | 76.7 | 9 | 20.9 | 34 | 79.1 |

+: positive bacterial growth, −: negative bacterial growth

Similar features were also seen in various studies [8–10, 25, 32]. Moreover, the results of the microbiological analysis revealed that the total CFU, MS, and lactobacilli counts gradually decreased during treatment (stages 1, 2, 3). This finding is also in agreement with those of many other studies which reported a substantial reduction of the cultivable flora [8–10, 21, 25, 30, 32]. The decrease in the bacterial counts was statistically significant between stage 1 and stage 2 and between stage 1 and stage 3 ($p < 0.05$), whereas no statistical difference was observed between stage 2 and stage 3 ($p > 0.05$) for both deciduous and permanent teeth. This result can be explained, as the procedures such as removing necrotic and infected dentin, calcium hydroxide application, and sealing the cavity with reinforced zinc oxide eugenol cement from the oral environment for 3 months in stage 1 reduced the viable microorganisms to a great extent. This finding supports the ideas of those who think that the second visit is unnecessary in IPT [11, 17, 18, 25, 31].

In the DCE group, although the caries was completely removed until hard dentin was reached at the cavity floor; bacterial growth was still detected in 25.6% of the dentin samples. This result is consistent with the findings of previous studies which indicated that the total counts of bacteria were largely reduced with excavation procedures, but some bacteria always appear to persist in clinically caries-free dentin [4, 22]. Moreover, Kidd et al. [20] claimed that the number of bacteria under $1 \times 10^2$ CFU/ml is clinically insignificant.

The clinical results in this study showed that the relation between humidity and consistency was statistically significant ($p=0.001$). As consistency changed from soft towards hard, the humidity of the dentin altered from wet to dry. Maltz et al. [25], who recorded only dentin color and consistency, found a statistical correlation between light brown color and medium hard consistency and also between dark brown color and hard consistency. Although our study includes a larger sample size, probably due to our color scale which includes extra units (five versus three), irregular distribution of data into scale units made it impossible for us to discover such a relationship.

There is no statistically significant difference between deciduous and permanent teeth in terms of total CFU, MS, and lactobacilli in any of the treatment groups. The lactobacilli counts are higher than the MS counts in one-visit IPT and in stage 1 of two-visit IPT in permanent teeth. This finding is consistent with the other studies which indicate that the lactobacilli species dominate deep carious lesions [14, 24]. In contrast, MS counts are higher than lactobacilli in deciduous teeth. Similar results were reported by Ayna et al. [3]. The authors stated that the smaller size and large opening of the lesions in deciduous teeth might easily have been affected by salivary flow, and the shallowness of the lesions could have permitted the diffusion of oxygen. These features affect the numbers of lactobacilli, which are strictly anaerobic and highly sensitive to pH changes [3].

The observed dentin consistency and humidity in one-visit IPT and in stage 1 of two-visit IPT were statistically different from each other. The dentin consistency was mostly medium hard (70.2%) and the dentin humidity was dry (70.2%) in one-visit IPT, whereas consistency was soft (48.9%) and humidity was wet (66.7%) in stage 1 of two-visit IPT. The bacterial counts were also significantly higher in stage 1 of two-visit IPT than the bacterial counts in one-visit IPT. In this study, it was impossible for the operator to excavate the carious tissue by being blinded to the treatment groups. In the two-visit IPT group, the operator knew that there would be a second visit and wanted to reduce the pulp exposure risk; thus, she might have been more cautious while deepening the cavity during the first excavation. However, the randomization should have been performed in two steps (at the first step, only DCE and IPT groups should have been constituted, then if IPT was chosen from the first draw of lots, a new draw of lots should be made after the first excavation to decide whether it will be one-visit or two-visit). If it was conducted like this, clinical characteristics and bacterial counts in one-visit IPT and in stage 1 of two-visit IPT might not be significantly different from each other. In our opinion, this bias was the limitation of this study.

Although not statistically significant, the results of this study revealed more dentin samples without bacterial growth in the stage 3 of two-visit IPT group (97.8%) than the DCE group (74.4%). This difference might be the result of the antibacterial effect of calcium hydroxide during the interval period. Similar results were found in the study of Maltz et al. which reported no growth of *Streptococcus mutans* and lactobacilli after 6–7 months application of calcium hydroxide and zinc oxide eugenol [25].

In conclusion, although none of the treatment methods completely eliminated the viable microorganisms at the initial excavations, a dramatic reduction of bacterial growth was detected during the treatment stages of two-visit IPT. Both the clinical changes in the color, consistency, and humidity of the dentin and the decreased numbers of bacterial counts in this group can be assessed as evidence of the arrestment of the carious process. Additional studies with larger study populations have to be conducted to ascertain these findings and to elucidate the issue.

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