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MICROBIAL EVALUATION FOLLOWING TWO IRRIGATION-MEDICATION PROTOCOLS IN SECONDARY INFECTION CASES

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ABSTRACT

Aim: The aim of this study was to clinically evaluate the antibacterial efficacy of two materials used as irrigants and intracanal medications (chlorohexidine, propolis) in secondary infection cases.

Materials and methods: Thirty-two patients with single-rooted, single-canal teeth associated with secondary infection were randomly assigned into four groups according to the type of irrigating solution and intracanal medication used. Group 1 (2% CHX irrigation and CHX gel intracanal medication), Group 2 (2% CHX irrigation and Propolis gel intracanal medication), Group 3 (30% Propolis irrigation and CHX gel intracanal medication), and Group 4 (30% Propolis irrigation and Propolis gel intracanal medication). The first microbial sample (S1) was obtained following complete aseptic removal of the primary filling material then the second microbial sample (S2) was obtained following chemomechanical preparation with various irrigant solutions. ProTaper Universal rotary system was used up to F4, or F5 according to canal size for root canal reinstrumentation. Finally, the third microbial sample (S3) was collected after removal of the intracanal medication. After cultivating the three samples, the growing colonies were counted and recorded as colony forming units (CFU).

Results: The third microbial sample after intracanal medication recorded the lowest microbial count in all groups. No statistical significant difference was recorded between the rate of reduction of S2 to S1 among the tested groups while there was statistically significant difference in microbial reduction of S3 to S2. Comparing between groups regardless the samples, there was no statistical significant differences between the groups.

Conclusions: Both CHX and Propolis irrigation and intracanal medication aid in microbial reduction, particularly in cases of secondary infection.

Keywords: Chlorohexidine, Colony forming units, Microbial reduction, Propolis.

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INTRODUCTION

Endodontic failure continues to occur despite advancements in endodontic materials, instruments, and techniques for a variety of reasons.[1] The presence of clinical signs and symptoms, as well as radiographic evidence of periapical bone destruction, indicates the need for retreatment. Nonsurgical endodontic retreatment is a predictable and dependable procedure with high success rates.[2]

Endodontic failure can be caused by a persistent or reintroduced intraradicular microorganism, an extraradicular infection, a foreign body reaction, or true cysts, or a combination of those factors.[3] Microorganisms found in root canals or periradicular lesions contribute significantly to the persistence of apical lesions following root canal treatment.[4]

Mechanical root canal preparation alone is ineffective at eradicating pathogenic microorganisms due to the complex root canal anatomy. As a result, additional chemical disinfection is critical to the success of a root canal retreatment.[5]

Irrigation is critical for successful root canal retreatment because it is the only way to clean areas of the root canal wall that are not accessible via mechanical instrumentation, such as isthmuses and lateral canals, as well as areas within oval and flat canals. [6,7]

Chlorohexidine (CHX) is frequently used as an irrigant and intracanal medication in retratment. [8] It is a potent antimicrobial agent that is particularly effective against *Enterococcus faecalis* (*E. faecalis*), the primary pathogen responsible for endodontic treatment failures.[9] It is bacteriostatic in low concentrations (0.2%) and bactericidal in high concentrations (2%). It possesses a unique substantivity as a result of its ability to bind to dentinal hydroxyapatite. It can be gradually released for up to 48-72 hours following root canal preparation and debridement.[10]

New alternatives of the currently available irrigating solution are necessary. Propolis is a new type of natural resin that is rich in flavonoids and is produced by bees from poplar or clusia flowers. This substance may be used intracanally or as a root canal irrigant.[11] Additionally, it possesses antibacterial, antifungal, and antioxidant properties.[12,13]

To maximise root canal disinfection in infected cases, intracanal medicaments can reduce remaining microorganisms and create a favourable environment for periapical tissue repair. [14] Both CHX and propolis have been used in various forms and concentrations as an irrigant and intracanal medication for disinfection.

Secondary infection is most frequently caused by facultative anaerobic and gram-positive bacteria. *E. faecalis* that is frequently isolated from previously endodontically treated teeth and persistent periapical lesions.[15]

This study aimed to assess the effect of different irrigations and intracanal medications on root canal microbiology in secondary infection cases

MATERIALS AND METHODS

Thirty-two patients requiring non-surgical retreatment were selected from the outpatient clinic at Tanta University's Faculty of Dentistry's Endodontic Department. The purpose of this study was explained to patients, and informed consent was obtained in accordance with the Research Ethics Committee at Tanta University's Faculty of Dentistry's guidelines on human research.

Patients requiring non-surgical retreatment of single-rooted, single-canal teeth who have been diagnosed with signs and symptoms of failure of primary root canal such as sensitivity to percussion, pain, swelling, or fistula, or teeth with radiographic signs of endodontic failure such as persistent periapical lesion or periodontal ligament widening were included in this study.

Fractured or non-restorable teeth that could not be isolated with a rubber dam, teeth with procedural

errors during primary root canal treatment such as ledge, broken instruments, or perforation that could complicate retreatment, as well as single-rooted teeth with multiple canals were excluded from this study.

All the steps of dental intervention were carried out in sterile conditions. A rubber dam* was used to completely isolate the tooth. OpalDam** was applied to flow and then cured for 10 seconds using light cure*** to prevent saliva entry. The operative field was disinfected with swabs moistened with 3% hydrogen peroxide**** until no further bubbling occurred, followed by a 1 minute rinse with 2.5 percent sodium hypochloride***** for 1 minute.[16]

The coronal restoration was removed with a carbide round bur size 3****** and the access cavity prepared with a long shank rose head bur size 3 attached to a high speed contraangle handpiece******* under copious water cooling and high suction.

ProTaperUniversalretreatmentfiles(D1,D2,D3)******** were used to remove the gutta-percha filling. Complete removal of primary root canal filling material was confirmed radiographically and clinically by absence of filling residues on the hand stainless-steel file. After selecting the appropriate initial file for the canal size, the working length was determined using an apex locator******** and confirmed with a digital radiograph********.

The first microbial sampling (S1) was performed immediately after gutta-percha removal and prior to chemomechanical preparation of the root canal. After irrigating the canal with 1mL of sterile saline solution, samples were taken using successive sterile paper points******** that were introduced into the canal for 60 seconds to absorb all the fluid inside it. The paper point was immediately placed in sterile tubes containing 1mL sterile saline and sent to the laboratory for processing at Microbiology and Immunology Department, Faculty of Medicine, Tanta University. The maximum time interval between sample collection and microbial laboratory processing was two hours. [17]

Root canal reinstrumentation was performed during the same appointment using ProTaper universal rotary system******** up to master apical file size F4 or F5 depending on the canal size in combination with the corrosbonding irrigating solution. After chemomechanical preparation, the second microbial sample (S2) was obtained and managed in the same manner as S1.

Group assignment

Thirty-two patients were randomly divided into four groups (n = 8) based on the type of irrigating solution and intracanal medication used:

Group 1: CHX******** irrigation solution / CHX intracanal medication

Group 2: CHX irrigation / Propolis******** intracanal medication

Group 3: Propolis irrigation / CHX intracanal medication.

Group 4: Propolis irrigation / propolis intracanal medication.

Finally, intracanal medication was applied for two weeks and then the third microbial sample (S3) was taken after removal of intracanal medication and managed as S1.

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******* NSK, Tokyo, Japan
******** Dentsply Maillefer, Ballaigues, Switzerland
******** Meta System Co. Ltd, Seongnam-si, Korea
******** Dr.Suni plus Digital Intraoral Sensor, Suni Medical Imaging, Inc., Sanjose, USA

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******** Cerkamed, Stalowa Wola, polland
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All microbial samples (S1, S2, S3) were collected using a sterile double ended calibrated loop* and streaked aseptically onto bile esculin media plates** for 48 hours[18]. Another sterile loop was used to transfer one micron of each sample into a tube containing 6.5 percent sodium chloride broth for confirmation of the presence of *E.faecalis*. The colonies that formed on each plate were then counted and multiplied to determine the colony forming units (CFUs)/mL of each specimen.[19]

Statistical analysis

Data of samples (S1, S2, and S3) were collected and tabulated S2 and S3 counts were expressed as microbial percentage regarding to S1. The mean and SD of CFU in each group were calculated and statistically analyzed using one-way analysis of variance with SPSS software version 20*** whenever a statistically significant results were recorded ($P \leq 0.05$) among the tested groups, Tukey's pairwise multiple comparison test was performed among the three groups.

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RESULTS

The third microbial sample recorded the least microbial count while the first microbial sample recorded the highest microbial count in all tested groups [Table 1].

It was necessary to compare the microbial percentage of S2 and S3 regarding S1 which is considered as 100%.

Two-Way ANOVA revealed statistically non-significant difference between the microbial percentage of S2 to S1 among the tested groups recording *P*-values of 0.473 while highly statistical significant difference of the microbial percentage of S3 to S2 and S3 to S1 among the tested groups recording *P*- values of 0.000 [Table 2].

Therefore, Tukey's pairwise comparison test was performed, in relation to S3 to S1, it revealed statistically significant difference between group 1 and 4 ($P = 0.031$), group 3 and 4 ($P = 0.004$) while there was no statistical significant difference between the other tested groups. In relation of S3 to S2, it revealed statistically significant difference between group 1 and 4, group 2 and 4, group 3 and 4 ($P<0.05$) while there was no statistical significant difference between the other tested groups group.

Table (1): The means and standard deviations of the number of CFU/mL for S1, S2 and S3 in all groups.

Group / Samples	Group 1 $\times 10^4$	Group 2 $\times 10^4$	group 3 $\times 10^4$	Group 4 $\times 10^4$	p-value
S1	9.11±1.99	9.80±1.98	12.20±2.49	11.33±2.46	0.051
S2	3.50±1.31	3.10±0.78	4.94±1.05	3.88±1.58	0.033*
S3	1.13±0.88	1.78±1.24	1.08±0.54	2.71±1.21	0.010*
P-value	0.000**	0.000**	0.000**	0.000**	

Table 2: The means of the microbial percentage of S2 and S3 in relation to S1

Mean of microbial	Group 1	Group 2	Group 3	Group 4	p-value
S2 to S1	38.61±14.11	33.11±10.48	40.73±5.21	34.60±11.17	0.473
S3 to S1	11.81±8.65	17.62±10.22	8.75±4.36	23.02±5.76	0.000**
S3 to S2	36.48±11.85	35.48±12.74	24.22±6.67	55.85±9.59	0.000**

DISCUSSION

The properly-designed obturation technique and the precise coronal sealing are not enough for successful root canal treatment if bacteria can survive in the complex root canal system or the periapical area [20] Anatomical variations in the number, size, shape, direction, and distribution of root canals add complexity to the root canal system, which contributes to endodontic failure either directly or indirectly. [21]

Enterococcus faecalis was chosen as the mono-infection bacterium in this study because it has been implicated in endodontic failures and is the most frequently isolated species from root filled teeth with apical periodontitis.[22] This bacterium is capable of forming biofilms on root canal dentin, which aids in their persistence following endodontic treatment. Thus, it is able to survive for extended periods of time without nutrients by invading dentinal tubules, where it can persist at depths exceeding 300 μm, where it is protected from the commonly used irrigating solutions.[23]

Mechanical instrumentation is the primary method used for eradication of bacteria from infected root canals.[24] ProTaper Universal retreatment files (D1,D2,D3) were used because they have large tapers and require less time during primary filling removal.[25]

Root canal reinstrumentation is a critical step in retreatment because it enables the removal of all root filling material, including gutta-percha and sealer remnants. [26] Any remaining material attached to the canal walls may obstruct effective removal of the inner, heavily infected layer of dentin and limit antibacterial agent penetration into the dentinal tubules. Thus, reinstrumentation aids in the enhancement of irrigation and intracanal medication effectiveness during retreatment. Also, reinstrumentation produces a shape to the canal that can be well obturated.[25]

Mechanical instrumentation alone is insufficient to eradicate microorganisms from root canals. Using a combination of mechanical instrumentation and irrigation, the number of microorganisms was further reduced by 100 to 1000 times. Due to the unique microflora found in failed endodontic treatment cases, this study utilized irrigant solutions such as chlorhexidine and propolis. [27]

Chlorhexidine digluconate (CHX) at a concentration of 2% was used in this study due to its antibacterial efficacy against *E. faecalis*,[28] and its substantivity, which prolongs its antimicrobial effects for days or weeks and prevents root canal reinfection between visits. [29]

Propolis was used as a natural irrigating agent in this study due to its therapeutic properties and its role in reducing microorganisms, particularly those found in endodontic failure cases.[30] Certain components of propolis extract, such as flavonoids, benzoic acid, and caffeic acid, likely act on the microbial membrane or cell wall site, causing functional and structural damage. [31]

A variety of intracanal medicaments between appointments have been used to disinfect the root canal completely and to create an environment conducive to periapical tissue repair, particularly in cases of endodontic retreatment because the majority of root canals contain viable microorganisms following completion of the chemomechanical preparation at the initial appointment.[32]

The number of CFUs was determined using a culture-based method because it is a simple, reliable, and rapid method for determining the presence of viable cells in a sample.[33]

To collect samples, sterile absorbent paper points were inserted into the canal to absorb all contaminated canal fluid. The paper points were then placed in tubes containing 1 mL of sterile saline, which cannot support bacterial growth, in order to maintain the concentration of bacterial load

within each tube, and bacteria were grown over bile esculin. [34]

Comparison between the samples in each group revealed that S1 has the highest microbial count because it was taken immediately after removal of the primary root canal filling material from the infected root canal that was filled with microorganisms before using any antimicrobial agents. While S3 has the lowest microbial count in all groups, this may be explained by the antimicrobial action of both irrigation and intracanal medications that were used.

Comparing between S2 and S1 revealed highly statistical significant difference in all groups. This may be attributed to the antimicrobial role of irrigating solutions used.

Regarding S2 sample in the tested groups, the highest microbial count was recorded for group 3 while the lowest microbial count was recorded for group 2. This may be attributed to the lowest antimicrobial effect of Propolis irrigation in comparison with CHX irrigation.[35]

Regarding S3 sample in the tested groups, the highest microbial count was recorded in group 4 while the lowest microbial count was recorded in group 3. This may be attributed to the lowest antimicrobial effect of Propolis gel in comparison with CHX gel. These was supported by Vasudeva et al.[35] who demonstrated the antibacterial efficacy of Propolis and 2% CHX along with other medicaments (Calcium hydroxide, Alovera gel) showing maximum microbial inhibition up to 200–400 micrometers depth occurred in CHX gel group, while Propolis exhibited the second highest antibacterial efficacy against *E. faecalis* among all medicaments.

These results are in agreement with Kayaoglu et al. [36], they demonstrated that the Propolis samples had remarkable antibacterial activities, but their activities were not greater than that of CHX, as established root canal irrigation but when used Propolis in the canal for 48 hours as intracanal medication did not report any antibacterial activity.

Moreover, these results were also supported by Bhandari et al.[37], that proved that 2% Chlorhexidine gel showed the maximum antimicrobial activity against *E faecalis* but Propolis can be used as an effective alternative intracanal medicament.

The result of this study are also in agreement with Evans et al.[38], they demonstrated that 2% chlorhexidine gel provided 100% inhibition of *E. faecalis* from day 1 to day 5 but Propolis doesn't provide their effect when used.

Kandaswamy et al.[10], Neelkantan et al.[12] and Gomes et al.[39] also supported these results. They reported that 2% CHX was more effective than Propolis against *E. faecalis*. Also this result was supported by Parolia et al [40], that also demonstrated the efficacy of CHX and Propolis.

The result of this study are also in agreement with Almadi et al. [41], that evaluate difference between the antibacterial efficacy of Propolis and CHX and proved that CHX had the same effect of Propolis as irrigation but had superior antibacterial efficacy against *E. faecalis* as intracanal medication.

In contrary, the result of this study were in disagreement with Saha et al.[42], they concluded that Propolis had better effect than CHX intracanal medicaments against *E. faecalis*. This contrary may be attributed to different methodology as the sample was taken early after one and two days of application of intracanal medicaments. Another study conducted by Piovesani et al.[43] concluding that none of the tested medicaments as CHX and $Ca(OH)_2$ assessed bactericidal effect as Propolis. This occurred due to different methodological techniques utilized for assessing microbial inhibition that included optical density and CFU counts.

Furthermore, these results were in disagreement with Madhubala et al.[44], they found that Propolis showed 100% reduction of *E. faecalis* on extracted human permanent incisors inoculated with pure culture of this bacteria and give better result than CHX and this may be attributed also to different time of application as it was applied only two days

inside the canal and then the sample was taken. Also, Oncag et al.[45] and Awawdeh et al.[46] observed that Propolis had good antimicrobial activity against *E. faecalis* compared with CHX and $Ca(OH)_2$ in the root canals of extracted teeth. This contrary may be attributed to short term application of it that ranged from 2 to 5 days.

CONCLUSION

Within the limitations of this study, it was concluded that:

Root canal instrumentation with different irrigations have major role in microbial reduction.

Both CHX and Propolis irrigation and intracanal medication have the ability to disrupt the microbial communities within the canal in cases of secondary infection. While none of them completely eliminate the microbes.

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