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ORIGINAL ARTICLE

Efficacy of Two Different Concentrations of Iodine-potassium Iodide Solution in Endodontic Retreatment: A Randomised Double-blinded Clinical Trial

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ABSTRACT

Objective: This study aimed to evaluate the antibacterial effectiveness of two concentrations of iodine-potassium iodide (IKI) used as the final irrigating solution during endodontic retreatment.

Methods: Thirty symptom-free root-filled anterior teeth with chronic apical periodontitis (<5*5mm) were included. They were divided into two groups consisting of 15 teeth according to the method of final irrigation. Group 1 were irrigated with 2% IKI, and Group 2 with 5% IKI. The direct bacterial viable count method was performed to determine the number of colony-forming units (CFUs) before and after disinfection. The reduction in bacterial count was assessed, and statistical analysis was performed using Mann–Whitney U tests with a 95% confidence level.

Results: Irrigation with 5% IKI resulted in significantly reduced bacterial counts than 2% IKI irrigation ($p<0.05$), indicating greater antibacterial effects.

Conclusion: The use of a 5% IKI solution as the final irrigating agent in endodontic retreatment cases with chronic apical periodontitis significantly reduces bacterial counts compared to a 2% IKI solution. The 5% IKI solution therefore exhibited a superior antibacterial effect. Consequently, 5% IKI solution application improves microbiological outcomes and enhances the overall disinfection of the root canal system.

Keywords: Antibacterial, colony-forming units, endodontic retreatment, iodine-potassium iodide, periapical lesion

HIGHLIGHTS

- Iodine-potassium iodide (IKI) at 5% concentration significantly reduced bacterial counts compared to 2% in endodontic retreatment.
- Final irrigation with 5% IKI after NaOCl and EDTA improved bacterial reduction in chronic apical periodontitis cases.
- This study supports integrating IKI into standard retreatment protocols for better infection control.

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INTRODUCTION

Oral inflammatory disease includes a variety of conditions, with one of the most prevalent being apical periodontitis (1). This infection is primarily caused by biofilm-forming bacterial communities adhering to the canal walls (2). Understanding the role of bacteria in oral inflammatory diseases is crucial for developing effective management and treatment strategies. The prevention and management of apical periodontitis are the primary objectives of root canal therapy (3). The treatment aims to eradicate existing infections and prevent re-infection of the pulp space and periradicular tissues. Effective endodontic treatment requires a deep understanding of the microbiological character-

istics of apical periodontitis (4), as residual bacteria in the root canal system have the potential to cause periapical lesions (5). Successful treatment involves reducing bacterial populations through the thorough cleaning and shaping of the root canal system, followed by effective irrigation to remove debris and disinfect the area (6). Intracanal medications and sealing materials also play an essential role in eliminating residual bacteria and preventing reinfection. Instrumentation limitations in root canal treatments present significant challenges and affect outcomes; more than 35% of the root canal surface is unreachable when using nickel-titanium instruments (7). To address this, chemical cleaning with irrigant solutions is critical for removing necrotic pulp, canal debris, bacteria, and their remnants, particularly in regions inaccessible to mechanical instruments (8). Smear layers comprise both organic and inorganic materials, which can accumulate in the canal after mechanical cleaning. These components can host bacteria capable of multiplying and infiltrating the dentinal tubules (9), reducing the efficacy of intra-canal medications and impacting root canal obturation sealing (10). Sodium hypochlorite (NaOCl) is often applied as an irrigant due to its antimicrobial effects and ability to dissolve organic tissue (9). In addition, the chelating irrigant ethylenediaminetetraacetic acid (EDTA) is employed in a 17% solution to remove inorganic components and further facilitate penetration into the dentinal tubules (10–12). The sequential use of NaOCl and EDTA effectively disinfects and cleans the root canal system by removing both organic and inorganic components of the smear layer (9, 10).

Recently, significant attention has turned to the endodontic therapeutic potential of iodine-potassium iodide (IKI). Pioneering research in the 1970s by Spangberg et al. (13) revealed its antibacterial effects and highlighted the lower cytotoxicity and irritation compared to NaOCl. IKI exhibits trichomonacidal and fungicidal properties and is effective against mycobacteria, the spores of bacilli and clostridia, enteric bacteria, enteric viruses, bacterial viruses, and protozoan cysts. The spectrum of action of IKI is influenced by the time of application and its concentration, underscoring its various therapeutic capabilities for combating a wide array of pathogens (14). Subsequent studies have demonstrated strong antibacterial properties, exceeding the effectiveness of NaOCl and chlorhexidine in terms of penetrating and disinfecting dentinal tubules (15). This highlights the potential of IKI as a powerful tool in medical and dental treatments. Despite its advantages, IKI is not capable of dissolving tissue, making it unsuitable as a primary irrigant (16). Various laboratory and clinical studies have reported conflicting results when comparing IKI solutions with other irrigation approaches. As a final irrigant, its effectiveness has been demonstrated in laboratory (17) and clinical (18, 19) settings; however, other clinical studies have failed to confirm its antibacterial efficacy (20, 21). These variable results underscore the complexity of treatment and the importance of further research in understanding the utility of IKI in dental applications. This study investigates the impact of two different concentrations of IKI solution (2% IKI and 5% IKI) used as a final irrigation solution on the microbial count in patients after unsuccessful endodontic treatment with periapical lesions. The null hypothesis posits that there is no difference in the antibacterial effect of final irrigation with 2% and 5% IKI during endodontic retreatment of teeth with chronic apical periodontitis.

MATERIALS AND METHODS

Study Design, Settings, and Ethical Approval

The study was a comprehensive, double-blinded, randomised clinical trial, utilising a two-arm parallel superiority design with a 1:1 allocation ratio. The research was conducted between May 2022 and August 2023 at the Damascus University Faculty of Dentistry. In adherence to ethical standards, the study was conducted following the guidelines outlined in the Declaration of Helsinki and received approval from the Local Research Ethics Committee of the Damascus University Faculty of Dentistry with the approval number DN-290122-18 dated 29/01/2022. The project was self-funded and registered at the ISRCTN registry under ID number ISRCTN34453184, dated 18/04/2024.

Sample Size Calculation

The sample size in this study was calculated with G* Power 3.1.9.4 (Heinrich-Heine-Universität, Düsseldorf, Germany). After careful analysis and based on the data of a prior study (22), it was determined that at least 30 participants, with 15 in each group, would be required. This sample size would ensure that the study could achieve statistically significant results at a significance level of 0.05 and a power of 95%. This method ensures that the study has sufficient statistical power to detect meaningful differences between the treatment groups.

Recruitment and Eligibility Criteria

During the study period, one hundred and nine patients aged between 21 and 49 years were referred to the Endodontic Department with symptom-free root-filled anterior teeth with chronic apical periodontitis. The principal investigator (M.S.) assessed the patients and reviewed their medical and dental histories. Subsequently, patients with signs of asymptomatic periapical periodontitis, such as minor percussion pain or large untreated caries on maxillary or mandibular anterior teeth, were included in the study. Sixty-seven patients met these inclusion criteria. Thirty-seven patients were further excluded after declining to participate, or due to the presence of systemic diseases that compromised general immune status, pregnancy, preoperative anxiety, antibiotic therapy within the past three months, open-apex teeth, multicanal teeth, or advanced periodontitis (defined as more than 5mm periodontal attachment and bone loss).

Finally, 30 patients were included in the study. All participants signed an informed consent sheet after being fully informed about the trial and its therapeutic nature.

Randomisation

Teeth were allocated into two groups based on IKI concentration at a 1:1 ratio using simple randomisation. Patients randomly selected one of 30 opaque, sealed envelopes (15 per group), each containing cards defining the final irrigation solutions randomly numbered using Excel's random allocation function (Microsoft, Washington, USA). Thus, patients were divided into two groups: Group 1 (n=15) received 2% IKI as a final irrigation solution, and Group 2 (n=15) received 5% IKI.

Blinding

Due to the interventional nature of the study, the treating clinician could not be blinded to the final irrigation type. However, the participants were fully blinded. Two PhD student researchers, blinded to the final irrigation used and trained in the assessment criteria, evaluated the treatment results.

Preparation of Research Solutions

Solutions of 5% and 2% IKI were carefully prepared to ensure accurate concentrations, typically maintaining a 1:2 ratio of iodine (I_2) to potassium iodide (KI). For the 5% IKI solution, 85 ml of distilled water was combined with ten parts KI (Adani Pharmachem Private Limited, India) and five parts I_2 (Loba Chemie PVT LTD, India). The 2% IKI solution required 94 ml of distilled water with four parts KI and two parts I_2. The solutions were filter-sterilised and stored in tightly sealed, amber-coloured bottles to prevent light degradation. The storage duration should not exceed one month to maintain stability and effectiveness (23).

Clinical Procedure

Periapical radiography was performed using a special sensor positioner (Vatech, Hwaseong, Korea) to assess periapical lesion size (mm), tooth anatomy, canal curvature, apex diameter, and the cause of the periapical lesion. These measures were used to identify teeth to be included and to estimate the working length. After determining the enrolled patients, the clinical procedure began with thorough scaling and cleaning of all teeth with supragingival plaque. This was followed by an oral antiseptic rinse containing 0.12% chlorhexidine (Aphamia, Hamah, Syria). The endodontic access cavity was prepared by removing any caries and previous restorations without breaching the pulp chamber.

The water spray was replaced with intermittent sterile saline cooling to minimise bacterial contamination, and a 30% hydrogen peroxide (H_2O_2) swab was used to sterilise the operative fields. Rubber dam isolation (Sanctuary, Perak, Malaysia) was also implemented, and the clamp and treatment tooth were disinfected by the same method, followed by a 2.5% NaOCl swab. Subsequently, a 5% sodium thiosulfate ($Na_2S_2O_3$) solution was used to neutralise residual NaOCl (24). Following this step, the access cavity was shaped using an Endo-Z bur (Dentsply Maillefer, Tusla, Oklahoma, USA), and the previous root filling was carefully removed using a sterile H-file (Mani, Tochigi, Japan). Removal was confirmed with radiography.

Notably, chloroform ($CHCl_3$) was omitted to preserve microbial viability (25). Canals were subsequently filled with saline solution, and a sterile K-file (#10) (Mani, Tochigi, Japan) was carefully inserted into the root canal according to the estimated working length. The file was used to meticulously address the mesial, lingual, distal, and buccal walls for one minute. For the initial root canal sampling (S1), three sterile paper points (Gabadent, Guangdong, China) were inserted into the canal, about 1 mm short of the radiographic apex, and left for at least 60 seconds to ensure thorough fluid absorption. These paper points were transferred into Eppendorf tubes containing 1 ml of peptone water (HIMEDIA, India) for transport. The S1 collecting method was similar to that described by Abbaszadegan et al. (21).

Once the glide path was achieved, the working length was measured using an electronic apex locator (Woodpex III, Woodpecker, China) with a K-file of appropriate size, and confirmed by radiography.

All canals were prepared using rotary files. After using the initial apical file (IAF), instrumentation was performed by three additional rotary files up to the master apical file (MAF), corresponding to sizes ranging from 30.04 to 40.04, depending on the initial apical size.

The canal was irrigated with 2 mL of 5.25% NaOCl (Merck) between each file use, for a total of 10 mL. After instrumentation was completed, the canals were flushed with 2 mL of 5% sodium thiosulfate followed by 5 ml of sterile saline. The canal was subsequently irrigated with 2 ml of EDTA (Produits Dentaires SA) for 2 minutes and then flushed with another 5 ml of sterile saline. At this stage, a second microbial sample (S2) was collected using three sterile paper points, as described by Abbaszadegan et al. (21).

These samples were then divided into two groups based on the final irrigant solution. Groups 1 and 2 were irrigated with 5 ml of 2% and 5% IKI, respectively, with the solution left in the canal for 5 minutes. The root canal was then rinsed with 2 mL of 5% sodium thiosulphate to neutralise the antibacterial activity of IKI, followed by 5 ml of sterile saline. Finally, a third microbial sampling (S3) was carried out using the Abbaszadegan et al. (21) methodology. Afterwards, the canals were filled using gutta-percha points and accessory cones (Gabadent, Guangdong, China) with a lateral condensation technique, and sealed with zinc oxide eugenol (Prime Dental Products Private Ltd., India). Finally, a radiograph was performed to check for overfilling. During the next visit, permanent restorations using suitable resin-bonded material were applied.

It is important to note that all clinical operations were carried out by a single clinician.

The steps, patient flowchart, and key elements of the study are summarised in Figure 1.

Microbiological Examination

All microbiological samples were processed within one hour of collection in a specialised laboratory. Each sample was carefully mixed and homogenised for 30 seconds in Eppendorf tubes using a vortex mixer (CSL-VORTEX, Thistle Scientific Ltd, Glasgow, United Kingdom).

After performing a series of dilutions, 100 µL of the highest dilution from each tube was plated onto nutrient agar plates (HIMEDIA, India). The plates were then incubated for two days at 37°C under aerobic conditions (Bacteriological Incubator 6640-01-071- 6596/National Appliance Heinicke Co. Tualatin, USA). Colony-forming units (CFUs) were counted in samples.

Photographs of bacterial cultures after 48 hours of incubation in aerobic conditions for the 2% IKI and 5% IKI groups are shown in Figure 2 and 3, respectively.

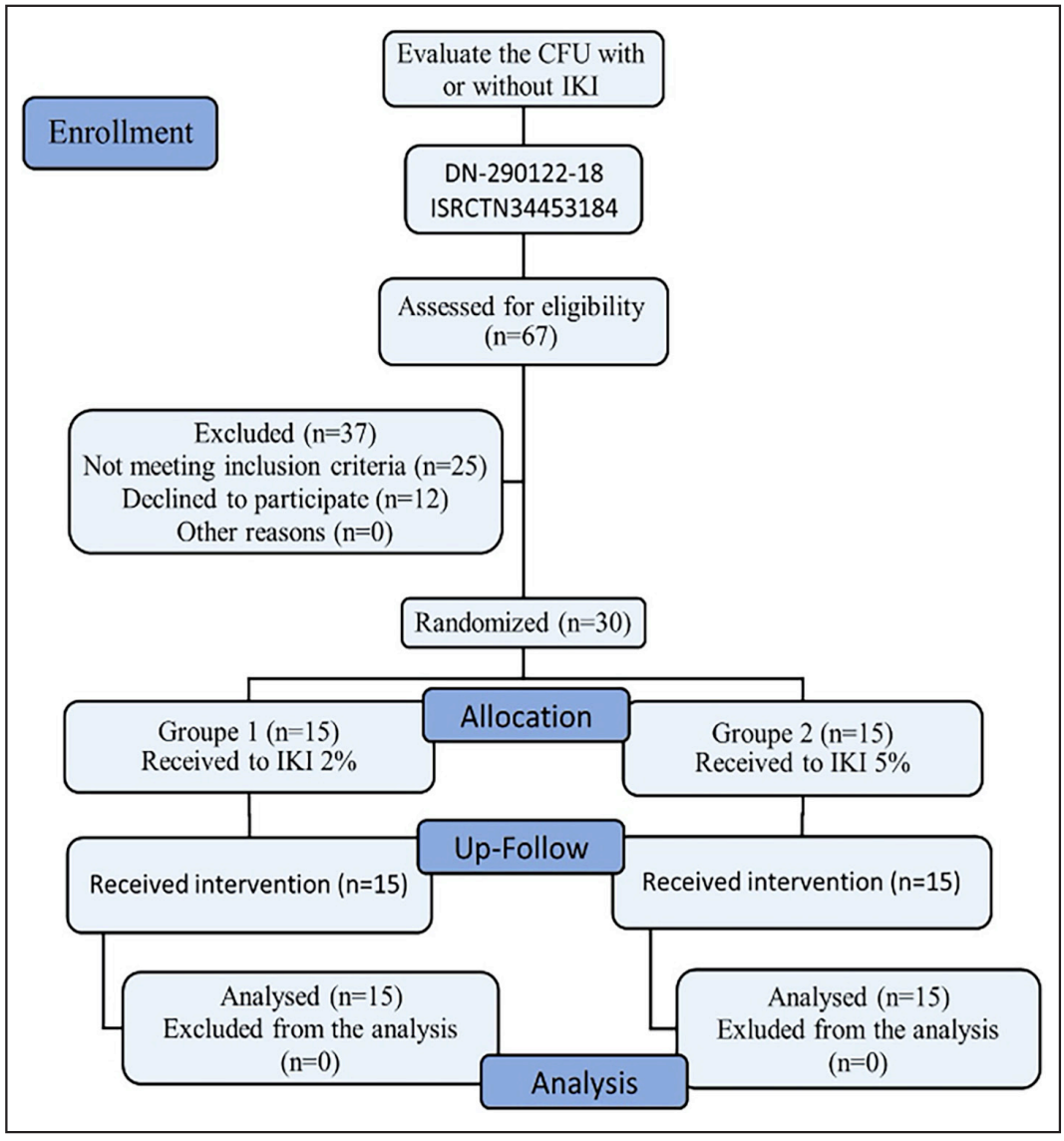

Figure 1. Flow diagram of the study design

CFU: Colony-forming unit, IKI: Iodine-potassium iodide, n: Number of teeth

Afterwards, the vital count of bacterial CFU per millilitre was calculated using the following equation: Number of bacteria/ml=number of CFU counted x reciprocal of dilution factor x 10 (where the dilution factor adopted in counting dishes was 10^{-3}). The bacterial unit number was converted into logarithmic values to facilitate bacterial colony counting and statistical analysis.

Statistical Analysis

Comprehensive statistical analysis was used to assess the impact of final irrigation on the reduction of culturable bacteria. Quantitative data from stages S1 and S2 with the logarithm of CFU values were utilised to calculate the impact of each IKI concentration.

The data were organised and analysed using SPSS software (SPSS Version 25, IBM SPSS Inc., Chicago, IL, U). The normality of the quantitative measurements was assessed by the Shapiro–Wilks test, while groups and CFU pairs were compared using the Mann-Whitney U test. A significance level of 0.05 was used to ensure a rigorous and reliable data evaluation.

RESULTS

This study included 30 symptom-free root-filled anterior teeth with chronic apical periodontitis, from 10 men and 20 women aged between 21 and 49 years (mean age=33.13). Random allocation of patients into different groups ensured unbiased results. No significant differences were reported between the groups regarding age or gender ($p>0.05$). The means of the bacterial counts in stages S1, S2 and S3, with standard deviations, are shown in Table 1.

Results comparing the samples taken at S2 vs S1 are shown in Table 2. The mean reduction in CFUs ranged from 17.67 to 35.53, with a mean±standard deviation of 23.98±4.8.

Moreover, a comparison between S3 and S2 samples revealed a higher mean reduction in the S3 sample (Table 3). Specifically, the reduction in the 2% IKI group ranged from 74.74 to 100.0, with a mean±standard deviation of 87.44±8.6. The reduction for the 5% IKI group ranged from 91.48 to 100.0, with a mean±standard deviation of 97.65±3.1.

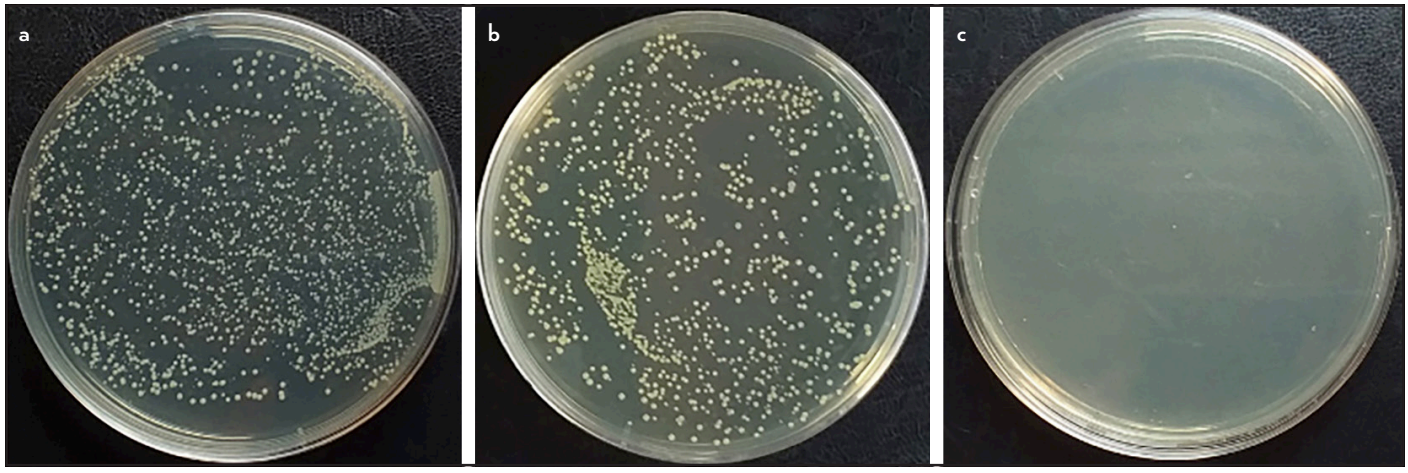

Figure 2. Photographs of aerobic bacterial cultures after 48 h of incubation from swabs taken from the root canals: (a) S1 - 2% IKI group, (b) S2 - 2% IKI group, (c) S3 - 2% IKI group

IKI: Iodine-potassium iodide

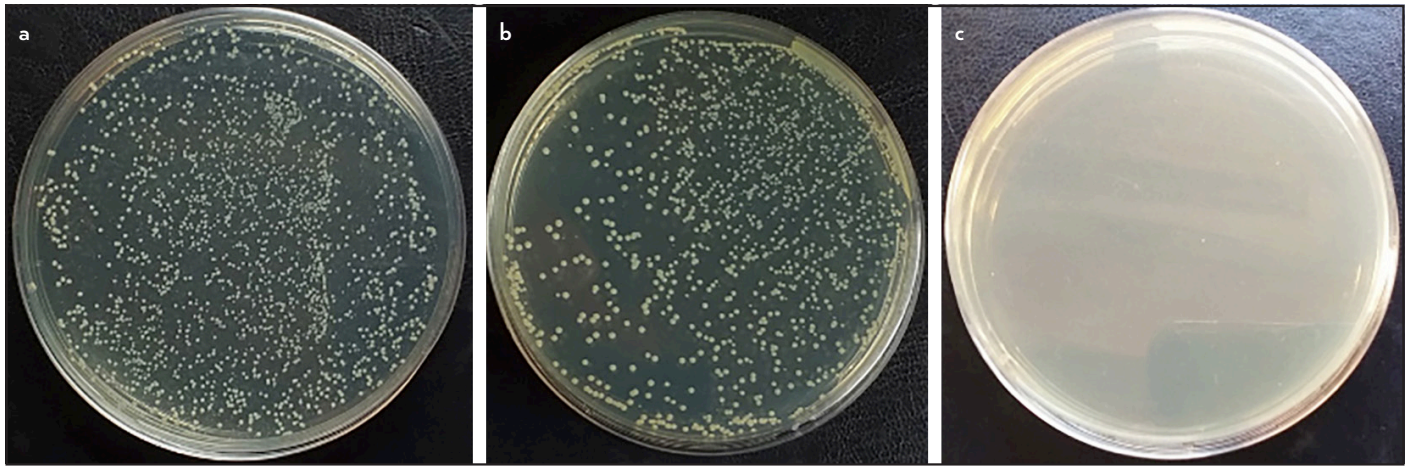

Figure 3. Photographs of aerobic bacterial cultures after 48 h of incubation from swabs taken from the root canals: (a) S1 - 5% IKI group, (b) S2 - 5% IKI group, (c) S3 - 5% IKI group

The decimal logarithm of the bacterial CFU count based on the final irrigation solution applied and the sample point is illustrated in Figure 4.

DISCUSSION

Previous studies have demonstrated that a significant number of necrotic teeth with apical lesions can show signs of healing after conventional nonsurgical endodontic treatment (26, 27). However, the presence of bacteria within the root canal system affects this healing process and can activate the immune response in surrounding tissues. Consequently, effective management of the bacterial load is essential for successful endodontic treatment (28). Furthermore, there is significant interindividual variation in intracanal bacterial communities of treated teeth, indicating that different combinations of bacteria may contribute to post-treatment disease (29). In some cases, teeth with inadequate previous therapy exhibit bacterial colonisation resembling primary infection, whereas adequately filled teeth have less diverse microbiota (29).

The treatment success is influenced by various factors, including cytokine activity, immune response, and bacterial load. It does not require completely bacteria-free canals, highlighting the need for understanding bacteria-immune interactions and healing processes for effective treatment (30, 31).

Researchers have emphasised a focus on the presence or absence of bacteria in canal cultures to evaluate treatment effectiveness (32). The use of isolation techniques and disinfection materials effectively reduces bacterial presence (33). According to Carrotte (34), root canal obturation after shaping and cleaning, irrespective of visit number, enhances the overall effectiveness of the endodontic procedure.

Recent studies comparing the antimicrobial effectiveness of 5.25% NaOCl and 17% EDTA with and without a final IKI solution rinse have provided insights into effective strategies. While negative cultures are associated with better outcomes (35).

However, a significant limitation of traditional methods is that only around half of the endodontic bacteria have been successfully cultured (36). Moreover, culturing techniques are unable to uncover bacterial virulence factors, which could be more influential than bacterial growth alone in cases of apical periodontitis (36). This study represents the

TABLE 1. Descriptive statistical evaluation of the bacterial counts across three studied periods: S1, S2, and S3

Studied period	Irrigation solution during preparation	Sample size	Mean±SD	Median	Interquartile range	Range
S1	–	30	6.72±0.32	6.66	1.65	6.08–7.36
S2	5.25% NaOCl	30	5.09±0.63	5.03	0.55	3.78–6.30
S3	%2 IKI	15	0.58±0.40	0.70	0.55	0.00–1.15
	%5 IKI	15	0.13±0.17	0.00	0.30	0.00–0.48

SD: Standard deviation

TABLE 2. Descriptive statistical evaluation of the intervention effectiveness by NaOCl during preparation in terms of reduction in bacterial counts

Studied period	Irrigation solution during preparation	Sample size	Mean±SD	Median	Interquartile range	Range
S2 vs. S1	5.25% NaOCl	30	23.98±4.8	22.60	6.33	17.67–35.53

TABLE 3. Descriptive statistical evaluation of the intervention effectiveness by IKI as the final irrigation solution in terms of reduction in bacterial counts between the two groups of study

Studied period	Final irrigation solution	Sample size	Mean±SD	Median	Interquartile range	Range	p[a]
S3 vs. S2	%2 IKI	15	87.44±8.6	85.24	13.00	74.74-100.0	0.0005[b]
	%5 IKI	15	97.65±3.1	100.0	5.37	91.48-100.0	

[a]: Mann-Whitney test, [b]: Significant difference. IKI: Iodine-potassium iodide

first randomised clinical trial to evaluate CFU values after a combination of 5.25% NaOCl and 17% EDTA followed by final irrigation with either 2% or 5% IKI during single-visit therapy for teeth with unsuccessful previous endodontic procedures, defined by apical lesions. This research sheds light on the efficacy of this combination in reducing bacterial load in root canals, potentially influencing and improving current endodontic treatment practices.

An obturation procedure was employed to prevent the presence of excess materials (gutta-percha and sealers) beyond the apex. All groups received a good filling at the apex—determined by the Apex Locator device and confirmed radiographically—with no overextension, an important factor for lesion healing post-treatment (37).

Pre-treatment root canals associated with apical periodontitis contain bacterial levels from 103 to 107 cell equivalents; higher bacterial counts are linked to inadequately treated canals (38). This study's findings align with Siqueira et al. (38), which showed a similar bacterial presence at 106 cells/millilitre. Our findings align with previous research, demonstrating that NaOCl in a chemomechanical preparation effectively reduces bacterial counts but does not completely eliminate bacteria. CFU values notably decreased after final irrigation, regardless of the concentration used. The Mann-Whitney U test revealed further significant differences ($p<0.05$) between the two used concentrations. This allows us to reject the null hypothesis and accept the alternative hypothesis that there is a difference in the antibacterial activity of 2% and 5% IKI when used as the final irrigating solution during endodontic retreatment of teeth with chronic apical periodontitis. Our results suggest that 5% IKI exhibits superior antibacterial activity.

The study supports the use of IKI in irrigation protocols to enhance disinfection, particularly in 5% solutions. Peciuliene et al. (19) also reported the efficacy of IKI in root canal system disinfection in retreatment cases accompanied by periapical lesions, when used as a final irrigation after 2.5% NaOCl (19). However, this contrasts with the results of Abbaszadegan et

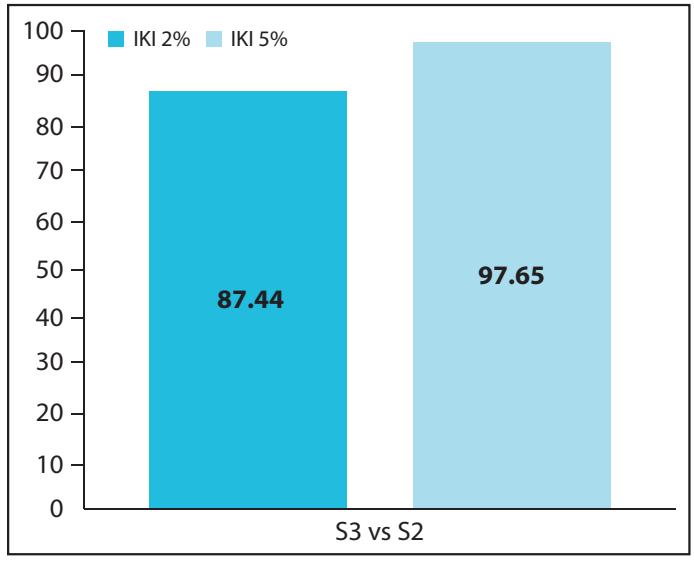

Figure 4. Decoding bacterial colony reduction in irrigation solutions by logarithmic shift

IKI: Iodine-potassium iodide

al. (21), who found IKI to be ineffective as an antibacterial solution. This is potentially attributable to the use of sterile saline during the preparation stages. This study focused on aerobic bacteria, but further investigation into anaerobic bacteria is necessary to understand their sensibility to IKI and their role in apical periodontitis.

Although we used G* Power software to determine an adequate sample size for statistical significance, research with larger populations is required to enhance the generalisability of our findings. Additionally, this study only examined immediate reductions in bacterial counts; long-term follow-up studies are needed to assess the sustained effectiveness of IKI in preventing reinfection and promoting periapical healing.

Advances in molecular microbiology diagnosis techniques are essential for the rapid and meaningful detection of bacteria and other microorganisms. The development of quick, sensitive, and precise assays could provide results in minutes to hours; this would greatly benefit clinicians and patients, particularly for the management of complicated abscess infections of endodontic origin. Identifying optimal treatment protocols for periapical lesions is crucial for successful treatment outcomes (39). Further research in diagnosis methods could improve treatment planning, patient outcomes, and endodontic care. Moreover, a greater understanding of the microbial profiles associated with endodontic infections could lead to targeted therapies and preventive strategies, benefiting patient care and public health. Therefore, continued research in this field is essential for advancing endodontics and improving the quality of life of patients.

CONCLUSION

In conclusion, within the limitations of this randomised clinical trial, we found that final irrigation with 5% IKI significantly reduces the CFUs of symptom-free root-filled teeth with chronic apical periodontitis compared to irrigation with a 2% IKI solution. Although irrigation with a 5% IKI solution may not entirely eradicate all bacterial species, it is a highly efficient antiseptic for such cases. This study supports the integration of IKI into standard endodontic protocols, particularly for cases involving complex infections and retreatments.

Disclosures

Ethics Committee Approval: The study was approved by the Damascus University Faculty of Dentistry Ethics Committee (no: DN-290122-18, date: 29/01/2022).

Authorship Contributions: Concept – M.S., H.A., S.A.A.; Design – M.S., H.A., S.A.A.; Supervision – H.A., S.A.A.; Funding – M.S.; Materials – M.S.; Data collection and/or processing – M.S.; Data analysis and/or interpretation – M.S., H.A., S.A.A., Ha.A.; Literature search – M.S., H.A., Ha.A.; Writing – M.S., H.A.; Critical review – M.S., H.A., S.A.A., Ha.A.

Conflict of Interest: All authors declared no conflict of interest.

Use of AI for Writing Assistance: The authors declared that they did not use artificial intelligence (AI) assisted technologies (such as Large Language Models [LLMs], chatbots, or image creators) in the production of submitted work.

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