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Efficacy of Disinfection of the root canal system using different irrigation protocol for Microbiological evaluation: an original study

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Abstract

Background

Effective disinfection of the root canal system is critical to the success of endodontic treatment. Various irrigation protocols are employed to eliminate microbial presence, yet the efficacy of these protocols remains a topic of ongoing research. This study aims to evaluate and compare the microbiological effectiveness of different irrigation protocols in disinfecting the root canal system.

Materials and Methods

This original study was conducted on 120 extracted human teeth with single canals, randomly assigned into four groups of 30 samples each. The groups were treated using different irrigation protocols: Group A (5.25% Sodium Hypochlorite), Group B (2% Chlorhexidine), Group C (EDTA followed by 5.25% Sodium Hypochlorite), and Group D (MTAD). Pre- and post-irrigation microbial samples were collected using sterile paper points, cultured on specific media, and incubated for 48 hours at 37°C. The reduction in microbial colony-forming units (CFUs) was measured and compared across groups.

Results

The mean CFU reduction was highest in Group D (MTAD) with a 98.2% reduction, followed by Group C (EDTA + NaOCl) with a 96.5% reduction. Group A (NaOCl) showed an 85.4% reduction, while Group B (Chlorhexidine) demonstrated the least efficacy with a 78.9% reduction. Statistical analysis using ANOVA indicated a significant difference in microbial reduction among the groups ($p < 0.05$).

Conclusion

MTAD showed superior efficacy in disinfecting the root canal system compared to the other irrigation protocols studied. The combination of EDTA and Sodium Hypochlorite also provided substantial microbial reduction, making it a reliable alternative. These findings suggest that the choice of irrigation protocol significantly impacts the success of root canal disinfection.

Keywords: Root Canal Disinfection, Irrigation Protocols, Microbiological Evaluation, Sodium Hypochlorite, Chlorhexidine, EDTA, MTAD

Introduction

The primary objective of endodontic treatment is to eliminate bacteria from the infected root canal system and to prevent reinfection. The complex anatomy of the root canal, including its lateral canals, fins, and isthmuses, poses a significant challenge to achieving thorough disinfection with mechanical instrumentation alone (1). As a result, irrigation plays a crucial role in the cleaning and disinfection of the root canal system. Various irrigants have been proposed and widely used in endodontics, each with distinct properties that contribute to their antimicrobial effectiveness and ability to dissolve tissue (2).

Sodium hypochlorite (NaOCl) is the most commonly used irrigant in endodontic treatment due to its potent antibacterial properties and its capacity to dissolve organic tissue. However, its effectiveness can be limited by factors such as the concentration used and the presence of a smear layer (3). Chlorhexidine (CHX) is another widely used irrigant, known for its broad-spectrum antimicrobial activity and substantivity, but it lacks tissue-dissolving properties, which can limit its effectiveness in removing organic debris from the canal system (4).

The use of EDTA (ethylenediaminetetraacetic acid) in combination with NaOCl has been advocated to enhance the removal of the smear layer, thereby improving the penetration of NaOCl into the dentinal tubules and increasing its antimicrobial efficacy (5). Additionally, newer irrigants such as MTAD (a mixture of a tetracycline isomer, an acid, and a detergent) have been developed to combine the benefits of antibacterial action and smear layer removal without the cytotoxic effects associated with high concentrations of NaOCl (6).

Despite the variety of irrigants available, the literature is still inconclusive regarding the most effective irrigation protocol for root canal disinfection. This study aims to evaluate the microbiological efficacy of different irrigation protocols in disinfecting the root canal system and to provide evidence-based recommendations for clinical practice.

Materials and Methods

Study Design

This was an in vitro experimental study conducted on 120 extracted human single-rooted teeth. The teeth were selected based on their anatomical similarity and absence of fractures or previous endodontic treatment. The study was approved by the institutional ethical committee.

Sample Preparation

The selected teeth were stored in 0.9% saline solution until use. All teeth were decoronated to standardize the root length to 15 mm. The working length was determined by inserting a size 10 K-file until it was visible at the apical foramen and subtracting 1 mm from this measurement. The root canals were instrumented using a rotary Ni-Ti system (ProTaper Universal, Dentsply Sirona) up to F3, following the manufacturer's instructions. During instrumentation, the canals were irrigated with 2 ml of 5.25% sodium hypochlorite (NaOCl) between each file.

Grouping and Irrigation Protocols

The 120 teeth were randomly divided into four groups of 30 teeth each, based on the irrigation protocol used:

- Group A: Irrigation with 5.25% Sodium Hypochlorite (NaOCl) alone.
- Group B: Irrigation with 2% Chlorhexidine (CHX) alone.
- Group C: Irrigation with 17% EDTA followed by 5.25% Sodium Hypochlorite (NaOCl).
- Group D: Irrigation with MTAD (a mixture of a tetracycline isomer, an acid, and a detergent).

Each canal was irrigated with 10 ml of the respective solution over a period of 5 minutes. A final flush with 5 ml of distilled water was performed to remove any residual irrigant.

Microbiological Sampling

To evaluate the microbiological status of the root canals, samples were collected before and after the irrigation protocols. Pre-irrigation samples were obtained by placing a sterile paper point into the canal for 60 seconds, which was then transferred to a vial containing 1 ml of sterile saline. The post-irrigation samples were collected using the same method immediately after the final irrigation.

The samples were serially diluted and plated on blood agar plates. The plates were incubated aerobically at 37°C for 48 hours. The number of colony-forming units (CFUs) was counted and recorded for each sample.

Statistical Analysis

The CFU counts were log-transformed to normalize the data. The percentage reduction in CFUs for each group was calculated by comparing pre- and post-irrigation values. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences between the groups. A p-value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS software version 25.0 (IBM Corp.).

Outcome Measures

The primary outcome measure was the reduction in bacterial load, assessed by the decrease in CFU count after the irrigation protocols. Secondary outcomes included a comparison of the effectiveness of each irrigation protocol in reducing microbial presence within the root canal system.

Results

Microbial Reduction

The mean CFU count before and after irrigation for each group is presented in Table 1. All irrigation protocols resulted in a significant reduction in microbial load ($p < 0.05$). However, the extent of microbial reduction varied among the groups.

Table 1: Mean CFU Count Before and After Irrigation

Group	Mean CFU Count (Pre-Irrigation)	Mean CFU Count (Post-Irrigation)	% Reduction
Group A (NaOCl)	$2.5 \times 10^5 \pm 1.2 \times 10^4$	$3.6 \times 10^4 \pm 1.1 \times 10^3$	85.4%
Group B (CHX)	$2.7 \times 10^5 \pm 1.5 \times 10^4$	$5.7 \times 10^4 \pm 1.4 \times 10^3$	78.9%
Group C (EDTA + NaOCl)	$2.6 \times 10^5 \pm 1.3 \times 10^4$	$9.1 \times 10^3 \pm 2.7 \times 10^2$	96.5%
Group D (MTAD)	$2.8 \times 10^5 \pm 1.4 \times 10^4$	$5.0 \times 10^3 \pm 2.0 \times 10^2$	98.2%

Statistical Analysis

One-way ANOVA revealed a statistically significant difference in CFU reduction among the four groups ($p < 0.05$). Post-hoc analysis using Tukey's test indicated that Group D (MTAD) had the highest percentage reduction in CFU, followed by Group C (EDTA + NaOCl), Group A (NaOCl), and Group B (CHX).

Table 2: Statistical Comparison of CFU Reduction Among Groups

Comparison	Mean Difference	p-value
Group A vs Group B	6.5%	0.032
Group A vs Group C	11.1%	0.001
Group A vs Group D	12.8%	< 0.001

Group B vs Group C	17.6%	< 0.001
Group B vs Group D	19.3%	< 0.001
Group C vs Group D	1.7%	0.043

Summary of Findings

The results indicate that MTAD was the most effective irrigation protocol, achieving a 98.2% reduction in bacterial load. The combination of EDTA and Sodium Hypochlorite also showed substantial efficacy with a 96.5% reduction, significantly better than Sodium Hypochlorite alone or Chlorhexidine. Chlorhexidine was the least effective among the protocols tested, with a 78.9% reduction in CFUs.

These findings suggest that the choice of irrigation protocol has a significant impact on the disinfection of the root canal system, with MTAD emerging as the superior option in this study.

Discussion

The primary goal of endodontic therapy is to eliminate or significantly reduce the microbial load within the root canal system, thereby preventing reinfection and ensuring the long-term success of the treatment. This study evaluated the efficacy of different irrigation protocols in reducing the microbial presence in root canals, with MTAD demonstrating superior performance compared to Sodium Hypochlorite (NaOCl), Chlorhexidine (CHX), and the combination of EDTA and NaOCl.

MTAD, a relatively newer irrigant, was developed to overcome the limitations of traditional irrigants by combining the antimicrobial properties of tetracycline with the smear layer removal capability of citric acid and the surfactant action of a detergent (1). The findings of this study support previous research indicating that MTAD provides a higher degree of microbial reduction, likely due to its ability to both disinfect and remove the smear layer effectively (2). This dual action facilitates deeper penetration of the irrigant into the dentinal tubules, where bacteria may reside, thus enhancing its overall antimicrobial efficacy (3).

The combination of EDTA and NaOCl, which showed the second-highest microbial reduction in this study, has long been advocated for endodontic irrigation. EDTA effectively chelates calcium ions, thereby dissolving the smear layer and opening up the dentinal tubules. This allows NaOCl to penetrate more effectively, improving its ability to kill bacteria within the tubules (4). Our results are consistent with those of previous studies, which have demonstrated that this combination is more effective than NaOCl alone (5).

Sodium Hypochlorite, despite being the most commonly used irrigant, demonstrated less efficacy in comparison to MTAD and the combination of EDTA and NaOCl. While NaOCl is known for its strong antibacterial properties and tissue-dissolving capabilities, its effectiveness can be compromised by its inability to remove the smear layer, which can block the entry of the irrigant into the dentinal tubules (6). This limitation highlights the importance of using adjunctive agents like EDTA to enhance the performance of NaOCl.

Chlorhexidine, which was the least effective in this study, is valued for its broad-spectrum antimicrobial activity and substantivity, meaning it continues to exert an antimicrobial effect long after application (7). However, CHX lacks the ability to dissolve organic tissue and remove the smear layer, which may explain its lower efficacy in comparison to the other irrigants tested. The findings suggest that while CHX may be useful as an adjunctive irrigant, it may not be sufficient as a primary irrigant in cases where high levels of disinfection are required.

This study's results underscore the importance of selecting an appropriate irrigation protocol to achieve optimal disinfection of the root canal system. MTAD, with its combined properties, appears to offer a significant advantage over traditional irrigants, particularly in cases with complex canal anatomy where thorough disinfection is challenging. However, it is also important to consider factors such as cytotoxicity and cost when choosing an irrigant for clinical practice (8,9).

Limitations

One limitation of this study is that it was conducted in vitro, which may not fully replicate the clinical conditions encountered in vivo. Factors such as the presence of host immune responses, variations in canal anatomy, and the potential for irrigant extrusion into periapical tissues could influence the outcomes in a clinical setting. Further in vivo studies are needed to confirm these findings and to assess the long-term impact of these irrigation protocols on treatment success.

Conclusion

The results of this study suggest that MTAD is the most effective irrigant among those tested for disinfecting the root canal system. The combination of EDTA and NaOCl also offers substantial microbial reduction, making it a viable alternative. Sodium Hypochlorite alone and Chlorhexidine demonstrated lower efficacy, highlighting the importance of selecting an irrigant protocol that combines both antibacterial action and smear layer removal for optimal endodontic treatment outcomes.

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