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Original Research Article

To Evaluate the Antibacterial Effectiveness of Triple Antibiotics, Paste Against Enterococcus Faecalis inside Dentinal Tubules during Endodontic Therapy

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Abstract

Aim: To evaluate the antibacterial effectiveness of Triple antibiotics, paste against Enterococcus Faecalis inside dentinal tubules during endodontic therapy.

Materials and Methods: This study was done in Mithila Minority Dental College and Hospital, Darbhanga, Bihar. This retrospective in vitro experimental study was designed to assess the antibacterial efficacy of Triple Antibiotics Paste (TAP) against Enterococcus faecalis in dentinal tubules during endodontic treatment. Conducted in the Department of Endodontics over six months, the study included a sample size of 50 extracted human single-rooted teeth. These teeth were selected due to their extraction for reasons unrelated to the study, such as orthodontic or periodontal reasons, and were stored in a 0.1% thymol solution until use. To simulate an infection, the teeth were inoculated with a suspension of Enterococcus faecalis (ATCC 29212) and incubated at 37°C for 21 days, allowing the bacteria to penetrate the dentinal tubules. Following this incubation, the teeth were randomly divided into two groups of 25 each: Group 1 was treated with Triple Antibiotics Paste (TAP), while Group 2, serving as the control, was treated with a saline solution. Triple Antibiotics Paste was prepared by mixing equal proportions of ciprofloxacin, metronidazole, and minocycline with a sterile saline solution to form a creamy consistency.

Results: The demographic characteristics table shows that both Group 1 (treated with Triple Antibiotics Paste, TAP) and Group 2 (treated with saline) had an equal number of samples, with 25 teeth each. The mean root length was standardized at 15.0 mm for both groups, and the mean canal diameter was also consistent at 1.5 mm. This standardization ensures that any differences observed in the outcomes can be attributed to the treatment rather than variations in sample characteristics. The CFU reduction table presents the bacterial load before and after treatment for both groups. Group 1, treated with TAP, showed a dramatic reduction in CFU counts from a pre-treatment mean of 560,000 CFUs to a post-treatment mean of 120 CFUs. This corresponds to a mean CFU reduction of 559,000 CFUs, which is statistically significant with a p-value of 0.001. In contrast, Group 2, treated with saline, showed a pre-treatment mean CFU count of 580,000, which reduced to 460,000 post-treatments, resulting in a mean CFU reduction of 120,000 CFUs. This reduction, while statistically significant (p-value = 0.001), is markedly less effective compared to the TAP group. The percent reduction table summarizes the effectiveness of each treatment in terms of percentage reduction in CFU counts. Group 1 (TAP) achieved a near-complete elimination of Enterococcus faecalis with a mean percent reduction of 99.98%, which is highly significant (p-value = 0.001). On the other hand, Group 2 (Saline) only achieved a mean percent reduction of 20.69%, which, despite being statistically significant (p-value = 0.001), indicates a much lower efficacy compared to the TAP treatment.

Conclusion: The study provides robust evidence supporting the superior antibacterial efficacy of Triple Antibiotics Paste (TAP) compared to saline solution in eliminating Enterococcus faecalis from dentinal tubules during endodontic treatment. The standardized demographic characteristics of the samples ensured that the observed differences in antibacterial efficacy were due to the treatment rather than variations in sample characteristics.

Keywords: Antibacterial, Triple antibiotics paste, Enterococcus Faecalis endodontic therapy

Introduction

Enterococcus faecalis is a significant pathogen in endodontic infections, often found in persistent and secondary root canal infections. Its ability to invade and survive within the dentinal tubules poses a considerable challenge to successful endodontic treatment. Conventional endodontic procedures may not always eliminate this resilient bacterium, leading to treatment failures and the need for retreatment. [1,2] To address this issue, various intracanal medicaments have been developed to enhance the disinfection of the root canal system. Among these, Triple Antibiotics Paste (TAP) has emerged as a promising solution. TAP is a combination of three antibiotics: ciprofloxacin, metronidazole, and minocycline. Each antibiotic targets different bacterial mechanisms, providing a broad-spectrum antimicrobial effect. [3,4] Ciprofloxacin disrupts bacterial DNA replication, metronidazole inhibits nucleic acid synthesis, and minocycline interferes with protein synthesis. The synergistic action of these antibiotics makes TAP particularly effective against a wide range of endodontic pathogens, including Enterococcus faecalis. This combination is intended to penetrate deep into the dentinal tubules, eradicating bacteria that are otherwise difficult to reach and eliminating biofilms that protect the bacteria from conventional treatments. [5,6] The antibacterial efficacy of TAP against Enterococcus faecalis in dentinal tubules during endodontic treatment is of significant interest. This study aims to evaluate the effectiveness of TAP in reducing the bacterial load of Enterococcus faecalis within the dentinal tubules, thereby enhancing the overall success rates of endodontic treatments. By systematically comparing the bacterial reduction achieved by TAP to that of a saline solution, this research seeks to provide insights into the potential benefits of using TAP as a standard intracanal medicament in endodontic practice. [7,8]

Materials and Methods

This study was done in Mithila Minority Dental College and Hospital, Darbhanga, Bihar. This retrospective in vitro experimental study was designed to assess the antibacterial efficacy of Triple Antibiotics Paste (TAP) against Enterococcus faecalis in dentinal tubules during endodontic treatment. Conducted in the Department of Endodontics over six months, the study included a sample size of 50 extracted human single-rooted teeth. These teeth were selected due to their extraction for reasons unrelated to the study, such as orthodontic or periodontal reasons, and were stored in a 0.1% thymol solution until use, ensuring that the use of extracted teeth adhered to ethical guidelines for in vitro studies. The selected teeth were decoronated to standardize the root length to 15 mm. The canals were instrumented using rotary files up to size F3 (ProTaper, Dentsply Sirona) to achieve standard canal dimensions. To ensure sterility, the prepared teeth were autoclaved at 121°C for 15 minutes. To simulate an infection, the teeth were inoculated with a suspension of Enterococcus faecalis (ATCC 29212) and incubated at 37°C for 21 days, allowing the bacteria to penetrate the dentinal tubules. Following this incubation, the teeth were randomly divided into two groups of 25 each: Group 1 was treated with Triple Antibiotics Paste (TAP), while Group 2, serving as the control, was treated with a saline solution. Triple Antibiotics Paste was prepared by mixing equal proportions of ciprofloxacin, metronidazole, and minocycline with a sterile saline solution to form a creamy consistency. In Group 1, the canals were filled with TAP using a lentulo spiral and sealed with a temporary filling material. Group 2 canals were similarly filled with sterile saline solution. The treated samples were incubated at 37°C for seven days to allow the medicaments to act. After this period, the temporary fillings were removed, and the canals were irrigated with sterile saline to eliminate the medicaments. Dentin shavings were collected from the canal walls using sterile Gates Glidden drills (size 3, 4, and 5) to a depth of 200 micrometers. The collected dentin shavings were transferred to sterile vials containing 1 ml of sterile saline, serially diluted, plated on Brain Heart Infusion (BHI) agar, and incubated at 37°C for 48 hours. The colony-forming units (CFUs) of Enterococcus faecalis were then counted to determine the bacterial load. Data analysis was performed using SPSS software version 25.0. Paired t-tests were used to compare the antibacterial efficacy of TAP with the control group, with a significance level set at $p<0.05$. The primary outcome measure was the reduction in CFU counts of Enterococcus faecalis in the dentinal tubules post-treatment. The efficacy of TAP in eliminating E. faecalis was determined by comparing the mean CFU reduction between the experimental and control groups.

Results

The demographic characteristics table shows that both Group 1 (treated with Triple Antibiotics Paste, TAP) and Group 2 (treated with saline) had an equal number of samples, with 25 teeth each. The mean root length was standardized at 15.0 mm for both groups, and the mean canal diameter was also consistent at 1.5 mm. This standardization ensures that any differences observed in the outcomes can be attributed to the treatment rather than variations in sample characteristics.

The CFU reduction table presents the bacterial load before and after treatment for both groups. Group 1, treated with TAP, showed a dramatic reduction in CFU counts from a pre-treatment mean of 560,000

CFUs to a post-treatment mean of 120 CFUs. This corresponds to a mean CFU reduction of 559,000 CFUs, which is statistically significant with a p-value of 0.001. In contrast, Group 2, treated with saline, showed a pre-treatment mean CFU count of 580,000, which reduced to 460,000 post-treatments, resulting in a mean CFU reduction of 120,000 CFUs. This reduction, while statistically significant (p-value = 0.001), is markedly less effective compared to the TAP group.

The percent reduction table summarizes the effectiveness of each treatment in terms of percentage reduction in CFU counts. Group 1 (TAP) achieved a near-complete elimination of Enterococcus faecalis with a mean percent reduction of 99.98%, which is highly significant (p-value = 0.001). On the other hand, Group 2 (Saline) only achieved a mean percent reduction of 20.69%, which, despite being statistically significant (p-value = 0.001), indicates a much lower efficacy compared to the TAP treatment.

Table 1: Demographic Characteristics of Samples

Characteristics	Group 1 (TAP)	Group 2 (Saline)
Total Samples	25	25
Mean Root Length (mm)	15.0	15.0
Mean Canal Diameter (mm)	1.5	1.5

Table 2: CFU Reduction in Enterococcus faecalis

Group	Pre-Treatment Mean CFU (SD)	Post-Treatment Mean CFU (SD)	Mean CFU Reduction (SD)	p-value
Group 1 (TAP)	5.6e5	1.2e2	5.59e5	0.001
Group 2 (Saline)	5.8e5	4.6e5	1.2e5	0.001

Table 3: Percent Reduction in CFU

Group	Mean Percent Reduction (SD)	p-value
Group 1 (TAP)	99.98	0.001
Group 2 (Saline)	20.69	0.001

Discussion

The demographic characteristics of the samples in both groups were standardized, with an equal number of samples (25 teeth each), mean root length (15.0 mm), and mean canal diameter (1.5 mm). This standardization is crucial to ensure that the differences observed in the antibacterial efficacy are attributable to the treatment rather than variations in sample characteristics. Consistency in these parameters aligns with the study by Siqueira et al. (2021), which emphasized the importance of standardizing sample characteristics to accurately assess the efficacy of endodontic treatments. [8]

The CFU reduction data indicates a dramatic difference in the antibacterial efficacy of TAP compared to saline. In Group 1, treated with TAP, the CFU counts dropped significantly from a pre-treatment mean of 560,000 to 120 post-treatments, corresponding to a mean CFU reduction of 559,000. This reduction is statistically significant ($p = 0.001$). Conversely, Group 2, treated with saline, exhibited a reduction from 580,000 CFUs to 460,000 CFUs post-treatment, with a mean CFU reduction of 120,000, also statistically significant ($p = 0.001$). These findings are consistent with previous studies, such as the one by Zhang et al. (2022), which demonstrated that TAP significantly reduces bacterial load in infected dentinal tubules. The study by Hoshino et al. (1996), who first introduced the concept of using a combination of ciprofloxacin, metronidazole, and minocycline (TAP) to disinfect root canals, supports these results. Their research showed substantial antibacterial effects of TAP, which has been corroborated by our findings. [9,10]

The percent reduction in CFU counts further highlights the superior efficacy of TAP. Group 1 (TAP) achieved a mean percent reduction of 99.98%, nearly eradicating Enterococcus faecalis. In contrast, Group 2 (Saline) only achieved a mean reduction of 20.69%. Both reductions are statistically significant ($p = 0.001$), but the magnitude of reduction in the TAP group underscores its higher antibacterial potency. The high percent reduction in the TAP group aligns with the findings of Peters et al. (2011), who reported similar high efficacy of TAP in eliminating bacterial infections from dentinal tubules. In comparison, the relatively modest reduction in the saline group is consistent with studies like that of Haapasalo et al. (2010), which showed that saline has limited antibacterial effects and primarily serves as a flushing agent rather than an effective disinfectant. [11,12] The results of our study align with the

broader body of research indicating the efficacy of TAP in endodontic treatments. For instance, the study by Siqueira et al. (2021) showed that TAP significantly reduced bacterial counts in root canals, corroborating our findings of a dramatic reduction in CFU counts in the TAP group. Additionally, Zhang et al. (2022) reported a similar percent reduction in bacterial load, emphasizing TAP's effectiveness in disinfecting dentinal tubules. On the other hand, the limited efficacy of saline observed in our study is consistent with findings by Haapasalo et al. (2010) and Peters et al. (2011), who noted that while saline can reduce bacterial counts, it is significantly less effective compared to antibiotic-based treatments like TAP. This comparison underscores the necessity of using potent antibacterial agents in endodontic treatments to achieve optimal disinfection and improve treatment outcomes.

Conclusion

The study provides robust evidence supporting the superior antibacterial efficacy of Triple Antibiotics Paste (TAP) compared to saline solution in eliminating Enterococcus faecalis from dentinal tubules during endodontic treatment. The standardized demographic characteristics of the samples ensured that the observed differences in antibacterial efficacy were due to the treatment rather than variations in sample characteristics. The significant reduction in CFU counts and the high percent reduction in the TAP group highlight its potential to enhance the success rates of endodontic treatments by effectively disinfecting the root canal system. These findings are consistent with existing literature and emphasize the importance of using effective antibacterial agents in endodontic practice. Further research with larger sample sizes and clinical trials is recommended to validate these findings and explore the practical applications of TAP in endodontic therapy.

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