ORIGINAL RESEARCH

Evaluation of Antimicrobial Efficacy of Etidronic Acid against *Enterococcus faecalis* in Primary Teeth: An *In Vivo* Study

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Abstract

Background: In pulpectomy, to remove the inflamed or diseased pulp tissues and smear layer, the root canal is disinfected with mechanical instrumentation and copious irrigation.

Aim: The purpose of this present study is to evaluate the antibacterial efficacy of etidronic acid as an irrigant in primary teeth.

Materials and methods: A total of 60 necrotic primary teeth in children aged 3–8 years were included. Of these, 30 were irrigated with 2% chlorhexidine (CHX) gluconate (group I) and 30 with 9% etidronic acid (group II). Two microbiological samples were collected with sterile paper points from the canal in both groups during the pulpectomy process—first after access opening and before the first irrigation (S1), and second after instrumentation and final irrigation, before filling (S2). The presence of *Enterococcus faecalis* was determined using colony-forming units per milliliter (CFU/mL) in all samples (S1 and S2).

Results: After analyzing the samples before and after irrigation in groups I and II, there was a statistically significant reduction in CFU/mL ($p < 0.05$). Group II had a statistically significant advantage when the two groups were compared after irrigation.

Conclusion: As a result, etidronic acid can be recommended as a pulpectomy irrigating solution for necrotic primary teeth.

Keywords: Antimicrobial efficacy, Etidronic acid, *Enterococcus faecalis*.

International Journal of Clinical Pediatric Dentistry (2024): 10.5005/jp-journals-10005-2834

Introduction

The normal development of permanent teeth depends on the preservation of primary teeth. The carious primary teeth can be maintained in the oral cavity by various treatment procedures such as restorations and pulp therapy. Various factors such as proper diagnosis, cleaning with intracanal medicaments, irrigation solutions to remove the necrotic debris, and obturation of the pulpal space followed by final restoration determine the success of endodontic treatment.[1] During pulp therapy, mechanical instrumentation alone cannot effectively eliminate the microorganisms from primary teeth due to unusual internal root configurations, and furcal, and horizontal inosculations.[2] Hence, chemical agents, in combination with instruments, completely remove all necrotic debris and microorganisms from the root canal for the success of endodontic therapy.

Endodontic irrigants available for disinfecting root canals include sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), etc. Although NaOCl has antibacterial and dissolving effects on necrotic tissues, it is highly toxic when inserted into periradicular tissues and has an unpleasant taste. Hence, CHX is being used as an irrigant as it has the property of substantivity and an antibacterial effect against *E. faecalis*.[3] *E. faecalis* has high viability and is present in 63% of necrotized primary teeth.[4] The ideal irrigating solution fulfilling all the properties to be used during pulp therapy in primary teeth is questionable.

Narrow and calcified root canals, and the elimination of *E. faecalis*, pose a challenge in the biomechanical preparation. This can be achieved using irrigating solutions containing chelators. Etidronate or 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), also known as etidronic acid, is considered a substitute for EDTA or citric acid (CA) and is used in combination with NaOCl.[5] Its use in permanent teeth is effective against *E. faecalis*; however, its effectiveness in primary root canals needs to be studied further as less literature is available.

Hence, the present study aimed to assess the antimicrobial effect of etidronic acid against *E. faecalis* in the primary root canal.

Materials and Methods

After approval from the Institutional Ethics Committee Board, the current study was carried out in the Department of Pediatric Dentistry. The study was also registered in Clinical Trials of India (ICMR) with designation CTRI/2020/09/027510. Children whose parents signed the informed consent were included in the study. The sample size

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How to cite this article: Supraja AN, Arali V, Rapala H, *et al.* Evaluation of Antimicrobial Efficacy of Etidronic Acid against *Enterococcus faecalis* in Primary Teeth: An *In Vivo* Study. Int J Clin Pediatr Dent 2024;17(4):433–436.

Source of support: Nil

Conflict of interest: None

was estimated based on data obtained from a study conducted by Arali et al.[6] Nonprobabilistic sampling was carried out, and the irrigant was chosen for each case randomly. Around 60 teeth from patients aged 3–8 years old requiring pulpectomy were included in the study. The study was a controlled, randomized, double-blind clinical trial wherein the operator and the participants were blinded.

Inclusion Criteria

- Patients in good optimal health, with no systemic medical condition, were included.
- Primary teeth which comprise at least one necrotic pulp canal, abscess, or sinus tract were included.
- The radiographic evaluation shows the presence of radiolucency in the furcation or periapical region, and at least two-thirds of the roots are present.
- A restorable tooth structure strong enough to support a rubber dam was required.[7]

Exclusion Criteria

- Individuals on antibiotics within two weeks prior to sampling or who had any type of systemic condition were excluded.
- Teeth with pathological root resorption, perforated pulpal floor, nonrestorable teeth, or excessive mobility were excluded.[7]

Preclinical Laboratory Procedure

Preparation of 9% Etidronic Acid

According to the manufacturer's instructions, two capsules weighing 0.246 gm each dissolved in 10 mL of 3% NaOCl were used to prepare 9% etidronic acid.

Methodology

A total of 60 pulpectomies were performed in necrotic primary teeth and were divided into two groups using the following irrigants:

- Group I (30): Around 2% CHX gluconate.
- Group II (30): Around 9% etidronic acid.

Demographic details of the patient such as name, age, and sex were taken prior to the procedure. Single-visit pulpectomy was performed, and periapical radiographs of the teeth were taken.

Isolation and Operative Field Disinfection

After achieving asepsis of the oral cavity, the inferior alveolar nerve block was achieved using 2% lidocaine for primary mandibular teeth and infiltration (palatal and buccal) for primary maxillary teeth. The modified protocol of disinfection by Ng et al.[8] was employed, wherein each tooth was cleaned using pumice and isolated with rubber dam. The dental crown, along with the rubber dam and clamp, was disinfected with 30% hydrogen peroxide, and then the operating field was disinfected with 5.25% NaOCl, followed by inactivation with 10% sodium thiosulfate. A round carbide bur (No. 3) in a slow-speed handpiece was used to remove the gross carious tissue. A new No. 3 round carbide bur was used to deroof the pulp chamber.

Collection of Microbiological Samples

After negotiation and determination of the working length, a baseline sample was taken from the largest canal (palatal for maxillary molar, distal for mandibular molar) using two sterile absorbent paper points compatible with the root canal diameter. The absorbent points were placed in each canal for 30 seconds. A graduated test tube containing brain heart infusion (BHI) broth, a growth media, was used to transport these paper points for microbiological evaluation. Routine biomechanical preparation was done following the baseline sample collection. The teeth were randomly divided into group I (0.5 mL of 2% CHX) and group II (9% etidronic acid) before obturation. No other irrigating solutions were used. The second sample was collected and transported by the same procedure similar to the baseline sample collection. Metapex was used to obturate the root canals, and an intraoral periapical radiograph was taken postoperatively to assess the obturation. Analysis of the samples was done for the growth of *E. faecalis*, and the cultures were expressed as colony-forming units per milliliter (CFU/mL).

Laboratory Procedures

The collected samples were streaked on Petri dish plates and placed in an anaerobic gas jar for 48 hours for bacterial culture. Gram staining of the magenta pink-colored colonies was done, and they were observed under the microscope. The turbidimetry method was used to count the colonies. Based on the degree of turbidity or density exhibited, McFarland's scale pattern approach was used to determine the CFU/mL.

Results

Statistical Package for the Social Sciences software version 20.0 and Microsoft Excel 2013 were used for statistical analysis. Intra- and intergroup comparisons were made using the paired *t*-test. The data obtained were tabulated as mean and standard deviation. The means of the two groups were compared using the independent *t*-test.

A total of 120 microbiological samples were obtained, of which 60 samples were collected from group I (30 preirrigation and 30 postirrigation, respectively) and 60 samples from group II (30 preirrigation and 30 postirrigation, respectively).

The bacterial load was comparable between the two groups before irrigation at baseline. The amount of CFU/mL in preirrigation samples was counted and compared. On comparison of data, it revealed a *p*-value of 0.605, indicating no significant statistical difference between groups at baseline (Fig. 1).

The baseline samples from the control group revealed a mean of 383,250.00 CFU/mL. A mean of 1,873.67 CFU/mL was discovered

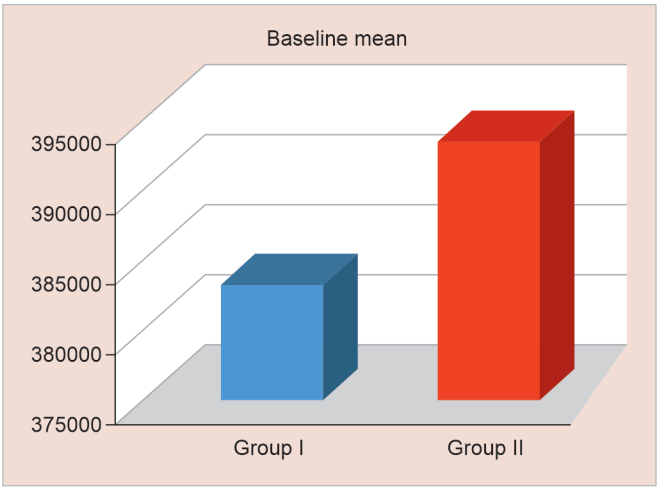

Fig. 1: Bar graph representing the baseline mean CFU/mL of both the groups

postirrigation. A statistically significant variation was observed between CFU/mL preirrigation and postirrigation (Fig. 2).

The baseline samples from the experimental group had a mean of 393,440.67 CFU/mL. A mean of 2,104.53 CFU/mL was discovered postirrigation. The difference between pre- and postirrigation CFU/mL was statistically significant (Fig. 3).

The antibacterial activity of both solutions used in this study was then compared using CFU/mL postirrigation. The study revealed that the experimental group had a substantial advantage (Fig. 4).

Discussion

Success of pulpectomy depends on the disinfection of the root canal system by chemomechanical instrumentation using an array of irrigants.[9] Due to the intricate canal structure in primary teeth, complete elimination of bacteria with an endodontic tool is impossible. This is where the adjunctive use of root canal irrigants in conjunction with mechanical instrumentation comes in.[1]

Hobson[10] noticed that three-fourths of root dentin in necrotic primary teeth were infected, revealing the importance of instrumentation and irrigation. Cogulu et al.[11] reported infectious bacteria, such as *E. faecalis*, *Porphyromonas gingivalis*, and *Treponema denticola*, as the causative organisms of endodontic lesions in primary teeth. *E. faecalis*, a gram-positive facultative anaerobic cocci, has been known to be present in periradicular infections and is considered the most resistant bacterial species in necrotized primary teeth.

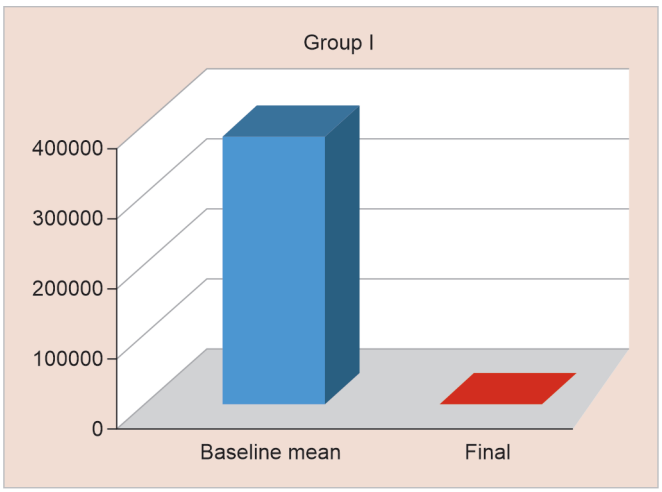

Fig. 2: A bar graph showing the comparison of baseline and final mean CFU/mL of group I

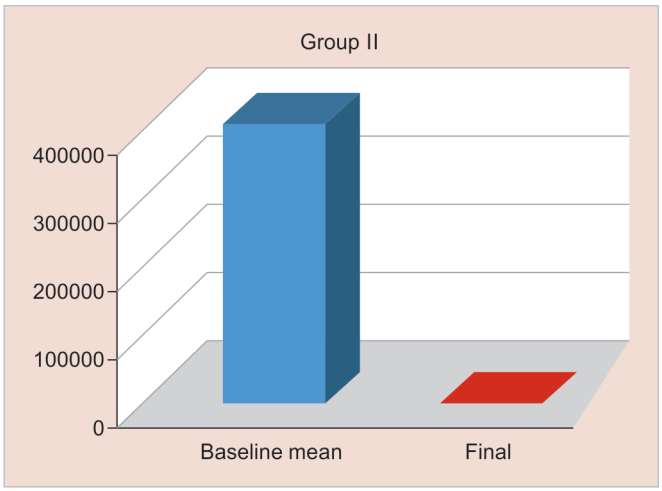

Fig. 3: A bar graph showing the comparison of baseline and final mean CFU/mL of group II

Apart from *E. faecalis* virulence, the difficulty of disinfecting the root canal system can be attributed to the variations in the complex anatomy of the root canal, such as the presence of accessory canals, intercanal communications, and apical ramifications, which favor bacterial growth. On the contrary, promising results are seen with altered high concentrations and formulations, treatments, and combinations of several root canal irrigants.[12] There is a need for a substance that can be utilized to diminish or eradicate *E. faecalis* with the fewest negative effects possible.

Although CHX, a biocompatible and effective irrigant during pulpectomy for the treatment of necrotic primary teeth, has been found to be bacteriostatic and bactericidal against a wide spectrum of bacteria for 48–72 hours, it cannot eliminate biofilms or organic debris.[13]

The results of our study were similar to the results of the study conducted by Ruiz-Esparza et al.,[7] that showed a significant difference using 2% CHX. In an *in vitro* study conducted by Zhang et al.[14] against *E. faecalis*, it was concluded that CHX had the strongest and longest antimicrobial activity compared to EDTA, MTAD, and Qmix. Jolly et al.[15] also found results similar to our study in the 2% CHX group and concluded that CHX has superior antimicrobial activity against both endodontic microflora in primary teeth.

Smear layer formation is an unavoidable result of mechanical instrumentation, independent of the equipment and techniques utilized to achieve root canal therapy success. The smear layer is considered harmful because it adheres to the root canal walls, partially or totally occluding the dentinal tubules and inhibiting irrigating solution penetration. The removal of the smear layer, on the other hand, is thought to remove the associated microbiota and their toxins from the root canal walls, limiting the likelihood of bacterial survival and reproduction.[16]

Despite being the most often used endodontic irrigant, NaOCl is incapable of dissolving inorganic smear layer components. Hence, chelating agents are recommended as adjuncts to root canal irrigants in removing the smear layer. Alternate use of NaOCl

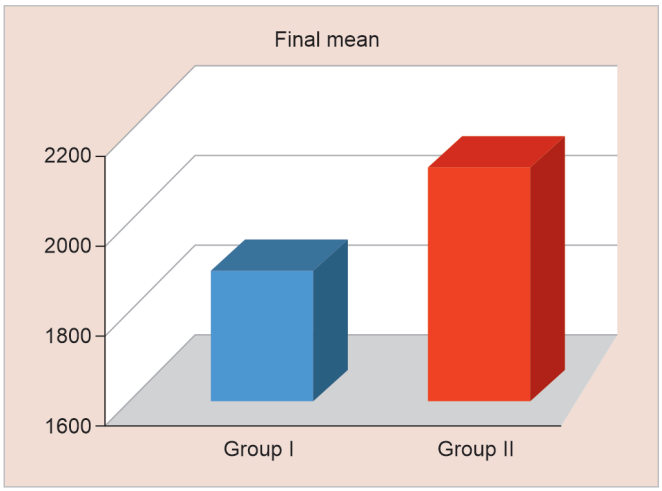

Fig. 4: A bar graph showing the comparison of the final mean CFU/mL of both the groups

and chelating agent appears to be a tedious task, especially in uncooperative children. In such cases, the use of etidronic acid, a mild chelator that contains NaOCl as the only solution, has the added advantage of both antimicrobial and chelating agents.[17]

Most of the literature available concerning etidronic acid has evaluated its efficacy on microhardness or mineral content of root dentin and its effect on the bond strength of resin sealers to dentin after various irrigation regimes. The literature search showed that studies evaluating the *in vivo* antimicrobial efficacy of etidronic acid against *E. faecalis* in both primary and permanent teeth are sparse. Arias-Moliz et al.[18] found that a combination of HEBP and NaOCl was the most effective irrigating solution for dissolving and destroying *E. faecalis* biofilms. The study's findings are similar to those of our research.

Another study which was also conducted by Arias-Moliz et al.[19] concluded that HEBP, when combined with NaOCl, does not restrict the ability of NaOCl to kill the *E. faecalis* within the dentinal tubules. Additionally, the addition of HEBP to NaOCl did not alter the chlorine content within 60 minutes. However, both these studies were in vitro studies, the results of which cannot be extrapolated to *in vivo* conditions.

Our study is perhaps the first to evaluate the *in vivo* efficacy of etidronic acid with NaOCl as an antimicrobial agent and was found to be acceptable in eradicating *E. faecalis* in the root canal of primary teeth.

A significant reduction in *E. faecalis* was observed in our study and can be attributed to the efficacy of both NaOCl, which is one of the components used along with 9% etidronic acid.

According to research, a combination NaOCl/HEBP solution may offer a number of advantages over a pure NaOCl solution, such as prevention of the formation of the smear layer and reduction of the formation and deposition of hard tissue debris during instrumentation of the root canal.

Why This is Important to Pediatric Dentists?

- Etidronic acid can remove the inorganic debris produced during root canal instrumentation.
- It can be used as an alternate irrigant, having both antimicrobial action and chelating action during the pulpectomy procedure in primary teeth.

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