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MICROBIAL EVALUATION OF THE EFFECT OF ENDO-SOLUTION AND SODIUM HYPOCHLORITE ON ENTROC OCCUS EACALIS ON NON - VITAL PRIMARY TEETH

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ABSTRACT

The aim of this study is to evaluate the microbial effect of endo – solution and sodium hypochlorite on Entrococcus Feacalis on non- vital primary anterior teeth.

Methodology: Forty teeth with early childhood caries were selected from Pedodontics Outpatients Clinic, Faculty of Dental Medicine, Boys, Cairo, Al- Azhar University, during period of 2014 - 2016. The teeth were classified into two equal groups (A&B) each group formed of 20 teeth. Group (A) teeth were irrigated with endo-solution (EDTA 15%) after pulpectomy procedure. Group (B) teeth were irrigated with sodium hypochlorite 2.25% after pulpectomy procedure. The sample taken before and after 48 hours post irrigation.

Results : Sodium hypochlorite decreased the bacterial count from 5708.33 to 1650 (P = 0.002). While EDTA decrease bacterial count from 13334.16 to 6471 (P =0.252).

Conclution: Both EDTA and sodium hypochlorite have positive effect on Efeacalis but sodium hypochlorite more effective than EDTA.

INTRODUCTION

Early childhood caries mainly occurs in maxillary primary anterior teeth and if untreated it can lead to pulpal involvement and destruction of coronal tooth structure, these teeth are difficult to restore. Elimination of microorganisms from infected root canals is a difficult task, numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments.

The antimicrobial solution that has had extensive use in endodontic as a root canal antimicrobial is sodium hypochlorite (NaOCl), in concentrations ranging from 0.5% to 5.25%. This is due to its antimicrobial and dissolving effects on necrotic tissues [(1)]. NaOCl is commonly used in concentrations between 0.5% and 6%. It is a potent antimicrobial agent, killing most bacteria instantly on direct contact. It is used as an unbuffered solution at pH 11 in the various concentrations mentioned earlier, or buffered with bicarbonate buffer (pH 9.0), usually as a 0.5% (Dakin solution) or 1% solution.3

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However, buffering does not seem to have any major effect on the properties of NaOCl, contrary to earlier belief [2].

There is considerable variation in the literature regarding the antibacterial effect of NaOCl. In some articles hypochlorite is reported to kill the target microorganisms in seconds, even at low concentrations, although other reports have published considerably longer times for the killing of the same species [3,4]. Such differences are a result of confounding factors in some of the studies. The presence of organic matter during the killing experiments has a great effect on the antibacterial activity of NaOCl. The limited antimicrobial effectiveness of NaOCl in vivo is also disappointing. The poorer in vivo performance compared with in vitro is probably caused by problems in penetration to the most peripheral parts of the root-canal system such as fins, anastomoses, apical canal, lateral canals, and dentin canals. Also, the presence of inactivating substances such as exudate from the periapical area, pulp tissue, dentin collagen, and microbial biomass counteract the effectiveness of NaOCl [5].

Ethylenediamine tetra acetic acid (EDTA).

EDTA is a chelating agent used for the removal of the inorganic portion of the smear layer. NaOCl is an adjunct solution for removal of the remaining organic components. Irrigation with 17% EDTA for one minute followed by a final rinse with NaOCl is the most commonly recommended method to remove the smear layer, Longer exposures can cause excessive removal of both peritubular and intratubular dentin [6]. EDTA (17%, disodium salt, pH 7) has little if any antibacterial activity. On direct exposure for extended time, EDTA extracts bacterial surface proteins by combining with metal ions from the cell envelope, which can eventually lead to bacterial death. EDTA is an effective chelating agent, which is widely used in endodontic preparation [7]. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentin to a depth of 20–30 μm in 5 min [8]. it effectively removes smear layer by chelating the inorganic component of the dentine. Therefore, by facilitating cleaning and removal of infected tissue, EDTA contributes to the elimination of bacteria in the root canal.

SUBJECTS, MATERIALS AND METHODS

1- **Study design:** This study was interventional and observational study.

2- **Study setting and sample size:** This study was carried out in period between November 2014 and November 2016, on 120 badly decayed primary anterior teeth that indicated for pulpectomy. These teeth were selected from child patients of Pedodontics Dentistry Outpatients Clinic, Faculty of Dental Medicine, Boys, Cairo, Al-Azhar University. The prçcedure was discussed in briefly for each patient s parent and informed consent was taken from each one.

A-Inclusion criteria:

1. Patient and parent cooperation.
2. Absence of any systemic disease which would contraindicate pulp therapy.
3. No previous history of antibiotic therapy for at least 2 weeks.
4. Presence of clinical signs or symptoms suggesting a vital or non- vital tooth.
5. Possibility for establishing a final restoration of the tooth.

Microbiological evaluation:

Microbial samples were taken before and after irrigation with sodium hypochlorite 2.25% or Endo-solution (EDTA 15%) to evaluate and compare the antimicrobial efficacy of the studied medicaments. Samples was obtained with sterile absorbent paper points and transferred into screw-capped tubes containing 5 ml of transport medium.

Sampling procedure:

1. Sample taking by paper point from infected root canal
2. Sample collection through transport medium.
3. Sample transfer into saline solution.

Medium for transportation of clinical specimens: The medium was normal saline. The pH of the medium was adjusted to 7.2±0.1. The medium was then distributed in 5ml in screw-capped tube. Specimens were immediately transported to microbiological lab at Microbiology Department, Faculty of Science, AI-Azhar University. Homogenized specimens were serially diluted down to 10^{-3} in sterile normal saline. From last dilution tubes, 1 ml aliquots were aseptically spread with a sterile bent-glass rod on the proper medium for organism.

Media for culturing of clinical specimens:

Enterococcus selective agar media (Entero-coccusi agar). Selective medium for the enrich-ment and isolation of enterococci specially E. Fa-cials from diverse clinical materials and of highly contaminated products of sanitary importance.

Bacterial count: Bacterial colonies were counted using light microscope and expressed as colony forming unit/ml(cu/ml).

Statistical Analysis:

The significant differences between groups was assessed using t-test and one-way analysis of variance (ANOVA). Values of P≤ 0.05 were considered significant. These analyses were done using SPSS 18.0 statistical software [9].

RESULTS

Comparison between EDTA 15% and sodium hypochlorite 2.25% pre and post-treatment: It was found the effect of both irrigation on Faecalis with positive value so mean value of pre and post - treatment by EDTA 15% was 6862.5 and mean value of pre and post- treatment by sodium hypochlorite 2.25% was 4058.33 (Table 1).

TABLE (1) Comparison between EDTA 15% and sodium hypochlorite 2.25% pre and post-treatment.

Group	Mean	S. D	S. E	Sig.
EDTA15%	6862.5	19654.86	5673.86	0.252
	4058.33	3449.23	995.7	0.002

DISCUSSION

Early childhood caries (ECC) is a serious public health problem in both developing and industrialized countries, ECC has been characterized by first affecting the primary anterior maxillary teeth, followed by involvement of the primary molars [10]. The microbiological investigation of the present study exhibited anaerobic microorganisms in the infected root canals mainly Enterococcus facials, this was seen through decrease in their count after irrigation with 2.25% Sodium hypochlorite by 71.09 %. This result is in concomitant with other studies [11,12].

It has been assessed the efficacy of 0.5%, 2.5% and 5.25% NaOCl as intracanal irrigants against E. faecalis within root canals and dentinal tubules, the results revealed 5.25% concentration was the most effective solution followed by 2.5% concentration [11]. Other study compared the efficacy of two different concentrations of NaOCl (5.25% and 1.5%) with 2% chlorhexidine (CHX) gel against E. faecalis, they concluded that 5.25% NaOCl and 2% CHX gel had good potential to keep a low E. faecalis count immediately and 7 days after instrumentation, where as 1.5% NaOCl reduced the E. faecalis only after instrumentation [12]. The antibacterial effect of 2.25% Sodium hypochlorite was strong against Enterococcus faecalis, this result is in concomitant with other studies [13,14].

According to other researchers, the degree of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue when using NaOCl solution or chlorhexidine (CHX gel) with real-time quantitative-polymerase chain reaction (RTQPCR) and culture techniques. Their results revealed that, using both identification techniques, the bacterial reduction in the NaOCl group was significantly greater than in the CHX group [13].

Another study [14] investigated the bacterial reduction after instrumentation using 2.5% NaOCl as an irrigant and further inter- appointment dressing with a calcium hydroxide (Ca(OH)2)/ camphorated paramonochlorophenol (CPMC) paste. They concluded that chemo- mechanical preparation with 2.5% NaOCl significantly reduced the number of bacteria in the canal but failed to render the canal free of cultivable bacteria in more than one-half of the cases. On the whole, it can be concluded that NaOCl, in both in vitro and In vivo conditions, exhibits excellent antibacterial activity, this powerful of antibacterial action of 2.25% NaOCl may be due to chlorine (a strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulfhydryl group) of essential bacterial enzymes [15].

The antimicrobial effectiveness of sodium hypochlorite, based in its high pH>11(hydroxyl ions action), is similar to the mechanism of action of calcium hydroxide. The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation observed in lepidic peroxidation [16].

In the present study, the antibacterial effect of 15% EDTA was weak against Enterococcus facials (38.53%), this result is in concomitant with another study [17-21].

In study of another investigators [17,18], demonstrated that combination of EDTA and 5% NaOCl had better antibacterial activity of NaOCl alone. In spite of EDTA is not irritating to pulpal or periapical tissue, self- limiting, and not corrosive to endodontic instrument. But has additional benefit, EDTA has been found to inhibit bacterial growth. This result was confirmed with other investigator [19] concluded that antibacterial activity for smear clear (mixture of 17% EDTA, cetrimide, polyoxymethylene [10] iso-octylclohexyl ether) with a 78% in decrease bacterial number compared to a 27 % decrease bacterial number for an irrigating solution only containing 17% EDTA.

In study of Patterson (1963) [18], EDTA had limited antibacterial activity. It seems that the antibacterial activity of EDTA is due to the chelation of cations from the outer membrane of bacteria. Antimicrobial effect of Na-EDTA was maintained as long as the chelators have not formed bonds with metal ions. In study of other searcher [21] Concluded that antibacterial activity of EDTA combined with ultrasonic activation clinically. After 7 days, without placing any intracanal medicament, most cases were bacteria-free.

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