ORIGINAL ARTICLE

Evaluation of antifungal efficacy of QMix 2in1 as a final irrigant: An *in vitro* study

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Abstract

Background: It is known that no specific antifungal agent exists at present for irrigation of infected root canals. QMix 2in1 was investigated to determine whether they could be an alternative for sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), and ethylenediaminetetraacetic acid (EDTA).

Objective: The aim of this *in vitro* study was to evaluate and compare the antifungal efficacy of QMix 2in1, 5.25% NaOCl, 2% CHX, and 17% EDTA as a final rinse against *Candida albicans* (*C. albicans*).

Materials and Methods: Ninety single-rooted mandibular premolar teeth were randomly divided into four experimental (n = 20) and two control (n = 5) groups. All root canals were instrumented with Mtwo rotary file system using crown-down technique to an apical size 40. Following root canal preparation, teeth were inoculated with *C. albicans* and incubated for 72 h. Teeth were irrigated with one of the following solutions as a final irrigant: (1) 5.25% NaOCl, (2) 2% CHX, (3) QMix 2in1, and (4) 17% EDTA. Aliquots from the samples were plated on 4% Sabouraud Agar, and colony-forming units were counted.

Results: QMix 2in1, 5.25% NaOCl, and 2% CHX were equally effective ($P > 0.05$) and significantly superior to 17% EDTA in eradicating *C. albicans* ($P < 0.05$).

Conclusion: QMix 2in1 proved to be effective against *C. albicans* when used as a final rinse. According to the findings of the present study, QMix 2in1 may be recommended as an alternative final rinse solution.

Key words: Antifungal, *Candida albicans*, endodontics, final irrigant, QMix

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Introduction

The main objectives of root canal treatment are the elimination of bacteria from root canals and the prevention of recontamination.[1] Pulpal and periapical infections are polymicrobial.[2] The most common fungi isolated from root canals is *Candida albicans* (*C. albicans*), which is found 21% primary[3] and 18% secondary infections.[1] The persistence of *C. albicans* in the root canals stems from its ability to penetrate into dentin tubules and resist antimicrobial agents.[4,5]

Antimicrobial activity is one of the most important qualities of an ideal endodontic irrigant.[6] While no specific antifungal agent exists at present for the irrigation of infected root canals,[2] many endodontic irrigants have been used in infected root canals with antifungal efficacy.[2,7-10] Sodium hypochlorite (NaOCl) is an endodontic irrigant that has been widely used for its tissue-dissolving and antimicrobial capacity.[11] However, not only is NaOCl highly toxic and capable of injuring periapical tissue, it also has an unpleasant taste,[12] and unable to sufficiency remove the smear layer.[13] Chlorhexidine gluconate (CHX), which also exhibits antimicrobial activity and is substantively less toxic than NaOCl, has been suggested as an alternative

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irrigant; however, CHX has no tissue-dissolution capacity.[14] Ethylenediaminetetraacetic acid (EDTA), a chelating agent that dissolves inorganic dentin components but not the organic components, and which is used mainly to remove the smear layer, may also act as an antimicrobial irrigant; however, dentin erosion has been reported with prolonged exposure.[6]

QMix 2in1 (Dentsply Tulsa, Tulsa Dental Specialties, OK, USA) is a new irrigation solution recently introduced to kill bacteria and remove the smear layer.[15] Its proprietary formulation contains EDTA, CHX, a nonspecified detergent, and water.[16] It is designed to combine the long-term antimicrobial properties of CHX with EDTA's ability to remove the smear layer in one formulation. Moreover, the surfactant in QMix 2in1 is included to decrease surface tension and increase wettability for better intracanal delivery of the solution.[17] QMix 2in1's unique chemical design has also eliminated the white precipitate that is usually produced when EDTA and CHX are mixed as well as the potentially carcinogenic brown/orange precipitate that occurs when CHX is combined with NaOCl.[18]

Previous studies[2,7-9] have reported on the antifungal efficacy of NaOCl, EDTA, and CHX on *C. albicans*; however, there is no study in the literature examining the antifungal efficacy of QMix 2in1 on *C. albicans* and comparing this with other endodontic irrigants. Therefore, the aim of the present study was to evaluate the antifungal efficacy of QMix 2in1, 5.25% NaOCl, 2% CHX, and 17% EDTA as a final rinse against *C. albicans in vitro*. The null hypothesis was that the ability to eliminate *C. albicans* would not vary significantly among the different irrigation solutions.

Materials and Methods

Ninety freshly-extracted, single-rooted mandibular premolar human teeth were stored in 0.2% sodium azide. All teeth were cleaned of superficial debris or calculus and radiographed to confirm the presence of a single canal. Teeth were decoronated to a standard 14 mm root length, and the root canals were instrumented with Mtwo (VDW, Munich, Germany) rotary file system to an apical size 40 from 1 mm short of the apical foramen. During the preparation, 1 mL of 5.25% NaOCl was used as an irrigant between each file change. Once instrumentation was completed, root canals were irrigated with 1 mL of 17% EDTA to remove the smear layer, followed by 3 mL of 5.25% NaOCl to remove residual irrigants, and the canals were flushed with 30 mL of sterile saline. Apical foramen of all roots were sealed with a temporary filling material (Cavit; 3M ESPE, Germany), and the root surfaces were coated with two layer of nail polish. All specimens were sterilized with ethylene oxide and placed in 1.5 mL centrifuge tubes. Specimens were randomly divided into four experimental ($n = 20$) and two control ($n = 5$) groups.

A suspension with a haze of 0.5 McFarland was prepared from *C. albicans* (ATCC 10231) manufactured at Sabouraud Dextrose Agar (SDA) (acumedia) and it was added on 300 μL centrifuge tubes in such a way that it covered to submerge all roots except the negative control ones and the mixture was stirred well. Specimens in negative control group were covered with equal amount of sterile saline.

Samples were incubated at 36°C for 72 h. Mixtures were refreshed with newly prepared 0.5 McFarland *C. albicans* suspensions every 24 h. Following incubation for 48 h, 10 μL was taken from each tube and planted on SDA as a reproduction control.

The presence of *C. albicans* in the root canal system was verified at 72 h, and the specimens were rinsed with 3 mL of following solutions for 1 min:

- Group 1: 5.25% NaOCl
- Group 2: 2% CHX (Drogsan, Ankara, Turkey)
- Group 3: QMix 2in1
- Group 4: 17% EDTA (Henry Schein Inc., Melville, USA)
- Group 5: (Positive control): Sterile saline
- Group 6: (Negative control): Sterilized teeth irrigated with sterile saline.

All solutions were delivered into root canals 2 mm short of the working length with sterile plastic syringes and 17-gauge needles until root canals and plastic test tubes were totally filled with them. All specimens were then flushed with 30 mL of sterile saline to prevent potential carry-over of the irrigants.

Three sterile paper points were used to collect the fluid from the canal carefully in order not to touch the outer surface of the canals, and the points were transferred to sterile tubes containing 1 mL of sterile saline solution. After vortexing, the tubes for 15 s, 100 μL of the contents were removed from the tubes and placed in petri dishes containing SDA. After incubating the dishes at 36°C 91% humidity for 48 h, the number of *C. albicans* colony-forming units (CFUs) were counted and recorded. All procedures were carried out under aseptic conditions.

Data were analyzed using Kruskal–Wallis and Mann–Whitney rank sum tests by using SPSS software (PASW Statistics 20; SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at $P < 0.05$.

Results

Mean *C. albicans* CFU following final irrigation with the tested solutions is presented in Table 1. No bacterial growth was observed in 5.25% NaOCl, 2% CHX, QMix 2in1, and negative control groups. These results show 5.25% NaOCl, 2% CHX, and QMix 2in1 to be equally effective ($P > 0.05$)

Table 1: Mean number and SD of *Candida albicans* colony-forming units (1×10^3/mL) in groups

	Mean number	SD
NaOCl	0	0
EDTA	2790*	34,311
QMix 2in1	0	0
CHX	0	0

*Statistically different from NaOCl, CHX, and QMix 2in1. SD=Standard deviation; NaOCl=Sodium hypochlorite; CHX=Chlorhexidine gluconate; EDTA=Ethylenediaminetetraacetic acid

and significantly superior to 17% EDTA ($P < 0.05$). All irrigation solutions were significantly superior to the positive control group, which exhibited bacterial growth in all samples ($P < 0.05$).

Discussion

This *in vitro* study evaluated the antifungal efficacy of QMix 2in1 and the commonly used root canal irrigants (5.25% NaOCl, 2% CHX, and 17% EDTA). There is little knowledge about the antimicrobial properties of QMix 2in1.[18-23] The present study, which, to our knowledge, is the first to eliminate the antifungal activity of QMix 2in1, found it to be equally effective as 5.25% NaOCl and 2% CHX and to perform significantly better than EDTA. Thus, the null hypothesis that there is no significant difference among the selected irrigation solutions in eradicating *C. albicans* has to be rejected.

Candida albicans was chosen as the test microorganism in the present study based on its various pathogenic characteristics. Not only does *C. albicans* have the ability to bind to dentin collagen, invade deep dentin tubules and form a biofilm, it is also known to activate host defenses and to show resistance to different antimicrobial agents used in endodontics. *C. albicans* cells have also been found in the resorption lacunae of periapical root surfaces and in periapical granuloma.[4,5] Moreover, oral candidiasis – a common infection of the oral mucous membranes in which *C. albicans* is frequently implicated – is highly prevalent in immunocompromised patients, whose compromised immune systems might increase the risk of fungi colonization of the root canal system.[9] For these reasons, an optimal solution for irrigation during cleaning and shaping of root canals should possess antifungal properties.

Previous studies showed that both NaOCl and CHX are able to eliminate *C. albicans* with contact time of under 1 min.[24,25] Similarly, the manufacturers of QMix 2in1 suggest it be used for 60–90 s as a final rinse. Therefore, the present study used 1 min of contact time for all the groups.

Sodium hypochlorite is the most widely used root canal irrigant, yet there is no consensus about its optimal concentration.[26] A past study have indicated that exposure to high concentrations of NaOCl is the most predictable method for eliminating intracanal bacteria and removing intracanal biofilm.[11] In the present study, 5.25% NaOCl was one of the most effective irrigants, with no bacteria counted in the NaOCl group. This finding is in line with previous studies.[18,20,27] With respect to antifungal efficacy, the present study found QMix 2in1 and 5.25% NaOCl to be equally effective. This is also in line with previous studies.[18,20,23] Besides, its unpleasant taste, NaOCl is also highly toxic, may cause severe irritation if inadvertently extruded into the periapical area, and unable to completely remove the smear layer. For these reasons, the use of QMix 2in1 as an irrigant may be a good alternative to NaOCl.

Ethylenediaminetetraacetic acid is recommended for removing the smear layer in root canal treatment.[28] However, disinfection of the dentin surface and dentin tubules may still be necessary. Due to its compatibility with dentin and potential residual antimicrobial effects, many researcher and clinicians have recommended soaking the root canal with 2% CHX following removal of the smear layer.[15] QMix 2in1 is a novel endodontic irrigant that combines the positive properties of both CHX and EDTA. Previous studies[18,20,22,23] have found the antimicrobial activity of QMix 2in1 to be better than that of CHX and EDTA. QMix 2in1 performed better than EDTA in the present study as well, whereas QMix 2in1 and CHX showed similar antifungal efficacy. Differences in the findings for CHX may be attributed to differences in methodology; especially, in the present study, the smear layer, a potential barrier to some irrigants, was removed before incubation of *C. albicans*, whereas earlier studies did not remove the smear layer.

Considering that endodontic infections are polymicrobial biofilm-based diseases, evaluating antifungal activity against only one organism represents a limitation to the present study, since the presence of multiple microorganisms might have altered the dynamics demonstrated by the present study.

Recent studies[18,29,30] have focused on new endodontic irrigants that can effectively clean root canals without exhibiting negative interactions with other irrigation solutions. QMix 2in1 has previously been shown not to interact with NaOCl[29] and to remove the smear layer as effectively as EDTA.[18] The present study found QMix 2in1 to exhibit antifungal activity similar to that of NaOCl and CHX and greater than that of EDTA. Given these finding, QMix 2in1 may be recommended as an alternative final rinse solution.

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