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The Anti-Bacterial Effect of Protocols of Different Irrigation Solutions

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ABSTRACT

The aim of study was to evaluate the anti-bacterial effect of four protocols of irrigation solutions (group 1: distilled water, group 2: hypochlorite sodium NaOCl 5.25% + EDTA, group 3: MTAD, and group 4: NaOCl 1.3% + MTAD). The clinical study consists of 52 anterior teeth from 29 adolescent patients (10-16 years). The studied bacteria were: ichirichia colli, SPP and anaerobic bacteria. Group 3 and 4 showed the reduction of an account of ichirichia colli, SPP and anaerobic bacteria after immediate irrigation solutions with superiority of group 3. Group 3 showed the reduction of an account of SPP after 48 hours of irrigation solution. Group 2 showed reduction of SPP + anaerobic bacteria after immediate irrigation solution and reduction of SPP bacteria after 48 hours of irrigation solutions. The new endodontic irrigation solution MTAD examined in this study had good anti-bacterial effect on many bacteria.

1. Introduction

Endodontic treatment aims to treat bacterial infection of the root canal system. Therefore, it is necessary to use anti-infection substances as perfusion solutions, and the main task of these substances is their effective effect on microorganisms located within the root canal system [1].

Complete resection and cleaning of the entire content of the root canal system and removal of dead tissue and dentinal residues formed from the preparation of the root canal is the prerequisite for successful endodontic treatment [2].

Although the chemical-mechanical preparation of the root canal system reduces the number of germs, the complete removal of spores is very difficult due to the complex internal anatomy of the root canal system [3]. Therefore, it is necessary to use cannula irrigation solutions such as:
Sodium hypochlorite which is most commonly used in endodontic as an anti-inflammatory with chemical properties for the breakdown and disintegration of pulp tissue and an effective antibacterial action, which is similar to organic substances in addition to deodorizing effects [4].

Sodium hypochlorite is not the ideal irrigation fluid, it cannot remove the pulp smear layer [5] and is

ineffective in eliminating enterococci [6] as its toxicity appears in high concentrations [7] away from thinking about its taste and unpleasant smell, as it has other disadvantages such as its inability to sterilize and disinfect the root ducts [8].

Endodontic treatment chelates were introduced as an adjunct in the preparation of calcified and narrow root ducts [8]. EDTA was the first chelating liquid solution used in dentistry for its ability to lubricate the dentin of the root canal [9]. Studies recommend the combination of decalcifying agents (general organic acids) and sodium hypochlorite to achieve the main goal [10]. To date there is no ideal irrigation solution, and for this reason the combination of irrigation solutions such as NaOCl and EDTA has been carried out with the aim of overcoming the limitations of the use of the irrigation solution alone.

Chelation agents remove dentin calcification by combining with calcium electrolytes from the tooth structure; this imbalance depends on the application time, the pH of the solution, and the concentrations used as well. The final washing EDTA, when used alternately with sodium hypochlorite, removes the pulp smear layer by its effect on the inorganic (mineral) components, and also affects the mineral salts of dentin, as it removes calcification 47 of the ductal and periductal dentin.

Based on the principle of root dentin cleansing, a new solution was developed called MTAD Bio Pure (Biopure, Dentsply Tulsa, Tulsa, OK, USA) to irrigate the root canal system [11]. It consists of 3% tetracycline molecule (doxycycline), 4.25% citric acid, 0.5% (polysorbate) and 80 detergent (detergent). The clinical success rates are very wide but have a high disinfection capacity when used alternately with sodium hypochlorite [6]. NaOCl is a broad-spectrum antibiotic, but it needs to be clinically tested [3], [12]. Initially, it was believed to use MTAD solution to remove traces of sodium hypochlorite, but it turned out that the most disinfecting method for the root canal system is washing with sodium hypochlorite solution NaOCl before abundant final rinsing with MTAD solution [11], which has a role in the disinfection of the ducts infected by germs and is considered a bio-harmonious substance [6].

The complete sterilization of the root canal system is one of the main objectives of the treatment of the root canal system [13]. Clinical findings have shown that a chemical-mechanical preparation with the use of antibacterial drugs will effectively reduce the microbial content of the ducts [14]. Despite these precise efforts, some microorganisms remain within channel [15], [16]. Since approximately 700 species of germs can be found in the oral fossa, anyone has about 100-200 species [17].

The intraductal microflora is often a mixture of a large number of bacterial species and it also contains a wide diversity of aerobic and anaerobic bacteria [18]. A clear difference has been observed between the spores in the root ducts of the dead pulp opened to the oral medium and the spores isolated from the root ducts with closed death and that all samples taken were germ containers [19], where it was proven that there were 169 different microbial species, and the number of microbial species in one channel ranged between 1 and 11 bacterial species. And that 80% of the germ species were anaerobic, as confirmed that the average total number of spores inside the canal is between 105 and 109 [20]. As for the ratio of aerobic bacteria to anaerobic bacteria located in the root canal, most studies have confirmed that forced anaerobic bacteria strict anaerobes are found in a greater proportion compared to strict aerobes and anaerobic bacteria Facultative anaerobes with a difference in the value of this ratio [21].

During endodontic treatment of the root canal system, perfusion solutions help to dissolve the pulp tissue, and have microbial activity within the root canals and their branches. However, the perfusion solutions can inadvertently touch the tissues around the root, for this histotoxicity it is important to consider in the context

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of cannula treatment [13]. The toxicity of perfusion solutions should be minimal due to the possibility that they may be pushed abruptly in the course of canal treatment beyond the apical foramen which occurs as a result of the surgeon's inexperience or anatomical differences in the apex of the root and thus resulting in tissue damage around the apex [13]. For this, the typical or ideal endodontic irrigation solution should be non-toxic and non-irritating for periodontal tissues [15].

When we decide to use irrigation solutions, it is necessary to take into account the physical, chemical and biological properties. MTAD is an alternative chemical compound but it is still being studied to prove its benefits, advantages and antibacterial property if it is used alone or in combination with other irrigation solutions within specific protocols, which is why this research was conducted. To examine the comparison of the antibacterial activity of perfusion solution protocols, we looked for three types of bacteria: First, Enteroenterofecal which was tested as resistant to endodontic treatment. It is most commonly found in endodontic treated teeth at large rates from 30% to 90% of cases [22]. The presence of lithogenesis for long periods of time can cause or maintain the peri-peak lesion intact [23]. Second, Streptococci comprises a relatively high percentage, approximately 20% (an average of 16-50%) of germs recovered from dental canals infected with recurrence treatment [24]. And third, gram-positive antennas that can cause permanent and continuous endodontic infections.

2. Materials and Methods

An informed written consent was obtained from the participants before starting the study. The clinical study sample consisted of 52 anterior teeth from 29 adolescents aged between 10 and 16 years, each of whom had one or more teeth that needed ductal treatment, the study sample was subject to the following criteria:

- Age from 10 to 16 years.
- Does not complain of general diseases.
- Has not been subjected to antibiotic treatment in the past two weeks.
- All teeth are not alive and teeth with a single channel and have not been subjected to previous canal treatment without any trace of fistula or open peaks or even absorption of the roots.

Attention is paid to recording all information related to the location of the treatment status for each of the studied teeth (the number of the treated tooth, its upper or lower location, its right or left side and its type).

The clinical study sample was divided into four main groups (N=13) according to the irrigation solution protocol used (control group for 25 minutes, sodium hypochlorite at a concentration of 5.25% for 24 minutes, EDTA solution 17% for 1 minute, MTAD solution for 5 minutes, distilled water for 20 minutes, sodium hypochlorite 1.3% for 20 minutes, then MTAD for 5 minutes).

The targeted bacteria were enterobacteriace, SPP and gram-positive anaerobic bacteria, then clean the tooth surface and then apply the rubber barrier and disinfect the area with a cotton pellet moistened with 1 ml sodium hypochlorite solution 5.25% for 30 seconds and then the necrosis was swept away and the endodontic chamber was opened by a sterile spherical spike with water spray, and then a sterile K file 15 cooler was inserted to clear the channel and calculate the working length using a determining device electronic peak [25].

2.1 Initial enumeration of units forming germ colonies

Two sterile paper funnels measuring 15 were inserted to the entire working length and left for a minute to absorb the fluids within the channel. We put one of the two paper funnels in 1 ml (thioglycolate broth is the carrier medium for the cultivation and incubation of gram-positive anaerobic bacteria, and the second

funnel we put in 1 ml of broth Heart and Brain Extract (BHI) is the medium that transmits fecal intestinal and streptococcal SPP.

2.2 Bacteriological implants and definition of techniques

For fecal intestinal bacteria implant, 15 microliters of heart-brain broth are taken with a micro Pipette and brushed using L-shaped metal rods on pre-prepared yellow Petri Agar-Agar-escolin dishes, after being incubated at 37° C for 24 hours. The sample was brushed using a metal rod specially prepared for research. After planting the germ colonies, they were incubated in an incubator at a temperature of 37° C for 24 hours. The colonies were filtered and purified by colony shape, gram coloring, catalase analysis and the colonies were counted CFU/ml. Figure 1 shows colony shape on yellow esculine medium. Then, it was placed under the microscope to examine the germs by magnification as shown in Figure 2. After that, the germ colonies were counted using the colony counting device.

Figure 1 Colony shape on yellow esculine medium

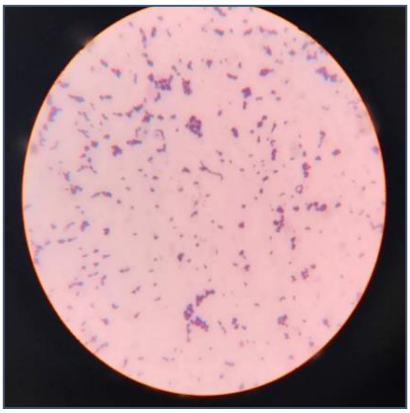

Figure 2 Gram-positive bacteria on the medium of yellow esculine using microscope

For streptococcal SPP implants, 10 μL of heart and brain broth was taken and brushed with L-shaped metal rods on potassium-rich salivary agar dishes and then the germs were incubated with anaerobic brood bags at a temperature of 37° C for 48 hours. The sample was spread on the potassium-rich Mitis implant medium with the metal rod and then petri dishes of the potassium-rich medium were placed in the anaerobic brood bags. Then, they were placed in the incubator with a temperature of 37° C. The purity of the implant is confirmed by gram-coloring, catalase analysis, and counting of bacterial colonies on this medium with a microbial counting device. The microbial colonies were then examined under a light microscope after making preparations and coloring them as shown in Figure 3.

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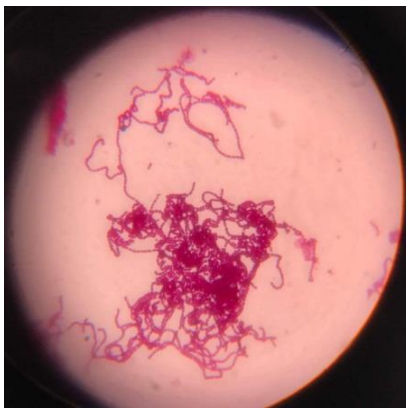

Figure 3 Streptococcus SPP under the microscope

For aerobic microbial implants, 10 µL of teoglycolate broth is brushed using L-shaped metal rods on Petri dishes from Wilkins-Chalgren agar, and then incubated for 48 hours in aerated brood bags, the different colonies are colored in gram coloring and are classified according to the shape of the colony and similar colonies are counted CFU/ml. Finally, they were examined under a light microscope as shown in Figure 4.

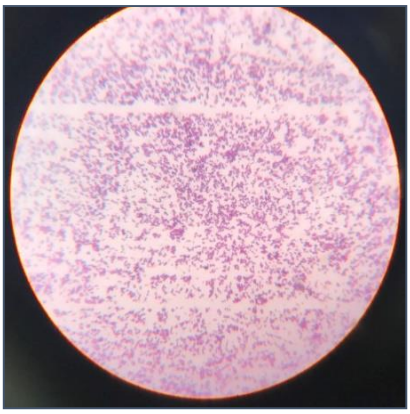

Figure 4 Gram-positive anaerobic bacteria under the microscope (Gram-positive cocci)

2.3 Perfusion protocols

Chemical mechanical preparation of the root canal system is carried out using the Step-Back technique using K files, and when each instrument is changed, it is irrigated with the irrigation solution of each group (sodium hypochlorite at a concentration of 5.25% for 19 minutes, EDTA solution 17% for 1 minute, sodium hypochlorite 1.3% for 15 minutes, then MTAD for 5 minutes, MTAD solution for the control group).

The sample was taken after drying the root ducts with sterile paper cones. The second sample is taken as the first sample was taken, and the root channels were left empty and a sterile cotton pellet was placed at the entrance to the canal and temporarily closed with zinc oxide and eugenol filling for 48 hours.

After 48 hours and with the same sterile conditions, the temporary filling was removed using circular spikes and at high speed. The third sample was taken as it was with the previous samples.

The average difference in the number of germs was calculated immediately after irrigation at the end of the first visit by subtracting the first sample from the second sample, and after 48 hours by subtracting the first sample from the third sample.

Finally, all the teeth were irrigated with natural saline solution as a final irrigation solution. AhPlus root channels were filled using quota cones by side condensation with filler and filling the entrance to the hole with composite filling.

The census of microbial colonies (CFU) was counted and the decimal logarithm of the census of germ colonies was calculated in three different implant media (Bile Esculin Azide Agar, Mitis Salivarious Agar Base, Wilkins Chalgren Agar) in three different stages (after tooth opening, immediately after irrigation, 48 hours after irrigation) For each of the teeth studied in the clinical study sample. Then, the amount of change in the number of germ colonies (in thousands) in each of the two stages was calculated (immediately after perfusion, 48 hours after irrigation) in each of the implant media studied for each of the teeth studied.

3. Results

The ANOVA single variance analysis test was performed to study the significance of the differences in the average amount of change in the decimal logarithm of the census of microbial colonies between the groups of the perfusion method used (irrigation using sodium hypochlorite and EDTA, irrigation using sodium hypochlorite and MTAD, irrigation using MTAD, irrigation using distilled water (control group)) in the clinical study sample, according to the studied stage and the studied implant medium.

Table 1 shows the arithmetic mean, standard deviation, minimum and maximum amount of change in the decimal logarithm of the census of bacterial colonies in the sample of the clinical study according to the method of perfusion used, the studied stage and the studied implant medium.

Table 1 Arithmetic mean, standard deviation, minimum and maximum amount of change in the decimal logarithm of the census of bacterial colonies in the sample of the clinical study according to the method of perfusion used, the studied stage and the studied implant medium

Planting medium	Studied stage	Irrigation method	Arithmetic mean	Standard deviation	Minimum limit	Maximum limit
Bile Esculin Azide Agar	Immediately after irrigation	NaOCl + EDTA	-0.28	0.81	-1.87	0.84
		NaOCl + MTAD	-1.18	0.76	-2.37	-0.04
		MTAD	-0.84	0.63	-1.90	-0.02
		Distilled water	-0.08	0.45	-1.48	0.38
	48 hours after quenching	NaOCl + EDTA	-0.71	0.85	-1.91	0.44
		NaOCl + MTAD	-0.68	1.04	-2.83	0.75
		MTAD	-0.52	0.94	-1.99	0.89
		Distilled water	-0.11	0.78	-2.62	0.52
Mitis Salivarious Agar Base	Immediately after irrigation	NaOCl + EDTA	-0.64	0.92	-2.21	0.16
		NaOCl + MTAD	-0.63	1.11	-2.37	1.36
		MTAD	-1.10	0.77	-2.05	-0.09
		Distilled water	0.06	0.15	-0.09	0.52
	48 hours after quenching	NaOCl + EDTA	-0.41	0.64	-2.13	0.16
		NaOCl + MTAD	-0.06	0.98	-1.97	2.10
		MTAD	-0.50	0.47	-1.63	0.11
		Distilled water	0.23	0.47	-0.75	1.17
Wilkins Chalgren Agar	Immediately after irrigation	NaOCl + EDTA	-0.15	0.31	-0.93	0.18
		NaOCl + MTAD	-0.55	0.87	-3.25	0.17
		MTAD	-0.91	0.88	-2.44	0.20
		Distilled water	0.03	0.11	-0.18	0.20
	48 hours after quenching	NaOCl + EDTA	-0.21	0.62	-1.90	0.69
		NaOCl + MTAD	-0.15	0.58	-1.10	1.03
		MTAD	-0.34	0.60	-1.77	0.18
		Distilled water	0.09	0.19	-0.34	0.33

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Table 2 shows the results of the bilateral comparison by LSD method to study the significance of the bilateral differences in the average amount of change in the decimal logarithm of the census of bacterial colonies between the groups of the perfusion method used, immediately after irrigation according to the studied implant medium, and after 48 hours of irrigation in the Mitis Salivarious Agar Base implant medium group from the clinical study sample.

Table 2 Bilateral comparison by LSD method showing the significance of the bilateral differences in the average amount of change in the decimal logarithm of the census of bacterial colonies between the groups of the perfusion method used, immediately after irrigation according to the studied implant medium

Planting medium	Studied stage	Group (I)	Group (J)	Difference between the two averages (I-J)	Significance level value
Bile Esculin Azide Agar	Immediately after irrigation	NaOCl + EDTA	NaOCl + MTAD	0.90	0.001*
			MTAD	0.56	0.039*
			Distilled water	-0.19	0.470**
		NaOCl + MTAD	MTAD	-0.34	0.209**
			Distilled water	-1.09	0.000*
		MTAD	Distilled water	-0.75	0.007*
Mitis Salivarious Agar Base	Immediately after irrigation	NaOCl + EDTA	NaOCl + MTAD	-0.01	0.971**
			MTAD	0.45	0.166**
			Distilled water	-0.70	0.034*
		NaOCl + MTAD	MTAD	0.47	0.156**

*significant; **not significant

4. Discussion

It can be seen that the significance level value is smaller than 0.05 when comparing the values of the magnitude change in the decimal logarithm of the census of microbial colonies, immediately after perfusion, between the perfusion group using sodium hypochlorite and EDTA, the perfusion group using distilled water (control group), the perfusion group using sodium hypochlorite and MTAD and the perfusion group using MTAD in the Bile Esculin Azide Agar implant medium group. Likewise, when comparing the values of the magnitude of change in the decimal logarithm of the colony census Bacterial immediately after irrigation between the distilled water irrigation group (control group), the irrigation group using sodium hypochlorite and EDTA, the irrigation group using sodium hypochlorite and MTAD and the MTAD irrigation group separately in the Mitis Salivarious Agar Base and Wilkins Chalgren Agar Base implant media group. The same happens when comparing the values of the magnitude change in the decimal logarithm of the census of microbial colonies after 48 hours between the distilled water irrigation group (control group). Both the perfusion group using sodium hypochlorite and EDTA and the perfusion group using MTAD separately in the Mitis Salivarious Agar Base implant medium group, that is, at the 95% confidence level there are statistically significant binary differences in the average amount of change in the decimal logarithm of the census of microbial colonies between the groups of the perfusion method used in the clinical study sample. By studying the values of the arithmetic means, we conclude that the magnitude of the change (in absolute values) in the decimal logarithm of the census of microbial colonies immediately after perfusion in both sodium hypochlorite and EDTA irrigation group and distilled water irrigation group (control group) was smaller than in the perfusion group using sodium hypochlorite and MTAD and the

perfusion group using MTAD in the Bile Esculin Azide Agar implant medium group. We conclude that the decimal logarithm values for the census of microbial colonies immediately after irrigation increased in the irrigation group using distilled water (control group) and decreased in both the irrigation group using sodium hypochlorite and EDTA, the perfusion group using sodium hypochlorite and MTAD and the perfusion group using MTAD separately in each of the Mitis implant medium group Salivarious Agar Base and Wilkins Chalgren Agar implant medium group. The values of the magnitude change in the decimal logarithm for the census of microbial colonies after 48 hours increased in the distilled water perfusion group (control group) and decreased in both the perfusion group using sodium hypochlorite and EDTA and the MTAD perfusion group separately in the Mitis Salivarious Agar Base implant medium group in the clinical study sample.

As for the rest of the studied binary comparisons, it is noted that the value of the significance level is greater than the value of 0.05, i.e. at the confidence level of 95%, there are no statistically significant binary differences in the average amount of change in the decimal logarithm of the census of microbial colonies between the groups of perfusion method used in the clinical study sample.

We agreed with the results of another study that the values of the decimal logarithm decreased in the bacterial population of colonies of fecal enterobacteriacetes immediately after irrigation [26]. This study included sodium hypochlorite perfusion solution and chlorhexidine perfusion solution, but in our study sodium hypochlorite perfusion solution was combined with MTAD perfusion solution.

We agreed with the study of [27], through which the researcher showed that the MTAD perfusion solution is effective in reducing the number of fecal intestinal bacteria. This study was done in the laboratory on extracted front teeth, and this is similar to our findings despite the different conditions of our study.

For gram-positive anaerobic bacteria, irrigation solutions in sodium hypochlorite, MTAD and MTAD alone showed a statistically significant decrease in logarithmic values of these bacteria by the end of the first session immediately after irrigation. There was no significant decrease in decimal logarithm of these spores' values after 48 hours. This is due to environmental changes resulting from biomechanical preparation, which removes most sensitive spores and provides suitable growing conditions for anaerobic bacteria [28]. Similar results were found due to insufficient sealing, which in turn provides an entry for germs as well as fluid leakage from the oral medium into the root canal [15].

For SPP bacteria, Perfusion solutions with sodium hypochlorite group, EDTA, sodium hypochlorite group, MTAD and MTAD perfusion solution group alone showed a statistically significant decrease in logarithmic values of these bacteria by the end of the first session immediately after irrigation. Sodium hypochlorite, EDTA, and MTAD perfusion solutions showed a significant decrease in decimal logarithm values after 48 hours. It seems clear that sodium hypochlorite and MTAD perfusion solutions are effective immediately after perfusion, but their effectiveness decreases and the decimal logarithm count of SPP colonies increases after 48 hours. This indicates the intrinsic efficacy of MTAD perfusion solution when used alone, and greater objectivity than other solutions. Thus agreeing with the study of Mohammadi, who proved the long-term effectiveness of MTAD against stubborn germs [29]. We can also explain this because the sodium hypochlorite irrigation solution when used in combination with the MTAD solution reduces the effectiveness of this solution, while both solutions have greater effectiveness when used independently and without mixing. The same result reached when sodium hypochlorite included alone in one group and MTAD in another group and a third group included the two solutions, and concluded for each solution better effectiveness if used alone and stronger than the combination of the two solutions [30].

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5. Conclusion

Within the conditions of this clinical study it can be concluded that the perfusion protocol used MTAD solution alone, the irrigation protocol used sodium hypochlorite and MTAD showed a decrease in the number of fecal enterobacteriace and anaerobic bacteria immediately after irrigation. The perfusion protocol used in MTAD alone showed a decrease in the SPP population 48 hours after irrigation. The perfusion protocol used sodium hypochlorite with EDTA showed a decrease in SPP population 48 hours after perfusion. The possibility of using MTAD as an irrigation solution according to any protocol used in this study, because there is no difference between its use alone or with sodium hypochlorite concentration of 1.3% in this study because of its effect on fecal intestinal bacteria and anaerobes immediately after irrigation. We emphasize the need to follow the instructions of the producing company regarding the method of applying irrigation solutions, and the instructions of the producing company to mix the MTAD solution and the time of its application. We recommend that MTAD not be left in the endodontic cavity to reduce the internal pigmentation of the dentin caused by tetracyclines present in this mixture.

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