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Efficacy of Magnetized Water as a Mouth Rinse in Comparison to Chlorhexidine Digluconate (0.2%) against *Candida albicans*: An *in vitro* Study

Sanaa Ammar Qassabbashe*, Afrah Khazal Al-Hamadany

Department of Pedodontics, Orthodontics and Preventive Dentistry, College of Dentistry, University of Mosul, Iraq

ABSTRACT

Background: Obvious elevation of yeasts occurs after insertion of orthodontic appliances, colonization with C. albicans is actually important to orthodontists due to the possible cariogenic effect of these microorganisms.

Objectives: The goal of this study was to estimate the antifungal effectiveness of magnetized water as a natural mouthrinse and the possibility of its use as alternative to the synthetic mouthrinse.

Materials and method: In glass containers, 100 ml of distilled water was prepared and each container was surrounded by two magnets. The chlorhexidine 0.2% mouthrinse (Wisdom-England) was used as control positive, while the distilled water was used as a control negative, as well as the experimental mouth rinses include magnetized water prepared at high magnetic power 3000 Gs and at low magnetic power 1000 G. The agar well diffusion method was used to investigate the antifungal susceptibility. One way (ANOVA) and the more sensitive Duncan's multiple range tests were used to evaluate the presence of significant difference between the groups.

Results: The chlorhexidine had the highest mean value followed by high magnetized water and low magnetized water respectively while the distilled water did not display any inhibitory effect against Candida albicans.

Conclusion: It might be conducted that magnetized water can be used as a natural, adjunctive antifungal mouthrinse as it has approximately comparable effect on Candida albicans as synthetic mouthrinse chlorhexidine.

Key words: Antifungal effect, C*andida albicans*, Chlorhexidine, Magnetized water, Mouthrinse

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Corresponding author: Sanaa Ammar Qassabbashe

e-mail✉**:** sanaa.20dep47@student.uomosul.edu.iq

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INTRODUCTION

One of the most prevalent oral health complications is malocclusion; this problem is often managed with orthodontic treatment. Orthodontic therapy comprises fixed and removable orthodontic appliances [1]. Using the acrylic removable orthodontic appliances (ROAs) has an important role in the progression of oral *C. albicans* colonization; this might be due to the reduced salivary flow and subsequently low PH levels as well as diminished oral hygiene. The use of ROAs offer an intensified situation for *C. albicans* development as they covering a huge part of mucosal tissues for a large period of time each day for a fairly prolonged time [2]. Also these ROAs preserve the yeast from the usual salivary flow and mechanical elimination effects of oral muscular structures, so that the collaboration of these influences can alter the balance in the patients using the ROAs to encourage *C. albicans* growth and propagation [2-4].

On the other hand foreign structures in the oral environment such as fixed orthodontic devices (FOAs) might seriously disturb the oral health by permitting plaque and food debris build up. Treatment with FOAs is linked with alterations in the oral microbiota, involving enhanced *C. albicans* growth. *C. albicans* can result in oral infections and lesions for example candidiasis as well as angular cheilitis; all these complications are deleterious for both patient as well as orthodontist. The obvious elevation of oral yeasts after insertion of (FOAs) has been demonstrated and the colonization of the oral cavity with *C. albicans* is actually important to orthodontists due to the possible cariogenic effect of these microorganisms, which has been confirmed *in vitro* and in vivo. Gingival inflammations and decalcification of enamel around FOAs are common complications when the appropriate prevention programs have not been employed. Therefore, obliteration of plaque is the chief goal to avoid and/or defeat the complications documented above [5-9]. Numerous strategies of chemical plaque resistor

have been implemented as supplemental treatments, concerning appropriate oral hygiene programs in association with several dentifrices, gels, and mouth rinses. The most commonly utilized antimicrobial mouthrinse which is presently contemplated as the gold standard is chlorhexidine (CHX), an antimicrobial agent that has been confirmed to decline the colonization of microorganisms in the oral cavity but its prolonged usage has some particular limitations like bitter flavor, light brown discoloration of teeth, evolution of resistant microorganisms, oral mucosal erosions and this has established the necessity for detecting alternatives that can be acceptable, affordable and appropriate as well [10,11]. To minimize the limitations of CHX that listed above, the therapeutic properties of other natural agents, herbs and plant extracts have been assessed by several previous studies . Based on Kasi, et al. study's observations, it can be concluded that magnetized water might be effectively utilized as a supplement to mechanical plaque control to prevent plaque as well as gingival inflammations, moreover MW has displayed an elimination in Streptococcus mutans (S. mutans) count, so that, MW may be utilized as an alternate mouthrinse to CHX [10].

If permanent magnets are retained in contact with suitable amount of water, for appropriate time, the water is not only affected by the magnetic flux but also gets to be magnetized and obtains magnetic properties and will has many impacts on human body. Magnetized water (MW) raises the pH level of saliva and gets rid of toxins, it is an energy -building, activating, cleaning, bacteriostatic and bactericidal agent [12,13]. As the MW is biocompatible, well accepted by all subjects without any side-effects as with CHX [10]. Therefore, this study was conducted to seek for natural mouthrinse (MW) as alternative to the available synthetic mouthrinse (as CHX) and understand the effect of MW on *C. albicans*.

MATERIALS AND METHODS

Preparation of magnetized water

The method of using static magnets near certain volume of water was used in the present study. In glass containers, 100 ml of DW was prepared and each container was surrounded by two magnets. Pairs of neodymium magnets were fixed in north-north poles and they were in a state of revulsion and the magnetic power was determined by Gauss meter [13]. The power of magnets utilized in the present study was 1000Gs and 3000Gs respectively. Each 100ml of distilled water (DW) was magnetized for the duration of 72 hours [12]. Then the PH and electrical conductivity were checked to make sure that the DW became magnetized [10,12]. MW was freshly prepared to be ready for use after 72 hours, avoiding any loss of magnetization as it has been found that best results were achieved when MW used immediately after magnetization.

Antifungal susceptibility

In this research the agar well diffusion procedure (Kirby Bauer Test) was utilized to assess the antifungal efficacy for the four mouthrinses. The CHX 0.2% mouthrinse (Wisdom-England) was used as control positive, while the DW was used as a control negative, as well as the experimental mouth rinses include HMW (prepared at high magnetic power 3000 Gs) and LMW (prepared at low magnetic power 1000 Gs). Pure culture of *C. albicans* was isolated from the oral cavity, and it was used in this study, the culture was carefully managed and prepared for the experiment. The whole experiment was performed in microbiological laboratory (RNA lab. In Mosul city). Sub-culturing of *C. albicans* was freshly adjusted 24 hours prior to the experiment. Suspension of *C. albicans* in 0.9 NaCl was adapted to 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the inoculum suspension and applied on the Müller-Hinton agar media in the petri dishes. Then four wells of 6mm diameter and 2mm depth at equidistance in each petri dish were opened by using standard sterile perforator, these wells were filled with 50µl of the mouth rinses solutions with micropipette untouched by human hands and kept in the incubator (NüveEN 400-Turkey) at 37° C for 24 hours, and after the end of the incubation period, each inhibition zone diameter around the holes in the petri dishes was evaluated by measuring millimetrically with a digital caliper (Tide machine tool supply-China), for the purpose of ensuring that the results could be replicated, the experiment was run three times [14-24].

Statistical analysis

All results were computed and expressed as mean, standard deviation (SD), range, minimum and maximum values from the three petri dishes (n = 3). Statistical analysis was accomplished by utilizing SPSS software (version 18.0) with analysis of variance (One-Way ANOVA) and post-hoc Duncan's multiple range test were utilized for comparing the significant difference between the groups. A ρ-value of ≤ 0.05 was contemplated as statistically significant.

RESULTS

The antifungal effect of the four mouth rinses CHX, HMW, LMW and DW, was analyzed and showed in (Table. 1), and it was found that both of the experimental mouthrinses HMW and LMW showed different degree of antifungal activity against *C. albicans* viewed in (Figure 1). It was found that CHX had the highest mean value followed by HMW and LMW respectively while the DW did not display any inhibitory effect against *C. albicans*. The result achieved from (ANOVA) statistical test revealed a significant difference at ($p \leq 0.05$) viewed in (Table 2). Analysis of the Duncan's multiple range test results manifested a significant difference among the mean values of the inhibition zone diameter of all the mouthrinses in this study ($p \leq 0.05$) was viewed in (Table 3).

Table 1: Descriptive statistic for the inhibition zone diameter of the study groups.

Groups	N	Mean	SD	Range	Minimum	Maximum
LMW	3	10.96	0.25	0.5	10.7	11.2
HMW	3	12.63	0.45	0.9	12.2	13.1
DW	3	0	0	0	0	0
CHX	3	22.6	0.7	1.3	22.1	23.4
N: Number of samples in each group						
LMW: Low Magnetized Water Group						
DW: Distilled Water Group						
CHX: Chlorhexidine Group						
SD: Standard Deviation						
HMW: High Magnetized Water Group						

Table 2: One Way (ANOVA) test for the mean values of inhibition zone diameter among the study groups.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	771.05	3	257.01	1358.69	0
Within Groups	1.51	8	0.189		
Total	772.57	11			
Df is degree of freedom					
F is F test					
sig. is significant level is ($p \leq 0.05$)					

Table 3: Duncan's multiple range tests for determining the significant difference between the groups.

Groups	N	Mean	SE	Duncan Groups
LMW	3	10.96	0.14	A
HMW	3	12.63	0.26	B
DW	3	0	0	C
CHX	3	22.6	0.4	D
N: Number of samples in each group				
LMW: Low Magnetized Water group				
HMW: High Magnetized Water group				
DW: Distilled Water (control negative) group				
CHX: is chlorhexidine (control positive) group				
SE: Standard Error of means. Different letters mean significant difference ($p \leq 0.05$)				

Figure 1: Antifungal effect of the mouth rinses used against *C. albicans*. LMW: Low Magnetized Water group. HMW: High Magnetized Water group. DW: Distilled Water.

DISCUSSION

There is well knowledge about the complex equilibrium of oral microbiota within oral microflora and the presence of any foreign appliance would result in the damage of this equilibrium, which cause emersion of microorganisms that are potentially pathogenic and the initiation of disease progress. Though, after the placement of orthodontic appliances either the removable or the fixed devices, patients deal with several difficulties such as alteration in the oral environment, elevation of plaque quantity, variations in the structure of the flora, and complication in maintaining adequate oral hygiene [8,20] .

The difficulty in cleaning during the treatment with orthodontic appliances causes a reduction in the effectiveness of performing mechanical oral hygiene techniques including brushing and flossing, so that although the mechanical cleaning is a way to prevent and eliminate the colonization of microorganisms but not enough to maintain adequate oral hygiene. Therefore the use of mouthrinses for supporting oral hygiene and providing antimicrobial efficacy should be recommended. Noticeable elevation of oral yeasts appears after insertion of orthodontic appliances, particularly colonization with *C. albicans* is of special importance for orthodontists because the possible cariogenic effect of these microorganisms, which has been assessed *in vitro* and in vivo [9]. *C. albicans* is the most frequent yeast isolated from the oral cavity. One of

the causes for the accumulation of these microorganisms and their consequent destructive activity is the deficient oral hygiene [19]. *C. albicans* also implicated in oral candidiasis, systemic infections and even dental caries, so that *C. albicans* was chosen for testing if MW has good inhibition properties against these microorganisms, then can be recommended for orthodontic patients have susceptibility to the fungal infection. There is a greater colonization of oral cavity by some species of candida with fixed appliances compared to the normal flora, and *C. albicans* is the most prevalent microorganism (35%), followed by C. tropicalis (23.5%) [6].

CHX mouthrinse known as the gold standard in plaque elimination so that it was chosen in this study as a control positive. CHX has many side effects such as bitter flavor, light brown discoloration of teeth, development of resistant microorganisms and oral mucosal erosions [10-11]. All the previously mentioned drawbacks of CHX limited its use and it should not be indicated for long-term use.

After magnetization, the water's surface tension is reduced, making the MW softer and more alkaline, with a PH as high as 9.2, it has been found that the force of magnets and time of magnetization have great effect on the results achieved from magnetization, another important factor is the magnetic field polarity used for magnetization of water whether the north (N) or south (S) pole of the magnet was the surface closest to the water. Various diseases are treated using the bio-north magnetic water [13]. Some studies claimed that the north pole of magnet retards and arrest the growth and activity of bacteria, fungus, virus and tumors, in a study conducted by Bajpai, et al. (2012) discovered that only the North Pole with 0.1 T magnetic powers impeded the development of E. coli and Staphylococcus epidermidis bacteria [21]. Another study by Brkovic, et al. used the north poles of magnets as a therapeutic pole has revealed beneficial influence of the North Pole magnetic fields on the decrease of the quantity of dental plaque microbes in vivo [22].

So that in this study water magnetization was prepared by north poles of a pair of magnets facing each other surrounding glass containers filled with 100ml of DW. The plaque inhibitory effect of MW and its effect on Streptococcus mutans also were studied in many researches [10,12,13] which were concluded that MW may be efficiently utilized as a supplement to mechanical plaque control to stop gingivitis and other oral disease. The antifungal activity of MW was not conducted by previous researches so that this study was conducted to assess the antifungal effect of MW by using the agar well diffusion technique which is created on the basis of measuring the inhibition zone diameter. Diffusion occurrence based on the physical and chemical properties of tested substances [16]. The greatest inhibition zone for *C. albicans* was noted in the CHX (Wisdom-England) mouthrinse, this may be due to its high chemical contents (Glycerin, limonene, and hydrogenated castor oil and sodium saccharin). CHX is efficient for impeding the adhesion of candida to the biological and inert surfaces. It performs a fungicide and fungi static effects, causing alterations in cell walls and the coagulation of nucleoproteins, which may allow cytoplasmic components to escape all through the plasma lemma [23].

Although the CHX had better antifungal effect but the HMW and LMW had also showed clear antifungal effect against *C. albicans* and therefore they can be used as suitable, biocompatible and friendly mouthrinse without any side effects as with CHX.

As this study provides findings of the antifungal activity of the HMW and LMW, but one certain point with this study was that it was performed *in vitro* and the clinical implication was not adjusted nor examined. There are many intra oral factors that could alter the efficacy of the tested mouthrinses such as temperature, PH, salivary flow and many other factors. Therefore further studies are required to realize the clinical effectiveness of the MW as mouthrinse.

CONCLUSION

According to the results of this study, it might be conducted that MW can be used as a natural, adjunctive antifungal mouthrinse as it has approximately comparable effect on *C. albicans* as synthetic mouthrinse CHX.

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