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Evaluating the Disinfecting Effect of Microwave and 0.1% Sodium Hypochlorite on Contaminated Toothbrushes

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ABSTRCAT

Background and Aim: Contaminated toothbrush has important role in transmission of infection and disease. The purpose of this study was to evaluate two methods of toothbrush disinfection by sodium hypochlorite 0/1% and microwave on three types of microorganisms: staphylococcus aureus, streptococcus mutans and candida albicans.

Materials and Methods: In this in vitro study, 90 toothbrushes were divided into 9 groups (n=10). Toothbrushes were contaminated by standard suspension of streptococcus mutans, staphylococcus aureus and candida albicans. Then, toothbrushes were disinfected with hypochlorite sodium %0.1 for 10 minutes and with microwave for 1 minute. Afterwards, remained bacteria on surface of toothbrush were cultured and counted. For analyzing Numbers of Colony-Forming Units remained on toothbrush one-way Kruskal-Wallis and Mann-Whitney tests were used.

Results: Results of this study showed that significant difference exists among disinfectant effects ($p<0.001$). Results of Mann-Whitney test showed hypochlorite sodium had more disinfecting effect on Streptococcus mutans and Candida albicans compared with distilled water or microwave ($p<0.05$).However disinfecting effect of microwave was more than two other disinfectants on Staphylococcus aureus($p<0.05$).

Conclusion: hypochlorite sodium had more disinfecting effect on Streptococcus mutans and Candida albicans however microwave was more effective on Staphylococcus aureus.

Key words: Microwave ; hypochlorite; Sodium; Toothbrushing

INTRODUCTION

Daily brushing of teeth has an important role in maintaining personal hygiene and effective removal of microbial plaque. Toothbrushes can get contaminated not only by oral microorganisms, but also by those with environmental origins. [1,2] This microbial contamination can cause infection by the toothbrush containing pathogenic organisms. As reported in primary studies by Cobb et al. toothbrush is the source of recurrent oral infections.[3] Svanberg et al. found that toothbrushes can seriously get contaminated by streptococcus mutans after 24 hours.[4] Based on the study performed by Glass et al., microorganisms not only stick to the toothbrush and proliferate, but also can cause and transmit localized and systemic diseases. The increasing growth of intestinal bacteria, yeasts and fungi on toothbrushes of children has been proven.[5]

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Toothbrush can get contaminated with bacteria, blood, saliva and even the remnants of toothpaste. Most people injure their gums when brushing. Disinfecting the toothbrush prevents the risk of re-infection or being infected by environmental pathogenic microorganisms. Hospital patients are classified as vulnerable, however, nurses are not provided with any sort of guideline for storing and disinfecting the toothbrushes. [6]

Although disinfecting the toothbrush is beneficial for ordinary people, it is highly necessary for hospital patients, children[7], elderly people, those with immune system deficiencies and patients who consume immune system suppressant medications such as transplant patients.[8] Several bactericidal substances have been offered to reduce the risks of contamination within the intervals between uses. Storing the toothbrush in alcohol solution was among the first suggestions for disinfecting. After that, other methods were suggested such as exposure to sunlight, salt tablet to absorb the moisture and storing the toothbrush in a tight lid container full of formaldehyde gas.[10] Among other methods are chlorhexidine usage, immersion in disinfectant solutions, antimicrobial mouthwashes and using 1% and 3% sodium hypochlorite.[11,12] Tetra sodium salt, EDTA (ethylenediamine tetra acetate), microwave and UV radiations have recently been introduced for disinfecting the toothbrushes. [6, 8 ,13] Devine et al. announced that for disinfecting belongings such as toothbrush, people need fast-acting, effective, affordable and non-toxic methods of disinfecting that are easy to apply.[9]

Considering the performed studies and regarding that most offered methods were expensive and difficult to apply, the current in-vitro study was carried out to evaluate the disinfecting effects of microwave and sodium hypochlorite on toothbrushes contaminated with three types of oral cavity microorganisms. The practical objective of this study was to provide an easy disinfecting method that is applicable by the individual at home.

Materials and Methods:

In this in-vitro experimental study, 91 toothbrushes of standard dimensions and bristles of Diamond model and soft type (Panberes, Bushehr Mesvak Co., mod Diamond, Soft, Iran) were selected. According to the 3 microorganisms studied and the 3 disinfecting agents (0.1% sodium hypochlorite, microwave, and sterile distilled water), toothbrushes were assigned into 9 groups (n=10) (Figure 1). Contamination was observed on one of the toothbrushes in the negative control group. So all the toothbrushes were sterilized in autoclave and the following steps were respectively done.

The first stage- preparing the standard suspension (106×1 cfu/ml); the standard strains of the following microorganisms were obtained from Pasteur Institute:

1.Streptococcus mutans ATCC35688 (398= ג nm and O.D=0.620)

2.Staphylococcus aureus ATCC6538 (490=ג nm and O.D=0.374)

3.Candida albicans ATCC18804 (530=ג nm and O.D=0.284)

These standard strains were first cultured in their specific culture medium on TSA (tryptic soy agar) plate for streptococcus and staphylococcus and sucrose agar plate for candida albicans. For cultivation, the samples were incubated for 24 hours at 35±1°C. The culture medium used for streptococcus mutans contained 5% CO2. The solutions of standard strains (106×1 cfu/ml) were read by Spectrophotometer (Beckman Coulter DU720, USA). Samples were homogenized and prepared. [11]

The second stage- preparing the culture medium, toothbrushes and contaminating them. 10ml of SB (sabouraud broth) and TSB (tryptic soy broth) were distributed into the test tubes and were placed within the autoclave for 15 minutes under the pressure of 15pound/inch2 to be sterilized.

After sterilizing toothbrushes with autoclave, one of them was cultured to ensure of its being sterile (negative control). For each microor

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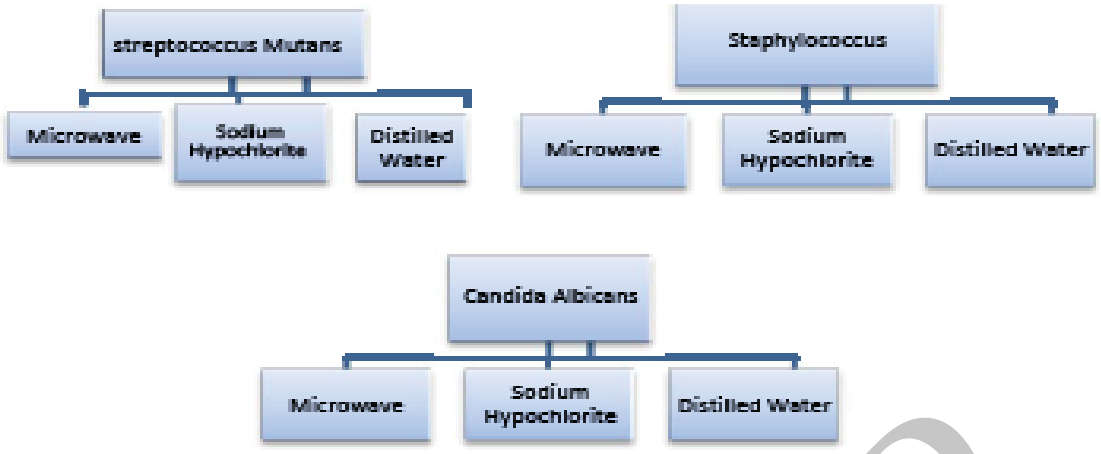

Schematic view of classification of study groups

For each microorganism under the study (streptococcus mutans, candida albicans, staphylococcus aureus) based on disinfectants (distilled water, microwave, sodium hypochlorite), 10 toothbrushes were assigned for distilled water (as positive control), 10 toothbrushes for microwave and 10 for sodium hypochlorite. They were all placed within sterilized test tubes.

Of each set of tubes, 10ml TSB (as liquid medium) was inoculated for contamination with streptococcus and staphylococcus and 10ml SB (as liquid broth) for contamination with candida albicans. Then 0.2ml of the prepared microbial suspensions was added to the culture medium. The toothbrush containing tubes were incubated at 35±1°C for 24 hours (CO2 environment was considered for streptococcus).[14]

The third stage- disinfecting the toothbrushes and quantifying the colony-forming units on toothbrushes in CFU unit.

Each microbial group was disinfected using one of the following methods of 0.1% sodium hypochlorite, microwave and distilled water.

Performing the disinfecting methods:

Storing toothbrushes in 0.1% sodium hypochlorite for 10 minutes

Storing toothbrushes in a water containing dish inside the microwave (power: 900watt, capacity: 50%) for 1 minute

Storing the toothbrushes in distilled water for 10 minutes as control group

Disinfecting with microwave: The head of each single toothbrush was aseptically immersed in a container containing a cup of water and was placed inside the microwave oven (MF45, Bu tane, Iran). The microwave was turned on for 1 minute (power 900watt, capacity 50%). At the end, toothbrushes were placed in sterile distilled water for 2seconds. Then they were transferred into the tubes containing 10ml sterilized physiological serum and were vibrated in Shaker (TS110, Arasteh co, Tehran, Iran) for 10 minutes for microbial separation. The suspension was diluted (10-1, 10-2, 10-3), then 10µl of it was cultured over the related medium of each microorganism to count the colonies. The cultivated plates were incubated at 35±1°C. After 24 hours the colonies of bacteria and fungi were quantified.

Disinfecting with sodium hypochlorite: 10ml of 0.1% sodium hypochlorite (Raga, Pakrood, Iran) was poured into the tubes of each group of microorganisms, in such a way that the bristles were entirely soaked. Then toothbrushes were placed in distilled water for 2 seconds to eliminate the effects of sodium hypochlorite. Toothbrushes were transferred to tubes containing 10ml of sterilized physiological serum. Similar to other disinfectant agents, the tubes were shaken in Shaker device (TS110, Arasteh Co.) for microbial separation. Other steps were per-

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formed exactly the same as the previous stage. Dilution series were prepared, cultivated on the specified medium and were incubated for 24 hours. After that the colonies of microorganisms were counted.

Disinfecting with distilled water (as control group): Similar to the previous stages, 10 tubes were prepared for each microorganism. The contaminated toothbrushes were placed in distilled water for 10minutes, then again in sterilized distilled water for 2 seconds. Toothbrushes were transferred to sterilized tube containing 10ml of sterilized physiological serum and were shaken on shaker device for 10 minutes. dilution series were prepared of each tube and cultivated on the specified medium. The colonies were finally counted.

Analyzing the number of colonies of tested microorganisms formed on toothbrushes (CFU) of each group was performed by Kruskal-Wallis test and Mann-Whitney test.

Results:

Diagram 1 represents the mean CFU on toothbrushes, divided based on the type of micro organism and the disinfecting agent. The highest mean value of the streptococcus mutans formed colonies was observed on the toothbrushes disinfected with distilled water, next on those disinfected with microwave and the lowest mean of colonies was detected on toothbrushes disinfected with 0.1% sodium hypochlorite ($P<0.001$). The lowest amount of staphylococcus colonies on toothbrushes was found on those disinfected by microwave followed by 0.1% sodium hypochlorite and distilled water ($P<0.001$). Candida albicans showed the highest amount of CFU on toothbrushes disinfected with microwave. Disinfecting with distilled water revealed lower amount of colonies of candida albicans in comparison to disinfecting with microwave, however, the least colonies were related to brushes disinfected with 0.1% sodium hypochlorite ($P<0.001$). The results of Kruskal-Wallis test revealed existence of a significant difference between the disinfectant agents ($P<0.001$). According to the results of Mann-Whitney test, the disinfecting effect of sodium hypochlorite on streptococcus mutans and candida albicans was higher than distilled water and microwave ($P<0.05$); while the disinfecting effect of microwave on staphylococcus aureus was more than the two other agents ($P<0.05$).

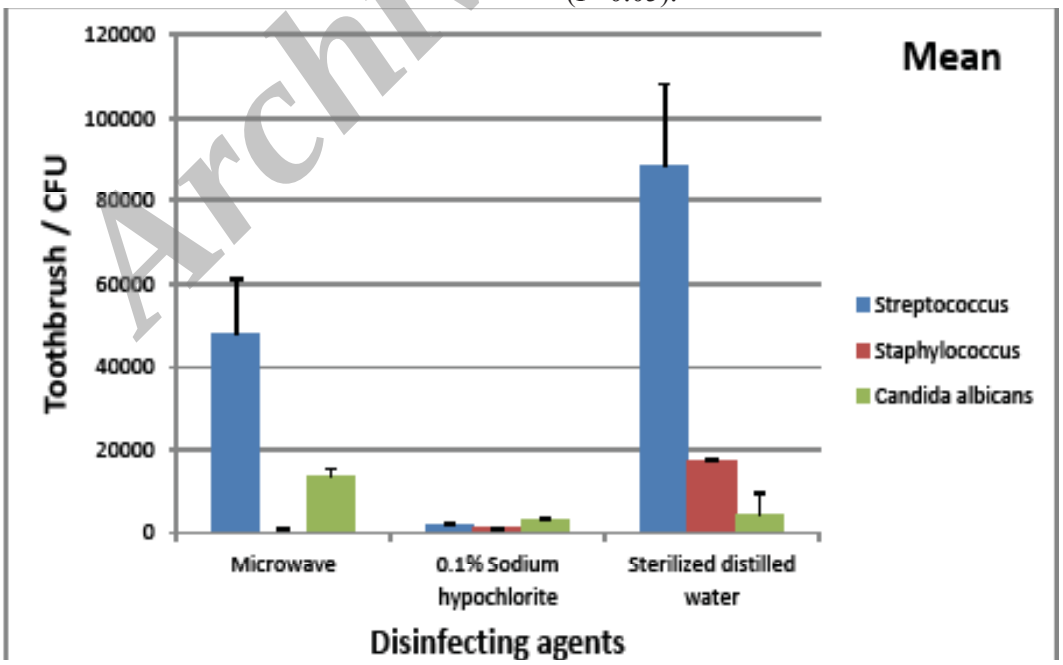

Diagram 1- Mean CFU on toothbrushes divided based on the type of micro organisms and disinfectants

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Discussion:

Contaminated toothbrushes damage the oral tissues more than sterile ones. The two disinfecting methods used in this study were immersion in 0.1% sodium hypochlorite for 10 minutes and irradiation in microwave for 1 minute. Then the amounts of CFU of three microorganisms were counted on toothbrushes, including candida albicans (the main cause of oral fungus), streptococcus mutans (one of the chief types causing dental caries) and staphylococcus aureus (which is in relation with severe human infections like pneumonia).

The results of this study showed that considerable difference exists between the disinfecting agents ($P<0.001$). Sodium hypochlorite is more effective on streptococcus mutans and candida albicans in comparison with distilled water and microwave ($P<0.05$); while microwave has more disinfecting effect on staphylococcus aureus than the two other disinfectants ($P<0.05$).

Devine et al. concluded that fast rate of action, affordability, non-toxicity and ease of use are among important characteristics of a disinfectant agent. Nevertheless, many of the proposed agents such as chlorhexidine gluconate, EDTA and UV radiation generally did not show acceptable results in terms of affordability and ease of application.[9]

Several studies have focused on immersion and have reported that immersing the toothbrush in 0.12% chlorhexidine for 2 and 20 hours and in 0.2% chlorhexidine for 24 hours are adequate for disinfection. Also disinfecting with 3% sodium hypochlorite has been reported to have 100% positive results and claim has been made that toothbrush placed in this solution for 30 minutes can be used for 3 months with no infection. [15] Having anti-microbial activity and efficacy, chlorhexidine is usually used as the first choice for disinfection before surgery. A study by Nelson-filho et al. also revealed that disinfecting the children's toothbrushes with 0.12% chlorhexidine for 20 hours destroys the microorganisms. Despite the observed efficacy of this substance, it seems that this method is costly for the general public and is not applicable regarding the suggested disinfecting time.[7] Sodium hypochlorite and microwave can be used for disinfecting the toothbrushes in different conditions because they are easily available and affordable in addition to showing favorable results. All three agents of distilled water, 0.1% sodium hypochlorite and microwave can be used to reduce the contamination of toothbrushes. With respect to the results of the current study, 0.1% sodium hypochlorite and microwave were more effective than rinsing with normal water in decreasing the streptococcus mutans and staphylococcus aureus bacteria. Generally, it can be claimed that sodium hypochlorite is more effective than the two other substance. However, the disinfecting effect of microwave is more than 0.1% sodium hypochlorite on environments contaminated specifically with staphylococcus aureus.

Some studies have attributed the disinfecting effect of microwave to its thermal effects, while some others believe the bactericidal effects of microwave to be caused by its non-thermal effects. Some molecules like nucleic acid absorb the relevant frequencies and this leads to cell death. The other proposed mechanisms are alterations in selective permeability and resonance of the cell wall which creates crack in the molecular wall and leads to removal of nucleic acid and proteins.[16] A preliminary report showed that microwave can be effective for disinfecting wet under wares contaminated with candida; it also mentioned that placing the dry cloth in microwave would not be effective.[17] The toothbrushes that were studied in this research were wet when irradiated in microwave so that the thermal effects of microwave could take the full effect. Nonetheless, the results showed that not only microwave could not be effective in reducing the growth of candida, but also it increased its growth. This inconsistency can be due to the difference in power, frequency or the duration of irradiation of the device.

The study performed by Dardanonl et al. revealed that at 72GHz and within 1 minute, microwave can decrease the growth of candida albicans, while continuous microwave radiation at 72GHz for 3hours can intensify its growth. This study has emphasized on the effect of frequency and modulation of microwave radiation on growth of candida. It seems that the rate of candida growth after being irradiated by microwave depends on several factors such as frequency and duration of irradiation in microwave. [18]

In current study, which the power of device was 450watt (power: 900watt, capacity: 50%), frequency was 2.45MHz and the radiation duration was 1minute, increased growth of candida albicans was observed compared to the distilled water group; however, the decrease was not statistically significant. In addition, researches have been carried out about the possibility of disinfecting the common oral bacteria by microwave. [17-19] In the present study, the disinfecting effect of microwave on staphylococcus was

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more than 0.1% sodium hypochlorite. Although the power of device and radiation duration was lower, the results obtained were similar to what was found in previously mentioned study. [19-22]

Comparing the efficacy of distilled water and sodium hypochlorite revealed the later to be more influential on staphylococcus. 0.6% sodium hypochlorite has been reported effective for disinfecting the impression taking tools.[23] Kondo et al. showed that 200ppm sodium hypochlorite can decrease 94-98% of staphylococcus aureus contamination in 1 minute.[24] Despite the 50ppm sodium hypochlorite used in the current study, the results obtained were similar to that of the previous study; i.e. sodium hypochlorite considerably reduced the staphylococcus aureus colonies. Nelson Filho et al. studied the disinfecting effects of 1% sodium hypochlorite and 0.12% chlorhexidine and observed that both substances could destroy all the bacteria.[7]

Another study assessed the disinfecting effects of 2% triclosan, 0.2% chlorhexidine, 3% sodium hypochlorite (neem3) and 1% hypochlorite on streptococcus mutans bacteria attached to the bristles of toothbrush. The results indicated the neem3 to be more effective in disinfecting the toothbrush. [25]Despite the fact that the dilution of sodium hypochlorite used in the current study was 1/10th of the above mentioned study, sodium hypochlorite was significantly influential in decreasing the streptococcus mutans colonies. It seems that dilutions of less than 1% can be used to disinfect the toothbrushes.

Using more diluted hypochlorite has advantages such as reduced pungent smell of this disinfectant for user and decrease in the amount of the chemical disinfectant substance used (in terms of toxicity and affordability). Furthermore, it is likely that decreasing the concentration of the disinfecting substance leave lower negative effects on toothbrush compared to the higher concentration. In the present study, sodium hypochlorite affected streptococcus mutans more than microwave.

Increased number of colonies of candida albicans on toothbrushes after using microwave as the disinfecting agent suggests that using sodium hypochlorite can be more beneficial for people living in humid environments in which fungi grow more commonly and widely.

Compared to the other two disinfecting agents (distilled water as control group and sodium hypochlorite), microwave had the highest effect on staphylococcus aureus. So microwave is preferred to be used for toothbrush disinfection in places like hospitals in which the amount and growth of this microorganism is high.

According to the results of the current study, the toothbrush disinfecting agents are better to be prioritized as sodium hypochlorite followed by microwave and in case these two were not available, water can be used.

Conclusion:

Based on the results of this study, sodium hypochlorite has higher disinfecting effect on streptococcus mutans and candida albicans, while microwave is more effective on staphylococcus aureus.

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