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Research Article

Comparing the Effect of Chlorhexidine and Hydrogen Peroxide on Peri-implantitis Associated Strain of *Staphylococcus aureus*

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Abstract:

Background: *Staphylococcus aureus* is one of the critical microbiotas, which can cause peri-implantitis and endanger dental implants success rate. The aim of this study was to compare the disinfectant properties of chlorhexidine (CHX) and hydrogen peroxide (H_2O_2) on peri-implantitis associated strain of *S. aureus*.

Materials and Methods: Totally, 15 implant titanium disks were prepared in the same thickness and diameter. The disks were randomly divided into three groups (n = 5) based on the experimental disinfectants (CHX 0.12% and H_2O_2 3%) and designated control groups. After the formation of a protein layer on disk surfaces, the specimens were exposed to *S. aureus* suspension. The decontamination procedure was completed during 5 min for both disinfectants. Trypsin protease 2% was applied to isolate the survived microorganisms at suspension of ½ and ¼. Muller Hinton agar culture was used for microbiota growth. After 48 h incubation, the standard colony forming unit was assayed. Finally, the collected data were analyzed by Kruskal–Wallis and Mann–Whitney tests using SPSS software version 22 at a significant level of 0.05.

Results: The Kruskal–Wallis test revealed the significant differences between study groups ($P < 0.001$). Furthermore, both groups presented significant differences with the control groups (all $P < 0.01$).

Conclusion: Both H_2O_2 and CHX are effective on S. *aureus*, nevertheless CHX seemed to be more lethal on studied bacteria but not significantly.

Key Words: Chlorhexidine, dental implants, hydrogen peroxide, periimplantatis, *Staphylococcus aureus*

Introduction

Nowadays, dental implants are proposed for replacing a missing tooth. However, the possibility of failure due to peri-implantitis (PI) is so concerning among clinicians. PI is an inflammatory response, which endangers implants and surrounding supportive tissues and brings about bone loss and implant failure finally.[1] The PI has a remarkable correlation with oral microflora and further immunological response. Therefore, the success of implant placement might highly depend on the colonization rates of microorganisms.[2] Many different microorganisms are involved in PI, but some of them seems to have more pathogenicity such as: *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Candida albicans* (C. *albicans*).[3,4]

In general, two methods are introduced to make significant decrease in bacterial biofilm on implant's surface: Mechanical (such as using dental curettes, ultrasonic scalers, and nano abrasions) and chemical (such as using citric acid, hydrogen peroxide [H_2O_2], chlorhexidine [CHX], and antibiotics).[5] Unfortunately, treatment protocol for PI remained uncertain but the decontamination of implant rough surface might be a hopeful solution.[6] Periodontal debridement of pathogenic biofilms on the surface of titanium implants is somehow impossible, due to the design and texture of screwed shape implants. Hence, effective antiseptic therapy as a non-surgical procedure is recommended for PI.[7] Bürgers *et al.*[4] evaluated the antiseptic effects of six disinfectants on three PI associated microorganisms (*S. epidermidis*, *Streptococcus sanguinis*, *C. albicans*). The results manifested the significant effect of sodium hypochlorite (NaOCl) on the mentioned microorganisms.[4] Another study evaluated the antiseptic effects of six antiseptic agents (NaOCl, H_2O_2 3%, CHX 0.2%, plax, listerine, and citric acid 40%) on PI. The final results reflected the highest bactericidal effects of NaOCl, H_2O_2, CHX, and listerine.[8]

The aim of this study was to compare the antiseptic properties of the H_2O_2 and CHX on PI associated strain of *S. aureus* which was cultured on titanium implants.

Materials and Methods

This study was approved by the Research Ethics Committee of Torabinejad Research Center and Dental Implant Research

Center, Isfahan University of Medical Sciences, Protocol # 294028.

Sample preparation

In this analytical-observational *in vitro* study, 15 implant titanium disks (XiVE, Dentsply, Friadent GmbH, Mannheim, Germany) with the same thickness (2 mm) and diameter (4.5 mm) were prepared and randomly divided into three main groups based on the type of disinfectant and considering the control group.

The disks were sterilized by autoclaving (121°C for 15 min). To form a protein layer on the surfaces, the disks were stored in separate sterile eppendorf which were poured previously with 1.7 mL diluted horse serum (Biowest, Nuaillé, France) by normal saline in 1:9 for 2 h at 37°C temperature.[9]

Contamination

After formation of the protein layer, the disks were transferred to *S. aureus* (ATCC 29213) microbial suspension (1.7 mL) and were remained for 60 h at 37°C temperature.

For many types of susceptible testing, standard inoculums of microorganisms must be used.

Hence, the standard inoculums were prepared according to 0.5 McFarland (1.5×10^8 colony forming unit [CFU]/ml) by transferring 1-2 colonies of 18-24 h culture strains to tryptic soy broth medium and incubating at 37°C.

In the next stage, the disks were expelled from the microbial suspensions and gently inserted in normal saline for three times to wash away any loosely attached microorganisms.[9] Without any delay or drying, the decontamination procedure was performed on wet surfaces of the disks.

Decontamination

CHX 0.12% (Shahdaru Laboratories, Tehran, Iran) and H_2O_2 3% (Shimiran, Tehran, Iran) were used for decontamination, separately during 3 min.

Microbiological assay

After decontaminating, each disk was washed by 3 ml sterile distilled water for 30 s to omit any chemical remnants and avoid culture contaminations or bias.

To isolate the survived microorganism as much as possible, trypsin protease 2% (AG Scientific Inc., CA, USA) was administered for 60 min. Then, the suspension of ½ and ¼ Trypsin were prepared by using 100 µl samplers. These samples were transferred and spread on Muller Hinton agar culture media (Sigma-Aldrich, MO, USA) by using Pasteur pipet, which was heated to make a 90° bend. After 48 h incubating, the standard CFU was assayed, and the collected data were analyzed by Kruskal–Wallis and Mann–Whitney tests using SPSS software version 22 at a significant level of 0.05.

Results

The Kruskal–Wallis test revealed significant differences among all of the study groups ($P < 0.001$). Table 1 represents the mean CFU/ml values of different microorganisms after decontamination. Based on the result, the highest amount of remained bacteria belonged to H_2O_2 at a concentration of 1 ($0.28 \pm 0.62 \times 10^2$ CFU/ml). The mean CFUs were descending as their concentration descended in all the groups.

Table 2 manifests the pairwise comparison of different groups with the exclusive control group by Mann–Whitney statistical test. Based on the results, both of the groups exhibited significant differences with the control groups (all $P < 0.01$). However, there was no significant difference between both test groups ($P = 0.07$).

Discussion

S. aureus is one of the microbiotas of the human oral cavity, which can bind to hard surfaces.[10] This bacteria can make pathogenic biofilms on various implant devises and has been turned to a major concern in dental implants failure.[11] *S. aureus* is able to colonize on the titanium implant surface just 30 min after implantation and survive under different environmental conditions.[12] Its pathogenicity is due to release of cytolytic toxins and virulence factors such as coagulase, catalase, and clumping factor A.[13] Based on analyzed data, there was no significant difference between CHX and H_2O_2, however, the CHX presented better decontamination. CHX is a diphenyl compound with wide spectrum antibacterial activity on both Gram-positive and Gram-negative bacteria. It makes an alteration in the bacterial cell membrane and results in leakage and cell destruction.[14] Furthermore, CHX inhibits glycosidic and proteolytic activities and reduces matrix metal-proteinase action in most oral bacteria.[15] Carcuac *et al.* surveyed the

Table 1: The mean quantitate values ($\times 10^2$ CFU/ml) of remained bacteria after decontamination.

Groups	Concentration	*S. aureus*
H_2O_2	1	0.28±0.62
	½	0.08±0.17
	¼	0.04±0.08
CHX	1	0.00
	½	0.00
	¼	0.00
Control	-	5.85±0.00

S. aureus: Staphylococcus aureus, CFU: Colony forming unit, CHX: Chlorhexidine

Table 2: Pairwise comparison (*P* value) of different groups with the exclusive control group.

Groups	Concentration	*S. aureus*
H_2O_2	1	0.004
	½	0.004
	¼	0.004
CHX	1	0.003
	½	0.003
	¼	0.003

S. aureus: Staphylococcus aureus, CHX: Chlorhexidine

antiseptic effect of CHX on different titanium implants. Their result reflected minor treatment outcomes of CHX, which was differ based on implant surface characterizations.[16] In the other hand, another study evaluated adjunctive effect of a dental water jet rinse mixed with CHX gel on PI.[17] With respect to the technique, the flushing pressure of water jet might play a synergic role in decontamination of implant surfaces.[18]

It has been reported that CHX disturbs streptococci attachment and its subsequent biofilm formation. Due to the fact that initial colonization of bacteria influences the later colonizers, CHX inhibits bacterial adherence and formation of biofilms.[19]

In vitro experiments encounter some limitations, as they are observed in a static system compared to *in vivo* studies, which are more comprehensive due to various dynamic factors such as different systemic status, complex bacterial biofilms, and different immunological responses.[20] Nevertheless, it might be considered that if the antimicrobial agent does not have activity *in vitro* it most likely will not work *in vivo*. Greater number of microbial species including indigenous and invaders species might reduce the accuracy of the antimicrobial testing. In the other hand, *in vitro* studies might provide a reference line for clinical studies.

Conclusion

Within the limitations of this *in vitro* study, it can be concluded that both H_2O_2 and CHX are effective on *S. aureus*, nevertheless CHX seemed to be more lethal on studied bacteria but not significantly.

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