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Potential Effect of 2% Chlorhexidine Gel in the Implant Screw Hole on Bacterial Count

Abd Khudair Ali[1]*, Ali Basima GH[2] and Al-Mizraqchi Abbas S[3]

[1] *Department of Periodontics, Master Student, College of Dentistry, University of Baghdad, Iraq*

[2] *Department of Periodontics, College of Dentistry, University of Baghdad, Iraq*

[3] *Department of Basic Science, College of Dentistry, University of Baghdad, Iraq*

**Corresponding e-mail: khudairali4@yahoo.com*

ABSTRACT

Background: *Microbial penetration inside the implant's internal hole creates a bacterial reservoir that is related with an area of inflamed connective tissue opposite the fixture-abutment junction and this can affect with the health of the peri-implant tissue. Chlorhexidine (CHX) has been used to prevent internal implant contamination as a 0.2% solution, a varnish or gel.* ***Aim:*** *To evaluate the aerobic/anaerobic bacterial count-reduction potential of 2% CHX gel placed, at the time of surgery, in the implant screw hole over a period of minimum 90 d, and to monitor the periodontal health status of all patients, throughout the study.* ***Material and methods:*** *Ten partially edentulous patients received 30 DI and these implants were randomly allocated in to: Group I (test) 15 implants applied by flap or flapless surgery with 2% CHX gel application. Group II (control) 15 implants applied by flap or flapless surgery without CHX gel application. All patients were examined clinically to determine their oral health status by examination of their plaque index, PLI, Gingival index GI, Bleeding on probing, BOP, and probing pocket depth, PPD, every two weeks throughout the study. Three months later, the plaque sample was collected from the internal hole of fixture and was sent for bacteriological examination.* ***Results:*** *The present study shows highly significant reduction of aerobic count of bacteria from 52.1% to 100%. Also, anaerobic bacterial count was reduced from 64.6% to 100% for group that received 2% CHX gel in screw hole of implants at time of surgery. When compared, the count of aerobic and anaerobic bacteria (CFU) between test and control group, a significant reduction was found.* ***Conclusion:*** *The use of 2% CHX gel at the time of placement can significantly reduce bacterial counts in the implant screw hole, and this effect can be maintained for 90 d or longer.*

Keywords: 2% Chlorhexidine, Screw hole, Flapless surgical implant placement, Bacterial count

INTRODUCTION

Studies of dental implants over the years have showed great dependability for the success rates of implant treatment [1]. Longstanding implant success is highly dependent on adaptation of the implant with oral soft and hard tissues. Commonly, the crestal bone at the implant-tissue interface is the early point of surgical trauma and tissue breakdown.

At this instant, there is no single flap design that functions as the main approach for all single implant surgery and there is a need for esthetics procedures with slightly invasive techniques that can ensure the changeability in flap design [2].

Implant dentistry is responsible for obtaining best esthetic, function, and phonetic outcomes and it is also responsible for receiving the stability of alveolar bone with the peri-implant soft tissues.

In deference to the several factors causing failure of dental implants, for example, bone condition, occlusion etc., numerous studies support that microbial infections could have serious effects leading to failure in dental implants prosthesis. For most implant systems, micro leakage in dental implants may cause mucositis within a short extent from the alveolar bone crest.

Microbial penetration inside the implant's internal hole creates a bacterial reservoir that is related with an area of

inflamed connective tissue opposite the fixture-abutment junction and this can affect with the health of the peri-implant tissue [3].

Microorganisms colonizing surfaces are progressively planned into complex biofilms. Species within the biofilm interrelate specially with each other. Such as, initial colonists, for example *Actinomyces* or *Streptococcus* species, are necessary for the adhesion of late-colonizing gram-negative species. A sufficient cooperative maintenance care for patients with dental implants has an essential function in attaining longstanding success for implant-supported restorations [4]; yet, conventional mechanical professional hygiene procedures are ineffective against the inner implant microflora.

Various attempts have been made to reduce the inner micro-bacterial population [5-8]. Chlorhexidine is found effective and used in practice as an antiseptic for treatment of periodontal disease [9]. This is justified by its efficient antimicrobial and antifungal function [10]. Chlorhexidine has been used to prevent internal implant contamination as a 0.2% solution [6] or as a varnish.

Aims of the study

1. To evaluate the aerobic/anaerobic bacterial count-reduction potential of 2% CHX gel applied at the time of surgery, in the implant screw hole of Flap and Flapless implant design with transmucosal healing abutments over a period of minimum 90 d and compare the bacterial count with control group.
2. To monitor the periodontal health status of all patients, throughout the study period.

MATERIALS AND METHODS

Study sample

This clinical prospective study was conducted from October 2016 to July 2017. The sample included patients who attended the Dental Implantology Unit, Department of Oral & Maxillofacial Surgery in the Teaching Hospital of College of Dentistry- University of Baghdad for the purpose of implant placement, Medical laboratory assistant for bacterial culture and Al-Maghreb Specialized Dental Center for radiographic examinations.

Total 32 DIs in 10 Iraqi patients, 6 males and 4 females, age ranged from 30 to 45 years that fulfilled the inclusion criteria were recruited for this study. Out of which 30 DIs were included, 2 were excluded - one failure and the other replaced again which require a long term follow up. The total 30 DIs were included in the study.

Inclusion criteria

- Patients who were partially edentulous (patients requiring at least two separated (not neighboring) teeth replacement in the anterior, premolar, and posterior region)
- Systemically healthy
- Free of active caries and active periodontitis
- Smokers: patients smoke less than 10 cigarettes per day (mild smokers)
- Patient who were well motivated for implant treatment and maintaining good oral hygiene and follow a regular periodontal maintenance protocol

Samples grouping

Total 30 dental implants DI were randomly allocated in to 2 groups. The first group (test group) consisted of 15 implants with 2% CHX gel to be added within the implant internal cavity at the time of surgery. The second group (control group), included 15 implants without CHX gel. Both flap and flapless design of implant placement were done.

Clinical examination

- Extraoral examination
- Intraoral examination

Clinical periodontal parameters examination done every 2 weeks (8 visits) for all patients include:

- Assessment of plaque index (PLI) [11]
- Assessment of gingival index (GI) [12]
- Assessment of bleeding on probing [13].
- Assessment of probing pocket depth [14].

Radiographic examination

A pre-operative orthopantomograph (OPG) was obtained for all patients.

Surgical procedure

Before surgery, patients were instructed to rinse with 0.12% CHX solution for 1 minute (min.) as part of the standard surgical protocol and the skin was scrubbed with povidone-iodine solution as antiseptic agent.

The surgical site was anesthetized, and implants were placed in site previously examined and according to a studied treatment plan with respect to the demand of the patients. Flap or Flapless surgical design was selected.

The surgeon randomly assigned the implant(s) either to one of the test group (with CHX 2% gel) or to one of the control group (without CHX gel) by randomly alternating the implant(s) to be enrolled in those groups, which enabled unbiased distribution of implants in each group. Before placement of the healing abutment, all implant screw holes were rinsed with about 20 ml of sterile saline solution by disposable syringe and dried using surgical suction, thus preventing further contamination of the screw hole with saliva or blood. For the test and control groups, healing abutments (gingival formers) were placed, on the implants and tightened using hex driver, as indicated by the manufacturers. After this step, in the test group, 2% CHX gel (GLUCO-CHX 2% gel, CERKAMED medical company), was placed on the entire thread portion of the healing abutments; the healing abutments were then screwed into place by hex driver with the same torqueing protocol as in the control groups in flapless implant.

For an implant required 2nd surgery (flap design) the 2% CHX gel applied one-month post-surgery and in the same manner as in flapless design implant according to Hahn [15].

Bacteriological examination

- Laboratory methodology
- After preparation of the following culture media
- Phosphate buffer saline (PBS)

Culture media preparation

- Brain Heart Infusion Agar (BHI)
- Mannitol Salt Agar

Specimen collecting

Plaque samples were collected from the internal screw holes under standard condition, approximately 3 months after surgical placement using sterile micro brush and then the brushes immediately dropped into screwed universal tube containing phosphate buffer saline (PBS).

Culturing technique

The collected sample were mixed using Vortex mixer for 2-3 minutes. Ten-fold steps of serial dilutions were prepared using sterile phosphate buffer saline, 0.1 ml was withdrawn from each dilution (10-3, 10-5) and then, inoculate in to the petri dishes contain Brain-Heart-Blood agar media, and spread by using sterile microbiological spreader on the plates of BHI-Blood agar and incubate aerobically and anaerobically.

Enumeration of bacterial colonies (count)

Identification of most common bacterial colonies

- Morphological characteristics
- Gram's stains

- Morphological examination of the microbial cells
- Catalase test (H_2O_2)
- Oxidase test
- Mannitol salt agar

Statistical analysis

Statistical analysis was done using SPSS version 21. Statistical analysis includes (minimum, maximum, median, and mean rank) in graphs and tables and Inferential analysis includes statistical test of non-parametric data as Friedman and Wilcoxon-sum rank tests were used.

RESULTS

Throughout all 8 visits, a dramatic decrease in PLI and GI with a highly significant change during visits was observed (Table 1). Median of PLI changed from (1.280) at the first visit to 0.9 at the eighth visit. While GI decreased from 1.595 at first visit to 1.045 at eighth visits, which is the lowest score.

Table 1 Descriptive and statistical test of PLI and GI change during visits

Index	Visits	Median	Mean Rank	Friedman test		
				Chi-square	df	P-value
PLI	1	1.28	7.65	49.717	7	0.00*
	2	1.02	6.00			
	3	1.00	5.55			
	4	1.00	4.55			
	5	1.00	4.85			
	6	0.900	2.45			
	7	0.900	2.85			
	8	0.900	2.10			
GI	1	1.595	7.50	36.926	7	0.00*
	2	1.37	6.00			
	3	1.24	5.50			
	4	1.165	4.65			
	5	1.095	3.35			
	6	1.09	3.95			
	7	1.055	2.70			
	8	1.045	2.35			
*Highly significant at P<0.01						

Table 2 reveals the descriptive and statistical analysis of BOP changes throughout all visits.

It appears that there is a highly significant decrease of BOP percentage during visits. The highest median in a first visit 59.990 decrease to 5.250 (the lowest median of BOP) at the last visit.

Table 2 Descriptive and statistical test of percentage of surfaces with bleeding on probing change during visits

Visits	Median	Mean Rank	Friedman test		
			Chi-square	df	P-value
1	59.99	7.90	65.734	7	0.00*
2	37.90	6.55			
3	25.00	6.20			
4	16.54	5.20			
5	10.20	3.95			
6	8.30	3.00			
7	6.10	1.80			
8	5.25	1.40			
*Highly significant at P<0.01					

Results in Table 3 illustrates the descriptive and statistical tests of both aerobic and anaerobic viable count (CFU/ml) in the test groups which that both median and mean rank of anaerobic viable count (0.220 and 15.87) respectively are higher than those of that count of aerobic (0.050 and 15.13) respectively, but with statistically not significant difference between those counts (P>0.05).

While, in the control group which that although both median and mean rank of anaerobic viable count (7.00 and 16.60) respectively are higher than those of that count of aerobic (3.250 and 14.40) respectively, but with statistically not significant difference between those counts (P>0.05).

Table 3 Descriptive and statistical test of viable count between aerobic and anaerobic bacteria in test and control groups

Group	Descriptive	Bacterial groups		Z	P-value
		Aerobic	Anaerobic		
Test Group	Minimum	0.00	0.00	0.23	0.838^{NS}
	Maximum	1.15	3.00		
	Median	0.05	0.22		
	Mean rank	15.13	15.87		
Control Group	Minimum	1.04	1.10	0.685	0.512^{NS}
	Maximum	51	98.00		
	Median	3.25	7.00		
	Mean rank	14.4	16.6		
NS: Not significant					

Results in Table 4 demonstrates the descriptive and statistical tests of aerobic viable count (CFU/ml) between control and test media that the median and mean rank of aerobic viable count in test media (0.050 and 8.20) respectively which are less than those count in the control media (3.250 and 22.80) with statistically highly significant difference between the two medias (P<0.01).

Table 4 Descriptive and statistical test of aerobic viable count (CFU) change between test and control groups

Descriptive	Groups		Z	P-value
	Test	Control		
Minimum	0.00	1.04	4.553	0.00*
Maximum	1.15	51.00		
Median	0.05	3.25		
Mean rank	8.20	22.8		
*Highly significant				

Findings in Table 5 shows the descriptive and statistical tests of anaerobic viable count (CFU/ml) between tests and control media that both median and mean rank of anaerobic viable count in the test media (0.220 and 8.73) respectively which are less than those of that count in the control media (7.00 and 22.27) respectively with statistically highly significant difference between those media (P<0.01).

Table 5 Descriptive and statistical test of anaerobic viable count (CFU/ml) change between test and control groups

Descriptive	Groups		Z	P-value
	Test	Control		
Minimum	0.00	1.10	4.215	0.00*
Maximum	3.00	98.00		
Median	0.22	7.00		
Mean rank	8.73	22.27		
*Highly significant				

The most common bacteria isolated from implant in test group, under aerobic condition were *Streptococcus* spp. (65%), *Staphylococcus aureus* (30%) and Gram –ve *diplococci* (5%). While under anaerobic condition were (25%),

(60%), (10%) respectively and Gram –ve *Bacilli* (5%).

In control group, under aerobic condition were *Streptococcus* spp. (50%), *Staphylococcus aureus* (36%), G –ve *diplococci* (8%), G +ve *Bacilli* (3%) and G –ve *Bacilli* (3%). While under Anaerobic condition were (30%), (50%), (10%), (3%), (4%) respectively and *Enterococcus faecalis* (3%) (Table 6).

Table 6 Percentage of the most common isolated aerobic and anaerobic bacteria in test and control groups

Aerobic bacteria		**Anaerobic bacteria**	
Test group			
Species	**Count (%)**	**Species**	**Count (%)**
Streptococcus spp.	65.00%	*Streptococcus* spp.	25.00%
Staphylococcus aureus	30.00%	*Staphylococcus aureus*	60.00%
G-ve *diplococcus*	5.00%	G-ve *diplococcus*	10.00%
-	-	G-ve *Bacilli*	5.00%
Control group			
Streptococcus spp.	50.00%	*Streptococcus* spp.	30.00%
Staphylococcus aureus	36.00%	*Staphylococcus aureus*	50.00%
G -ve *diplococcus*	8.00%	G -ve *diplococcus*	10.00%
G +ve *Bacilli*	3.00%	G +ve *Bacilli*	3.00%
G –ve *Bacilli*	3.00%	G -ve *Bacilli*	4.00%
-	-	*Enterococcus faecalis*	3.00%

DISCUSSION

Implants inserted with the closed technique near half of it become contaminated during the surgical procedure [7]. Following healing abutments placement, the microbial flora level extremely increases, and most screw cavities are contaminated 6 weeks after they are exposed, suggesting that both surgery and microleakage contribute to the contamination.

In a semi-closed system, for instance the inner implant hole, CHX gel may remain longer, successfully decreasing the microbial colonization for an elongated time.

The present study showed the beneficial effect of internal implant decontamination of 2% CHX gel in terms of bacterial count reduction.

These results are in agreement with Groenendijk, et al. in 2004, they reported that after application of a 0.2% CHX solution, the gingival index and crevicular fluid flow were lower than in saline solution-treated controls [7].

As no statistical difference between aerobic and anaerobic culture was documented, the bacterial contamination is thought to be mostly facultatively anaerobic. To identity the frequency of occurrence of Gram-negative species, bacterial Gram staining was conducted on the cultured samples. Unexpectedly, samples presented the Gram-positive coccoid species were the most common species (*Staphylococcous* and *Streptococcus*). However experimental error cannot be excluded, the common of initial colonizers belong to streptococcal species, for example *Streptococcus gordonii* and *Streptococcus sanguinis*.

Modern studies have also directed that in healthy implant sites, Gram-positive cocci have the greatest proportion of finding [16], which is corresponding with our results.

Many studies have examined the bacterial species found in the biofilm present on the restorative components and in the internal parts of implants [17,18].

These studies indicated that these restorative components and internal parts were highly contaminated. All implants in the study were regarded to be free of peri-implantitis and radiographic estimation did not exhibit signs of important bone loss beyond what is corresponding with crestal bone regeneration. Also, the oral hygiene of the patient remains good and this help in maintaining the health of the peri-implant tissue over the observative period.

Any bacteriological colonization should permanently be regarded a longstanding risk factor and should be removed if possible. The results of the present study suggest that this can be successfully achieved utilizing 2% CHX gel.

Furthermore, this inhibition should persist through utilization of 2% CHX gel to the screw cavity to the restorative phase of implant treatment whenever it is open for taking an impression or abutment insertion/removal.

CONCLUSION

Based on the results of this study, the use of 2% CHX gel at the time of placement can significantly reduce bacterial counts in the implant screw hole, and this effect can be maintained for 90 d or longer (average 110 d). We propose that prevention of implant screw hole contamination should be included in the standards of care in implant dentistry.

DECLARATIONS

Ethics approval and consent to participate

The ethics committee approved the study. Written informed consent was obtained from all participants.

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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