# Comparative Evaluation of the Efficacy of 0.2% Chlorhexidine v/s Aqueous Ozone Solution Irrigants in the Control of Dental Plaque Microorganisms at Submerged Single Implant Area - A Randomized Controlled Clinical Trial.

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## Abstract:

**Background**: The aim of this randomized control clinical trial of the study was to analyze and compare two irrigating agents (4 ppm ozonated water and 0.2% chlorhexidine (CHX)) for their antimicrobial and wound healing efficacy over a 2 weeks period immediately after implant placement.

**Materials and Methods**: The study was a single blinded, parallel arm, randomized controlled clinical trial. 20 patients undergoing dental implant placement in a single-tooth edentulous area were enrolled in the study. 10 patients in each group were randomly assigned to either the test (4 ppm ozonated water irrigation) or the control (0.2% chlorhexidine) group. Patients were evaluated at baseline, day 3, day 5, day 7 and day 14. Clinical and microbiological assessment was carried out at each follow up day. The primary objective was to assess the total microbial count and the secondary objectives were to assess the Wound healing index (WHI), the gingival variables (GV), Modified gingival index (MGI) and the Plaque index (PI).

**Results**: Chlorhexidine group showed a significantly higher microbial count on day 5, 7 and 14 compared to the ozonated water group. Both the groups showed an increase in the colony forming units (CFUs) but, the CHX group showed a significant increase from day 0 to all time intervals. From day 3 to day 5, both the groups showed significant increase in CFUs, from day 3 to day 7, the ozonated water group showed significantly improved in both the groups. Gingival variables such as inflammation, oedema and granulation tissue were similar in both groups at all time intervals. MGI significantly reduced to 0 in both the groups. PI significantly

increased in both the groups. On comparison between both the groups WHI, GV, MGI and PI were similar. *Key Word: Dental implant, ozonated water, chlorhexidine, antimicrobial, colony forming units* 

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## I. Introduction

Dental implants, also known as oral or endosseous implants have revolutionized dentistry by providing state of the art restorative options for lost teeth. They have reported high success rate (over 97% for the past ten years)<sup>(1)</sup>. The success of dental implants largely depends upon the meticulous surgical protocol, management of the early post operative period and the healing of the soft tissues at the implantation site.

Maintaining a high level of oral hygiene during and after implant placement is critical to its success. Bacterial contamination of implant can disrupt early wound healing and disrupt osseointegration leading to early failure<sup>(2)</sup>. The risk of early implant failure due to infection is greatest before re-epithelialisation of wound. Bacterial activity around implanted biomaterials appears to be antibiotic-resistant and, in the worst-case scenario, may persist until the device is removed<sup>(3)</sup>. A surgical wound harbours plaque in this extremely delicate zone. For these reasons, agents capable of potentially preventing periodontal pathogen recolonization may provide significant therapeutic benefits<sup>(4)</sup>. Effective plaque control following implant therapy is critical to ensure the complete success of this rehabilitation procedure<sup>(2,5)</sup>.

Post surgery, it is extremely uncomfortable and difficult for the patient to perform mechanical oral hygiene at the healing site, which is fragile and must not be injured. Various procedures have been tried to prevent such contamination. Mouthwashes are useful as they contains various antimicrobial agents to complement mechanical oral hygiene measures<sup>(6)</sup>. Microbial load can be decreased by 95% via a pre operative rinse with 2% chlorhexidine<sup>(2)</sup>. Chlorhexidine digluconate (CHX) is an established antimicrobial agent (bacteriostatic and bactericidal agent<sup>(7-9)</sup>, anti-plaque and anti- gingivitis agent<sup>(10)</sup>. It acts against a wide array of bacteria including Gram positive and Gram negative bacteria, dermatophytes, viruses, fungi and yeasts. When

compared to other chemical agents, CHX is most effective in plaque control<sup>(11,12)</sup>. However, chlorhexidine has side effects like mucosal desquamation, tooth staining, increased calculus deposition, altered taste sensation, impaired wound healing and fibroblast attachment to the tooth surfaces<sup>(13-15)</sup>. There is a need for alternative antimicrobial agents with high antimicrobial potential and minimal side effects.

Ozone therapy has been shown to reduce the growth of A. actinomycetemcomitans, P. gingivalis, and T. forsythia significantly<sup>(16)</sup>. Furthermore, positive clinical effects of ozonated water on gingivitis and periodontitis have been observed in clinical studies<sup>(17)</sup>. It is a known fact that low redox potential favors anaerobic bacterial growth<sup>(18)</sup>. By altering the redox potential, the environment can be made non conducive for the growth of anaerobic microorganisms such as the application of an oxygenating agent in a surgical site. The antimicrobial action of Ozone<sup>(19)</sup> is due to the damage to the cytoplasmic membrane of cells as a consequence to ozonolysis of dual bonds and modification of intracellular contents because of secondary oxidant effect that leads to oxidation of protein and loss of organelle function.

Therefore, the aim of the present study is to evaluate and compare the effect of oral irrigation with 4 ppm ozonated water and 0.2% chlorhexidine on microbial load after dental implant placement.

# II. Material And Methods

#### Study design

The single blinded, parallel arm, randomized control clinical trial was conducted in the volunteers who satisfied the following inclusion / exclusion criteria.

#### Inclusion criteria

1. At least 18 years of age.

2. Patients referred to the Department of Periodontics for dental implant placement in a single-tooth edentulous area with the presence of healthy teeth adjacent to the healed extracted site (tooth without fixed prosthetic restoration, without failed dental restorative materials or restored cervical abrasion, abfraction, resorption lesion).

3. Non-smokers.

4. Patients demonstrating good oral hygiene.

#### Exclusion criteria

1. Uncontrolled diabetes and severe cardiovascular or infectious diseases.

- 2. Intravenous and oral bisphosphonate therapy.
- 3. Presence of severe, moderate or mild untreated periodontal disease.
- 4. Unwillingness to return for the follow-up examination.
- 5. Patients who were psychologically unable to participate.

Patients visiting the Outpatient Section of the Department of Periodontology and Implantology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru from February 2021 to January 2023 were selected. The study protocol adhered to the provisions of Helsinki declaration and was approved by the institutional ethics review board KCDS/EsC/008/2020-21. Informed consent was obtained from all the patients.

## Intervention

In a presurgical visit, 3-4 weeks before the implant placement, patients were educated and motivated

about oral hygiene maintenance. Scaling and root planning was done. After local anaesthesia (2% Lignocaine Hydrochloride with 1:80000 adrenaline)\*, a mucoperiosteal flap was elevated at the edentulous ridge. Osteotomy was performed according to the manufacturer guidelines. A dental implant† was placed in osteotomy site according to the standard procedures, with the implant shoulder 2-3 mm apical to mid buccal mucosal margin (Figure 1a). The platform of the implant was placed 2-3 mm below CEJ of adjacent teeth. The surgical wound was coapted with mattress and single interrupted sutures (Figure 1b). After suturing, the flap on the randomly selected group was irrigated either with ozonated water (Test) or chlorhexidine mouthrinse (Control) (Figure 2a-c).

Ozonated water of 4ppm/mL was generated using a medical grade ozone generator (TechGreen Ozonator)<sup>‡</sup>. The solution was prepared according to the manufacturer's instructions. The ozonator was connected to 1000 mL of distilled water in an ozone resistant bottle for 15 minutes with a power of 30W. The analysis of the ozonated water was done at a water testing laboratory (Indian Analytical Testing Laboratory, Nayandanahalli, Bengaluru,

<sup>\*</sup> Lignox 2% Indoco remedies, Warren Klitch drugs India, Boisar, India

<sup>&</sup>lt;sup>†</sup> Neodent helix acqua by Straumann® - International Headquarters Straumann Holding AG Peter Merian-Weg 12, 4002 Basel Switzerland

<sup>&</sup>lt;sup>‡</sup> TechGreen Solution, #4, Hoysala Road, Vijanapura Ward, Ramamurthy Nagar, Bangalore 560016

560039, Karnataka). This ozonated water was now transferred to a 10 ml syringe with a 20 gauge blunt bent needle and the surgical site was irrigated for 5 minutes (Figure 1c). Similar protocol was followed in the control group. However, the irrigating solution used was 0.2% chlorhexidine mouthwash § (Figure 2a-c).. All patients were prescribed Ibugesic (400 mg)|| as an analgesics to be taken for 5 days twice daily and Amoxicillin (500 mg)\*\* thrice daily for 5 days. Patients reported back on 3rd, 5th, 7th and 14th day post implant placement and the designated irrigating agent was used for 5 minutes. Patients were adviced not to perform brushing and inter dental cleaning of the implant surgical area for the post surgical two weeks. The submerged implants included in this study were restored 3–4 months after implant placement.

# Microbiological assessment

Microbial samples were collected from the sutured incision lines with the help of a gentle swab of a sterile microbrush for 30 seconds (Figure 3a). It was transferred into a glass tube, containing thioglycollate broth (Figure 3b). Collected specimens were transferred immediately to the Department of Microbiology. The glass tubes containing the specimens were incubated at 37°C for 1 hour and vortexed at 60 seconds to allow complete mixing and release of the biofilm from the microbrush into the solution. 50  $\mu$ L of the specimen was transferred under aseptic conditions onto the dried Brucella Blood agar plate (Figure 3c). Innoculum was spread evenly using a sterile L- spreader (Figure 3d). Plates were incubated in an anaerobic jar with gas pak at 37°C for 48 hours. Each petridish was removed from the incubator after 48 hours and digital images were taken for bacterial colony counting. Colonies were observed for their morphology and total bacterial count was calculated manually (Figure 4 a-c & Figure 5 a-c). Colony counts were recorded based on the dilutions used. The data were entered in a computer database.

# Data collection

Clinical and microbiological parameters were assessed at baseline, 3rd, 5th, 7th and 14th day. The primary outcome assessed was the total microbial count. The secondary outcomes that were assessed were the Wound healing index, Gingival variables, Patient Acceptance questionnaire, Modified gingival index and Plaque index. Total microbial count and Plaque index was assessed at baseline, day 3, day 5, day 7 and day 14. Other parameters were evaluated at day 3, day 5, day 7 and day 14.

#### Statistical analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk,

NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Mann Whitney Test was used to compare the mean PI and wound healing indices scores between 2 groups at different time intervals. Friedman's test followed by Wilcoxon Signed Rank Post hoc test was used to compare the mean PI, stain and wound healing indices scores between different time intervals in each study group. The level of significance [P-Value] was set at P<0.05.

## III. Result

A total of 20 patients were included in the study. 1 patient in both the groups was lost to follow up on day 5 and day 7 and 3 patients in both the group did not report for the 14th day follow up. Immediately after implant placement, 10 were randomly irrigated with ozonated water (TEST) and 10 were randomly irrigated with 0.2% chlorhexidine (CONTROL). A total of 10 patients (8 males, 2 females with a mean age of  $35.30 \pm 8.23$  (26 - 49) years) in the test group and 10 patients (10 males with a mean age of  $40.50 \pm 14.32$  (26 - 62) years) were enrolled (Table 1).

			Test	С			
Variable	Category	Mean	SD	Mean	SD	p-value	
Age	Mean	35.30 8.23		40.50	14.32	0.765	
	Range		26 - 49	2	0.70a		
		n	%	n	%		

Table 1 - Age and gender distribution among 2 groups

<sup>§</sup> Chlohex ADS, Dr Reddy's Laboratories Ltd, Hyderabad, India

TAB BRUFEN 400 mg – Abbott India Ltd.

<sup>\*\*</sup> CAP Cipmox 500 mg - CIPLA Ltd.

Sex	Males	8	80%	10	100%	0.14b	
	Females	2	20%	0	0%	0.140	
Parameters	Groups	N	Mean	SD			
Loc10 CEU/mLo	Test	10	6.721	0.755			
Log10 CF0/IIILS	Control	10	6.588	0.490			
PI	Test	10	1.00	0.61			
	Control	10	0.92	0.48			

Note: a. Mann Whitney Test & b. Chi Square Test

At baseline, both the groups presented with similar microbial count with test group recording Log10 6.721 CFU/mL and control group Log10 6.528 CFU/mL (Graph 1).





The PI scores were similar (1.00) in test group and (0.9) in control group. At all follow up intervals, the total microbial count increased significantly from baseline in the control group. Although, the test group also showed increase in the total microbial count at all follow up intervals, it was not significant. Between the time intervals, significant increase was seen in both the groups from day 3 to day 5. A significant increase was also noted in the test group from day 3 to day 7 (Table 2 and 3).

Table 2 - Comparison of mean Log10 CFU/mLs & other clinical parameters between 2 groups using
Mann Whitney Test; (* - Statistically Significant )

Parameters	Days	Groups	N	Mean	SD	Mean Diff	p-value	
Log10 CFU/mLs	BASEI INE	Test	10	6.721	0.755			
	DAGEERI(E	Control	10	6.588	0.490	0.133	0.47	
	D 1	Test	10	7.442	0.354	0 107	0.21	
	Day 5	Control	10	7.549	0.234	-0.107	0.31	
	Day 5	Test	9	7.503	0.288	-0.368	0.002*	

		Control	9	7.871	0.117			
	D 7	Test	9	7.617	0.373	0.247	0.02*	
	Day /	Control	9	7.963	0.130	-0.347	0.02*	
	D 14	Test	7	7.611	0.375	0.665	0.01*	
	Day 14	Control	7	8.276	0.519	-0.665	0.01*	
	D 1	Test	10	3.00	0.82	0.000	0.27	
	Day 3	Control	10	3.30	0.82	-0.300	0.27	
		Test	9	3.78	0.44	0.22	0.54	
XX /1 XX	Day 5	Control	9	3.56	0.73	0.22	0.54	
WHI		Test	9	4.67	0.50	0.24	0.45	
	Day /	Control	9	4.33	0.87	0.34	0.45	
	D 14	Test	7	5.00	0.00	0.00	1.00	
	Day 14	Control	7	5.00	0.00	0.00		
	Day 2	Test	10	2.00	1.15	0.500	0.40	
	Day 5	Control	10	2.50	0.53	-0.500	0.40	
	Day 5	Test	9	1.78	1.09	0.11	0.78	
	Day 5	Control	9	1.67	0.50	0.11		
MGI	Day 7	Test	9	0.56	0.53	0.11	0.06	
	Day /	Control	9	0.67	0.87	-0.11	0.96	
		Test	7	0.00	0.00			
	Day 14	Control	7	0.00	0.00	0.00		
	BASELINE	Test	10	1.00	0.61			
		Control	10	0.92	0.48	0.08	0.76	
PI		Test	10	1.45	0.63			
	Day 3	Control	10	1.24	0.72	0.21	0.59	
		Test	9	1.71	0.36	0.07	0.15	
	Day 5	Control	9	1.78	0.62	-0.07	0.49	
		Test	9	1.77	0.54	0.50	0.55	
	Day 7	Control	9	1.97	0.55	-0.20	0.35	
		Test	7	2.04	0.42	0.51	0.00	
	Day 14	Control	7	2.29	0.32	-0.24	0.09	

# Table 3 - Inter group comparison of change in the parameters at each time intervals

			Comparisons between times								
P-value		T0 vs T1	T0 vs T2	T0 vs T3	T0 vs T4	T1 vs T2	T1 vs T3	T1 vs T4	T2 vs T3	T2 vs T4	T3 vs T4
Log10 CFU/mL	Test	0.94	0.69	0.51	0.25	0.28	0.02*	0.003*	0.38	0.11	0.19
	Control	0.005*	0.01*	0.005*	0.04*	0.13	0.03*	0.47	0.29	1.00	1.00

PI	Test	0.17	0.02*	0.02*	0.02*	0.26	0.37	0.39	0.86	0.18	0.04*
	Control	0.21	0.007*	0.02*	0.02*	0.02*	0.07	0.04*	0.51	0.07	0.35
WHI	Test				1.00	0.006*	<0.001*	0.28	0.001*	0.47	
wni	Control					1.00	0.03*	0.009*	0.37	0.01*	0.62
MCI	Test			_		0.61	0.03*	0.03*	0.03*	0.03*	0.03*
MGI	Control					0.008*	0.005*	0.02*	0.01*	0.01*	0.10

Log10 CFU/mL, (Plaque index) PI, Wound Healing Index (WHI) Scores, Modified Gingival Index (MGI) - Wilcoxon Signed Rank post hoc Test

The improvement in WHI scores from day 3 to day 5 was not significant in both the groups. However, a significant improvement was seen from day 3 to day 7 and from day 3 to day 14 in both the groups. From day 3 to day 5, no significant difference was seen in both the groups. However, from day 5 to day 7, a significant improvement was seen, again from day 7 to day 14 the improvement was not significant in the both the groups. Between the groups, at all time intervals, the WHI scores were similar with no statistically significant difference (Table 2 and 3). No significant differences between the two groups were found for any of the gingival variables on day 3, day 5, day 7 and day 14 (Table 4).

Variables	Groups	Significance and power between	Follow-up times						
		groups	Day 3	Day 5	Day 7	Day 14			
	Test		8/2 (80.0%)	8/1 (88.9%)	5/4 (55.6%)	0/7(0%)			
Inflammation P/A(P%)	Control		10/0 (100.0%)	9/0 (100.0%)	4/5 (44.4%)	0/7(0%)			
<b>F</b> / <b>A</b> ( <b>F</b> 70)		P-value OW versus CHX	0.14	0.30	0.64				
Oedema P/A (P%)	Test		8/2 (80.0%)	5/4 (55.6%)	1/8 ( 11.1%)	0/7(0%)			
	Control		10/0 (100.0%)	6/3 ( 66.7%)	0/9(0%)	0/7(0%)			
		P-value OW versus CHX	0.14	0.63	0.30				
	Test		8/2 (80.0%)	8/1 (88.9%)	5/4 (55.6%)	0/7(0%)			
Inflammation around Suture	Control		10/0 (100.0%)	9/0 (100.0%)	5/4 (55.6%)	0/7(0%)			
		P-value OW versus CHX	0.14	0.30	1.00				
	Test		7/3 (70.0%)	8/1 ( 88.9%)	1/8 ( 11.1%)	0/7(0%)			
Granulation Tissue	Control		10/0 (100.0%)	6/3 ( 66.7%)	2/7 ( 22.2%)	0/7(0%)			
		P-value OW versus CHX	0.06	0.26	0.53				

Table 3 - Comparison of Gingival variables (GV) between 2 groups at different time intervals

In both the groups, MGI reduced to 0 on day 14 from day 3 which is statistically significant. Significant reduction in MGI was seen at all time intervals except from day 3 to day 5 in test group, whereas, in the control group, statistically significant reduction in MGI was observed at all time intervals except from day 7 to day 14. Comparative evaluation between both the groups showed no difference in the MGI scores at all time intervals between both the groups, the PI scores were similar with no

statistical difference. Within both the groups, there was a statistical significant increase in the PI score from baseline to day 14 (Table 2 and 3). None of the patients reported any bad taste, food taste alterations, changes in salt taste perception or any other adverse effects in either of the two groups over the 2 weeks study period.

# **IV. Discussion**

The antimicrobial effectiveness of aqueous ozone was more effective than the established antiseptic chlorhexidine digluconate (CHX) against periodontal pathogens in in-vitro studies<sup>(20)</sup>. Ozone therapy reduces the growth of P. gingivalis and T. forsythia. When ozonated water was used in the treatment of gingivitis and periodontitis, higher reduction in plaque, gingival inflammation and bleeding was noted when compared to chlorhexidine. An appreciable reduction in Aa was seen while there was no change seen when using CHX<sup>(17)</sup>. Contradictory studies have also reported incomplete efficacy of ozone on viable bacteria<sup>(21)</sup>. Despite the promising in-vitro evidence by ozone, as a potent antimicrobial agent, its clinical role as a therapeutic agent has not yet been fully realized. There is a need for randomized control trials, analyzing and comparing ozone with standard antimicrobial therapeutic regimen in periodontal and implant therapy. Therefore, this current trial aims to compare and analyze ozone with CHX as an antimicrobial therapy for implant surgeries. Information regarding the application time or dose of ozone, the exact concentration of ozone in water and contact time are not standardized and are rarely reported in the literature. There is ambiguity in the standardization of ozone concentration in ozonated water. Various authors have reported various concentrations such as 1000 mg/L (Prabhakar AR, 2019)<sup>(22)</sup>, 0.082 mg/h (Dodwad V, 2011)<sup>(23)</sup>, 2.4 g/L (Anumula L, 2017)<sup>(24)</sup>, 0.02 ppm (Lauritano, V 2020)<sup>(25)</sup>, 4 ppm (Bocci V, 2011)<sup>(26)</sup>, 300 mg/h (Niveda R, 2019)<sup>(27)</sup>, 1.5 mg/L (Hayakumo S, 2013)<sup>(28)</sup>, 2.5 ppm (Leewanthawet, 2019)<sup>(29)</sup> and 20 µg/mL (Al Habashneh, 2015)<sup>(30)</sup>. The current trial used ozonated water at 4ppm concentration; Bocci V(26) generated ozonated water by using a glass cylinder which was about 3/4 th filled with deionized and bidistilled water through which the gas mixture (oxygen-ozone) was bubbled continuously for atleast 5 minutes to achieve saturation. The ozonated water was irrigated using a 5 ml syringe with a blunt 21 gauge needle. The same protocol is followed in the current study. The present study demonstrated that, 4 ppm ozonated water significantly reduced CFUs in total anaerobic culturing when compared to 0.2% CHX during the peri operative period of implant surgery. Also, the test group did not have any side effects and was agreeable to patients, similar to CHX. The current clinical trial supports the efficacy of ozonated water, showing no difference in WHI, gingival variables like inflammation, oedema, granulation tissue and inflammation around the sutures, MGI and PI. Most of the studies show similar antimicrobial properties between ozonated water and CHX<sup>(17,24, 31-33)</sup>. All the above mentioned results are carried out in either gingivitis or periodontitis patients wherein, ozone therapy has been used as an adjunct to SRP. There is no literature analyzing ozone therapy as an antimicrobial agent in periodontal and implant surgeries. However, ample studies have evaluated the wound healing efficacy of ozone therapy on periodontal and implant surgeries. Thus, it is difficult to compare the outcomes of our study as it is first of its kind trial analyzing the effect of ozone therapy on the microbial count of a healing peri operative periodontal / implant surgical wound. Between the groups, at all time intervals, the WHI scores were similar. Positive outcomes of surgical wound healing after the use of ozone therapy have been frequently reported in the literature<sup>(34-39)</sup>. However, comparison between CHX and ozone therapy is rare. Comparative evaluation between both the groups showed no difference in the MGI scores at all time intervals. Kshitish  $D^{(17)}$  observed a greater reduction in GI (29%) for ozone group as opposed to the current trial where, no difference in the groups was noted. Issac  $AV^{(40)}$  noted significantly greater improvement in the GI with ozone therapy when compared with SRP alone. Dodwad  $V^{(23)}$  also noted higher percentage of GI reduction (72%) in the ozone group as compared to the CHX group, which is contradictory to the present trial. In the current trial, at all time intervals the PI scores were similar between both the groups with no statistical difference. Within both the groups, there was a statistical significant increase in the plaque score from baseline to day 14. In contrast to our findings, Dodwad V<sup>(23)</sup> and Kshitish D<sup>(17)</sup> observed greater reduction in plaque index (PI).

After conclusion of this trial, a similarity between CHX and ozone as irrigants after implant surgery during the perioperative period (0 to 14 days) was noted. Since 3 patients were lost to follow up, the final analysis of 7 patients in each group is relatively a very small sample size to derive any conclusion about the antimicrobial and wound healing efficacy of ozone and its superiority/inferiority quotient when compared to CHX.

# V. Conclusion

Within the limits of the present study, it can be concluded that 4 ppm ozonated water was comparable to 0.2% chlorhexidine as an antimicrobial agent. Ozonated water significantly reduces the total anaerobic count in a healing implant surgical wound. Wound healing, gingival variables, modified gingival index and plaque index were similar in both the groups. Application of ozonated water showed no adverse effects and was well tolerated by the patients. The use of ozonated water could be of use in patients undergoing surgery and are not

CHX tolerant. If further randomized control trials can put forth standardized criteria for ozonated water application as an irrigant, it can be used as a substitute for CHX post surgeries. Ozonated water may also be used during supportive periodontal therapy as it will a valuable antimicrobial adjunct to mechanical periodontal therapy.

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Figure 1 - Test group - a) Implant placement b) Suturing c) Ozonated water irrigation



Figure 2 - Control group - a) Implant placement b) Suturing c) Chlorhexidine irrigation



Figure 3 - Microbiological analysis. a) Swabbing the incision line with microbrush b) Transferring the specimen to thioglycollate broth c) Transferring to Brucella blood agar plate d) Innoculum spread using L-spreader



Figure 5 - Total Microbial count (Control group) - a) Baseline b) Day 3 c) Day 5 d) Day 7 and e) Day 14

#### FIGURE LEGENDS

FIGURE 1 : Test group procedure Implant placement Suturing Ozonated water irrigation FIGURE 2 : Control group procedure Implant placement Suturing Ozonated water irrigation FIGURE 3 : Microbiological analysis protocol Swabbing the incision line with microbrush Transferring the specimen to thioglycollate broth Transferring to Brucella blood agar plate Innoculum spread using L-spreader FIGURE 4 : Total microbial count Test group Day 0 Day 3 Day 5 Day 7 Day 14 FIGURE 5 - Total microbial count - Control group Dav 0 Day 3 Day 5 Day 7 Day 14

#### TABLE / GRAPH LEGENDS

Table 1 : Age and gender distribution among 2 groups

Table 2 : Comparison of mean Log10 CFU/mLs & other clinical parameters between 2 groups

Table 3 : Comparison of Gingival variables (GV) between 2 groups at different time intervals

Table 4 : Inter group comparison of change in the parameters at each time intervals

Graph 1 : Mean CFUs [Log10] values between 2 groups at different time intervals

Dr. Apeksha Vikas Kamble, et. al. "Comparative Evaluation of the Efficacy of 0.2% Chlorhexidine v/s Aqueous Ozone Solution Irrigants in the Control of Dental Plaque Microorganisms at Submerged Single Implant Area - A Randomized Controlled Clinical Trial." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 22(3), 2023, pp. 04-13.

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