

Comparative Evaluation of Antibacterial Efficacy of Cetylpyridinium Chloride and Chlorhexidine Gluconate Mouthwashes after Periodontal Surgery - A Randomized Clinical Study

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ABSTRACT

Background: Periodontitis affects 20-50 percent population is and has various systemic interrelationship. Treatment of periodontal disease requires a holistic approach. Prevention of bacterial accumulation at surgical site is crucial to minimize post-operative complications. The principal cause of post-surgical infection is bacterial colonization due to the wicking effect of the sutures. Despite numerous factors, pathogenic biofilm formation continues to play a crucial role in disease progression. Hence, a comprehensive approach with the goals of infection control, periodontal tissue regeneration, and function restoration becomes a necessity. In this study, we assessed the antibacterial efficacy of Chlorhexidine (CHX) and Cetylpyridinium Chloride (CPC) mouthwashes after periodontal surgery.

Material and Methods: In this single-centric, prospective, randomized study, patients undergoing various periodontal surgeries including flap procedures, crown lengthening, resective osseous surgeries, implant placement and periodontal plastic procedures following placement of simple interrupted sutures using monofilament PTFE sutures to reduce the wicking effect. Patients included were >18 years of age, while those with any systemic diseases, pregnant and lactating women, smokers or any other deleterious habits, immune and mental disorders, unwilling to participate were excluded from the study. A total of 46 patients were randomized using envelope method to either one

of the two types of mouthwashes CHX or CPC for 10 days post-periodontal surgeries and the sutures were removed after 10 days for CFU analysis.

Results: Out of total 46 participants more than half were males (26) and the mean age of participants was 32.53 years (range 18-65 years). Maximum periodontal surgeries were flap procedures (21) and crown lengthening (15) while remaining included osseous surgery (5), perioplastic procedures (3) and implant procedures (2). There was a statistically significant difference seen for the values between the groups. Group I CPC had average CFU count (Mean 1.2×10^{10}) Group II CHX had (Mean 1.2×10^8) The p value of Mann-Whitney U test was <0.01.

Conclusion: Though the bacterial load was reduced by both types of mouthwashes, our study observed better antibacterial effectivity with CHX as compared to CPC. The findings of the study also highlighted the importance of careful adjuvant therapies regardless of the type of periodontal surgery.

Keywords: Periodontal surgery, Cetylpyridinium chloride, Chlorhexidine gluconate, mouthwashes, periodontitis, biofilm, Plaque, Wound healing.

INTRODUCTION

According to AAP workshop 2017, Periodontitis is defined as a microbially-associated, host mediated inflammation that

results in loss of periodontal attachment and identified by clinical attachment loss.¹ Periodontitis, is a chronic inflammation progressive degradation of connective tissue attachments, and alveolar bone loss and recession.² It affects 20-50 percent world's population.³ It is associated with age, smoking, poor oral hygiene maintenance, systemic diseases, medication, hereditary factors, stress levels, and several contributing variables.³ Common pathogens causing periodontitis include *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetem-comitans* and *Fusobacterium nucleatum*.⁴ The holistic treatment includes non-surgical therapy, mechanical therapy, surgical therapy, and local therapy with adjuncts like antibiotics, antiseptics.⁵ Despite numerous risk factors, the formation of pathogenic biofilm continues to play a crucial role in disease onset and progression.^{6,7} Localized Pain, bleeding, swelling, mobility of teeth, root hypersensitivity, trismus, delayed wound healing, bacteremia, and bruising are common post-surgical problems. Complications with local anaesthesia, flaps, sutures, grafts, periodontal packs also alter the surgical outcomes.⁸ Therefore, a comprehensive approach with the goals of infection control, periodontal tissue regeneration, and function restoration is a necessity. Primary closure of wound is a prerequisite for uneventful wound healing. Multifilament sutures placed following a periodontal surgery accumulates more plaque and bacteria, called the wicking effect. Monofilament sutures do not cause wicking effect and it has been proven that bacterial accumulation with monofilament sutures is low leading to less inflammation at surgical site⁹. Moreover, reduced plaque retention at the surgical site reduces inflammation and promote better tissue repair. Monofilament sutures like polytetrafluoroethylene (PTFE) have fewer tissue abrasion and lower risk of infection than other sutures.¹⁰ Adjunctive therapy employing mouthwashes bearing anti-plaque and anti-gingivitis features can enhance the

effectiveness of plaque control.¹⁰ Bacterial colonization at the surgical site highlights the necessity for plaque management during the initial critical period for uneventful wound healing.^{10,11} The commonly used adjunct for chemical biofilm control has been Chlorhexidine gluconate mouthwash (CHX).¹¹ It is considered gold standard due to bactericidal antiseptic component containing bis-biguanide that suppresses bacterial colonization. However, its prolonged use has been associated with several side effects like bacterial resistance and staining of teeth.¹¹ Cetylpyridinium Chloride (CPC), a quaternary ammonium compound, is an antiplaque and antibacterial agent since it generates cytoplasmic leakage and disintegrates the bacterial membrane. Hiromi Taninokuchi et al studied positive effects of a CPC-, GK2-, and TXA-based mouthwashes after implant placement by scanning electron microscopy. A statistically significantly greater number of CFU in the placebo group especially for gram negative anaerobes were noted. Moreover, no statistically significant in-vitro resistance for *P. gingivalis*, *S. aureus*, and *P. aeruginosa*.¹² Mono-filamentous sutures showed less microbial accumulation which further depends upon type of surgery, periodontal diagnosis, and antibiotic consumption.¹² Alberto Pulcini et al. evaluated the efficacy of a 0.03% CHX and 0.05% CPC mouth rinse and demonstrated adjunctive benefits in peri-implant mucositis (PiM) though complete disease resolution could not be achieved in every case.¹³ Similar observations by Rösing et al. were presented where CPC and zinc lactate had significant anti-plaque and anti-gingivitis effects.¹⁴ Fei Teng et al. noted gingival inflammation reduction significantly slower in the CPC group than control group.¹⁵ Guerra et al. demonstrated addition of CPC allowed reduction of CHX percentage in mouthwash formulation keeping equal efficacy and less side effects; while Anti-Discoloration System (ADS) addition decreased CHX efficacy in reducing plaque and bleeding.¹⁶ The most popular antibacterial mouthwash is chlorhexidine

digluconate (CHX), a bisbiguanide that binds to cell membranes and increases permeability and leakage of intracellular components in order to inhibit and prevent the formation of bacteria. The primary cause of its impact is substantivity in the mouth.^{17,18,19} Multiple research studies have clinically evaluated the performance of ingredients in mouthwashes and recommend the use of CPC/CHX with mono-filamentous absorbable sutures in majority of the periodontal surgery cases. In support of this valid observation The aim of our study was to compare the effectiveness and antibacterial efficacy of Cetylpyridinium and Chlorhexidine mouthwashes after periodontal surgery through a randomized clinical study. The objective was to evaluate the antibacterial efficacy based on the colony forming units (CFU/ml).

MATERIALS & METHODS

Study Design: This study design was a single-centric, investigator initiated, in-vivo prospective study, to comparatively evaluate the antibacterial efficacy of CPC and CHX mouthwashes after placement of PTFE sutures post periodontal surgeries.

Study setting: The study was conducted abiding all human ethical principles as per the Declaration of Helsinki and Good Clinical Practice guidelines. In this in-vitro human clinical study, the patients were screened initially and eligible patients were recruited by convenience sampling. The study was performed over a span of 7 months, following ethical clearance from the local Institutional ethical committee and was registered with CTRI.

Study Population: All patients >18 years of age who underwent periodontal surgeries such as flap surgery, crown lengthening procedure, gingivectomy, implant surgery, mucogingival surgery, osseous resective surgery, bone augmentation surgery who agreed to be a part of study by signing informed consent form were included; while the patients with systemic diseases, pregnant and lactating women, smokers or deleterious habits, immune disorders and mental

disorders were excluded from the study. Also, patients who refused to participate and who exited from the study on their will were excluded from the study. A total of 46 patients meeting the inclusion criteria were prescribed either one of the two mouthwashes for 10 days post-surgery and the sutures were removed after 10 days for CFU analysis.

Study Procedures: Study procedures initially included periodontal surgery and placement of sutures in the patients satisfying the inclusion criteria. Poly-tetrafluoroethylene (PTFE) sutures were placed using simple interrupted sutures with a surgeon's knot following the periodontal surgery. The randomization of patients was done by envelope method and the name/brand of the mouthwashes were masked before giving it to the patients. After the informed consent, patients were randomly allocated to 2 groups: Group I: Cetylpyridinium chloride (0.075%) and Group II: Chlorhexidine gluconate mouthwashes (0.2%). The patients were given post-surgical oral hygiene instructions and prescribed either of the mouthwashes for 10-days. Patients were advised to use of 10 ml of mouthwash in undiluted form to be used twice a day, half an hour after brushing. The patients were advised to avoid eating anything for 20- 30 minutes after mouthwash use. On the 10th day, sutures were removed using sterile scissors and collected in a conical sterile polypropylene centrifuge tubes under all aseptic precautions. The formulations were prepared using the reagents/chemicals such as Phosphate buffer solution (PBS) and normal saline. For microbiological analysis, the tubes collected were centrifuged for 60 seconds in order to detach the plaque from the sutures. After this, the sample were serially diluted and colony forming units (CFU/ml) were checked by visual method. The colony forming units (CFU/ml) count were assessed at 3 dilutions of 10^{-3} , 10^{-4} , 10^{-5} to comparatively evaluate the antibacterial efficacy of the CPC and CHX mouthwashes.

Outcomes: To compare the evaluation of antibacterial efficacy of CPC and CHX mouthwashes after periodontal surgery.

Data Collection and Data Management: Total 46 suture samples were collected from patients satisfying the inclusion criteria, after post-surgical mouthwash regimen. The antibacterial efficacy of both mouthwashes were comparatively evaluated. The statistical analysis was performed by a blinded statistician.

Sample Size Calculation: The Sample size was determined using the estimates of mean and standard deviation values from literature using the formula,

$$n = \frac{2(Z_{\alpha} + Z_{\beta})^2 [s]^2}{d^2}$$

where Z_{α} is the z variate of alpha error i.e. a constant with value 1.96, Z_{β} is the z variate of beta error i.e. a constant with value 0.84.²⁰

Approximate estimates: 80% power, Type I error to be 5%, Type II error to be 20%; True difference of at least 0.25×10^6 units between the groups and Pooled standard deviation of 0.30×10^6 units.

Substituting the values, $n = \frac{2(2.8)^2 [0.30]^2}{(0.25)^2}$

$n = 22.57$.

Thus, approximately 23 samples per group were completed in the study.

STATISTICAL ANALYSIS

The data collected was compiled on MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States) and was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). The Normality of numerical data was checked using Shapiro–Wilk test or Kolmogorov-Smirnov test. Depending on the normality of the data, statistical tests were determined. For numerical continuous data following a

normal distribution, inter-group comparison (2 groups) was done using a t-test, or a non-parametric Mann-Whitney U test was used wherever required. The Intra-group comparisons for numerical continuous data following a normal distribution were done using paired t-test (for 2 observations) or repeated measures ANOVA for >2 observations, else a non-parametric substitute like Wilcoxon signed rank test or Friedman’s test for >2 observations was used. The descriptive statistics like frequency (n) & percentage (%) of categorical data, mean & Standard deviation of numerical data in each group was depicted & may be compared using chi-square test. Keeping alpha error at 5% and Beta error at 20%, power at 80%, $p < 0.05$ was considered statistically significant.

RESULTS

Table 1: Baseline characteristics of both groups

Mean age of patients				
Age	n	Minimum	Maximum	Mean
	46	18	65	32.53
Distribution as per Gender				
Gender	Frequency			
Female	20			
Male	26			
Total	46			

Table 1 depicted baseline characteristics of participants in Group I: Cetylpyridinium chloride (CPC) and Group II: Chlorhexidine gluconate (CHX) in terms of age and gender distribution and periodontal surgical procedures. Out of total 46 participants, more than half were males (26) and mean age of participants was 32.53 years (range 18-65 years). Maximum periodontal surgeries under the study were cases of flap procedures (21) and crown lengthening (15) while remaining were resective osseous surgery (5), perioplastic procedures (3) and implant procedures (2).

Table 2: Procedures performed

Sr no.	Procedures	Number of procedures
1	Crown lengthening procedures	15
2	Flap procedures	21
3	Implant placement	2
4	Periodontal plastic procedures	3
5	Resective osseous surgery	5

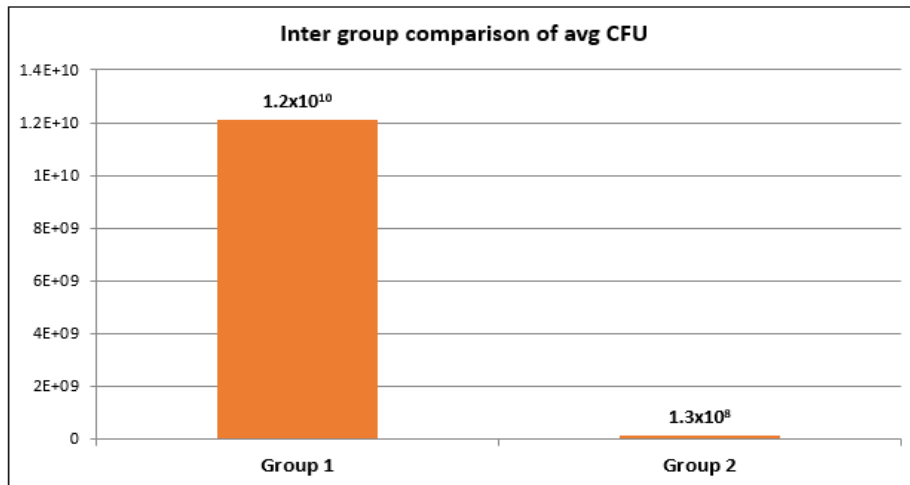
Table 2 depicted the procedures and numbers of procedures performed.

Table 3: Intergroup comparison of average CFU count

	Group	N	Mean	Std. Deviation	Std. Error Mean	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
CFU/ml	I	23	1.2x10 ¹⁰	2.2x10 ¹⁰	4.6x10 ⁹	5305000000	0.000	-5.745	0.000**
	II	23	1.3x10 ⁸	2.5x10 ⁸	5.3x10 ⁷	25000000			

Table 3 depicted intergroup comparison of average CFU counts between two groups. There was a statistically significant difference seen for the values between the groups. Group I (CPC) had average CFU count (Mean 12110909090.9091 ± 2.20847*10 while Group II (CHX) had (Mean 139040909.090909 ± 2.52774*8). The p value of Mann-Whitney U test is <0.01.

Graph 1: Intergroup comparison of average CFU count



Graph 1: Depicted the Intragroup comparison of colony forming units (CFU/ml) in Group I(CPC) and Group II(CHX)



Image 1: depicted the suture samples collected in polypropylene tubes



Image 2: depicted Colony forming units (CFU/ml) checked at 3 dilutions of 10⁻³, 10⁻⁴, 10⁻⁵

DISCUSSION

Our study aimed to compare the effectiveness and antibacterial efficacy of CPC and CHX mouthwashes after periodontal surgery through a randomized clinical study. As the oral cavity serves as a reservoir for numerous bacteria, the chances of contacting infection even increase when any dental procedures like periodontal surgeries are performed. Post these procedures, there may be an increased plaque build-up and bacterial colonization which can disrupt the wound healing process. In addition, with the standard post-surgery strategies, the plaque retention at the surgical site will be decreased by using mouthwash as a supplement to mechanical plaque treatment. The choice of in situ plaque collection was thought to be a suitable method for simulating the formation of a typical biofilm, which is distinguished by high complexity and the presence of various bacterial strains.^{21,22}

Deus et al. conducted a systematic review to conclude that CHX was primarily a supplement to diverse dental treatments. When mechanical prophylaxis proved impractical for short periods of time, mouthwash was the best option for preventing gingivitis. With little negative side effects, CHX products are frequently used in periodontics, post-oral surgical treatments, and as a prophylaxis for invasive procedures due to its antibacterial characteristics made a perfect preventative measure at concentrations between 0.12% and 0.2%. CHX chips were advised for long-term therapy, particularly in periodontitis patients (stage I–III) receiving nonsurgical treatments.²³ According to systematic review by Solderer et al, CHX after surgery significantly reduced plaque (means of 29–86% after 1 week) and bleeding (up to 73%) compared to using a placebo with no further periodontal probing depth reduction over any specific placebo or control solution could be found. It was determined that CHX represented chemo-preventive strategy just after surgery. The most successful outcomes involved rinsing with less concentrated

formulations (e.g., 0.12%), which reduced CHX's adverse effects while maintaining comparable clinical effects.²⁴

Likewise, a mono-cationic quaternary ammonium molecule, Cetylpyridinium Chloride is a quaternary nitrogen linked to one or more hydrophobic side chains. Numerous in vitro research has looked into the antibacterial effectiveness of CPC that focused on microorganisms, although bacteria trapped in biofilms show completely different characteristics from their planktonic counterparts.²⁵ For illustration, when screening streptococcal isolates for Minimum Biofilm Inhibitory Concentrations (MBICs) against CPC and MICs measured in cultures, the researchers discovered median MICs of 0.12 or 0.24 g/ml and median MBICs of 7.81 to 15.63 g/ml.²⁶

There are multiple studies in literature which proves that CHX and CPC are excellent mouthwashes to be used pre or post periodontal surgeries either alone or in combination. CHX solutions reduced plaque and gingivitis in randomised research by Quirynen et al. Streptococcus mutans was suppressed in all species with further decreases in the CFU/ml of aerobic and especially anaerobic species. The outcomes showed the potential of a novel non-alcoholic CHX 0.05% + CPC 0.05% formulation as a long-term antiplaque agent with minimal subjective side effects.²⁷ Becker et al conducted similar study to compare the CHX and CPC mouthwashes and it had shown similar results as this study.²⁸ According to Bollain et al., there were statistically significant differences between the groups and a larger reduction in bleeding during probing. After expert mechanical debridement and the additional use of a mouthwash containing 0.03% CHX and 0.05% CPC, the control of gingivitis can be improved.²⁹ Pulcini et al. assessed the effectiveness of a mouthwash containing 0.03% CHX and 0.05% CPC in the treatment of peri-implant mucositis (PiM). The trial mouth-rinse showed additive advantages in the management of PiM.¹² These results are in line with those of Schwarz et al. (2011,

2012), who did not discover any differences in clinical outcomes between the combined surgical therapy for advanced peri-implantitis lesions and the approach of surface debridement and decontamination. It was indicated that factors other than the technique of surface debridement and decontamination may have an impact on the long-term stability of the clinical outcomes.^{30,31} De Waal on implant surface decontamination with CHX/CPC on microbiological and clinical parameters demonstrated both decontamination procedures that resulted in significant reductions of bacterial load on the implant surface, but the test-group showed a significantly greater reduction than the placebo-group. Multilevel analysis showed no differences between both groups in the effect of the intervention on bleeding, suppuration, probing pocket depth and radiographical bone loss over time.³² In our study, CHX resulted in significant reduction of bacterial colonization at postoperative sites. Multiple studies mentioned formerly in this article are in similarity with previously conducted trials on CHX and CPC mouthwashes that established CHX had superior antibacterial efficacy than CPC alone with better effects when used in combination. In fact, numerous factors, including anatomical and technical aspects, patient compliance, plaque control, and cigarette smoking, affect postoperative success. Lower plaque has been demonstrated to improve complication management and lower the risk of infection. Inevitably, these considerations also result in stringent protocols, which are primarily justifiable by infection prevention and uneventful wound healing. One common practice that could contribute to a positive outcome is the adjunctive use of supplementary antibiotics or anti-inflammatory drugs during regeneration therapies. However, the frequency and duration of the maintenance phase with antiseptic mouthwashes tend to increase with increased interventional efforts.³³

Our investigation used PTFE sutures to demonstrate antibacterial efficacy of both CHX and CPC mouthwashes. We noted that CHX mouthwash had significant antibacterial efficacy in periodontitis as compared to CPC. Hence, we reinforce CHX containing chemical plaque control (mouthwash) as an adjunct to mechanical plaque control as a requisite. We recommend more in-vitro and in-vivo randomized controlled trials with large representation of sample size to justify the use of CHX, CPC or combination of CHX+CPC mouthwash post periodontal procedures in future researches.

Strength and Limitations:

Our study had some limitations. The investigation was conducted using a very limited sample size. There was also a risk of human error because the colony forming units (CFU/ml) were counted manually by visual method, which may or may not have affected the results of the current investigation. Additionally, we could not compare the features of various suture materials since we only included one suture material due to cost-effective methods.

CONCLUSION

In conclusion, there is a statistically significant difference between the antibacterial effectiveness of mouthwashes containing CPC and CHX. The bacterial load was reduced by both mouthwashes in an equivalently effective manner, but CHX showed significant reduction in bacteria. The findings of the current study also highlight the importance of providing adjuncts after therapies, such as mouthwashes regardless of the type of surgery performed or suture materials used. In order to completely comprehend the physical and biological components of bacterial colonization following periodontal surgery, additional randomized controlled in-vivo and in-vitro researches with numerous parameters and longer time periods are required.

Declaration by Authors

Ethical Clearance Statement: The study protocol and other related materials were approved by the local Institutional Ethics Committee of Mahatma Gandhi Mission's Dental College & Hospital, Junction of NH-4 and Sion-Panvel Expressway, Sector-18, Kamothe, Navi Mumbai- 410209 and was conducted conferring to ethical principles of Declaration of Helsinki, Good Clinical Practices, ICMR guidelines and applicable local regulations.

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Informed Consent Statement: Informed consent was explained and obtained from all the involved subjects those who voluntarily agreed to be a part of the study procedures

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