

# Clinical and Microbial Comparative Evaluation of 0.1% Chlorine Dioxide Mouthwash Versus 0.2% Chlorhexidine Mouthwash after Periodontal Surgery: A Randomized Clinical Trial

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**Abstract:-** Postsurgical mouthwash is routinely used in daily clinical practice. Chlorhexidine gluconate (CHX) is considered gold standard for chemical plaque control regime. Extensively studied Chlorine dioxide (ClO<sub>2</sub>) formulation has shown to have antiplaque, antibacterial effect and effective against oral malodor. Unlike CHX it does not cause teeth staining. Chlorine dioxide could be the possible alternative to CHX. The aim of the study is to clinical and microbial comparative evaluation of 0.1% chlorine dioxide mouthwash versus 0.2% Chlorhexidine mouthwash after periodontal surgery. Forty-five patients scheduled for periodontal flap surgery were randomly assigned in three groups depending on the post-surgical mouthwash. Patients belonging to Group A and Group B were asked to rinse with 0.2% CHX mouthwash and 0.1% Chlorine dioxide respectively, twice a day for 2 weeks after periodontal surgery while patients belonging to Group C were asked to rinse with saline solution. On 7<sup>th</sup> and 14<sup>th</sup> day, Plaque index (PI), Gingival index (GI), halitosis and early wound healing index were recorded. Microbial analysis was performed by determining colony forming unit on blood agar plates cultured using plaque samples from the site. The data obtained from these were statistically analysed. Both the test groups demonstrated statistically significant reduction in colony forming unit, PI, GI, and halitosis from baseline while Saline group showed non-significant reduction in colony forming unit, PI, GI, and halitosis from baseline. The result of the present study supports alternate use of chlorine dioxide mouthwash to promote early wound healing after periodontal surgery.

**Keywords:-** Chlorine dioxide, Chlorhexidine, Halitosis, Perio-mouthwash, Post-surgical mouth rinses.

## I. INTRODUCTION

Various surgical procedures are performed in Periodontics to treat periodontal pockets, correct mucogingival deformities or replacement of missing teeth by placement of implants. The success and the favorable clinical outcomes of all this procedures depend on how meticulously the bacterial contamination is avoided at these operated sites to ensure uneventful healing.<sup>[1]</sup> Immediately post operatively, these operated sites are predominated with inflammatory phase of healing that harbors biofilm formation and accumulation.<sup>[2]</sup> It has been observed that good plaque control at these operated sites could fasten healing, while in absence of good maintenance, chances and risk of secondary infection and delayed wound healing are high.<sup>[3]</sup>

Maintaining plaque free zone post-operatively is sine quo non and this is been extensively documented gold standard in re-establishment of periodontal and peri-implant health.<sup>[4,5,6,7,8]</sup> Despite of the extremely convincing evidence that support the positive effects of patient-performed plaque control post-surgically, the studies evaluating the effect of plaque control is sparse.<sup>[9]</sup>

The post-operative chemical plaque measures are suitable and complaint for patients. Among the various chemical plaque control measures that are available, chlorine dioxide mouthwash is one of the suitable alternatives that have been reported to reduce oral malodor. The chlorite anion in the mouthwash induces oxidative consumption of precursors of VSCs - Volatile sulphur compounds. <sup>[10]</sup> In this way, the post-operative use of chlorine dioxide mouthwash, a strong oxidizing agent, reduces oral malodor by reduction of cysteine and methionine like VSCs amino acids. The activity of Chlorine dioxide is easily lost. "Stabilization" process improves its stability by converting it to molecular chlorine dioxide at a low pH of 7.<sup>[11,12]</sup> However, its clinical trial are poorly documented.

Chlorhexidine (CHX) is the most studied antimicrobial chemical plaque control agent. Results from two case series type of study evaluating halitosis patients suggested a significant effect of 0.20% or 0.12% CHX rinsing.<sup>[14,15]</sup> Though the gold standard and most suitable, there are various side effects on its prolonged prescription to patients such as staining, altered taste and reduced taste sensation<sup>[16,17]</sup>

Considering the well documented CHX and poorly documented chlorine dioxide, the two most potent chemical plaque control agent, the clinical and microbial comparative evaluation of 0.1% chlorine dioxide mouthwash versus 0.2% chlorhexidine mouthwash was evaluated post-operatively in this study.

## II. MATERIALS & METHOD

Forty-five patients scheduled for flap surgery were selected for this randomised controlled clinical trial. Every subject received verbal and written information about the study and the signed consent was obtained from each of them. The study protocol was approved by the Committee of Ethics Affairs of dental college and was conducted according to the principles outlined in the Declaration of Helsinki for experiments involving human subjects.

The selected patients were systemically healthy, non-smokers and with no history of systemic antibiotics up to 6 months prior to enrolment. Female subjects with pregnancy and breastfeeding history were excluded. Subjects requiring reconstructive periodontal surgery as treatment were excluded from the study. All the patients diagnosed with chronic periodontitis were treated with initial periodontal therapy for 1 month prior to enrolment. Patients with an indication for periodontal surgery in at least one sextant exhibiting residual probing depths (PD) of  $\geq 6$  mm at the end of 1 month following nonsurgical therapy were only selected for the study.

A total of 45 subjects were randomly assigned in three groups (15 patients in each group). Group A was asked to rinse with 0.2% Chlorhexidine mouthwash\*, Group B with 0.1% chlorine dioxide mouthwash † and Group C with normal saline twice a day for 30 seconds for 2 weeks after periodontal surgery.

### A. Clinical procedures

Subjects satisfying the periodontal re-evaluation criteria continued for this trial. Open-flap debridement procedure were performed with root surface debridement with either hand or ultrasonic instruments. Reflected flaps were repositioned by direct loop interrupted sutures.

### B. Experimental design

With the help of computer generated randomization table, the subjected were allotted to the groups. Subjects were encouraged to strictly follow the post-surgical maintenance regime advised to them.

### C. Post-operative maintenance

Patients in the Group A, B and C were instructed to rinse with 15 ml CHX 0.2 % mouth rinse , 0.1% Chlorine dioxide mouthwash and normal saline respectively for 30 s twice daily. They advised to refrain from gargling, eat, or drink anything for subsequent hour. Oral hygiene reinforcement for non-operative sites were encouraged which included brushing twice daily from days 3 to 14 to ensure efficient plaque control and uneventful healing. 7 days post-operatively, sutures were removed.

### D. Clinical assessment

The clinical parameters were recorded at baseline, 1 week and at 2 weeks after surgery by the single calibrated examiner (K.S). Plaque index (PI),<sup>[18]</sup> Gingival index (GI),<sup>[19]</sup> halitosis using halimeter and early wound healing index were recorded. Halitosis was evaluated using HC-212SF Breath Checker. The HC-212SF uses a Semi-Conductor gas sensor to measures the amount of volatile sulfur compounds (VSCs) given off by bacteria. Plaque samples were collected and Microbial Analysis was performed using Blood agar as culture media. Post-operative healing was assessed by the early wound healing index (EHI)<sup>[20]</sup> differentiating between the following 5 degrees:

- Complete flap closure–no fibrin line in interproximal area
- Complete flap closure–fibrin line in interproximal area
- Complete flap closure–fibrin clot in the interproximal area
- Incomplete flap closure–partial necrosis of interproximal tissue
- Incomplete flap closure–complete necrosis of the interproximal tissue

### E. Evaluation of patient acceptance

Patient acceptance was determined by the use of visual analogue scale (VAS) questionnaires. Post-operative pain, irritation of taste and dentine hypersensitivity have been assessed using a scale of 10 cm. Additionally, patients have been asked to mark subjectively how much teeth have been stained using VAS with 0 cm representing none and 10 cm all teeth, respectively

### F. Statistical Analysis

For all tests,  $P < 0.05$  was considered statistically significant. Statistical analysis was performed using “INSTATS” software version 3.06.

## III. RESULT

The use of chlorine dioxide was observed and reported to be safe among Group B subjects with no adverse tissue reaction and good patient compliance. The subjects were regularly evaluated for clinical parameters to assess healing and improvement in the condition (Fig 1).

The Clinical Parameters like Halitosis, Plaque index and Gingival Index were evaluated at baseline, 7 days and on 14 days (Table1). The Halitosis result of Group A i.e. Chlorhexidine group showed statistical significant result

only between 7 days to 14 days' time period whereas the Group B (chlorine dioxide) showed statistical significant results throughout the study. The use of saline in Group C had no clinical relevance.



Fig 1:- Clinical pictures of immediate suture placement and 14 days post-operative healing pictures of Group A (Chlorhexidine), Group B (Chlorine Dioxide) and Group C (Saline)

Clinical Parameters	Time Interval	Group A		Group B		Group C	
		Mean	P value	Mean	P value	Mean	P value
Halitosis	Baseline -7 days	0.6667	>0.05	1.467	<0.001	0.845	>0.05
	7 days - 14 days	1.000	<0.05	2.533	<0.001	0.774	>0.05
	Baseline -14 days	0.3333	>0.05	1.067	<0.001	0.798	>0.05
PI	Baseline -7 days	1.4000	<0.001	0.833	>0.05	0.593	>0.01
	7 days - 14 days	0.8450	<0.05	0.743	>0.05	0.560	>0.05
	Baseline -14 days	1.867	<0.001	0.723	>0.05	0.593	>0.05
GI	Baseline -7 days	0.2327	<0.05	0.466	<0.05	0.133	>0.05
	7 days - 14 days	0.6800	<0.05	0.000	<0.05	0.333	>0.05
	Baseline -14 days	0.3007	<0.05	0.467	<0.05	0.466	<0.05

Table 1:- Comparative results of clinical parameters - Halitosis, Plaque Index (PI) and Gingival Index (GI) of Group A (Chlorhexidine), Group B (Chlorine Dioxide) and Group C (Saline)

The Plaque Index was found to be statistically significant only for Chlorhexidine group throughout the study timeline. Both Group B and C had very little effect on plaque maintenance. Group A and B, both showed significant result for Gingival Index. However, the Group C showing better results only from baseline to 14 days could be attributed to reduced inflammation post-operatively. (Table 1)

Zone of inhibition and Total colony forming unit was performed (Fig 2). For Zone of inhibition Blood Agar plates were plated separately by periodontal bacteria. Wells were prepared on agar plate using punch and filled with mouthwash. Plates were incubated for 24hrs at 37<sup>0</sup> C and later assessed for zone of inhibition and it was found that zone of inhibition for normal saline was 0mm, chlorhexidine mouthwash it was 18mm and for chlorine dioxide it was 9mm. For total colony forming units Plaque samples were collected using curette and inoculated on blood agar plates for 24 hours and CFUs were analyzed per 1ml.(Fig 3)

The analysed CFU colony unit for both Group A and B showed significant result. However, Group A was found to be better than Group B, and, Group C showed no effect. Similar type of results were found for Early Healing Index. (Table 2)

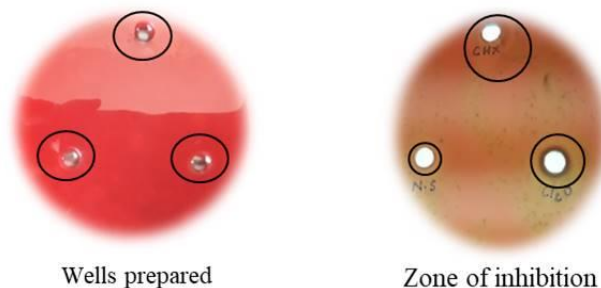


Fig 2:- Preparation of wells and Zone of inhibition for microbiologic evaluation.

Clinical Parameters	Time Interval	Group A		Group B		Group C	
		Mean	P value	Mean	P value	Mean	P value
CFU	7 days - 14 days	0.3407	<0.001	0.204	<0.05	0.762	>0.001
EHI	7 days - 14 days	0.4667	<0.05	0.066	<0.05	0.666	<0.05

Table 2:- Comparative results of microbiologic parameter CFU- Colony Forming Units and EHI – Early wound Healing Index of Group A (Chlorhexidine), Group B (Chlorine Dioxide) and Group C (Saline)

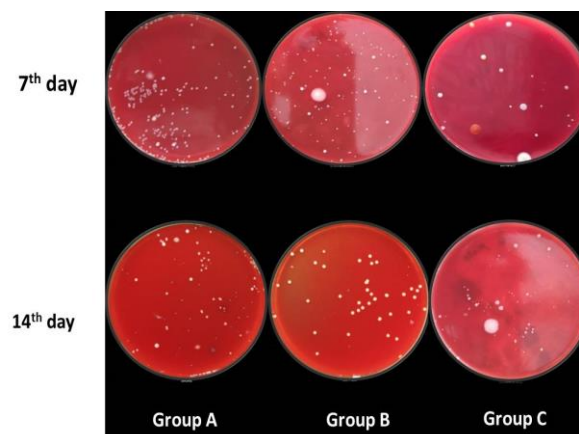


Fig 3:- Microbiologic parameter CFU- Colony Forming Units assessed in Group A (Chlorhexidine), Group B (Chlorine Dioxide) and Group C (Saline) at 7<sup>th</sup> and 14<sup>th</sup> day post-operatively.

#### IV. DISCUSSION

In present study, chlorine dioxide (0.1%) mouth rinsing showed good compliance with patient. In this randomized controlled clinical trial, 0.2 % CHX and 0.1% chlorine dioxide mouthrinses, showed relevant clinical finding in terms of reduction in probing depth, pathogens and oral mal-odour. However, saline mouthwash was comparative better than them in terms of early wound healing.

The major set-back of gold standard CHX, is the poor patient compliance in terms of side effects noted and documented. This includes altered taste, pigmentation on mucosa and irritation. Owing to these side-effects, poor patient acceptability is observed. In such clinical scenario, the subjects may become irregular or even discontinue using the mouthrinse.<sup>[21]</sup>

Chlorhexidine mouthwashes has anti-plaque, anti-septic, anti-gingivitis property and they inhibit formation of VSCs<sup>[22]</sup>. The result of gingival index in the current study was not in accordance to above mentioned study. The findings by Gürgan et al in their study concluded side effects of CHX in just one week rather than two weeks in some population.<sup>[16]</sup> The U.S. Food and Drug Administration (FDA) has approved Sodium chlorite (NaClO<sub>2</sub>) as a non-toxic antimicrobial agent which is equivalent to ClO<sub>2</sub>.<sup>[23]</sup> From the results obtained in this study, it can be concluded that ClO<sub>2</sub> was well tolerated by patients with no alteration in taste, discoloration of mucosa and also reduced malodor. Frascella et al, in his clinical trial testing the effectiveness of a ClO<sub>2</sub> mouthwash, concluded its clinical improvement in VSCs level and malodor. These parameters were tested at different time intervals of 96 hours and were compared to placebo.<sup>[11]</sup>

The maximum recommended dose allowed by FDI is 5,000 ppm for sodium chlorite<sup>[11]</sup> that is above the experimental level of mouthwash. Owing to this, the mouthwash may be advantageous with efficient clinical result and safety. Kimoto et al evaluated cytotoxicity and anti-bacterial property of ClO<sub>2</sub> on human cells and declared it to be safe for human cells and dental implants.<sup>[24]</sup>

ClO<sub>2</sub> along with the chlorite anion (ClO<sub>2</sub><sup>-</sup>) reduces cysteine and methionine like amino acids by directly oxidizing VSCs.<sup>[25]</sup> The chlorite anion is potent bactericidal to periodontal pathogens<sup>[11,26,27]</sup>. ClO<sub>2</sub> mouthwash when prescribed to healthy individuals, showed reduction in malodor in 4 hours.<sup>[27]</sup> Various studies support the clinical efficacies of ClO<sub>2</sub> on oral malodor.<sup>[12,28]</sup> However, the studies evaluating the microbiological efficacy are sparse. Considering the effective in vitro testing of antimicrobial action of ClO<sub>2</sub> mouthwash, the in vivo results were found to efficacious too.

The Group A results obtained from this study are in accordance to this data. The 7 – 14 days data obtained from Group A for inhibition of orally produced volatile sulfur compounds was significant and also corresponding with the study done by Young A and co-workers.<sup>[29]</sup> On contrary, Chlorine dioxide mouthwash in Group B showed better halitosis control, equilavent gingivitis control and poor plaque control in comparison to Group A. Given that the patient with excellent oral re-enforcements and with tendency of VSC, Chlorine dioxide seems to be a better option for such group of patients.

The limitation of present study was Small sample size, Specific microbial colony count was not performed, No

histological analysis was done to evaluate the healing mechanism.

## V. CONCLUSION

With the limitations both mouthwash inhibited plaque formation up to 7 days however after 14 days Chlorine dioxide mouthwash was found to be a less effective in plaque inhibition than chlorhexidine. Chlorhexidine have better antibacterial efficacy in comparison to chlorine dioxide mouthwash. The results conclude of ClO<sub>2</sub> mouthwash improving post-operative bad breath in the subjects.

## FOOTNOTE

\* chlorhexidine mouthwash

† chlorine di oxide mouthwash (freshchlor, Group pharma)

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