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COMPARISON OF CLINICAL AND MICROBIOLOGIC EFFICACY OF 0.12% CHX SOLUTION AGAINST 1% CHX GLUCONATE GEL APPLIED USING TRAYS FOR RELIEVING GINGIVITIS AND INHIBITION OF DENTAL PLAQUE FORMATION: A PILOT STUDY

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ABSTRACT

Chlorhexidine is an effective antibacterial agent and is used in dental treatment in several formulations. The aim of the study is to compare the clinical and microbiological efficacy of 1%CHX gel applied using trays with 0.12% CHX gluconate mouthwash in patients with chronic gingivitis. 30 participants are randomly divided into two groups: test group (1% CHX gel) and control group (0.12% Chlorhexidine mouthwash). Participants were provided with CHX products and were instructed to use each product for 2 week. Clinical and microbiological evaluation was done by recording gingival index (GI), plaque index (PI) and anaerobic colony forming units (CFU). The results were analyzed using independent t test ($p<0.001$). The GI and PI were decreased more in the test group compared to the control group 2 weeks later. In both the groups, anaerobic colony forming units (CFU) also decreased ($p< 0.001$) after 2 weeks, the CFU score decreased relatively more in CHX gel group than CHX solution group. These results suggest that 1% CHX gel is more effective in reducing gingivitis than 0.12% CHX solution. Therefore, 1% CHX gel can be preferred to be used in gingivitis and for non-surgical treatment of periodontal disease patients.

KEYWORDS: Participants were provided with CHX products and were instructed to use each product for 2 week.

INTRODUCTION

Oral cavity is the gateway to general health, and if this gateway is diseased, general health is also hampered. Amongst numerous diseases of oral cavity, the most common diseases are gingivitis and periodontitis after caries. Bacterial plaque is considered as the principle causative factor in gingival and periodontal diseases and the most common rationale towards prevention of these diseases is regular removal of plaque by an effective oral hygiene protocol.[1]

Home care regimes which are available for removal of plaque include mechanical methods and adjunctive use of chemical agents. Mechanical methods such as toothbrushing, flossing though effective are technique sensitive and time consuming and its effectiveness depend upon skills and techniques of the individual. A number of chemical agents which are commonly used are bisbiguanides/mouthwash, essential oils, quaternary ammonium compounds, sanguinarine, triclosan which are either available as a toothpaste/ dentifrice or in the form of mouthwash.

Amongst mouthwash, Chlorhexidine is considered "gold standard," as it is most potent in reducing oral biofilm. The mechanism of action of chlorhexidine begins with rapid attraction of a cationic CHX molecule to the surface of negatively charged bacterial cell containing phosphate and sulphate groups. CHX forms specific and strong adsorption to phosphate containing molecules. Penetration through the bacterial cell wall occurs, as a result of passive diffusion, damaging the cytoplasmic membrane of the cell. This results in an outflow of low molecular weight cytoplasmic components, such as potassium ions, and inhibition in activity of some of enzymes associated with the cytoplasmic membrane.[2]

Calderini et al 2013 demonstrated effectiveness of Chlorhexidine mouthwash in reducing periodontal pockets and gingival bleeding in patients with chronic periodontal disease.[3] Likewise, Berchier et al 2010 in the systematic review reported that both 0.12% CHX and 0.2% CHX mouthwash were effective in helping reduce gingival index and dental plaque.[4]

Chlorhexidine is used in different formulations such as solution, spray, gels, creams or toothpaste. It has been

reported by Stoken et al 2007 that chlorhexidine solution can be more effective than spray for control of dental plaque.[5] However, chlorhexidine solution has shown certain side effects, most commonly causes staining of dental surfaces in oral cavity when used for a long period of time. Contradictory to CHX solution, CHX gel has not shown any tooth staining as reported by Supranato et al 2015 in his study.[6] However, there is limited research done on CHX gel, thus limiting its use in routine oral hygiene care. Therefore, it is necessary to actively study effectiveness of CHX gel.

Hence, this study was carried out to compare the clinical and microbiological efficacy of 1%CHX gel applied using trays with 0.12% CHX gluconate mouthwash in patients with chronic gingivitis.

MATERIAL AND METHOD

The study was a prospective interventional comparative study and included a total of 60 participants. The institutional ethical committee approval was obtained before proceeding with the study.

Subject Selection

The study included 60 systemically healthy patients with plaque induced gingivitis, who were referred for treatment to the department of periodontology. The total number of subjects were determined based on the discussion held with biostatician, keeping him informed about various parameters and the groups involved in the study.

Patients were included in the study after having signed an informed consent.

Randomization

60 participants were randomly divided into two groups with 20 participants in each group.

The two groups were as.

1. Group 1: Chlorhexidine mouthwash
2. Group 2: Chlorhexidine gel

1st group (control group)- patients were advised to use Chlorhexidine mouthwash (Hexidine ICPA Health Product LTD) 10ml 0.2% for 60 sec twice a day for 2 week.

2nd group (test group)- patients were advised to use Chlorhexidine gel (Hexigel, ICPA Health Product LTD) 1% applied using periotrays twice a day for 2 week.

Selection criteria

Inclusion Criteria

1. Patients with chronic gingivitis and having GI and PI measurable at baseline.
2. At least 20 measurable teeth in patients oral cavity.
3. Systemically healthy patients
4. Age > 20 yrs
5. Patient who gave proper consent for the study.

Exclusion Criteria

1. Patients who are having an allergic reaction to CHX.
2. Patients having periodontitis OR even a single tooth attachment loss.
3. Pregnant / nursing or who have systemic condition/ disease with influence on periodontal health.
4. Patients who have habit of smoking or those who consume any forms of tobacco products.
5. Patients with high caries index.
6. Patients on antibiotic or those using other oral health supplements.

Instruments and equipments

For clinical study

1. Mouth mirror and explorer (GDC Fine Crafted Dental Pvt. Ltd, India)
2. Hand scalers (Hu Friedy Mfg. Co. LLC. Chicago, USA)
3. Ultrasonic scaler (Woodpecker India Pvt. Ltd)
4. Williams graduated periodontal probe (Hu-friedy)
5. Alginate impression material (IMPRECEED)
6. Impression trays
7. Bowl and spatula
8. Dental stone (Ultrastone)
9. Essix sheet
10. Vacuum forming machine (Bioart PT Plastovac)
11. Chlorhexidine mouthwash (Hexidine, ICPA Health Product LTD)
12. Chlorhexidine gel (Hexidine gel, ICPA Health Product LTD)
13. Sterile container for plaque sample collection

For microbiological study

1. Blood agar (Hi Media Laboratories Pvt. Ltd)
2. Colony counter (Hi Media Laboratories Pvt. Ltd)
3. Petri dish
4. Micropipette
5. Plaque sample
6. Anaerobic culture plates (BHI agar)

Screening and examination

- A total of 60 patients were screened for moderate to severe gingivitis in the age group of 20-50 years.
- Following a clinical examination, gingival index (GI), plaque index (PI) were recorded. Both groups received thorough dental prophylaxis at the beginning of the test period.
- Plaque samples were collected to evaluate anaerobic microorganisms colony forming unit CFU.

Parameters recorded at baseline and 2 weeks

1. Plaque index (PI) (Loe and Silness 1967)

The teeth surfaces were examined with the help of mouth mirror and explorer.
The six teeth were,
16, 12, 24, 36, 32, 44

Calculation of index

The indices for each tooth were added and then divided by the total number of each tooth examined.

2. Gingival index (GI) (Silness and Loe 1963)
The index teeth were
16, 12, 24, 36, 32, 44

Calculation of the index
For each individual, the indices for each of the teeth were added and then divided by the total number of teeth examined.

3. Total colony count (anaerobic colony forming units)

- Plaque samples which were collected using Gracey curette were immediately placed inside sterile tubes containing a reduced transport medium of thioglycolate.
- The tube containing thioglycolate transport media with plaque samples was centrifuged and vortexed for 30 sec.
- 100 µl aliquots of the vortexed samples were placed in a new sterile containing 1ml of thioglycolate.
- These diluted samples were then transferred to BHI agar plates under aseptic conditions.
- Then these agar plates were placed in an anaerobic sealed jar with Gas Pak system and were incubated for 48 hours at 37° C.
- Eventually, the number of bacterial colonies in each plate were counted and reported as CFU/ml.

Methods of using chlorhexidine mouthwash and chlorhexidine gel

1. Chlorhexidine mouthwash
Procedure:

- Use 10ml of 0.2% chlorhexidine mouthwash
- Swish it around the oral cavity followed by spitting, half an hour before toothbrushing, eating and drinking for 30 seconds twice in a day.
- Do not swallow the mouthwash and mix it with other substance

In all patients, plaque index and gingival index were recorded at baseline and 2 week. Plaque sample were collected for assessing colony forming unit count at baseline and 2-week time period.

2. Chlorhexidine gel
Procedure

- Impression was taken of both the arches using alginate impression material and cast were poured.
- The periotrays were prepared in vacuum forming machine (Bioart PT Plastovac) using Essix sheets
- Take 1% chlorhexidine gel and apply it using periotrays in the oral cavity twice a day for 2 week.
- Patients were asked not to brush teeth, or not to eat and drink immediately after using chlorhexidine gel.

RESULTS

Table 1: Comparison of baseline gingival index between chlorhexidine gel and chlorhexidine solution using Independent T test.

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	2.0323	.75206	-.09677	-.50362	.31008	-.476	.636 (NS)
CHX Sol	2.1290	.84624					

NS = Not significant

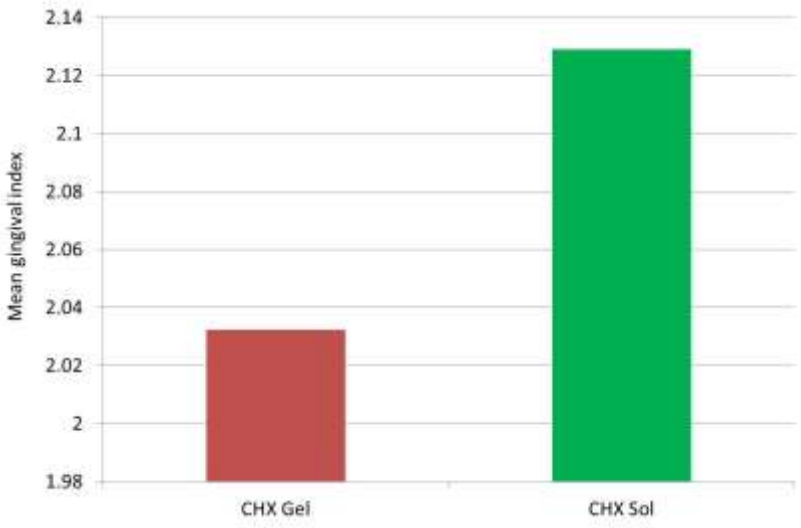


Table 2: Comparison of gingival index after 2 weeks between chlorhexidine gel and chlorhexidine solution using Independent T test.

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	.5161	.50800	-.87097	-1.18690	-.55503	-5.527	**.000 (HS)**
CHX Sol	1.3871	.71542					

HS = Highly significant (p<0.001)

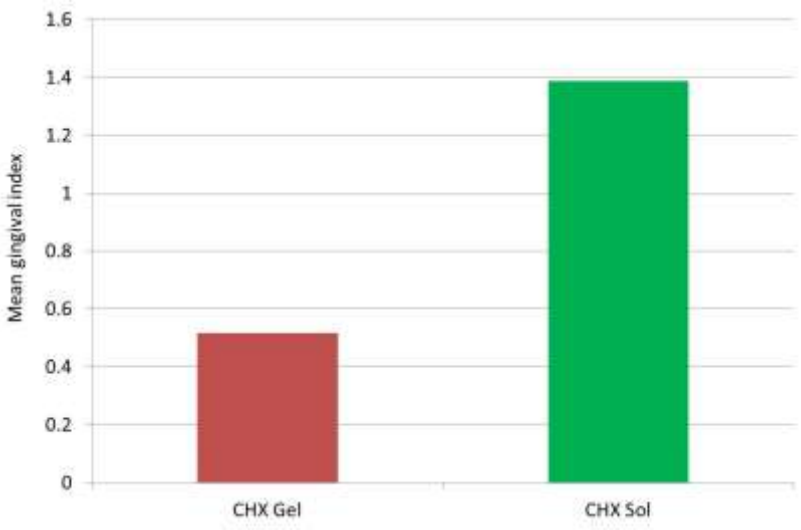
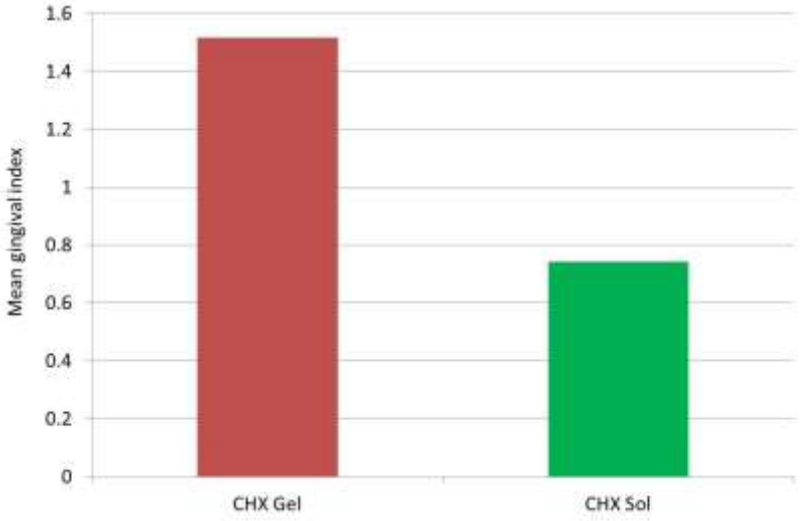


Table 3: Difference in reduction of gingivitis between chlorhexidine gel and chlorhexidine solution using Independent T test. (Baseline mean value- 2 week mean value)

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	1.5161	.76902	.77419	.42855	1.11984	4.488	**.000 (HS)**
CHX Sol	.7419	.57548					



Difference in reduction of gingivitis **(Baseline mean value- 2 week mean value)**

Table 4: Comparison of baseline plaque index between chlorhexidine gel and chlorhexidine solution using Independent T test.

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	2.2581	.77321	.06452	-.31106	.44009	.344	.732 (NS)
CHX Sol	2.1935	.70329					

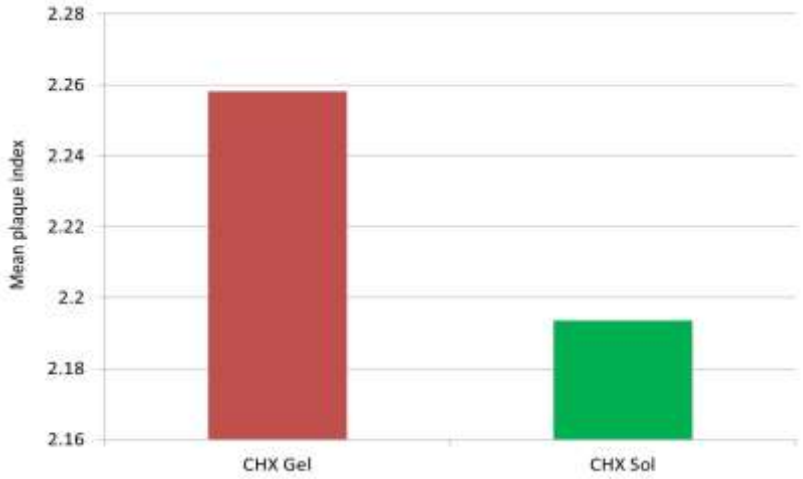


Table 5: Comparison of plaque index after 2 weeks between chlorhexidine gel and chlorhexidine solution using Independent T test.

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	.6129	.49514	-1.03226	-1.28161	-.78291	-8.281	**.000 (HS)**
CHX Sol	1.6452	.48637					

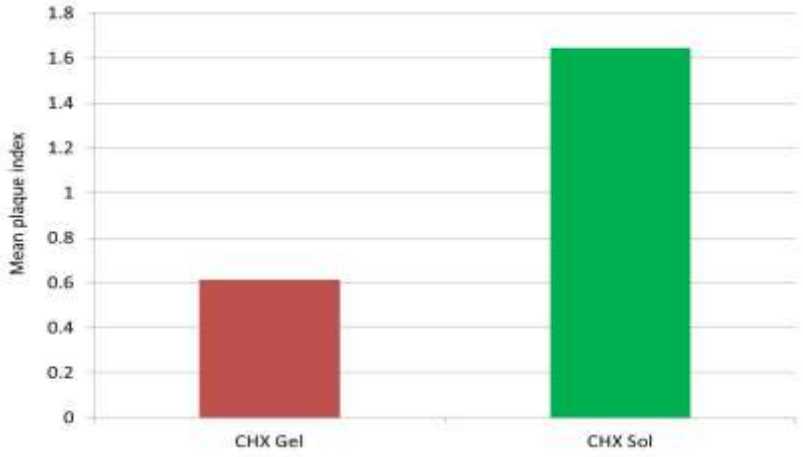
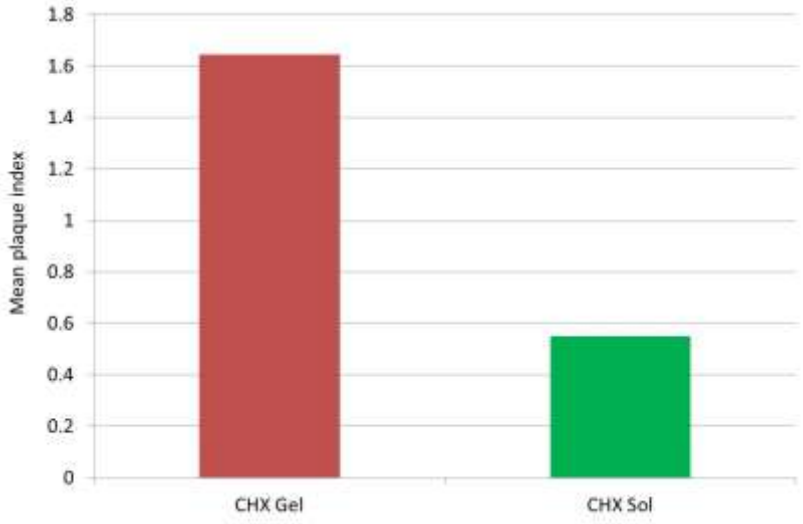


Table 6: Comparison of plaque index between chlorhexidine gel and chlorhexidine solution using Independent T test. (Baseline mean value- 2 week mean value)

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	1.6452	.70938	1.09677	.78307	1.41048	7.009	**.000 (HS)**
CHX Sol	.5484	.50588					



Difference in plaque index **(Baseline mean value - 2 week mean value)**

Table 7: Comparison of baseline anaerobic colony forming unit between chlorhexidine gel and chlorhexidine solution using Independent T test.

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	157.3226	78.41700	-12.93548	-51.32895	25.45799	-.674	.503
CHX Sol	170.2581	72.58465					

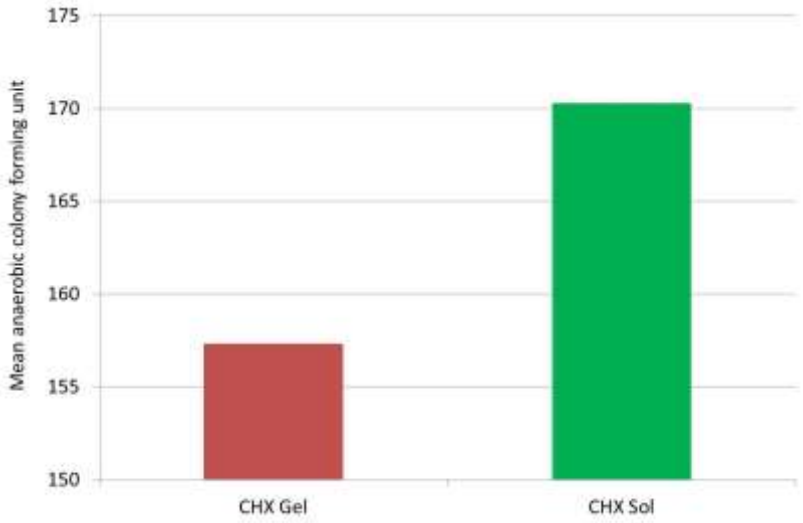


Table 8: Comparison of anaerobic colony forming unit after 2 weeks between chlorhexidine gel and chlorhexidine solution using Independent T test.

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	95.9032	56.31658	-48.67742	-79.40604	-17.94880	-3.170	**.002 (S)**
CHX Sol	144.5806	64.33443					

S = Significant ($p<0.05$)

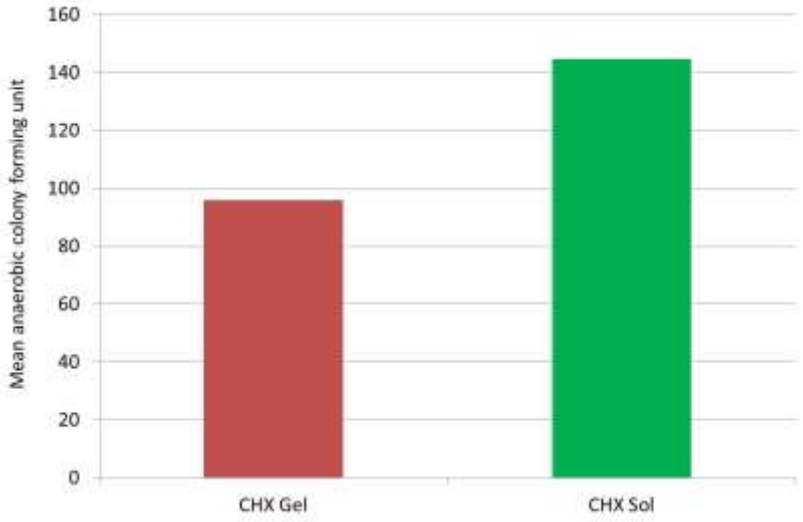
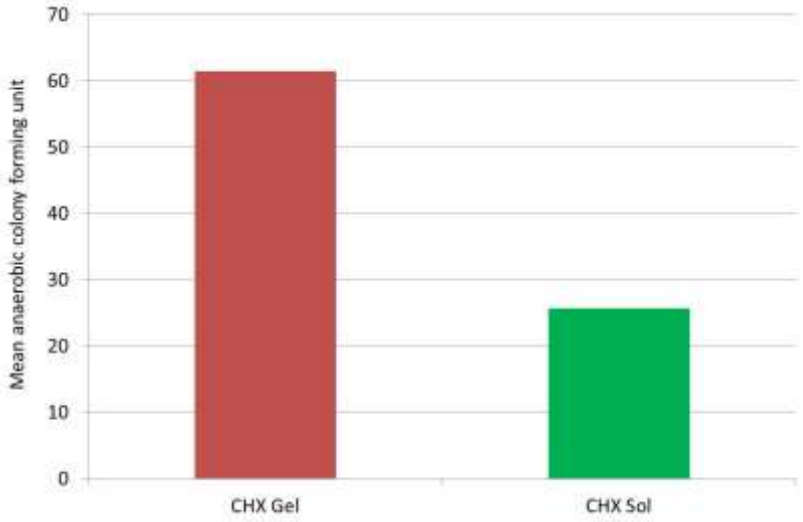


Table 9: Difference in anaerobic colony forming unit between chlorhexidine gel and chlorhexidine solution using Independent T test. (Baseline mean value- 2 week mean value)

Groups	Mean	Standard deviation	Mean difference	95% Confidence Interval of the Difference		t	p
				Lower	Upper		
CHX Gel	61.4194	30.62219	35.74194	22.41657	49.06730	5.381	**.000 (HS)**
CHX Sol	25.6774	20.74189					



Mean gingival, plaque and anaerobic colony forming units (CFU) were recorded for both the groups. Independent t test was used to assess the changes according to treatment period. At baseline GI, PI and anaerobic colony forming units (CFU) were almost same in both the groups with no significant difference for both the index in both groups.

Changes in gingival index and plaque index according to treatment period

Gingival index and plaque index were significantly decreased in both the test group (CHX gel) and control group (CHX solution) after 2 weeks **Highly significant (p<0.001).** As a result, when comparing for difference in reduction of gingivitis & plaque index, it was shown that there existed a highly significant difference in test and control groups depending on the period of treatments (Baseline mean value- 2week mean value).

Bacterial changes pre-intervention and post-intervention through Anaerobic Colony Forming Units (CFU)

Anaerobic colony forming units (CFU) were found to be decreased in both the groups after 2 weeks period, but a significant difference was found in test group (CHX gel) in reduction of anaerobic colony forming units (CFU) as compared to control group (CHX solution) after 2 weeks **significant (p<0.05).**

DISCUSSION

Chlorhexidine is considered as gold standard agent in effectively relieving gingivitis and reducing dental plaque. However, when used as mouthwash, it has shown certain side effects like taste perturbation, tooth discoloration, oral ulcerations, unilateral or bilateral parotid swelling.[7] Thus, despite of mouthwash being effective in reducing bacterial load in the mouth it cannot be prescribed on long term basis. On the contrary, newer Chlorhexidine formulation in gel and varnish form have shown superior antibacterial activity with no reported side effects.

In dental literature, CHX gel has been effectively used in dental treatment at various concentrations such as 0.2, 1, and 2%. 1% CHX gel is mainly applied for periimplantitis mucosititis treatment and plaque formation prevention.[8,9] A study done by Wang et al suggested that 2% CHX gel is an effective root canal disinfectant.[10] According to Haraji et al, 0.2% CHX gel was reported to be effective in controlling dry socket prevention in third molar surgery.[11]

The result of the clinical trial showed that, the CHX gel group showed statistically significant higher effect in GI reduction after 2 weeks than mouthwash group, which indicates that CHX gel has a long-term beneficial effect in reducing gingival inflammation. This difference is attributed to the fact that gel formulation has a viscous nature and is adsorbed onto the dental tissues and is gradually released over prolonged period of time at the treatment area whereas mouthwash washes off easily.

Similar results were seen in studies done by Vadiati et al[12] in 2017, who observed that injecting CHX gel with scaling and root planning resulted in a greater improvement in periodontal clinical indices than SRP alone. A study done by Asbi et al reported that a single application of 1% chlorhexidine gel reduced inflammation and interleukin -1β levels in the peri-implant soft tissue.

In the present study while evaluating the PI reduction after 2 weeks, there was significantly higher reduction in plaque score in CHX gel than in mouthwash group. This results were in accordance with study done by Slot et al 2010, who concluded that, with three day non brushing research design, 1% CHX gel application via trays were more effective than 0.12% CHX dentifrice gel in inhibiting plaque accumulation.[9] The significant reductions observed in plaque and gingival scores from the results of this study are consistent with the results observed by Vinholis[14] when they evaluated the effect of subgingival irrigation with a 0.2% chlorhexidine gel in periodontal pockets as an adjunct to scaling and root planning.

The most common microorganisms for causing gingivitis were aerobic bacteria but if the gingivitis is left untreated it progresses to periodontitis and thus there is a shift from aerobic to anaerobic bacteria. Thus, in this study assessment of anaerobic colony forming units (CFU) was performed to assess the effect of CHX formulation to see the effect on decrease in bacterial load. Both CHX solution group and CHX gel group showed statistically significant decrease in anaerobic CFU. However, anaerobic colony forming units (CFU) score was statistically significantly decreased in CHX gel group after 1 week compared to CHX solution group.

A study done by Manthena et al[15] to compare the effectiveness of CHX varnish and gel and concluded that subgingival application of highly concentrated CHX varnish and gel following SRP is beneficial in reducing microbial count in moderate to deep pockets. According to Paolantonio et al[16] xanthan-based chlorhexidine gel can be used during scaling and root planning with better clinical and microbiological outcomes.

Various studies have suggested that CHX gel is an effective medication due to its broad antimicrobial spectrum. According to De Siena[17] et al, 1% chlorhexidine gel is beneficial in treatment of peri-implant mucositis. Heitz Mayfield et al[18] stated that chlorhexidine gel can be useful against mucositis after non-surgical debridement. According to Rusu et al[19] both soluble chlorhexidine gel and gingiva adhering chlorhexidine gel present an improvement of clinical parameters after scaling and root planning compared to no topical therapy. One of the significant advantage of this study was that higher patient acceptance was seen while delivering the CHX gel via periotrays as it was convenient to use and the gel thoroughly distributed over the gingival surface.

In recent years, the use of CHX has become more and more common in various medical fields. In particular as proven from the study, CHX gel which has a long-lasting effect, can be actively used in dental clinics.

Limitations of the study

It is difficult to generalize the study results due to small number of subjects in a particular group. However, being a short-term study, there were no subjects who complained about side effects of the CHX gel during the study period but the results can be used as a baseline data for future studies with similar study design.

CONCLUSION

This study confirmed that 1% CHX gel has long lasting antibacterial activity and has been shown to reduce gingival inflammation and bacterial count more than 0.12 % CHX solution. Therefore, the 1% CHX gel is expected to be actively used for non-surgical treatment of gingivitis patients.

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