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Measuring Chlorhexidine Tooth Staining and Efficacy of Hydrogen Peroxide Mouthwash in Common Whitening Procedures

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Abstract

Objective: Chlorhexidine (CHX) mouthwash exerts a broad antibacterial spectrum against Gram-positive, Gram-negative bacteria, and yeasts but is not without its adverse effects, with staining being a prevalent side effect. This study aimed to determine the extent of discoloration induced by CHX mouthwash and investigate the potential impact of hydrogen peroxide mouthwash in mitigating this staining effect.

Methods: This study was a double-blind, randomized two group parallel experiment, using a 14-day non-brushing half-mouth model. Patients with mild to moderate gingivitis requiring treatment with chlorhexidine mouthwash referred to the periodontology clinic of Urmia University of Medical Sciences. The test group was randomly assigned to the mixed 0.12% CHX and 1.5% H2O2 mouthrinse, whereas the control group used 0.12% CHX. The patients received scaling and polishing 2 weeks prior to the experiment and then rinsed with the allocated mouthrinses twice daily for 2 weeks. The extent and intensity of stain scores were evaluated and recorded by a calibrated investigator.

Results: Thirty subjects completed the study (CHX + H2O2 n=15/CHX n = 15). There were more females than males in both groups (females: CHX: 60%, CHX + H2O2: 53.3%) with mean age of was 21.3 ± 2.3 years old. A significant decrease (Mean ± SD) in staining scores was observed in both the gingival (0.245 ± 0.35 vs. 0.694 ± 0.50, P=0.001) and body (0.178 ± 0.32 vs. 0.501 ± 0.48, P=0.001) regions of the CHX/H2O2 group compared to the CHX group. However, the extent staining did not differ significantly (CHX 0.61 ± 0.34 vs. CHX + H2O2 0.62 ± 0.31, p = 0.938) between groups. A significant reduction in staining extent was also observed in both the gingival (0.311 ± 0.43 vs. 1.056 ± 0.87, P=0.001) and body (0.189 ± 0.33 vs. 0.656 ± 0.74, P=0.001) regions of the CHX/H2O2 group compared to the CHX group.

Conclusions: In the absence of oral hygiene practice, the mixed CHX + H2O2 mouthrinse was slightly superior in reducing stain scores and stain with CHX alone.

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Introduction

Gingivitis, a localized inflammatory process affecting the gingival tissues [1], can advance into periodontitis if left untreated or in predisposed individuals [2]. Periodontal disease, a prevalent and historically established infectious disorder within the oral cavity, is a leading cause of tooth loss [3]. Mechanical plaque removal, primarily through toothbrushing and interdental cleaning, represents the

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primary preventive approach [4]. However, a comprehensive review indicates that solely mechanical methods may not adequately prevent periodontal disease development or recurrence in a subset of the population [5]. In contrast, the utilization of oral hygiene products, particularly mouthrinses containing chemical plaque control agents, has been demonstrated to produce statistically significant reductions in gingival bleeding, inflammation, and plaque indices [6].

Chlorhexidine (CHX) mouthwash, a cationic bisbiguanide, exerts a broad antibacterial spectrum against Gram-positive, Gram-negative bacteria, and yeasts [7]. Its high substantivity allows it to adhere to oral soft tissues and teeth, releasing its active form over an extended period [8]. Daily rinsing with a 0.2% CHX mouthwash for 4 to 6 weeks consistently demonstrates a reduction in gingivitis symptomatology [9]. However, its impact on pre-existing oral microbiota is limited, with no significant bacterial alterations observed after 48 hours of application [10]. CHX is commonly employed for short-term use in conjunction with standard oral hygiene practices, before periodontal surgery, or during the post-surgical healing phase to minimize mechanical brushing trauma [11].

Despite its therapeutic benefits, chlorhexidine (CHX) mouthwash is not without its adverse effects, with staining being a prevalent side effect [12]. This extrinsic brown discoloration can develop on teeth, prostheses, composite restorations, and even the tongue within a short period of CHX usage [12]. To address this staining concern, incorporating 1.5% hydrogen peroxide (H2O2) into CHX mouthwash has been explored [13]. H2O2 exhibits antimicrobial activity by releasing nascent oxygen, effectively targeting both Gram-positive and Gram-negative microorganisms [14]. Previous research has established the ability of H2O2 to whiten dental stains [15, 16], while others have demonstrated that hydrogen peroxide mouthwash does not impair the plaque-reducing and anti-inflammatory properties of chlorhexidine [17]. Accordingly, this study sought to determine the extent of discoloration induced by CHX mouthwash and investigate the potential impact of hydrogen peroxide mouthwash in mitigating this staining effect.

Materials and Methods

After the Research Ethics Committee approval of Urmia University of Medical Sciences (Approval no. IR.UMSU.REC.1396.87.) and obtaining written informed consent from patients, the study employed a randomized, double-blind, parallel-group clinical trial with a 14-day experimental period utilizing a half-mouth non-brushing model. Patients with mild to moderate gingivitis requiring treatment with chlorhexidine mouthwash referred to the periodontology clinic of Urmia University of Medical Sciences. Participants were randomly assigned to either a control group rinsing with 0.12% CHX or a test group rinsing with a combination of 0.12% CHX and 1.5% H2O2. The inclusion criteria were individuals with at least 12 anterior teeth, mild to moderate gingivitis with BOP ranging from 10 to 30%, and no history of allergy, smoking, pregnancy, orthodontic appliances, movable prostheses, or non-removable pigment. Participants were excluded if they experienced hypersensitivity, severe irritation, discomfort, or failed to adhere to the experimental protocol. All participants received written information about the mouthwashes and the study's aims before consenting to participate.

Clinical examination

To assess the level of tooth surface staining, the Lobene Color Index [18] was employed, a tool for evaluating the severity and extent of extrinsic discoloration on the facial surfaces of anterior teeth. The facial surfaces of the teeth were categorized into two distinct areas: gingival and trunk (Figure 1). The gingival area consisted of a 2-mm crescent-shaped band along the facial surface, stretching from the free gingival margin to the distal edge of the adjacent interdental papilla. The trunk region comprised the remaining portion of the tooth's facial surface.

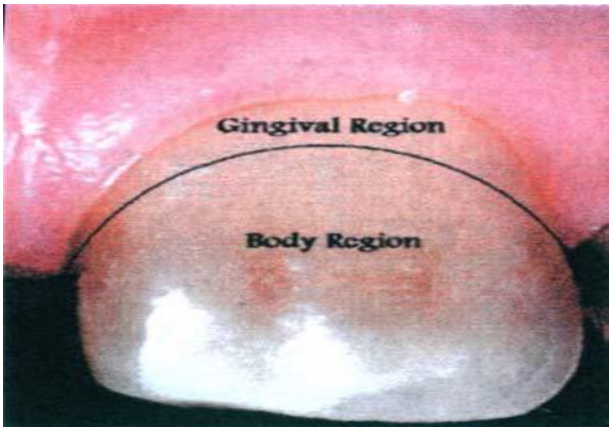

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Figure 1. *The division of the tooth surface based on the stain index provided by Lobene*

The intensity and extent of the color of the trunk area and the gum area are graded separately, the degree of tooth color ability is divided from zero to three, which is as follows: zero: no color. 1: Light color (yellow to light brown or gray). Two: medium color (medium brown). Three: dark color (dark brown to black) (Figure 2).

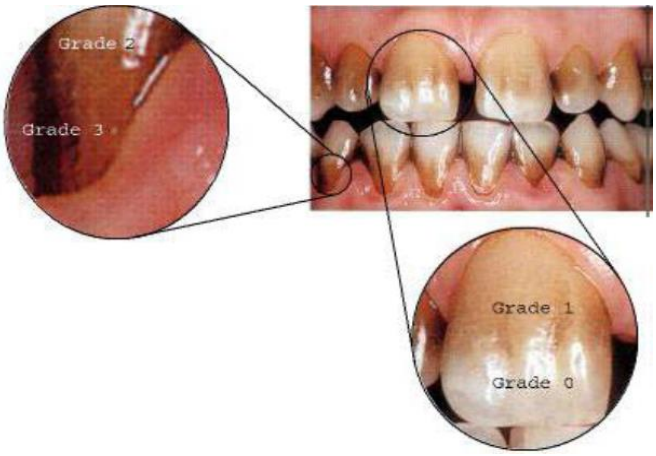

Figure 2. *Color intensity according to Lobene color index*

The staining extent was categorized as score 0 = no stain (color has not covered an area), 1 = stain over a third of the region, 2 = stain over two thirds of the region, and 3 = stain over more than two-thirds of the region. (Figure 3)

Figure 3. *Distribution of color intensity and extent according to Lobene color index*

Gradings are averaged separately for color intensity (sum of all color intensity grades/total graded areas) and for color width (sum of all color breadth grades/total graded areas).

Experimental design

Initial Visit: At the outset of the study, all subjects received comprehensive oral prophylaxis, involving scaling, polishing, and root planing. Subsequently, participants were educated on the prescribed mouthwash usage for a two-week period. Following this, subjects were randomly assigned to either the control or test group (n=30) via randomization procedures. During this interval, individuals were instructed to refrain from using any mouthwash. Second Visit: After two weeks, participants were recalled for assessment by the same examiner. The Lobene Color Index was employed to evaluate the severity of tooth surface staining.

Intervention

At the start of the study, participants were provided with two amber glass bottles of mouthwash (labeled A and B), each corresponding to their assigned group, and a 15-ml measuring cup. The control group performed 60-second rinses with 15 cc of 0.2% chlorhexidine mouthwash twice daily (morning after breakfast and evening before bedtime). The experimental group followed the same regimen for 0.2% chlorhexidine mouthwash, but then rinsed for an additional 60 seconds with 15 cc of 1.5% hydrogen peroxide mouthwash. Participants used Chlorhexidine gluconate 0.2% mouthwash from Vi-One brand and Nanosil hydrogen peroxide mouthwash. Rinsing with water was not permitted following mouthwash usage. However, if food became lodged between teeth during the experimental period, participants were permitted to floss.

To evaluate compliance, all bottles were required to be returned at the next visit. Participants were also provided with a rinsing diary to record the time of each mouthwash session. If any participant experienced hypersensitivity, severe irritation, or discomfort during the study period, they were instructed to immediately stop mouthwash usage, report the side effect to the investigators, and be excluded from the study.

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Third Visit:

After two weeks, participants returned for the final assessment. The blinded examiner repeated the clinical measurements, and the teeth were cleaned and polished. Additionally, participants were interviewed regarding any side effects experienced, including taste disturbances, tongue numbness, or irritation.

Statistical Analysis

The statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The descriptive analysis of the demographic data is presented as mean ± standard deviation (SD) for the quantitative variables, whereas qualitative data are presented as frequency analyses and percentages. The normal distribution of clinical measurements were assessed using the One-Sample Kolmogorov-Smirnov Test. For the clinical measurements is presented as mean ± SD to evaluate the efficacy of two mouth rinses. The age and sex distribution between the two studied groups were compared using independent sample t-test and chi squared test respectively. The differences within each group were assessed by Mann-Whitney Test. the Significant differences were defined as a =0.05.

Sample size calculation:

According to the study by Rahmani et al. [19], the mean and standard deviation of the width in the gingival area are 2.68+0.51 and 2.11+0.56, respectively, with a confidence of 95% and a test power of 80 %, according to the bellow formula. The number of 15 samples was estimated for each group.

$$n = \frac{(1.96 + 0.84)^2 * (0.51^2 + 0.56^2)}{(2.68 - 2.11)^2} = 14$$

Results

Thirty patients participated in this study that, 15 participants was in the control group (CHX) and 15 were in the test group (CHX + H 2 O2). Twelve teeth have been studied for each person (Fig. 1). The subject demographics in the two groups were not statistically significant (Table 1). The mean age of the subjects was 21.3 ± 2.3 years old (range 20–30 years old). There were more females than males in both groups (females: CHX: 60%, CHX + H2O2: 53.3%). No significant difference was observed in gender distribution between two groups. The mean (SD) age of CHX and CHX/H2O patients was 39.93 (10.58) and 39.29 (10.06) years, respectively. The age distribution of two groups was similar and not statistically significant (P=0.86)

Table 1. comparison of age and sex distribution between two studied groups

Groups Variables	CHX n=15	CHX/ H 2 O n=15	P value
Gender (%) male	6(40)	7(46.7)	0.71*
female	9(60)	8(53.3%)	
Mean (SD) age years	39.93(10.58)	39.29(10.06)	0.86**

*chi-square test, **independent sample t-test

Table 2 compares the severity and distribution of tooth surface staining in the gingival and body regions between the two study groups. A remarkable decrease (Mean ± SD) in staining scores was observed in both the gingival (0.245 ± 0.35 vs. 0.694 ± 0.50, P=0.001) and body (0.178 ± 0.32 vs. 0.501 ± 0.48, P=0.001) regions of the CHX/H2O2 group compared to the CHX group. These reductions were statistically significant.

A significant reduction in staining extent was also observed in both the gingival (0.311 ± 0.43 vs. 1.056 ± 0.87, P=0.001) and body (0.189 ± 0.33 vs. 0.656 ± 0.74, P=0.001) regions of the CHX/H2O2 group compared to the CHX group. This reduction was also statistically significant.

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Table 2. *Comparison of Gingival / Body region severity and Extent of the gingival/Body region between two studied groups.*

	CHX (n=15)		CHX/ H 2 O (n=15)		P value *
Indexes	Mean (SD)	Median (Min ,Max)	Mean (SD)	Median (Min ,Max)	
Gingival region severity	0.694(0.50)	0.67 (0.5-1)	0.245(0.35)	0.40 (0-0.83)	0.001
Body region severity	0.501(0.48)	0.50 (0.42-0.67)	0.178(0.32)	0.17 (0-0.58)	0.001
Extent of the gingival region	1.056(0.87)	1.17 (0.58-1.58)	0.311(0.43)	0.33 (0-1.33)	0.001
Extent of the body region	0.656(0.74)	0.58 (0.5-1)	0.189(0.33)	0.17 (0-0.83)	0.001

* Mann-Whitney U test

Discussion

Despite being widely regarded as the benchmark for plaque and gingivitis control in conjunction with conventional oral hygiene practices, chlorhexidine gluconate (CHX) has been linked to several potential local side effects, including staining, supragingival calculus buildup, oral lesions, and taste disturbances [20, 21]. To address these concerns, this randomized, double-blind, parallel-group trial investigated the impact of a CHX + H2O2 mouthwash relative to a CHX mouthwash on the prevention of staining and gingivitis.

One of the potential adverse effects of using chlorhexidine gluconate (CHX) mouthwash is discoloration of the teeth and a burning sensation. This discoloration can be visually unappealing and, when paired with an unpleasant sensation, may deter patients from using the mouthwash and reduce their adherence to its use [22, 23]. The current study revealed that the combination of chlorhexidine and hydrogen peroxide (H2O2) mouthwash resulted in significantly reduced staining intensity and extent compared to CHX mouthwash alone in both gingival and body regions. This finding corroborates the findings of a systematic review [24] that suggested that combining CHX with an oxidizing agent, such as H2O2, can effectively mitigate staining. This reduction is likely due to the inhibitory effect of H2O2 on CHX-induced staining. In contrast, Sarembe et al. [25] proposed that CHX discoloration arises from exposure to ferric and stannic sulfides present in food and beverages. In an oxidized environment, free radicals released from H2O2 break down the electron-rich alkene double bonds, converting sulfide compounds into water-soluble sulfates, which appear grayish/white. Similar results were observed in a study by Jhingta et al., who demonstrated that a combination of chlorhexidine and peroxyborate (CHX + PER) resulted in less staining than CHX alone after 14 consecutive days of rinsing [26].

The experimental group rinsed with 0.2% CHX mouthwash for 60 seconds, followed by 1.5% H2O2 mouthwash for an additional 60 seconds twice daily. This regimen aligns with previous studies that prescribed alternating rinsing with CHX and OA mouthwashes [19, 27]. An alternative approach, involving a mixture of CHX and H2O in a single rinse, has also been investigated [28].

Beyond staining, common CHX side effects, including tongue numbness and taste disturbances [29], were observed with similar frequency in both study groups. Taste impairments were more prevalent at higher CHX concentrations [30]. While H2O-related oral irritation was more frequent, most participants experienced mild symptoms that did not significantly interfere with their daily activities. Two individuals withdrew from the study due to severe burning sensations and gingival desquamation in the initial days of rinsing.

Due to the need to mitigate the well-documented adverse effects of chlorhexidine gluconate (CHX), such as a bitter taste and staining, commercially available CHX mouthwashes are now available in lower concentrations, including 0.12%, 0.1%, 0.05%, and 0.06%. These lower concentrations are an

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alternative to the more commonly used 0.2% CHX mouthwash prevalent in Europe. Nonetheless, the data presented in the present study support the effectiveness of a CHX and H2O2 combination mouthwash in reducing staining, regardless of the CHX concentration utilized [31]. While CHX concentration does not seem to significantly impact efficacy, the dose appears to be a crucial determinant of mouthwash performance [32, 33]. An optimal CHX dose of about 20 mg twice daily is generally regarded as balancing efficacy with local side effects and user acceptability [10]. Concentrations of 0.12% CHX can be equally effective as 0.2% CHX if the rinse volume is increased from 10 ml to 15 ml, delivering a dose of 18 mg per rinse [34]. In contrast, a study by Najafi et al. demonstrated that higher concentrations of CHX were associated with more pronounced tooth discoloration [35].

Limitations

The present study has certain methodological constraints. The recruitment of only gingivitis patients limits the applicability of the findings to this particular patient population. Further, reliance on returned bottles for compliance assessment may not accurately reflect actual compliance behavior. Additionally, the study duration of two weeks was relatively brief. Prolonging the study to one month in future studies would be beneficial; however, it is recognized that clinical improvements can be discerned within two weeks of initiating a gingivitis-related intervention protocol.

Conclusion

The inclusion of the oxidizing mouthwash **to CHX** resulted in a remarkable reduction in the proportion of stained surfaces. This suggests that the combined approach can effectively accelerate the elimination of extrinsic tooth discoloration.

Statements and Declarations

Funding support:

The authors did not receive support from any organization for the submitted work

Competing interests:

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval:

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Urmia University of Medical sciences (No. IR.UMSU.REC.1396.87).

Consent to participate:

Informed consent was obtained from all individual participants included in the study.

Author contributions:

R S: Conceptualization, the original draft writing, investigation, writing including reviewing and editing and investigation and formal analysis; A M: Conceptualization, supervision, and project administration

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