# In vitro evaluation of the effect of chlorhexidine mouthwash concentration(0.12, 0.2) and the presence of Anti Discoloration System (ADS) on the tooth discoloration

***Original Article***

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## Abstract

### Introduction:

Chemical control of plaque is performed in a variety of pharmacological ways, including the administration of biguanides. Among these bisbiguanides, chlorhexidine is the most common and well-known type of them. The results of different studies on tooth discoloration due to chlorhexidine use are different. The aim of this in vitro study was to compare the effect of concentration and presence of anti-discoloration system in chlorhexidine mouthwashes on tooth discoloration.

### Materials and methods:

This experimental study was performed in-vitro on 32 anterior teeth extracted from patients referred to Guilan University of Dental school, regardless of age and sex. We organized 4 groups including Chlorhexidine group with 0.2% Curasept anti-discoloration system, chlorhexidine 0.12%, chlorhexidine 0.2% and water as negative control. The baseline colors and after treatment colors were recorded by using Vita Easyshade V spectrophotometer and eventually ΔE was calculated.

### Results:

The mean coloration intensity of chlorhexidine group with ADS, was 2.10 ± 1.43, group of 0.20% chlorhexidine was 2.42 ± 1.09 and 0.12% chlorhexidine was 3.25 ± 1.15 and water was 2.87 ± 1.28. There was no statistically significant difference between all the groups (p = 0.295).

### Conclusion:

There was no difference between the two concentrations and the presence of anti-discoloration system in chlorhexidine mouthwash. None of the mouthwashes changed the color of the teeth significantly.

### Key words:

***• Color changing • Chlorhexidine mouthwash • Anti-discoloration system.***

## Introduction

Dental plaque is a common microbial biofilm which its existence depends on the host oral hygiene (1). Since the role of microbial plaque in pathology of dental caries and periodontal diseases has been proven and mechanical plaque control methods have some limitations, chemical plaque controls systems have also been proposed. Using mouthwashes can be an effective auxiliary method along with mechanical methods to reduce dental plaque (2, 3). Chlorhexidine is the most commonly used mouthwash and it is known as gold standard in chemical plaque control systems (4). The effect of this molecule in reducing microbial plaque and microorganisms in oral cavity and improving the condition of the gums has been studied in many researches (5-7). Of all the biguanides, chlorhexidine is the most widespread and well-known molecule which has broad antibacterial properties and low toxicity. The mechanism of action of chlorhexidine is that it affects internal cytoplasmic membrane of the microorganisms and has an intense tendency to bind to mucous membranes and also viruses. Studies have shown that daily use of this mouthwash beside toothbrushes, compared to toothbrushes and dental floss causes less interproximal plaque and therefore it is used in patients with high risk of caries (8-11). However, some of its side effects, such as unpleasant odor, change in taste, burning sensation in the oral mucosa ,stains on teeth, restorative materials and tongue surface, tongue numbness, unilateral and bilateral swelling of the parotid and formation of extra supragingival calculus, has led researchers to study various aspects of this mouthwash (12). Chlorhexidine is used in dentistry as a solution (water + chlorhexidine) in various concentrations (0.2 and 0.12%) or as a gel (1 and 0.5%) or more recently in tablet or spray form. One of the most common side-effect is the appearance of yellow-brown coloured stains that turn blackish on the back of the tongue and on the teeth. The mechanisms that determine the dental discoloration caused by the chlorhexidine are still being disputed. The tests available indicate the causes of staining to be: non-enzyme browning, also known as the Maillard reactions, or processes of condensation and polymerization of carbohydrates, peptides and proteins, that causes the formation of brown-staining substances, which are known as melanoidins; the deterioration of the chlorhexidine molecule in order to release parachloranilin; the protein denaturing with the formation of metal sulphides; and also reaction between the adsorbed chlorhexidine and the chromogens present in food and drink (tea, coffee and soft drinks) including polyphenols. It is now claimed that a chlorhexidine (Curasept) with anti-discoloration system can reduce teeth stains. The ADS system means a formulation that, according to the manufacturer, has been found to be optimal in preventing the main staining reactions that can occur after using mouthwashes containing chlorhexidine. This formulation was obtained by adding two active principles, sodium metabisulphite and ascorbic acid. According to the manufacturer, one of the components of the ADS (patented system) reacts with di-actos-amine, and stops the production of melanoidin by elimination of the di-actos-amine. (11) Since the duration of use, the number of uses in a day, the time of use and the concentration of the mouthwash have been influential factors in several studies, so in this study we wanted to examine two concentrations of chlorhexidine mouthwash (0.2 and 0.12%) and the presence of an anti-discoloration system with the recommended time and number of uses to control caries in high-risk patients (once daily for 30 seconds and for 2 consecutive weeks) (13).

## Materials and Methods

This study was performed experimentally , in-vitro on the human anterior teeth that were extracted due to periodontal disease from patients referring to the dental department of Guilan University of Medical Sciences, regardless of age and sex. Among the teeth collected and stored in 10% formalin, 32 central incisor teeth without any caries, cracks, anomalies, and discoloration caused by internal or external factors were selected. One week before the start of the examination, the samples were cleaned of any soft tissue and debris using a dental scaler and placed in distilled water. The roots of the teeth were covered with two layers of nail polish and then divided into four groups of 8: Group 1: Teeth receiving Curasept mouthwash which contains 0.2% chlorhexidine

digluconate and anti-discoloration system. Group 2: Teeth receiving Behsa chlorhexidine mouthwash with 0.12% concentration. Group 3: Teeth receiving Behsa chlorhexidine mouthwash with 0.2% concentration. Group 4: Teeth placed in water as negative control.

To make all samples recognizable, the teeth were marked using color coding system along the roots (red color stands for Curasept 0.2% chlorhexidine mouthwash, orange stands for Behsa 0.2% chlorhexidine mouthwash, blue stands for Behsa 0.12% chlorhexidine mouthwash, and finally green stands for Water). In addition, the code for each tooth was engraved on the root using a round bur. Then baseline colorimetric analysis of samples, were done by Vita Easyshade V spectrophotometer.

Color evaluation was done, with a standard white background, the device was placed at a 90-degree angle to the coronal middle third of each tooth, and the L, a, and b parameters associated with that tooth, were recorded. After recording each tooth color, the device was recalibrated and then another tooth was assessed.

The teeth were then placed in closed containers and treated with different concentrations and types of chlorhexidine and water for two consecutive weeks for 30 seconds in each day.

After each time, the teeth were rinsed with water and a toothbrush for about 2 minutes and placed in distilled water until the next time. The distilled water in which the teeth were kept was replaced daily. After two weeks and doing the cycles for 14 times, the samples` colors were re-measured under the same conditions and by the same operator by spectrophotometer and their color changes were compared before and after treatment. Finally, using the formula $\Delta E=\sqrt{(\square \Delta L\square ^2+\square \Delta a\square ^2+\square \Delta b\square ^2}$ ) the amount of color change (ΔE) was calculated (13-16).

## Results

The amount of discoloration (ΔE) caused by placing teeth in chlorhexidine solutions and water is given in Table 1. The mean discoloration intensity of chlorhexidine group with ADS, was 2.10 ± 1.43, group of 0.20% chlorhexidine was 2.42 ± 1.09 and 0.12% chlorhexidine was 3.25 ± 1.15 and water was 2.87 ± 1.28. The normality of the data was assessed using the Shapiro Wilk test and also data homogeneity was assessed using the Levene test. All the studied groups had normal distribution. Homogeneity of variance within groups was confirmed by Levene test ($p = 0.681$, Levene = 0.506).The ANOVA test did not show a significant difference in the mean color change between the groups of study ($p = 0.295$) (Table 1).

Table 1. Comparison of the mean ΔE in the four groups

| Group | Mean(SD) | P value |
|---|---|---|
| ADS | 2.10 ± 1.43 | 0.295 |
| 0.12 CHX | 3.25 ± 1.15 | |
| 0. 2 CHX | 2.42 ± 1.09 | |
| Water | 2.87 ± 1.28 | |

## Discussion

Chlorhexidine is considered to be one of the most common antiplaque and antigingivitis agents, which is gold standard to compare with other antiplaque agents. Chlorhexidine is also a bisbiguanide cationic molecule with broad antibacterial properties and low toxicity.

In the present study, the effect of two concentrations of chlorhexidine mouthwash (0.2 and 0.12%) and the presence of anti-discoloration system (ADS) on the amount of discoloration of extracted teeth was investigated in vitro.

tooth color evaluation is performed using several methods such as spectrophotometry, colorimetry and computer image analysis (7). In the present study, a spectrophotometer was used to measure and record samples colors and their changes after examination. In a study by Marcus RT (17), the accuracy of spectrophotometer was reported 82% and it was beyond other methods. The advantages of this device over other subjective measurements, is the ability to measure and record the light transmitted from the tooth more accurately and objectively.

The results of this present study showed that the use of chlorhexidine mouthwash with 0.2% and 0.12% concentration and also chlorhexidine containing ADS did not cause significant color changes compared to the control group (water).

This is in agreement with the study of Maleki et al. (18) who examined the effects of common mouthwashes on the color stability of teeth whitened with 38% hydrogen peroxide, and showed that the use of chlorhexidine mouthwash (twice a day and 1 minute each time), does not lead to color changes in the teeth after 3, 7 and 14 days usage, compared to keeping the teeth in the water.

However, in some studies, discoloration of teeth (yellow-brown stains) is known as the most common complication of chlorhexidine (19, 20).

However Chlorhexidine staining potential has also been reported to be dependent on its concentration (11).

In the study of Abed et al. (21), there was no difference between chlorhexidine mouthwash with 0.2% and 0.12% concentrations in terms of tooth staining in the patients using them which this result was similar to the present study that showed that there was no significant difference between the two concentrations of this mouthwash in the rate of tooth discoloration.

Contrary to the results of our study, Bernardi et al. (11) in the study which compared the intensity of anti-plaque property and tooth discoloration in patients using 0.2% chlorhexidine mouthwash with ADS to 0.2% chlorhexidine mouthwash without ADS (2 times a day, 1 minute each, 14 Day), found out that there was no significant difference between the two types of mouthwashes in terms of anti-plaque ability, while the presence of ADS reduced the intensity of tooth staining.

Reasons for discoloration of the teeth following the use of chlorhexidine include non-enzymatic browning and production of melanoidin (known as the Maillard reaction), accelerating the polymerization of carbohydrates and peptides, also denaturating proteins and the formation of metal sulfide (19, 20).

The presence of an anti-discoloration system with adding ascorbic acid and sodium metabisulphite may stop the production of melanoidin and also reduce the discoloration of teeth following using chlorhexidine mouthwash by inhibiting the formation of iron sulfide.(6)

On the other hand, Graziani et al. (22), in a study investigate the effect of 3 mouthwashes including 0.2% chlorhexidine with and without alcohol and 0.2% chlorhexidine with ADS in vivo (2 times a day, 1 minute, 35 days). They didn`t find any significant difference in color measures between chlorhexidine mouthwash with and without ADS , also all three mouthwashes caused significant color changes compared to the control group (normal saline).

The reasons of inconsistent results in various studies include differences in the study platform, including laboratory or clinical conditions, the length of the test period, the duration of usage in each time, the frequencies of mouthwash usage in a day, its brand, and the way of tooth washing. The chlorhexidine mouthwashes on the market may differ in terms of side effects.

The present study was performed in vitro on sound extracted teeth. With the conditions the study performed and the duration of using 0.2 and 0.12% chlorhexidine mouthwash with Behsa brand without anti discoloration system, they didn`t make a significant change in teeth`s color. It is recommended that future studies be performed in conditions closer to clinical and with more dental immersion duration and more number of times which the mouthwash is used.

## Conclusion

According to the conditions of the present study, it can be concluded as follows:

1.There was no difference between two concentrations and the presence of an anti-discoloration system in chlorhexidine mouthwash.

2.None of the mouthwashes significantly changed the color of the teeth.

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